Study of magnesium bioavailability using stable isotopes and the inductively-coupled plasma mass spectrometry technique in the rat: single and double labelling approaches

BY CHARLES COUDRAY[1]*, DENISE PEPIN[2], JEAN CLAUDE TRESSOL[1], JACQUES BELLANGER[1] AND YVES RAYSSIGUIER[1]

[1]*Centre de Recherche en Nutrition Humaine d'Auvergne, Laboratoire Maladies Métaboliques et Micronutriments, INRA de Theix/Clermont-Ferrand, 63122 Saint Genès Champanelle, France*
[2]*Laboratoire d'hydrologie, Institut Louise Blanquet, Faculté de Pharmacie, Université d'Auvergne, 63000 Clermont-Ferrand, France*

The present work aimed to investigate the feasibility of using stable isotopes and inductively-coupled plasma mass spectrometry (ICP/MS) to study Mg absorption in rats. Male Wistar rats, aged 7 weeks and weighing 180 g, were used. They were fed on a semi-purified diet containing 1070 mg Mg/kg, and had free access to feed and distilled water. In the first experiment, after a 16 d adaptation period, two doses of enriched ^{25}Mg (6 and 12 mg) were administered by oral intubation, faeces and urine were collected daily and blood was sampled. Isotope ratios were determined by ICP/MS. 'True' absorption values, using the faecal isotope data, were 0·63 and 0·56 in rats receiving 6 and 12 mg ^{25}Mg respectively, while apparent absorption was 0·50 for two successive periods of metabolic balance studies. Moreover, the oral isotope administration resulted in a measurable isotopic enrichment in plasma within hours which was still detectable on the third day following the isotope administration. In the second experiment, investigating the double labelling technique, similar rats were dosed simultaneously with 5 mg ^{26}Mg orally (premixed with diet) and 0·29 mg ^{25}Mg intravenously. The calculated Mg true absorption values were very similar when calculated from blood or urine data (0·38) but were lower than that obtained from faecal data (0·50). The possible causes of such an unexpected difference and limits of the double labelling technique for Mg absorption are discussed here. Together these results indicate that although ^{25}Mg and ^{26}Mg isotopes have high natural abundance, the described methodology permits meaningful investigations of Mg bioavailability and metabolism.

Magnesium: Absorption: Stable isotopes: Inductively-coupled plasma mass spectrometry

Mg plays an essential role in a wide range of fundamental biological and cellular functions and it is not surprising that Mg deficiency may lead to serious biochemical and symptomatic changes (Durlach, 1988; Rayssiguier *et al.* 1993*a*). Recent studies indicate an important role of Mg deficiency in the aetiology of cardiovascular pathology (Rayssiguier *et al.* 1993*b*; Gueux *et al.* 1995). Such Mg deficiency may result from inadequate intake and/or impaired intestinal absorption of Mg. An extensive recent review (Hardwick *et al.* 1990*a*) discussed the intestinal sites of Mg absorption and it was the consensus that the primary site of Mg absorption is the distal intestine, the ileum (Phillips *et al.* 1991). However, little information about Mg absorption and metabolism is available. This lack of data is largely due to restrictions in the use of radioisotopes and methodological problems

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inherent in balance studies (Mertz, 1987). A short half-life has so far limited *in vivo* use of [28]Mg. Moreover, absorption studies involving human subjects raise ethical questions regarding the use of radioisotopes. The relatively recent use of stable isotopes in nutrition research offers a safer alternative (Janghorbani & Ting, 1990; Yergey, 1996). The measurement of absorption based on this methodology requires that accurate isotopic determinations be carried out for the stable isotopes [24]Mg, [25]Mg, and [26]Mg. These measurements have previously been performed by expensive and time-consuming neutron activation or different mass spectrometry techniques (Schwartz & Giesecke, 1979; Liu *et al.* 1989; Stegmann & Karbach, 1993). The recent availability of inductively coupled plasma mass spectrometry (ICP/MS) has indicated the potential for a new and reliable method for conducting mineral bioavailability studies (Schuette *et al.* 1988; Sandström *et al.* 1993; Vanhoe, 1993; Crews *et al.* 1994). It offers high sensitivity, rapid throughput and simple sample preparation. This technique may be useful to study a large number of nutritional and antinutritional factors with true potential to affect Mg absorption. So far, only a few human Mg absorption studies have been performed with stable isotopes and ICP/MS (Cary *et al.* 1990; Schuette *et al.* 1990, 1994; Benech & Grognet, 1995). However, because of their high cost these studies have often used small numbers of individuals. We propose, therefore, to validate this approach using experimental animal models which, to our knowledge, has not yet been done. This should facilitate its use to study a large number of nutritional absorption factors. Accordingly, we have investigated the feasibility of studying Mg absorption using single and double labelling techniques with Mg stable isotopes in rats and compared the results with those determined using metabolic balance techniques.

MATERIALS AND METHODS

Materials and reagents

Enriched Mg isotopes ([25]Mg and [26]Mg) as MgO were obtained from Chemgas, Boulogne, France. HNO_3 (ultrapure), Mg and Be standard solutions (1 g/l) were obtained from Merck, Darmstadt, Germany. All other chemicals were of the highest quality available. Distilled water was used throughout. A Plasmaquad II system (Fisons Instruments, Manchester) with a Meinhard nebulizer was used for isotopic measurement and a Perkin Elmer 560 (Perkin Elmer, St-Quentin en Yvelines, France) was used for total Mg measurement.

Animals and diet

Male Wistar rats, aged about 7 weeks, weighing 180 (SE 2·5) g, were used. They were derived from the colony of laboratory animals of the Institut National de la Recherche Agronomique (INRA of Clermont-Ferrand/Theix, France). The rats were housed under conditions of constant temperature (20–22°), humidity (45–50%) and a standard dark cycle (20.00–08.00 hours). They received human care in compliance with the guidelines formulated by the European Community for the use of experimental animals (L358-86/609/EEC). Animals first went through an adaptation period of 16 d during which they received *ad libitum* a semi-purified diet containing 1070 mg Mg/kg and distilled water. The semi-purified diet contained (g/kg): casein 200, starch 650, maize oil 50, fibre 50, AIN-76 mineral mixture (American Institute of Nutrition, 1977) including MgO 35, AIN-76A vitamin mixture (American Institute of Nutrition, 1977) 10, DL-methionine 3, choline

bitartrate 2. Powder diet (100 g) was made up with 100 ml distilled water to form a kind of semi-liquid food prepared on site.

Expt 1: single labelling and comparison with balance method

Stable isotope administration. The isotopic analysis of ^{25}Mg as MgO yielded the following atom percent figures: ^{24}Mg 0·95 %, ^{25}Mg 98·82 %, ^{26}Mg 0·23 %. ^{25}Mg (100 mg) as oxide was first moistened with 1 ml distilled water, and then 1 ml 12 M-HCl (ultrapure) was added to transform the oxide into the soluble chloride of Mg. The solution was then diluted with 18 ml distilled water and adjusted to pH 6 with powdered $NaHCO_3$. Ten rats were divided into two groups of five animals each. On day 0, at 08.00 hours the animals in group 1 received 6 mg ^{25}Mg and the animals in group 2 received 12 mg ^{25}Mg (1·2 or 2·4 ml Mg solution) by oral administration in the postabsorptive state.

Collection of samples. The faeces and urine of each rat for each day (before and for 6 d after isotope administration) were collected quantitatively. Animals were lightly anaesthetized by exposure to diethyl ether and blood was sampled from the retro bulbar sinus before and at 0, 4, 8, 12 h and 1, 3, and 6 d after stable-isotope administration.

Metabolic balance study periods. Two successive 6 d balance periods were performed, one before and another after the administration of stable isotope. Daily Mg intake was determined and the faeces and urine of each rat were quantitatively collected.

Expt 2: double labelling method

Stable-isotope administration. The isotopic analysis of ^{26}Mg as MgO yielded the following atom percent figures: ^{24}Mg 0·41 %, ^{25}Mg 0·18 %, ^{26}Mg 99·41 %. ^{26}Mg (100 mg) as oxide was dissolved as indicated earlier for ^{25}Mg isotope. The ^{25}Mg solution, designed for intravenous injection in this experiment, was rendered isotonic by adding a sufficient quantity of NaCl to a final concentration of 9 g/l. On the evening of day −1, 5 ml diluted ^{26}Mg isotope solution (1 mg/ml) was mixed with 5 g diet without Mg. The oral dose was then allowed to equilibrate overnight before being administered. On day 0, at 08.00 hours, ten fasted animals received their meal diet containing the ^{26}Mg isotope over 2 h in the dark. The remaining diet was weighed and the exact amount of ^{26}Mg isotope ingested by each animal was determined. At 10.00 hours, animals received 0·25 ml ^{25}Mg (1·15 mg/ml) intravenously. The dark cycle in this experiment was 22.00–10.00 hours and the rats therefore received their labelled meal in the dark.

Collection of samples. Faeces and urine of each rat were collected before isotope administration (baseline), and then 12 h collections were made quantitatively during the first 2 d and daily for the next 3 d after isotope administration. Animals were lightly anaesthetized by exposure to diethyl ether and blood was sampled from the retro bulbar sinus before and at day 2 after stable-isotope administration.

Sample treatment and analysis

Erythrocytes were separated from plasma by centrifugation, washed twice with saline, (9 g NaCl/l) solution and then lysed into ten volumes of distilled water. Urine volume was determined and 10 ml urine was sampled and acidified with 100 µl 16 M-HNO_3. Mg concentration and isotope ratios in plasma and urine were determined by ICP/MS after appropriate dilution with 0·16 M-HNO_3 using Be as internal standard. Lysed erythrocytes were first dried overnight and then ashed at 500° for 10 h. The ash was dissolved in 0·2 ml

16 M-HNO_3 and 9·2 ml distilled water. The faeces were freeze-dried, powdered and then ashed at 500° for 10 h. The ash was dissolved in 0·2 ml 16 M-HNO_3 and 9·2 ml distilled water. In each case an appropriate dilution with 0·16 M-HNO_3 was performed before the ICP/MS analysis. Mg concentration and isotope ratios were determined by ICP/MS. The mass spectrometer settings and plasma conditions were optimized with a solution of 10 μg In/l and the instrument operating conditions were as follows: radio frequency (RF) generator 27·12 MHz, forward RF power 1350 W, reflected RF power < 3 W, outer Ar flow rate 14 litres/min, intermediate Ar flow rate 0·7 litres/min, nebulizer Ar flow rate 0·76 litres/min, mass resolution 0·9 Da, at 10 % of peak height. Data collection variables were as follows: total replicates per integration 5, signal integration time per replicate 40 s, dwell time per sweep 20·4 ms, scanning mode: peak hopping, five point per peak, sample uptake rate 0·6 ml/min. Total Mg was determined by flame atomic absorption spectrometry (Perkin Elmer 560).

Calculations

Mg has three stable isotopes with the following natural abundance: ^{24}Mg 78·90 %, ^{25}Mg 10·00 % and ^{26}Mg 11·10 % (De Bièvre & Taylor, 1993). Isotopic percent enrichment was obtained from the following equation:

$$((^{25}\text{Mg} : {}^{24}\text{Mg measured ratio} - {}^{25}\text{Mg} : {}^{24}\text{Mg baseline ratio})/$$
$$(^{25}\text{Mg} : {}^{24}\text{Mg baseline ratio})) \times 100,$$

given that isotopic natural baseline ratios are as follows:

$$^{25}\text{Mg} : {}^{24}\text{Mg } 0·1267$$
$$\text{and } {}^{26}\text{Mg} : {}^{24}\text{Mg } 0·1407.$$

The calculation of non-absorbed ^{25}Mg isotope present in the faecal sample (coming only from the label ^{25}Mg isotope) was made as follows:

$$(\text{total faecal Mg} \times (^{25}\text{Mg} : {}^{24}\text{Mg sample ratio} - {}^{25}\text{Mg} : {}^{24}\text{Mg baseline ratio}))/$$
$$(1·267 + (^{25}\text{Mg} : {}^{24}\text{Mg sample ratio} - {}^{25}\text{Mg} : {}^{24}\text{Mg baseline ratio})),$$

where total faecal Mg (mg) is determined by atomic absorption spectrometry, and 1·267 is the result of 1/0·789 to convert ^{24}Mg faecal quantity to total faecal Mg. The calculation can also be made directly from ICP/MS data. The two modes of calculation give the same results when the ICP/MS quantitative procedure is used.

The apparent absorption was calculated according to the following equation:

$$\text{Mg apparent absorption (MgAA)} = ((\text{Mg intake} - \text{faecal Mg})/(\text{Mg intake})),$$

whereas balance was determined as follows:

$$\text{balance (mg Mg/d)} = \text{daily Mg intake} - (\text{daily faecal Mg} + \text{daily urinary Mg}).$$

On the same basis, Mg 'true' absorption, based on faecal isotopic enrichment (single labelling method), was calculated from the following formula:

$$((\text{administered } {}^{25}\text{Mg} - \text{non-absorbed } {}^{25}\text{Mg excreted in the faeces})/(\text{administered } {}^{25}\text{Mg})).$$

Mg true absorption, derived from blood or urine data (double labelling method) was calculated according to the formula of Yergey et al. (1987):

$$(^{26}\text{Mg nat} \times \text{i.v.} {}^{25}\text{Mg dose} \times \text{enrich } {}^{26}\text{Mg})/(^{25}\text{Mg nat} \times \text{oral } {}^{26}\text{Mg dose} \times \text{enrich } {}^{25}\text{Mg}),$$

where nat is the ratio of the two isotopes in nature, i.v. ^{25}Mg and oral ^{26}Mg refer to the exact dose given intravenously or orally, and enrich ^{25}Mg or ^{26}Mg represents the degree to which a particular ratio differs from natural levels (isotopic enrichment percentage) at 48 h after isotope administration.

Endogenous faecal Mg was calculated as follows (mg/d):

(daily Mg intake − (daily Mg intake × apparent absorption)) − (daily Mg intake − (daily Mg intake × true absorption)).

Or simply:

endogenous faecal Mg (mg/d) = daily Mg intake × (true absorption − apparent absorption).

Statistical analysis

Throughout the present study, results are expressed as means with their standard errors (SE). The statistical significance of differences ($P < 0.05$) between means was calculated by use of Student's t test.

RESULTS

Expt 1: single labelling experiment

Mg 'true' absorption was calculated from the faecal isotopic enrichment and total non-absorbed ^{25}Mg isotope. The isotope ratio measurements were performed on an ICP/MS instrument which permitted within- and between-run % residual standard deviations as follows: ($^{25}Mg:^{24}Mg$ 0.34 %, 0.81 %); $^{26}Mg:^{24}Mg$ 0.40 %, 0.94 %) respectively. Table 1 shows the faecal isotope enrichment and the cumulative excretion of ^{25}Mg in faeces. The two doses used (6 and 12 mg) led to considerable enrichment of ^{25}Mg in faecal samples. The majority of faecal non-absorbed ^{25}Mg (> 85 %) was excreted within 24 h of isotope administration in the rat group receiving 6 mg ^{25}Mg isotope. 'True' absorption values calculated on the basis of faecal non-absorbed ^{25}Mg during the first 3 d were 0.63 (SE 0.036) and 0.56 (SE 0.049), and during the totality of the experiment (6 d) were 0.63 (SE 0.035) and 0.54 (SE 0.049) in rats receiving 6 and 12 mg ^{25}Mg respectively.

Metabolic balance studies. Two successive balance periods were investigated. The results, shown in Table 2, indicate that the apparent absorption of Mg obtained from the two balance periods was 0.50, and that the Mg balance was +7 mg in both periods.

Endogenous faecal magnesium. The calculation takes into account the daily intake of Mg, the apparent and 'true' absorption as indicated above. The calculated endogenous faecal Mg varied from 0.44 to 4.08 mg/d with a mean of 2.53 mg/d, or from 2 % to 18 % with a mean of 11.5 % daily Mg intake in the rat group receiving 6 mg ^{25}Mg isotope. The rat group receiving 12 mg ^{25}Mg isotope showed similar results (2.35 (SE 0.72) mg/d and 10.6 (SE 3.50) % daily Mg intake). Taking the results of both groups, the endogenous faecal Mg in the present study was 2.44 (SE 0.49) mg/d and represented 11.02 (SE 2.29) % daily Mg intake.

Plasma and urine isotopic enrichments. Fig. 1 shows the appearance and disappearance of administered ^{25}Mg in the plasma and urine in the two groups of rats receiving 6 or 12 mg ^{25}Mg. The highest isotopic enrichment in plasma ^{25}Mg was observed after approximately 4 h ^{25}Mg administration, reaching 84 % and 144 % enrichment in rats

Table 1. *Cumulative faecal ^{25}Mg excretion and percentage isotopic enrichment, and 'true' magnesium absorption in rats**

(Mean values with their standard errors for four rats)

	Rat receiving 6 mg† enriched ^{25}Mg‡						Rats receiving 12 mg enriched ^{25}Mg‡					
	Faecal enrichment (%)		Faecal excretion (µg)		'True' absorption§		Faecal enrichment (%)		Faecal excretion (µg)		'True' absorption§	
Time (h)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0–24	136	17·6	1943	194	0·67	0·03	291	27·8	3254	959	0·69	0·08
24–48	44·4	16·2	2080	226	0·65	0·04	135	38·1	4723	599	0·58	0·05
48–72	7·25	1·08	2169	215	0·63	0·04	15·8	3·46	4923	585	0·56	0·05
72–96	2·58	0·72	2197	207	0·63	0·04	7·95	0·68	5014	594	0·55	0·05
96–120	1·88	0·69	2213	207	0·63	0·04	6·00	1·23	5076	585	0·55	0·05
120–144	0·98	0·41	2223	207	0·63	0·04	2·50	0·43	5432	577	0·54	0·05

* For details of procedures, see pp. 958–961.
† Values of 'true absorption in rats receiving 6 mg ^{25}Mg were not significantly different from those obtained in rats receiving 12 mg ^{25}Mg ($P > 0.05$).
‡ 1 mg enriched ^{25}Mg represents 0·9885 mg ^{25}Mg.
§ Mg 'true' absorption = (ingested ^{25}Mg − faecal non-absorbed ^{25}Mg)/(ingested ^{25}Mg).

Table 2. *Magnesium intake, and faecal and urinary excretion of magnesium in two successive metabolic balance periods in rats**

(Mean values with their standard errors for four rats)

	Mg intake (mg/d)		Faecal Mg excretion (mg/d)		MgAA‡		Urinary Mg excretion (mg/d)		Mg balance (mg/d)	
Balance period†	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	21·96	0·76	10·73	0·95	0·51	0·07	4·10	0·49	+7·14	0·61
2	22·62	0·56	11·51	0·63	0·49	0·02	4·27	0·24	+6·83	0·35

* For details of procedures, see pp. 958–960.
† There was no significant difference between the two balance periods.
‡ Mg apparent absorption = (ingested Mg − faecal non-absorbed Mg)/(ingested Mg).

receiving 6 or 12 ^{25}Mg respectively. Absorbed ^{25}Mg was rapidly excreted in urine, and the urine highest ^{25}Mg percentage enrichment was in the fraction 0–12 h after ^{25}Mg administration. Considering the 6 mg Mg dose, plasma and urine isotopic enrichments at 48 h after isotope administration should be sufficiently high to permit accurate isotopic ratio determinations (< 15%).

Expt 2: double labelling experiment

Calculation of magnesium true absorption. In order to calculate true absorption from blood and urine data, blood was sampled at day 2 and the isotopic enrichments in ^{26}Mg and ^{25}Mg were determined by measuring isotopic ratios and comparing them with those in samples taken before isotope administration (day 0). The plasma isotopic enrichment of ^{26}Mg obtained 2 d after oral administration was about 20%. However, the plasma isotopic enrichment of ^{25}Mg obtained 2 d after the intravenous administration was only about 4%.
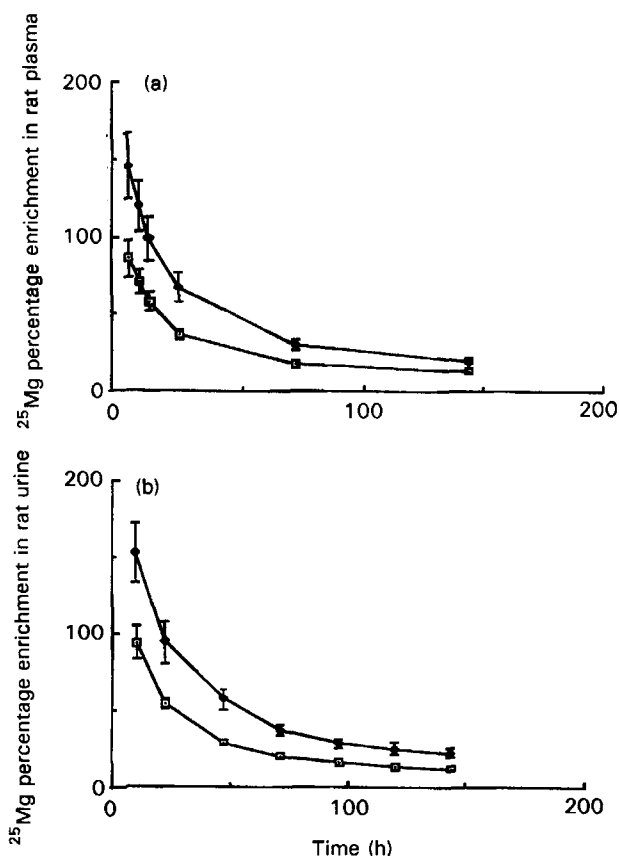
Fig. 1. The appearance and disappearance of administered ^{25}Mg in (a) plasma and (b) urine in two groups of rats receiving 6 (□) or 12 (●) ^{25}Mg. After a 16 d adaptation period, animals were divided into two groups and received orally either 6 or 12 mg ^{25}Mg isotope. Blood was sampled from the retro bulbar sinus at the indicated times, and urine was collected quantitatively over the entire period of the experiment (6 d). For further details of procedures, see pp. 958–960. Values are means for four animals, with their standard errors indicated by vertical bars. All values from rats receiving 6 mg ^{25}Mg were significantly different from those obtained in rats with 12 mg ^{25}Mg.

Similar isotopic enrichments were observed in erythrocytes obtained 2 d after isotope administration. As expected, the true absorption values, determined using either the plasma mean 0·38 (SE 0·021) or the erythrocyte mean 0·40 (SE 0·053) data, were very similar in the two media (Table 3). Because regular urine collection is easy, the isotopic enrichment was determined on samples of 12 h up to 48 h and then on those of 24 h up to 120 h. As indicated in Table 4, the highest ^{26}Mg enrichment of about 50 % was observed in the first two fractions (0–12 and 12–24 h), falling gradually to 8·6% in the last fraction (96–120 h). In the 36–48 h fraction, used to calculate the true absorption, the ^{26}Mg enrichment was 23 %, which is slightly higher than that observed in the plasma at 48 h. Concerning the intravenous isotope, the ^{25}Mg enrichment was very high (35·5 %) only in the first fraction (0–12 h), falling very rapidly to less than 10 % in the next fraction (12–24 h). In the last fraction (day 5) the ^{25}Mg enrichment was less than 2 %. However, in the 36–48 h urine fraction the ^{25}Mg enrichment was 4·5%, which is also slightly higher than that observed in

Table 3. ^{25}Mg and ^{26}Mg blood enrichment and magnesium true absorption (MgTA) values calculated 48 h after isotope administration in individual rats*

Rat...	1	2	3	4	5	6	8	9	10	Mean	SE
Plasma											
$^{25}Mg:^{24}Mg$ enrichment	2·96	4·39	3·47	3·79	3·85	3·93	3·54	3·91	3·54	3·71	0·13
$^{26}Mg:^{24}Mg$ enrichment	21·93	30·78	16·64	19·27	15·54	15·6	18·47	13·39	16·65	18·7	1·72
MgTA†	0·554	0·460	0·327	0·343	0·280	0·265	0·487	0·338	0·370	0·381	0·033
Erythrocytes											
$^{25}Mg:^{24}Mg$ enrichment	5·19	4·03	2·86	4·95	4·57	4·51	4·48	4·22	4·79	4·40	0·23
$^{26}Mg:^{24}Mg$ enrichment	23·68	30·57	17·21	21·34	16·36	25·4	36·99	17·42	16·4	22·8	2·40
MgTA†	0·341	0·498	0·411	0·291	0·249	0·376	0·752	0·407	0·269	0·40	0·051

* For details of procedures, see pp. 958–961.

† MgTA = $((^{26}Mg$ nat × i.v. ^{25}Mg × enrich $^{26}Mg)/(^{25}Mg$ nat × oral ^{26}Mg × enrich $^{25}Mg))$, where nat is the ratio of the two isotopes in nature, i.v. ^{25}Mg and oral ^{26}Mg refer to the exact dose given intravenously or orally, and enrich ^{25}Mg or ^{26}Mg represents the degree to which a particular ratio differs from natural levels (isotopic enrichment percentage) at 48 h after isotope administration.

Table 4. ^{25}Mg and ^{26}Mg urinary excretion, percentage isotopic enrichment and magnesium true absorption (MgTA) in rats†

(Mean values with their standard errors for nine rats)

Time after isotope administration (h)	Isotope excretion (μg)				Percentage enrichment				MgTA‡	
	^{25}Mg		^{26}Mg		$^{25}Mg:^{24}Mg$		$^{26}Mg:^{24}Mg$			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0–12	43·4	5·97	72·8	11·8	35·5	2·54	49·9	2·20	0·11*	0·09
12–24	20·8	1·23	111·8	7·11	9·91	0·44	49·3	1·19	0·37	0·01
24–36	10·5	1·08	62·9	5·17	6·16	0·19	32·1	0·95	0·39	0·02
36–48	11·3	0·50	63·6	4·93	4·56	0·21	23·0	0·77	0·38	0·02
48–72	7·82	2·00	41·6	9·43	3·30	0·20	15·5	0·44	0·36	0·02
72–96	5·81	1·48	29·1	6·62	2·29	0·14	11·0	0·51	0·37	0·02
96–120	3·42	0·72	19·1	4·06	1·73	0·14	8·55	0·35	0·38	0·02

* Mean values was significantly different from those at all other times, $P < 0·05$.

† For details of procedures, see pp. 958–961.

‡ MgTA = $((^{26}Mg$ nat × i. v. ^{25}Mg × enrich $^{26}Mg)/(^{25}Mg$ nat × oral ^{26}Mg × enrich $^{25}Mg))$, where nat is the ratio of the two isotopes in nature, i.v. ^{25}Mg and oral ^{26}Mg refer to the exact dose given intravenously or orally, and enrich ^{25}Mg or ^{26}Mg represents the degree to which a particular ratio differs from natural levels (isotopic enrichment percentage) at 48 h after isotope administration.

the plasma at 48 h (3·7 %). Table 4 also shows the calculation of true absorption on the different fractions of urine. This confirms that the first urine fraction (0–12 h) could not be used in the calculation of Mg true absorption. The calculation of Mg true absorption from this fraction gives a value of 0·11, which differs considerably from the other values (means 0·36–0·39). As expected, these later values are in agreement with those obtained from blood samples (Table 3). This means that true absorption could be calculated on blood or urine spots 24 h after isotope administration (Table 4).

Table 5. ^{25}Mg and ^{26}Mg percentage isotopic enrichment, faecal excretion and 'true' absorption of magnesium (Mg 'TA') in rats†

(Mean values with their standard errors for nine rats)

| Time after isotope administration (h) | Percentage enrichment | | | | Isotope excretion | | Cumulative excretion | | Mg'TA'‡ | |
| | ^{25}Mg : ^{24}Mg | | ^{26}Mg : ^{24}Mg | | ^{26}Mg (µg) | | ^{26}Mg (µg) | | | |
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0–12	0·75	0·28	98·7	44·9	454	176	454	176	0·90*	0·04
12–24	2·97	0·81	430	153	1036	107	1491	161	0·66*	0·03
24–36	1·32	0·43	60·4	11·6	460	51·1	2030	153	0·56	0·03
36–48	0·67	0·16	13·2	9·21	179	54·5	2077	156	0·52	0·02
48–72	0·48	0·15	4·98	1·18	73·1	21·4	2150	155	0·50	0·02
72–96	0·20	0·09	1·15	0·27	22·5	5·36	2173	155	0·50	0·02
96–120	0·33	0·13	1·17	0·39	15·3	5·81	2188	157	0·49	0·02
3 d pool	1·17	0·25	58·5	6·46			2143	145	0·50	0·02
4 d pool	0·82	0·11	39·7	2·9			2164	151	0·50	0·02

* Mean values were significantly different from that at 48–72 h, $P < 0.05$.
† For details of procedures, see pp. 958–961.
‡ Mg 'TA' = (ingested ^{26}Mg − faecal non-absorbed ^{26}Mg)/(ingested ^{26}Mg).

Magnesium 'true' absorption. To determine Mg 'true' absorption derived from the faecal ^{26}Mg isotope data, individual daily faecal samples for each rat were analysed and faecal pools of the first 3 d after isotope administration were made and analysed. As shown in Table 5, the highest ^{26}Mg enrichment of about 430 % was obtained in the second faecal fraction (12–24 h). Moreover, the oral dose of 5 mg ^{26}Mg yielded a faecal isotopic enrichment of 58·5 % in the 3 d faecal pool (Table 5) and, as expected, the enrichment measured in the 4th day fraction was as low as 1 %. This shows clearly that 3 d collection of faeces is appropriate to determine 'true' absorption of Mg on the basis of faecal data. When the faecal quantity of non-absorbed ^{26}Mg was determined, the 'true' absorption of Mg was 0·50 for the 3 d and 4 d faecal pools. Table 5 indicates that about 1% of the non-absorbed ^{26}Mg was excreted in the faeces on the fourth day after oral ^{26}Mg administration. However, a large part of this faecal ^{26}Mg could come from previously absorbed ^{26}Mg which is re-excreted in the faeces. The results of this experiment show that 'true' absorption based on the faecal excretion of non-absorbed isotope (0·50) can yield a different value from true absorption based on the urine and plasma double labelling technique (0·39).

DISCUSSION

Mechanisms underlying the effects of dietary components on Mg absorption are generally poorly understood (Brink & Beynen, 1992). The use of stable isotopes in mineral nutrition research is considered to be an important and valuable tool from the point of view that there is no exposure to radiation and no decay over time. Mg has three natural stable isotopes: ^{24}Mg (78·90 %), ^{25}Mg (10·00 %) and ^{26}Mg (11·10 %) (De Bièvre & Taylor, 1993), two of which can be used in bioavailability studies. Although thermal-ionization mass spectrometry (TIMS) is still the reference technique for stable-isotope measurements, inductively coupled Ar plasma mass spectrometry (ICP/MS) has many advantages and could supplant TIMS in many cases (Sandström *et al.* 1993; Crews *et al.* 1994). In the

present work the different methods of determining Mg absorption were compared, the feasibility of using stable isotopes and the ICP MS technique to study Mg absorption and metabolism was explored, and the appropriate dosage of Mg isotopes was investigated. To do so, two complementary experiments were conducted in adult rats.

Single labelling and metabolic balance study

The results of the first experiment show that the mean daily food intake was about 22 g per rat, which corresponds to about 23 mg Mg/d. In a classical balance study, the apparent absorption is calculated as Mg intake minus Mg faecal excretion and this includes endogenous faecal Mg. The apparent absorption was found to be about 0·50 in both balance periods. Previous work in rats (Brink *et al.* 1991, 1992; Verbeek *et al.* 1993) reported similar apparent absorption values for Mg (0·40–0·60, depending on diet composition). As expected, 'true' absorption of Mg (0·64), obtained from the faecal non-absorbed isotope measurement, was higher than apparent absorption (0·50) by about 15 % when the dosage of ^{25}Mg was 6 mg. This difference was expected and is essentially due to the fact that apparent absorption is calculated as intake minus faecal excretion and this includes endogenous faecal Mg. Moreover, the 'true' absorption, obtained here, was non-significantly lower in the rats receiving 12 mg ^{25}Mg than in those receiving 6 mg ($P > 0·05$). This slight decrease may be a consequence of a high alimentary level of Mg (Hardwick *et al.* 1990*a*, *b*; Kayne & Lee, 1993; Spencer *et al.* 1994). Schuette *et al.* (1990) obtained, in infants, a lower true absorption with 60 mg *v.* 20 mg ^{25}Mg dose. The time-course of faecal ^{25}Mg excretion, reported in this experiment, showed that more than 75 % of non-absorbed ^{25}Mg was recovered over 24 h and 99 % of non-absorbed ^{25}Mg was recovered during the first 3 d from rats receiving 6 mg ^{25}Mg. Faecal Mg is a mixture of non-absorbed Mg from the diet and non-reabsorbed endogenous excreted Mg. Endogenous faecal Mg was assessed from the difference between total faecal Mg (non absorbed Mg + endogenous Mg) calculated from the apparent absorption, and the non-absorbed Mg calculated from 'true' absorption. Endogenous faecal Mg was mean 2·44 (SE 0·49) mg daily, which represents 11 % of Mg daily intake in the present study. Similar measurements in animals were not found in the literature because of the lack of such studies. However, in some human trials, Schwartz *et al.* (1978) found excretion values of about 25–50 mg/d or about 10 % of daily Mg intake in adult man. However, Sojka *et al.* (1996) found endogenous faecal Mg of only 12·6 mg or about 5 % of the 250–350 mg Mg consumed by adolescent girls. In any case, one should bear in mind that these endogenous faecal Mg estimations are very approximate, because their reliability depends largely on the precision of the apparent and true absorption determinations. Consequently, non-random errors in these later determinations could translate to large variations in endogenous faecal Mg excretion values (Schuette *et al.* 1990).

The stable-isotope enrichment in plasma and urine traces the appearance and disappearance of ^{25}Mg in the two rat groups receiving 6 or 12 mg ^{25}Mg. These results show that Mg was rapidly absorbed and its plasma concentration peaked before the fourth hour following isotope administration. This absorbed ^{25}Mg was rapidly eliminated by the kidney. There are only a few studies that have investigated blood levels after oral ingestion of tracer Mg. In the present experiment the highest plasma ^{25}Mg enrichment was observed at about 4 h reaching 84 % and 144 % in rats receiving 6 or 12 mg ^{25}Mg respectively. Others have also observed a peak of ^{28}Mg 4 h after isotope administration in rats (Aikawa, 1981; Hardwick *et al.* 1990*b*). The plasma and urine enrichment levels at 24 h (35 %, 54 %) and at 72 h (15 %, 20 %) respectively were sufficiently high to permit accurate isotopic

measurements and their use to calculate several kinetic constants of Mg metabolism, particularly if used in conjunction with intravenous administration of a second Mg stable isotope (^{26}Mg) as in the next experiment.

Double labelling technique

In the next part of the present work, the double labelling technique was investigated and Mg absorption was obtained from faecal, urine and blood data. Based on the results of the first experiment, a target dose of 5 mg ^{26}Mg was administered orally and a dose of 0·29 mg ^{25}Mg was injected intravenously. In this second experiment the ^{26}Mg isotope was premixed with 5 g diet and allowed to equilibrate for 16 h before administration. To permit rapid consumption, rats were fasted for 16 h before receiving their labelled meal. The intravenous injection (0·25 ml) was performed immediately after the consumption of the labelled diet (over 2 h). The choice of this intravenous dose (0·29 mg ^{25}Mg per rat) was made to ensure sufficiently high isotopic enrichment, and the ^{25}Mg quantity injected in the present experiment was about twice the total plasma Mg of a rat and thus the maximum that we could administer in a bolus.

It was noted that 48 h after the intravenous administration of 0·29 mg ^{25}Mg the percentage enrichment in blood and urine was less than 5 %. Despite this low enrichment the calculation of true absorption using the formula of Yergey et al. (1987) was still possible because of the good analytical performance of the ICP/MS technique. Moreover, the calculated Mg true absorption was very similar whatever the biological sample, i.e. plasma, erythrocytes or urine (Table 6). This was to be expected and indicates the robustness of the present methodology. Additionally, the urine data showed that the true absorption could be determined as early as approximately 24 h after isotope administration where we obtained a higher percentage enrichment than at 48 h, which allowed more accurate isotopic ratio determinations.

In this experiment, the Mg 'true' absorption obtained from the faecal data was about 0·50. However, the 'true' absorption obtained from faecal data was considerably higher than that obtained from blood and urinary data (0·38). In the double labelling technique it is assumed that there is no difference in the distribution of the two tracers once they have

Table 6. *Individual magnesium true absorption values in rats determined using blood and urine samples obtained 48 h after isotope administration, and using faecal samples collected over 5 d†*

Rat...	1	2	3	4	5	6	8	9	10	Mean	SE
Plasma‡	0·55	0·46	0·33	0·34	0·28	0·27	0·49	0·34	0·37	0·38*	0·02
Erythrocyte‡	0·34	0·50	0·41	0·29	0·25	0·38	0·75	0·41	0·27	0·40	0·05
Urine‡	0·41	0·48	0·35	0·38	0·32	0·27	0·50	0·35	0·30	0·38*	0·02
Faeces§	0·51	0·54	0·48	0·48	0·46	0·40	0·57	0·54	0·52	0·51	0·02

* Mean values were significantly different from that obtained from faecal data, $P < 0.05$·

† For details of procedures, see pp. 958–961.

‡ Mg true absorption = ((^{26}Mg nat × i.v.^{25}Mg × enrich ^{26}Mg)/(^{25}Mg nat × oral ^{26}Mg × enrich ^{25}Mg)), where nat is the ratio of the two isotopes in nature, i.v. ^{25}Mg and oral ^{26}Mg refer to the exact dose given intravenously or orally and enrich ^{25}Mg or ^{26}Mg represents the degree to which a particular ratio differs from natural levels (isotopic enrichment percentage) at 48 h after isotope administration.

§ Mg 'true' absorption = (ingested ^{26}Mg − faecal non-absorbed ^{26}Mg)/(ingested ^{26}Mg). Data were calculated on the basis of 3 d collection of faeces.

entered the circulation by the two routes of administration. In other words, the absorbed portion of the oral ^{26}Mg will be distributed and excreted exactly as the entire intravenous ^{25}Mg dose. However, a large dose of tracer (0·29 mg) was injected which induced a moderate hypermagneseamia responsible for rapid urinary excretion of the ^{25}Mg. In fact, it is known that urinary excretion of Mg plays an important role in the control of Mg homeostasis (Quamme, 1993). This means that small changes in serum Mg are accompanied by a rapid increase or decrease in urinary Mg excretion. Therefore, an important part of intravenously injected ^{25}Mg could be excreted immediately or very rapidly in urine and thus not behave like the absorbed ^{26}Mg isotope. The results obtained in the present study are in agreement with such an hypothesis. In this regard, we noted that only 18 % of the total urinary ^{26}Mg was excreted in the first fraction (0–12 h), whereas more than 41 % of the total urinary ^{25}Mg was excreted in the first fraction (0–12). Given the high natural abundance of available Mg isotopes (10 %), it was necessary to inject a large dose of tracer in comparison with the body pool of Mg. Thus, it is preferable that intravenous Mg isotope should be given over a long period of time or divided into two or more small successive injections. The likely effect of perturbation of body pools needs further consideration in relation to the results obtained.

Finally, the results of this second experiment also showed a significantly ($P < 0·005$) lower 'true' absorption (0·50) than in the first experiment (0·63), suggesting that administration of ^{26}Mg premixed with the diet could be responsible for a decrease in fractional Mg absorption in comparison with the results of the first experiment where the ^{25}Mg isotope was given in liquid form by oral administration. This was confirmed in an other study in our own laboratory (Coudray *et al.* 1996). Schuette *et al.* 1990 found that Mg absorption was consistently less after bolus *v.* distributed administration of the same dose of Mg isotope in infants. Indeed, free ^{25}Mg given in liquid form in the first experiment cannot necessarily be assumed to have distributed in the intestine in a manner similar to ^{26}Mg equilibrated with or Mg bound in food (Hardwick *et al.* 1990*a*).

In conclusion, this work compared apparent absorption with true absorption, and optimized the dosages of Mg isotopes required in an experimental rat model. Mg 'true' absorption can be determined by the single labelling technique using ICP/MS and faecal monitoring. In this regard, doses as low as 2–3 mg Mg isotope (^{25}Mg or ^{26}Mg) can be sufficient. The advantage of using the double labelling technique is that it does not require faecal collections similar to those performed for mass balance. Because of the high renal regulation of Mg, isotope enrichment calculations should, however, preferably be performed on biological samples 24 h after isotope administration. However, the rapid urinary elimination of the intravenous tracer may lead to poor estimation of Mg true absorption. This technique may be more convenient for comparative studies on Mg bioavailability.

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