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MASTER

RADIOISOTOPE DETERMINATION OF WHOLE BODY MAGNESIUM

BY IN VIVO NEUTRON ACTIVATION*

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1. Introduction

In vivo neutron activation analysis has been used in man for the determination of total body Ca, Na, Cl, N, and P (1-3) and in mice for the determination of total body N and P (4). This technique has been shown to be both precise and accurate in the determination of various elements in-vivo (3,4) and studies utilizing this technique can provide information as to the state of a system and the progress of this system under normal and perturbed conditions. Radioisotopic tracer techniques have also been employed in the measurement of various body elements in vivo, but they are of limited utility since the mixing of the tracer with the stable isotope may not be complete. Chemical analysis, although an acceptable method of determining total body elemental composition, is destructive in nature and cannot be used in serial studies on the same animal.

In vivo studies utilizing ^{28}Mg provide an incomplete description of the effects of endocrine and metabolic factors on Mg homeostasis and conservation since 75% of body Mg does not exchange with the injected radioisotope. Neutron activation analysis has been employed in vitro for the determination of magnesium (5) and the present study is addressed to the problem of the determination of total body Mg in vivo. The 11% of Mg which exists as ^{26}Mg is capable of being transmuted by thermal neutron irradiation to ^{27}Mg [$^{26}\text{Mg} (n,\gamma) ^{27}\text{Mg}$], a radionuclide which emits gamma rays at 0.842 and 1.01 Mev with a half-life of 9.5 minutes. The 0.842 Mev photons are emitted 70 percent of the time as opposed to 30 percent for the 1.01 Mev photons. The 0.842 Mev photons were used for detection of Mg because of the greater sensitivity which can be

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obtained. The determination of total body Mg by in vivo neutron activation analysis requires the satisfaction of three conditions. The thermal neutron flux distribution within the body must be uniform in order to achieve a uniform activation of each ^{26}Mg atom independent of its position within the body. The induced gamma activity must be measured quantitatively, the response of the detector to the induced ^{27}Mg being relatively geometry independent. A suitable correction must then be applied to the data for interfering reactions such as the $^{55}\text{Mn}(n,\gamma)^{56}\text{Mn}$

($T_{1/2} = 2.54$ hr.) which produces a 0.847 Mev gamma photon and for the Compton photon production due to the other elements activated during the procedure.

2. Irradiation Procedure and Uniform Thermal Neutron Distribution

The neutron source for the irradiation procedure is the patient port facility of the Brookhaven Medical Research Reactor (6). Thermal neutrons are used and are constantly being generated by the thermalization of fast neutrons as they pass through the phantom or rat. In order to achieve a uniform thermal flux distribution, a lucite moderator 1.5 cm. thick is placed in front of the irradiation apparatus, and the lucite cylinder containing the rat or phantom rotates in the apparatus at 2 rpm. All irradiations are monitored using gold foils placed on the center surface of the cylinder.

The uniformity of depth flux distribution was examined in a lucite rat phantom 11.2 cm long and 6.0 cm in diameter, by placing gold foils at various levels within the phantom. The determinations were made in two individual irradiations at 3 megawatts for 2 minutes and are presented

in Table 1. The uniformity of thermal flux as a function of depth was found to be ± 4.5 percent. The incident thermal neutron flux in back of the lucite moderator was measured over the 20 cm length of the cylinder and found to be ± 1 percent.

3. Measurement of the Induced ^{27}Mg Activity

The geometric response of the detector was determined using equal amounts of ^{54}Mn (0.835 Mev) in three different size polyethylene phantoms placed within the rotating cylinder. The phantoms were 13.0 cm long and 5.5 cm diameter, 10.5 cm long and 4 cm diameter and 9.8 cm long and 3.5 cm diameter, respectively. The geometric response of the detector was found to be ± 1 percent of the mean value. The linearity and sensitivity of the system was determined over a range 80-500 mg of Mg in irradiated Nalgene phantoms containing water and MgSO_4 , and counted in the rotating cylinder of the counting apparatus (Fig. 1). The response of the system to both the 0.842 Mev and the 1.01 Mev photons was determined and found to be linear (Fig. 2).

After the irradiation, the rat or phantom is removed from the irradiation facility, transferred to a non-irradiated lucite cylinder, and then placed in a rotating counting apparatus (Fig. 1) which is positioned above a 25 cm^3 Ge(Li) semiconductor detector. The axis of rotation of the counting cylinder is at a position 7.0 cm from the center of the detector and normal to the axis of the detector. The pulses from the detector pass through a linear amplifier, and then go to a biased amplifier associated with a 1024 channel pulse height analyzer*. The stored counts are printed on a teletype printer. At 4-5 minutes post irradiation, the animal or phantom is counted sequentially for 400

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*Nuclear Data Corporation

second counting intervals for the first half hour and the counting procedure is repeated at 1 hour post irradiation for an additional half hour. A Mg standard of known quantity is irradiated and counted under the same conditions for the first half hour post irradiation.

4. Calibration Procedure

Phantoms were prepared in 250 ml Nalgene bottles containing 40-240 mg of Mg together with known quantities of Mn, Na, Cl, N, and Ca, and a standard containing 240 mg without contaminants was also prepared. The phantoms and standard were irradiated and counted three times each in the sequential manner described and in addition three male Sprague Dawley rats were irradiated and counted three times to establish the reproducibility of the technique in rats. The spectrum from one of these animals in the 0.842 Mev region can be seen in Fig. 3.

The gross data for each phantom and animal in the 0.842 Mev region was plotted as a function of time and the half life of the long lived component was determined to be 125 minutes. The counts $\bar{G}_{t_n}$ at times sufficiently long to ensure complete decay of ^{27}Mg ($t > 40$ min) were extrapolated to $t = 0$ using a $T_{1/2}$ of 125 min. The average of these extrapolated counts at $t = 0$ ($\bar{G}_{t_0}$) was then used to determine the contribution of the $t > 40$ min component to the first two gross counts. This contribution was then subtracted from the first two gross counts post irradiation (GC_{t_1} , GC_{t_2}) and the corrected counts (C_{t_1} and C_{t_2}) were extrapolated to t_0 using a $T_{1/2}$ of 9.5 min, the $T_{1/2}$ of ^{27}Mg . This $T_{1/2}$ was verified in the animal and phantoms runs. ~~The average $\bar{G}_{t_0}$ of these extrapolated counts was then used for subsequent calculations.~~
(See Appendix).

The $\bar{C}_{t_0}$ count was then used together with the thermal neutron flux (ϕ_{th} in $n\text{ cm}^{-2}\text{ sec}^{-1}$) and a calibration factor (CF) to calculate the amount of Mg (m) in the contaminated phantoms and animals. The calibration factor $CF = m \phi_{th} / \bar{C}_{t_0}$ is determined from an irradiated standard containing a known amount of Mg and irradiated under the same conditions as the contaminated phantoms or animals. The counts of the standard at times t_1 and t_2 , post irradiation, corrected for background, are extrapolated to t_0 and the average of these extrapolated counts $\bar{C}_{t_0}$ then used to obtain CF. The results of the contaminated phantom irradiations are found in Table 2 and the results of the rat irradiations are found in Table 3.

5. Discussion

The thermal neutron depth flux distribution measured in the lucite rat phantom and the response of the Ge(Li) detector to various size phantoms containing equal amounts of ^{54}Mn homogeneously dispersed, were found to be suitable for total body Mg determinations. Although Mg is not dispersed homogeneously in the rat, a homogeneous distribution was simulated by the use of a rotating counting cylinder. In any case, relative serial counts on the same animal can be obtained and the errors due to inhomogeneity of Mg distribution would be relatively constant throughout such studies.

The resolution of the detection system was 4.5 Kev at 0.842 Mev and this was sufficient to resolve the 0.847 Mev photopeak of ^{56}Mn from the 0.842 Mev photopeak of ^{27}Mg . The effective T-1/2 due to all interfering elements activated during the procedure (in the channels of interest) was found to be 125 minutes and followed first order kinetics. The "goodness" of the technique was tested statistically by determining the mean and S.E. of the gross counts at $t > 40$ min. extrapolated to time t_0 post irradiation.

Since the 80, 160, and 240 mg Mg phantoms had the same amount of contaminants, the counts from these 3 phantoms normalized for thermal flux were used for the determination. The mean extrapolated count ($\bar{G}_{t_0}$) was found to be 1018 ± 23 (S.E.) for 16 individual counts.

A limitation of the technique is the sensitivity which is on the order of 6-7 counts/400 sec at t_0 /mg Mg (see Table 2). The sensitivity can be increased by using a Ge(Li) detector of larger volume or possibly using two or more detectors surrounding the counting cylinder. Alternatively, the counts can be increased by increasing the ϕ_{th} but the increased dead time of the detector system and the increased dose delivered to the animal would make this alternative less desirable than the former alternative. Under the conditions of the irradiation, with a $\phi_{th} = 6.67 \times 10^9$ n cm⁻² sec⁻¹, a whole body rad dose of about 78 rads due to fast neutrons and 42 rads due to gammas is delivered (7). Although physiological changes can occur at this dose (cataract formation, hematological changes, etc.), the use of suitable controls would allow the technique to be used serially in rats. Some rats which had been irradiated up to 6 times during the development of the technique had not shown any visible changes such as weight loss or anorexia. The reproducibility of the technique in the contaminated phantoms indicated a precision of about $\pm 16\%$ in the 40 mg to $\pm 13\%$ in the 240 mg phantoms. The amount of Mg determined in the rats was on the order of 82.9 ± 3 mg (Table 3) and the reproducibility of the technique in animals was found to be $\pm 16\%$. In the 40-160 mg Mg range of the irradiated contaminated phantoms, a difference of 20 mg Mg was observed between the actual amount of Mg added and the amount of Mg determined. This difference can be corrected for by applying a suitable factor to the observed data.

Further studies are being carried out in the 40-160 mg Mg region and will be reported subsequently.

6. Summary. Total body Mg determination in rats using in vivo activation analysis has been demonstrated to be feasible. The technique is nondestructive and multiple determinations of whole body Mg in the same rat are possible. A thermal neutron depth flux variation of $\pm 4.5\%$ was observed in a lucite rat phantom when it was irradiated rotationally in a specially designed holder apparatus. The geometric response of the Ge(Li) detector system was found to be relatively independent of geometry when examined under certain conditions. After correction of the data for a component with a $T_{1/2} = 125$ min., the corrected data yields counts with a $T_{1/2}$ of 9.5 min, the $T_{1/2}$ of ^{27}Mg . The poor sensitivity of the detection system limits the application of the technique in its present form.

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Appendix

Correction Technique for the Determination of ^{27}Mg Activity

G_{t_n} = Gross counts observed in the 0.842 Mev region at a time $t > 40$ min with a decay constant λ_g .

$\bar{G}_{t_0}$ = Average of the extrapolated counts at time t_0 .

$$\bar{G}_{t_0} = \frac{\sum_{n=1}^N G_{t_n} e^{\lambda_g t_n}}{N} \quad (1)$$

G_{t_1} = Gross count at a time t_1 post irradiation

G_{t_2} = Gross count at a time t_2 post irradiation

C_{t_1} = Net count due to Mg at t_1 with a decay constant λ_c .

C_{t_2} = Net count due to Mg at t_2 with a decay constant λ_c .

$$C_{t_1} = G_{t_1} - \bar{G}_{t_0} e^{-\lambda_g t_1} \quad (2a)$$

$$C_{t_2} = G_{t_2} - \bar{G}_{t_0} e^{-\lambda_g t_2} \quad (2b)$$

$$\bar{G}_{t_0} = \frac{C_{t_1} e^{\lambda_c t_1} + C_{t_2} e^{\lambda_c t_2}}{2} \quad (3)$$

Table 1. Depth flux distribution
in lucite rat phantom

Depth (cm.)	Position along length (cm.)		
	-3.2	0	+ 3.2
- 3.0	96.19	97.69	95.12
- 2.4	97.04	93.11	96.61
- 1.2	93.50	91.12	93.27
0	90.81	90.92	91.79
+ 1.2	91.19	93.72	93.69
+ 2.4	94.36	98.48	100.0
+ 3.0	91.48	98.89	98.98

The distribution was measured using gold foils in a lucite phantom 11.2 cm long with a 6.0 cm diameter in two individual irradiations. ($\phi_{th} = 6.67 \times 10^9 \text{ n cm}^{-2} \text{ sec}^{-1}$). The data are normalized and expressed as percent of maximum.

Table 2. Experimental determination of magnesium in rat phantom¹

Run	ϕ_{th} (10^9 n cm ⁻² sec ⁻¹)	$\bar{C}_{t_0}^2$ (counts/400 S) (at $t = 0$)	Added Mg (mg)	Calc Mg ³ (mg)
1-1	6.790	311	40	46.2
1-2	6.528	402	40	56.5
1-3	6.629	468	40	65.1
$\bar{X}$			40	55.9 $\pm$ 5.1 ⁴
2-1	6.739	520	80	77.8
2-2	6.753	746	80	101.4
2-3	6.880	930	80	124.6
$\bar{X}$			80	101.3 $\pm$ 8.9
3-1	6.655	1130	160	171.1
3-2	6.379	1270	160	182.8
3-3	6.487	1309	160	186.1
$\bar{X}$			160	180.0 $\pm$ 5.4
4-1	6.977	1633	240	235.9
4-2	6.773	1571	240	212.9
4-3	6.669	1996	240	276.0
$\bar{X}$			240	241.6 $\pm$ 13

¹Phantoms 2-4 contained 1.2 g of NaCl, 35.8 g of Ca (NO₃)₂, 4 H₂O, 24.9 g of NH₄NO₃ and 44.25 mg MnSO₄ dissolved in distilled water. Phantom 1 contained one-half these amounts. ⁴All phantoms were 250 ml nalgene bottles.

² $\bar{C}_{t_0}$ = the average of the first two counts post irradiation due to Mg and extrapolated to time = 0.

³Based on $\bar{C}_{t_0}$.

⁴The mean $\pm$ S.E. was determined from 6 individual counts.

Table 3. Experimental Determination of Magnesium in Rats¹

Animal	Run	Weight (g)	ϕ_{th} ($10^9 n \text{ cm}^{-2} \text{ sec}^{-1}$)	$\bar{C}_{t0}$ ² (counts/400 s) at $t = 0$	Calc Mg^3 (mg)
A-1	1	441	6.703	521	67.1
	2		7.562	651	78.0
	3		6.624	730	94.6
$\bar{X}$					79.9 ± 6.2^4
A-2	1	484	6.996	617	76.1
	2		6.724	638	86.1
	3		6.522	749	89.9
$\bar{X}$					84.9 ± 5.5
A-3	1	379	6.841	570	71.8
	2		7.106	716	91.5
	3		6.625	640	85.6
$\bar{X}$					83.9 ± 4.7

1 Male Sprague - Dawley rats 220 days old were used for the irradiations.

2 $\bar{C}_{t0}$ = the average of the first two counts post irradiation due to Mg and extrapolated to time $t = 0$.

3. Based on $\bar{C}_{t0}$

4. The mean $\pm$ S.E. was determined from 6 individual counts.

FIGURE LEGENDS

Fig. 1.: The rat is positioned in the rotating cylinder above the Ge(Li) detector.

Neg.No.4-828-

Fig. 2.: Response of the counting system to 0.842 Mev and 1.01 Mev gamma photons.

Fig. 3.: The gamma ray spectrum of an irradiated rat at 4 minutes post irradiation following an exposure to a thermal flux of 6.7×10^9 n cm⁻² sec⁻¹.

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15.4 IN TO THE CENTER OF 40 IN ID
DIA. R. 1.0
RUSSELL & SEASH CO.

Cnts/400S

Response of Ge(Li) Detector

Response of Ge(Li) Detector

2

Counts / 400 sec.

400

200

100

^{27}Al (6843 MeV)

^{56}Mn (6847 MeV)

Gamma Ray Spectrum From

Thickened Pt
(gamma postirradiation)

^{27}Mg (1.0 MeV)

50

100

150

200

250

Counts / 400 sec.

400

200

2