DETERMINATION OF WHOLE-BODY PROTEIN IN SMALL LIVE ANIMALS BY IVNAA

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ABSTRACT

Measurement of nitrogen in small live animals (Weighing about 3 kg) can be used to determine the whole- body protein in the animals. Nitrogen may be measured by IVNAA in which the capture gamma rays of neutron induced are detected and counted. The neutron flux is provided by a collimated 740 GBq (20Ci) Pu- Be source. The 10.83 MeV thermal neutron capture gamma rays from ^{14}N are detected by a 12.7 cm by 10.2 NaI (Tl) detector. The nitrogen of a live rabbit was measured and recorded each day for a period of two months. The statistical error and reproducibility of measurement were around 10%. For a 1000 s irradiation the dose equivalent was 300μ Sv.

Keywords: Nitrogen Activation analysis In vivo Live animals Protein

I. INTRODUCTION

The measurement of whole- body protein in small live animals and domestic fowls lies on the measurement of body element- nitrogen by the in vivo neutron activation analysis (IVNAA) as the mass of nitrogen bears a fixed ratio to the mass of protein ($\lg N = 6.25$ g protein).

The measurement of body protein gives important information about the nutritional status. Using the in vivo technique to sdudy nitrogen in small live animals may provide the changes in protein levels in growing animals. Thus it may be applied in the scientific breeding of domestic animals. In addition it may be very useful in the diagnosis and treatment of little children or small babies for the malnourished disease in hospital.

The technique of IVNAA has been proved successful for measurement of whole body nitrogen of adult by the fast neutron reaction ^{14}N (n,2n) ^{13}N using cyclotron as neutron source. H.C.Biggin et al[1]. Mernagh et al[2] reported the determination of nitrogen in the section of a chest 20×20 cm^2 in area by the thermal neutron capture gamma rays from the reaction ^{14}N (n,γ) $^{15}N^*$ using four 185 GBq (5 Ci) ^{238}Pu- Be neutron sources. All subjects are irradiated bilaterally.

The present paper describes a facility for measurement of whole body nitrogen (i.e.protein) in small live animals (mass about 3 kg), which is approximately the bodies of only a tenth or twentieth the mass of adult.

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Ⅱ. EXPERIMENTAL METHOD

Typically, the facility for the measurement of nitrogen in the body of adult is bilaterally irradiated to improve the uniformity of neutron flux in the subject. Because non- uniformity is caused by the absorption of neutrons. However, non- uniformity in small animals is small. Thus all the neutron sources are put in a low collimator.

From the nuclear counting statistics, the fractional statistical error is given by equation $E=(S+2B)^{1/2}/S$. Where S is the net signal counts, B is the background counts. In order to reduce the fractional statistical error, the best way is to decrease the background counts by heavy shielding with borated wax and lead.

The irradiation facility is shown in Fig.1. The collimator is a block of borated wax with a funnel- shaped hole from the centre to one side. 740 GBq (20 Ci) Pu- Be sources are fixed in an aluminum pipe (2.54 cm in diameter) to run from the outside of collimator to its centre. Around the collimator some pieces of borated wax and lead are installed to prevent neutrons and γ - rays. On the top of the collimator surrounding the hole there are five layers of lead bricks and one layer of borated wax blocks. One NaI (Tl) detector was used, of 12.7 cm diameter and 10.2 cm depth. The detector is also shielded heavily by borated wax and lead to attenuate any neutrons and γ - rays scattered into the detector from the animal or other scatterers.

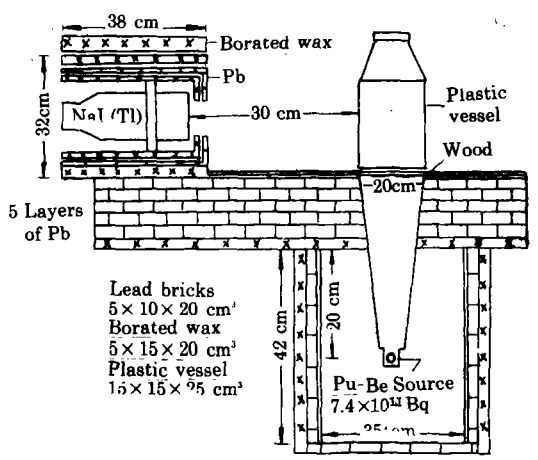

Fig.1 The irradiation facility

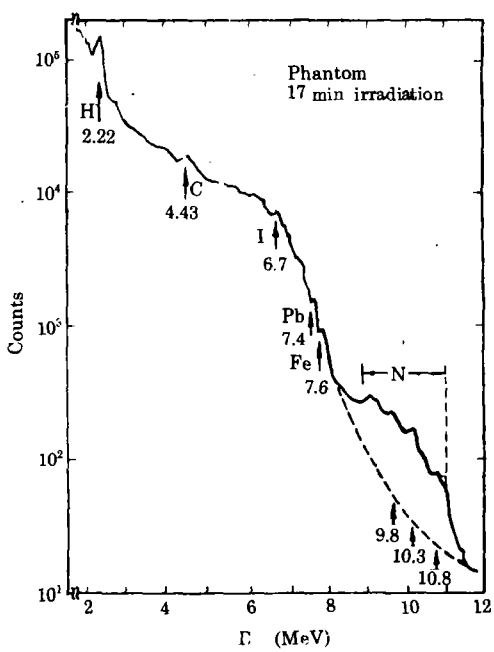

Fig.2 The gamma spectrum irradiated by neutron sources for a nitrogen phantom

A gamma spectrum was obtained for a 1000 s irradiation with 740 GBq (20 Ci)

Pu– Be neutron sources for a nitrogen phantom and this was superimposed on a gamma spectrum from the background, as shown in Fig.2.

Peaks at 2.2 MeV (hydrogen), 6.2 MeV (chlorine), 6.7 MeV (iodine), 7.4 MeV (lead), 7.6 MeV (iron) could be seen. Using these peaks in conjunction with the peaks from ^{137}Cs(0.663 MeV) and ^{60}Co (1.173 MeV and 1.333 MeV) sources, the energy window (10.1– 11.0 MeV) for the nitrogen region was obtained. For calibration, the phantoms (solutions of urea in water) were made up in volumes and shapes approximating to those of the different live animals investigated.

If the phantom with known the netrogen mass and the live animal investigated are irradiated and the γ – ray counts are measured in the same condition, we then have:

$$W_x/W_s = C_x/C_s \quad (1)$$

Where W_x is the nitrogen mass in the live animal, W_s the nitrogen mass in the phantom, C_x the net integral counts on nitrogen energy window in the live animal, and C_s the net integral counts on nitrogen energy window in the phantom.

From the equation (1) we can calculate the nitrogen mass in the live animal.

Ⅲ. RESULTS AND DISCUSSION

1. The linearity measurement of the nitrogen contents

Water phantoms (1000ml) containing various amounts of urea (nitrogen) were irradiated for the same period of time (1000 s) and counted under the identical conditions. The results are shown in Table 1. This showed that for a constant sized phantom the nitrogen net counts is almost proportional to the amount of nitrogen present.

Table 1

Linearity measurement of the nitrogen contents

Weight of urea (g)	Mass of N (g)	Net counts
600	280	338
700	327	445
800	374	733
900	420	879
1000	467	951
1200	560	1126
1400	654	1498
1600	747	1560
1800	841	1692
2000	934	1881
2200	1027	2384
2600	1214	2384
2800	1308	2582

An investigation was carried out in order to see how the net nitrogen counts vary

with the depth (or thickness of the phantom) of the urea solution. Three solutions of urea were prepared by dissolving 2000 g of urea (934 g of N) in 4000ml, 5000ml and 6000ml of water resulting in 5700ml, 6730ml and 7520ml of urea solutions respectively. The results from the 1000 s. irradiation are shown in Table 2.

Table 2

Net counts for three different volumes of the urea solution

5700 ml sol	6730 ml sol	7520 ml sol
1598	1442	1354
1563	1490	1403
1583	1517	1477
1558	1554	1463
1581	1435	1404
Average 1566±20	1488±50	1420±50

It was observed from Table 2 that the net counts decrease with increasing phantom thickness. This is due to a reduction in the thermal neutron flux as a result of the attenuation of the neutrons by the hydrogen in the water.

2. Reproducibility and accuracy of the technique

The reproducibility of the system was studied by means of phantom irradiations

Table 3

Reproducibility of net counts of phantom

1424	1404	1447	1482	1393
1432	1354	1463	1468	1581
1403	1358	1408	1421	1501
1477	1470	1482	1466	1356

Table 4

Measurement of ground beef and phantom

Background counts	Net counts (phantom)	Net counts (beef)	Mass of N (g)	% by mass of N
829	252±44	603±48	223	3.3
829	267±44	598±47	209	3.0
829	258±44	700±49	253	3.7
829	247±44	626±48	236	3.4
805	268±43	609±47	212	3.1
805	260±43	519±46	190	2.8
805	258±43	642±47	232	3.4
805	255±43	611±47	224	3.3
811	244±43	526±47	239	3.5
811	259±43	735±49	265	3.9
811	259±43	653±48	235	3.4
811	265±43	638±48	225	3.3

carried out during a two- week period. Altogether twenty irradiations of the phantom

(934 g of nitrogen) were done at different times under the same conditions of irradiation and counting. Each irradiation lasted for 1000 s. The results are shown in Table 3. The average net counts is 1440. The relative standard deviation is 4.3%. It is in consistent with counting statistics.

The accuracy of the procedure was established from analysis of a sample of ground beef weighing 6855g. The ground beef was irradiated at different times under the same conditions. Each irradiation lasted for 1000 s. The results are shown in Table 4.

The phantom was a solution of 200g of urea dissolved in 7000 ml of water, that is, of roughly the same volume as the sample.

The average mass of N in the beef is 229 g with a standard deviation of 20 g, the statistical counting error is 7.6%, the average % by mass of N in the beef is 3.3%.

Chemically, the small sample from the same ground beef was analysed in duplicate by the Microkjeldahl method (AOAC 1980)[3]. The % by mass of N in the beef is 3.01% $\pm$0.04%. The difference is well within the accuracy of the two methods.

3. In vivo measurement of nitrogen (protein)

Table 5

Contents of nitrogen in rabbit (weighing 2356g)

Background counts	Net counts (phantom)	Net counts (rabbit)	Mass of N (g)	% by mass of N
852	252±44	211±44	78.1	3.3%
808	268±43	223±43	77.6	3.3%
806	287±44	239±43	77.7	3.3%
796	298±43	246±43	77.0	3.3%
799	327±44	210±43	60.0	2.5%
824	348±45	227±43	60.9	2.6%
803	356±44	244±43	63.9	2.7%

Table 6

Contents of nitrogen in rabbit for long period (weighing 2356 g)

Background counts	Net counts (phantom)	Net counts (rabbit)	Mass of N (g)	% by mass of N
843	336±45	253±44	70	3.0
843	336±45	244±44	68	2.9
843	337±45	220±44	61	2.6
843	337±45	262±44	73	3.1
814	320±44	230±43	67	2.8
814	293±44	249±43	79	3.4
814	306±44	237±43	72	3.1
814	331±44	246±43	69	2.9
820	336±44	260±44	72	3.1
820	296±44	258±44	81	3.4
820	315±44	189±43	56	2.4
820	308±44	284±44	86	3.7

A live rabbit weighing 2356 g was measured seven times a day. Each irradiation

was lasted for 1000 s. The dose received was approximately 300 μ Sv (30 mrem) in each irradiation. The phantom was made by dissolving 200 g of urea in 4000 ml of water. The results of the experiment are shown in Table 5.

The average mass of nitrogen in the rabbit is 70.7 ± 8.6g. The average % by mass of nitrogen in the rabbit is $3.0\% \pm 0.4\%$.

The rabbit was raised in the same condition for two months, and during this period the nitrogen is measured for twelve times. The data are shown in Table 6.

The average mass of nitrogen in the rabbit is 71 ± 8g. The average % by mass of nitrogen in the rabbit is $3.0\% \pm 0.4\%$.

4. Summary

The work on the measurement of nitrogen in small live animals can probably be extended to the designing of a facility for hospitals to use Pu– Be sources to research the nutritional status of little children or small babies.

The radiation dose for the time of 1000 s is approximately 300 μ Sv (30 mrem). This is quite small and would allow the measurement for a sick child once every few days without poisoning and serious radiation hazard.

The difficulties encountered in the in vivo measurement of nitrogen are mainly due to the high background in the nitrogen region. In this work heavy shielding of both the collimator and detector provided a possible method for improving the sensitivity of measuring the nitrogen levels in small live animals.

The work demonstrates the feasibility of measuring nitrogen in small live animals (mass about 3 kg) by means of IVNAA, the statistical error and reproducibility of measurement were found to be around $\pm 10\%$.

In conclusion, in vivo neutron activation analysis of the nitrogen (protein) in small live animals may be added to the armamentarium of medical researchers in future.

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