Studies on magnesium in ruminant nutrition

3.* Distribution of ^{28}Mg in the gastro-intestinal tract and tissues of sheep

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(*Received* 31 *October* 1960—*Revised* 23 *March* 1961)

Assessment of dietary requirements of the ruminant for magnesium involves the determination of the availability of Mg in feeding-stuffs and supplements. The main reason why this determination has not been achieved is that the faecal Mg contains Mg derived not only from the unabsorbed food Mg but also from the intestinal secretions, and it is very difficult to determine the relative proportions of these two fractions. Two general techniques using radioactive tracers have been developed for this purpose: the comparative-balance method of Hansard, Comar & Plumlee (1954) and the isotope-dilution technique of Visek, Monroe, Swanson & Comar (1953). Recently both these methods have been adapted for ^{28}Mg, the former by Field (1959) and the latter by Macdonald, Care & Nolan (1959). However, because of the good agreement between the values obtained with the two techniques, there may be a tendency to overlook the assumptions inherent in them.

In the comparative-balance technique it is assumed that the ^{28}Mg given orally as the chloride distributes itself uniformly with the stable Mg in the ingesta in the gastro-intestinal tract before absorption. If such distribution does not occur the figures for availability of dietary Mg will be inaccurate and more applicable to the availability of ionic Mg than to that of total dietary Mg. There is no information in the literature on this point, but since the availability of dietary Mg is low (Field, McCallum & Butler, 1958) it is possible that this Mg may exist in chemical forms which are not readily exchangeable with ^{28}Mg, and that some of the feed Mg may remain in the solid phase of the ingesta out of physical contact with ^{28}Mg and thus be discontinuous in distribution. In the isotope-dilution method it is assumed that there is no exchange between exogenous and endogenous Mg in the gastro-intestinal tract, i.e. that the specific activity of the reabsorbed intestinal secretions is the same as that of the remainder of the secretions, and both Kjeriff-Jensen (1941–2) and Moore & Tyler (1955*b*) have questioned the validity of this assumption. In both methods it is assumed that there is no exchange of ^{28}Mg between the wall and contents of the gastro-intestinal tract.

The objective of the experiment described here was threefold; first, to investigate the validity of some of the above assumptions by studying the distribution of ^{28}Mg between the liquid and solid phases of the ingesta along the tract after oral or intravenous administration of a single dose; secondly, to obtain information on the sites of absorption and secretion of Mg and, thirdly, to measure the rate at which ^{28}Mg

*Paper no 2: *Brit. J. Nutr.* (1961), **15**, 287.

in the plasma exchanges with the stable Mg in the tissues. The results for bone have been briefly reported elsewhere (Field, 1960*a*).

Moore & Tyler (1955*a*) observed a marked diurnal variation in the concentration of calcium and phosphorus in the faeces of pigs. They found that these elements pass along the tract more rapidly than the dry matter and that hence the distribution of calcium and phosphorus in the tract is dependent upon the time between the last meal and the killing of the pig. To find if the same is true for Mg in sheep, the diurnal variation in the Mg concentration in the faeces was investigated.

EXPERIMENTAL

Two 5-year-old wethers received daily (in two equal portions at 10 a.m. and 4 p.m.) 1000 g of grass nuts containing 1·2 g Mg. After 6 days on the diet a single dose of 40–60 μc carrier-free ^{28}Mg, as the chloride, was given at noon by stomach tube to sheep A and by intravenous injection to sheep B. The ^{28}Mg was produced in a cyclotron by the bombardment of KCl with protons. The sheep were killed by exsanguination under nembutal anaesthesia 10 h later, immediately after sampling of the blood. The abdominal cavity was opened at once and double or single ligatures were tied with as little disturbance of the contents as possible around the junction between the omasum and abomasum, the duodenum immediately adjacent to the pylorus, at numerous points along the small intestine, the ileo-caecal valve, the junction between the caecum and the colon adjacent to the ileo-caecal valve, at two points along the spiral colon and the junction between the distal colon and rectum.

The contents of the gastro-intestinal tract were then removed. Those of the abomasum and sections of the small intestine were weighed, the liquid and solid phases separated by centrifuging at 1400 ***g*** for 20 min and the volume of the liquid phase and weight of the solid phase recorded. The two phases of the rumen and caecum contents were obtained by wrapping a representative sample in surgical gauze and squeezing in a hand press. No attempt was made to separate the phases in the contents of the colon and rectum. All liquid phases were centrifuged at 60000 ***g*** for 30 min before sampling for determination of radioactivity and Mg content.

The walls of the gastro-intestinal tract were washed free from contents, and after the lengths of the sections of small intestine had been measured, the mucous membranes were stripped from the walls of the small intestine, caecum, colon and rectum by the method of J. G. Brotherston, N. J. L. Gilmour and J. M. Samuel (1960, personal communication). In sheep B the walls of these structures were divided into an anterior and posterior portion. The mucous membranes were stripped from the walls of the rumen and abomasum by hand.

Duplicate samples of kidney, liver, spleen, gastrocnemius muscle, bile and various bones were taken from sheep B. The samples of soft tissue were taken before the alimentary tract was removed from the abdominal cavity to minimize contamination with blood and ingesta.

The radioactivity of the samples was determined with a well-type scintillation counter and a scaling circuit. All determinations were corrected for physical decay

of the isotope and were completed on the day of collection. For the solid samples, duplicate tubes were filled to a 5 ml mark and weighed, and radioactivity and Mg were determined in the contents of each. Duplicate portions of the liquid samples were taken for the determination of radioactivity (5 ml) and Mg (20 ml).

To investigate the diurnal variation in the Mg concentration in faeces, samples were collected over 3 h periods, for a total of 24 h, from four wethers that had been on the same dietary régime as sheep A and B for the previous 15 days.

The Mg content of the samples was determined by the method used in previous work (Field *et al.* 1958).

RESULTS

Oral administration of ^{28}Mg

Table 1 shows the distribution of ^{28}Mg and stable Mg in the mucosa and contents of the selected sections of the gastro-intestinal tract of sheep A 10 h after administration. The specific-activity values, as elsewhere, are expressed as percentage dose $\times 10^2$/mg of total Mg in the sample.

The specific activities of the contents of all sections were higher than that of the plasma (1·1), which was expected in view of the poor absorption of Mg from the gut. There was a fall in the specific activity of the liquid phase from the rumen to the abomasum and from the abomasum to the first part of the small intestine. It must be due either to secretion of Mg or the preferential absorption of Mg compounds of a higher specific activity relative to the mean for the liquid phase of the previous section. The fall in specific activity of the liquid phase from the rumen to the abomasum could not arise from a more complete mixing of the active Mg in the liquid phase of the rumen digesta with inactive Mg in the solid phase because the specific activity of the solid phase of the abomasum was lower than that of the rumen. There was a progressive increase in the specific activity of the liquid phase from sections 1 to 4. This must be due to Mg compounds of a relatively low specific activity being either selectively absorbed or transferred to the solid phase.

The specific activity of the liquid phase was generally higher than that of the solid phase of the same section. It should be noted that the observed differences are lower than the true values since the solid phase still contained some of the liquid phase. Of more practical importance was the difference between the specific activity of the liquid phase and that of the total contents, since it measures the failure of ^{28}Mg administered as chloride to act as a tracer for dietary Mg. It may be seen that the specific activity of the liquid phase was generally higher and that the difference varied with the section (Table 1).

Excluding the third section of the small intestine, the specific activities of all the mucosa segments of the gastro-intestinal tract were similar and were all higher than the specific activity of the plasma. In theory such an increase may be due to two factors, absorption of ^{28}Mg and exchange of ^{28}Mg in the intestinal contents with the stable Mg in the mucosa. The latter process is probably the main cause since there was no significant difference in specific activity between the mucosa of the abomasum, which

Table 1. *Distribution of ^{28}Mg and total Mg in the contents and walls of the gastro-intestinal tract of sheep A 10 h after oral administration of ^{28}Mg*

Site	Length of section (ft)	Mucosa S.A.*†	Liquid phase: Volume (ml)	Liquid phase: Concentration of Mg (mg/100 ml)†	Liquid phase: S.A.*†	Solid phase: Dry weight (g)	Solid phase: Concentration of Mg as percentage of dry weight†	Solid phase: S.A.*†	Total contents S.A.*†
Rumen	—	1·36	—	4·7	5·04	—	0·101	4·63	—
Abomasum	—	1·46	140	8·5	3·88	11·0	0·069	3·35	3·64
Small intestine:									
Section 1	8	1·37	45	10·0	2·96	3·0	0·063	3·16	3·02
Section 2	12	1·68	120	11·5	3·48	10·3	0·083	3·11	3·33
Section 3	15	3·17							
Section 4	24	1·62	125	11·5	4·04	13·1	0·125	2·79	3·37
Section 5	11	1·41	100	12·9	4·20	15·8	0·152	3·56	3·78
Caecum	—	1·79	—	19·5	4·15	—	0·197	3·64	—
Colon:									
Section 1	—	1·90	—	—	—	—	—	—	3·78
Section 2	—	1·88	—	—	—	—	—	—	4·04
Rectum	—	—	—	—	—	—	—	—	3·83

* Specific activity expressed as percentage of dose × 10^2/mg total Mg.
† Mean of two determinations.

appears to secrete Mg, and those of the other sections. The increased specific activity of the mucosa of the first section of the small intestine can only mean absorption into the mucosa or a greater exchange. It does not necessarily indicate increased passage through the mucosa.

The concentration of total Mg in the liquid phase showed a progressive increase along the tract from the rumen to the caecum, the last section whose contents were separated.

Intravenous administration of ^{28}Mg

Walls and contents of the gastro-intestinal tract. Table 2 shows the distribution of ^{28}Mg and stable Mg in the mucosa and contents of the selected segments of the tract of sheep B, 10 h after administration of ^{28}Mg. The specific activities of the contents of all sections were lower than that of the plasma, owing to the mixing of endogenous with stable exogenous Mg in the lumen of the gut.

There was an increase in the specific activity of the liquid phase from the rumen to the abomasum and from the abomasum to the first section of the small intestine. It must have been due either to secretion of Mg or to the preferential absorption of Mg compounds of a lower specific activity relative to the mean for the liquid phase of the previous section. Secretion takes place in the abomasum through the gastric juice (Garton, 1951) and in the first section of the small intestine through the bile and pancreatic juice (Field, 1960*b*), but absorption of Mg of a low specific activity cannot be excluded.

There was a progressive decline in the specific activity of the liquid phase from the mean for sections 2 and 3 to section 6 of the small intestine (as numbered from the pylorus). Since the specific activity of the solid phase did not increase from sections 2 and 3 to 6, there was no evidence of exchange with endogenous Mg of relatively high specific activity, and the fall presumably was due to preferential absorption of Mg compounds of high specific activity.

A marked variation in the specific activity of the mucosa from organ to organ and from section to section in the same organ was observed. The values for the small intestine, for example, ranged from 2·82 for the first part of section 5 to 8·53 for the first part of section 1. Section 1 and the first part of section 3 had specific activities greater than the plasma (5·70). The specific activity of the mucosa is dependent not only upon absorption and secretion but also upon the rate at which the Mg in the mucosa exchanges with the ^{28}Mg in either the plasma or the liquid phase of the ingesta. For this reason no conclusions could be drawn on the cause of the variation in specific activity along the tract.

The specific activity of the liquid phase was always higher than that of the solid phase of the same section. The difference was not a constant from section to section; it was greatest in those sections where secretion was occurring and least in sections 5 and 6 of the small intestine, where reabsorption of intestinal secretions was practically complete.

The pattern of variation in the concentration of total Mg in the liquid phase along the tract was similar to that for sheep A but the values were generally smaller.

Table 2. *Distribution of ^{28}Mg and total Mg in the contents and walls of the gastro-intestinal tract of sheep B 10 h after intravenous administration of ^{28}Mg*

Site	Length of section (ft)	Mucosa S.A.*†	Liquid phase: Volume (ml)	Liquid phase: Concentration of Mg (mg/100 ml)	Liquid phase: S.A.*	Solid phase: Dry weight (g)	Solid phase: Concentration of Mg as percentage of dry weight	Solid phase: S.A.*	Total contents S.A.*
Rumen	—	1·96	—	2·95	0·41	—	0·096	0·12	—
Abomasum	—	5·10	439	3·42	1·67	22·1	0·046	0·82	1·32
Small intestine:									
Section 1	24	8·53 6·43	104	7·30	5·07	4·2	0·066	3·50	4·65
Section 2	8	5·00 4·45	147	7·40	4·53	7·1	0·074	3·08	4·06
Section 3	15	7·67 4·40							
Section 4	11	3·98 —	93	8·22	2·34	4·17	0·121	1·56	2·03
Section 5	10	2·82 3·07	134	9·00	1·75	9·75	0·105	1·53	1·65
Section 6	17	4·75 4·18	93	8·55	1·14	3·71	0·166	1·05	1·07
Caecum	—	3·00 3·45	—	17·05	1·50	—	0·150	1·30	—
Colon:									
Section 1	—	2·74 2·96	—	—	—	—	—	—	1·10
Section 2	—	3·99 5·53	—	—	—	—	—	—	1·11
Rectum	—	—	—	—	—	—	—	—	0·20

* Specific activity expressed as percentage of dose × 10^2/mg total Mg.
† The first of the two values applies to the anterior and the second to the posterior part.

Body fluids, soft tissues and bones. The values obtained for the specific activity and the relative specific activity of the selected soft tissues and body fluids are given in Table 3. Relative specific activity is the ratio of the specific activity of the tissue to that of the plasma and is a measure of the proportion of stable Mg that has exchanged during the 10 h period between administration of ^{28}Mg and the death of the sheep. The fact that the values for kidney and bile were greater than 1 may be due to two factors: (1) the falling specific activity of the plasma (Care, Macdonald & Nolan, 1959), and (2) failure of ionic ^{28}Mg to exchange with the bound Mg in the plasma, thereby providing a fraction of relatively higher specific activity for exchange with intracellular Mg.

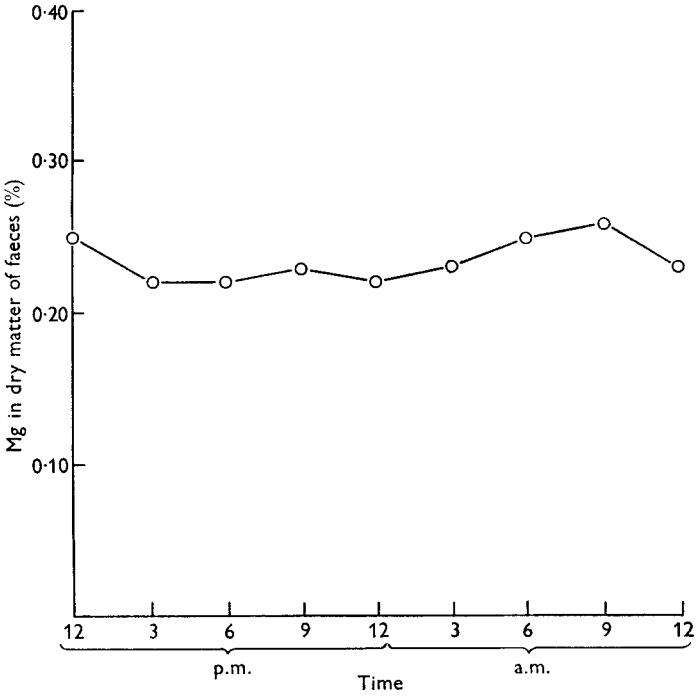

Fig. 1. Diurnal variations in the mean magnesium content of the faeces of four sheep.

Table 3. *Specific activity and relative specific activity of samples of tissues and bile of sheep B*

Sample	S.A.*	R.S.A.†	Sample	S.A.*	R.S.A.†
Bile	7·1	1·24	Femur, shaft	0·048	0·0084
Kidney	6·4	1·12	Femur, epiphysis	0·13	0·023
Liver	5·1	0·89	Rib, shaft	0·079	0·014
Spleen	4·0	0·70	Rib, sternal end	0·13	0·023
Muscle	0·23	0·04	Lumbar vertebra	0·10	0·018

* Specific activity expressed as percentage of dose $\times 10^2$/mg total Mg.

† Relative specific activity $= \frac{\text{S.A. of tissue Mg}}{\text{S.A. of plasma Mg}}$.

A striking feature was the variation in specific activity in different tissues; the values ranged from 6·4 for kidney to 0·23 for skeletal muscle. Thus the rate at which ^{28}Mg exchanges varies with different tissues, and their Mg may exist in different chemical forms.

The specific activities and relative specific activities for the samples of bone are given in Table 3. The overall proportion of bone Mg in equilibrium with the Mg in body fluids was low, but there was marked variation from bone to bone. It was greater in cancellated than in compact bone. Thus the sternal end of the rib and the epiphysis of the femur showed the greatest exchange and the femur shaft the least.

Diurnal variation in Mg concentration in faeces

The mean Mg content of the faeces of four wethers over a 24 h period is shown in Fig. 1. Although the mean values tended to be higher in the morning than in the afternoon the trend was not significant. Thus there was no evidence for a diurnal variation similar to that reported for calcium and phosphorus in pigs (Moore & Tyler, 1955*a*), and the results reported above for the distribution of Mg along the tract were therefore independent of the interval between the last feed and the killing of the sheep.

DISCUSSION

The use of ^{28}Mg in the ionic form as a tracer for the passage of dietary Mg through an animal is complicated by the fact that not all the Mg in the food is in the ionic form. Therefore, ^{28}Mg should be incorporated in, and allowed to equilibrate with, the Mg of the diet before feeding. The half life of ^{28}Mg is too short to allow this equilibration, so that the only possibility was to give ^{28}Mg separately in the hope that before absorption the ^{28}Mg and stable dietary Mg would mix and exchange uniformly in the rumen. The results described above show clearly that this exchange did not take place, since the specific activities of the solid phase of the contents in the various sections of the tract were generally lower than those of the corresponding liquid phase from which Mg is absorbed. Hence the figures previously obtained for availability (Field, 1959) are more relevant to the availability of ionic ^{28}Mg than to that of dietary Mg. It is essential that before the comparative-balance technique is used, the assumption of equilibrium between ^{28}Mg and dietary Mg in the gastro-intestinal tract be tested for the diet under investigation.

In the isotope-dilution method it is assumed that the secreted Mg is reabsorbed from the tract at the same specific activity. This assumption implies that no exchange of ^{28}Mg takes place in the lumen of the tract during the period between secretion and reabsorption. A progressive decrease in the specific activity of the liquid phase from sections 2 and 3 to 6 of the small intestine was observed in sheep B, which is consistent with the selective absorption of secreted Mg at a higher specific activity than that of exogenous Mg. However, since exogenous Mg was not isolated and its radioactivity not measured, limited exchange cannot be excluded.

Another major source of error in the two methods is the exchange of ^{28}Mg between the contents and mucosa of the gastro-intestinal tract, its direction depending upon

their relative specific activities. There is no way of differentiating with certainty exchange from absorption after oral administration and from secretion after intravenous administration and, for this reason, both methods lead to an overestimation of availability and endogenous faecal excretion, the extent of which is unknown.

When the Mg content in the gastro-intestinal tract of sheep was raised to abnormal levels with large doses of Mg salts, Stewart & Moodie (1956) found that, although absorption occurred from the rumen to the caecum, the main site was the small intestine. My observations suggest that the main site of Mg absorption in adult wethers is the middle third of the small intestine, but no definite conclusions can be drawn regarding other sites because of the complicating factor of isotopic exchange.

As expected, it was found that the main sites of secretion of Mg from the body into the lumen of the gastro-intestinal tract are the abomasum and the first part of the small intestine. The ^{28}Mg found in the rumen after intravenous administration is probably derived from the saliva.

In the isotope-dilution method the proportion of endogenous Mg in the ingesta is calculated by assuming that the specific activity of the gastro-intestinal secretions is equal to that of the plasma. However, this assumption appears to be invalid under my experimental conditions, since the specific activities of the mucosa of the sections that secrete Mg were higher than that of the plasma. It was possible, however, to calculate from the progressive fall in the specific activity of the total ingesta from section 1 to section 6 of the small intestine that about 75% of the secreted Mg was reabsorbed in the lower region of the small intestine. Thus factors that interfere with the process of reabsorption may have profound effects on Mg balance and possibly on homeostasis.

The uptake of ^{28}Mg by the tissues after its intravenous administration clearly shows that there is a constant movement of Mg into and out of cells. Further, the rate at which exchange occurred, which is measured by the specific activity, varied in different tissues. The specific activities of the tissues and fluids were in the descending order: bile (7·1), kidney (6·4), liver (5·1), spleen (4·0), muscle (0·23), bone (0·13–0·048). These findings are similar to those obtained with rats by other workers. On the basis of the specific-activity curves, Rogers & Mahan (1959) showed that organs fell into two classes: in the liver, kidney and heart the Mg exchanged rapidly and completely with the ^{28}Mg in the plasma, whereas in the brain, testes, erythrocytes and skeletal muscles there were two forms of Mg, one rapidly and one more slowly exchangeable. MacIntyre (1959), using a similar technique, confirmed these findings and also showed that the Mg in bone was only slowly exchangeable and that the specific activity of the liver was always higher than that of the plasma. I observed a specific activity higher than that of plasma for bile, kidney and parts of the wall of the gastro-intestinal tract but not for liver. Since Mg in the plasma exists both free and bound, it is possible that the bound Mg exchanges only slowly with ^{28}Mg and that the higher specific activity of the tissues results from exchange with ionic ^{28}Mg in the plasma. It is noteworthy that the rate of ^{28}Mg exchange in a tissue is not a measure of its Mg reserves, since Watchorn & McCance (1937) and Blaxter, Rook & MacDonald (1954) could find no evidence of Mg depletion in those soft tissues with high rates of exchange.

A marked variation in the specific activity from bone to bone was observed, and was greater in regions of rapid bone metabolism than in compact bone. Thus the sternal end of the rib and the epiphysis of the femur showed the greatest exchange and the femur shaft the least. A similar variation has been observed with the uptake of calcium by the same bones in cattle (Hansard, Comar & Plumlee, 1952). It is of interest that this variation in the specific activity of individual bones was in the same order as the degree of Mg depletion in the same bones of Mg-deficient calves (Smith, 1959).

SUMMARY

1. Two 5-year-old wethers were given a diet of grass nuts providing 1·2 g Mg daily for a period of 6 days. They were slaughtered 10 h after receiving a single dose of ^{28}Mg by stomach tube (sheep A) or by intravenous injection (sheep B). ^{28}Mg and stable Mg were determined in the liquid and solid phases of the ingesta and in the mucosa of the various sections of the gastro-intestinal tract. Selected tissues of sheep B were also analysed.

2. The main site of absorption of ^{28}Mg appeared to be the middle third of the small intestine.

3. ^{28}Mg was secreted into the lumen of both the abomasum and the first section of the small intestine and was apparently reabsorbed from the lower segments of the small intestine.

4. For both sheep the specific activities of the mucosa, apart from those sections where absorption or secretion was suspected to be predominant, differed from that of the plasma, which was tentatively attributed to exchange of ^{28}Mg between the contents of the tract and the walls.

5. After oral administration, the ionic ^{28}Mg and dietary Mg did not distribute uniformly in the gastro-intestinal tract. The specific activity of the liquid phase was generally higher than that of the corresponding solid phase, but the difference was not constant from section to section. After intravenous administration there was no evidence of appreciable exchange between the ^{28}Mg in the secretions with the stable exogenous Mg in the lumen of the gut.

6. There were marked variations in the specific activity between soft tissues, between bones and between parts of the same bone. The order of decreasing specific activity was bile, kidney, plasma, liver, spleen, skeletal muscle and bone.

7. The results are discussed in relation to the use of ^{28}Mg to determine endogenous faecal Mg excretion and availability of dietary Mg by the comparative-balance and isotope-dilution methods and it was concluded that these techniques are of doubtful value.

8. Samples of faeces were collected over 3 h periods, for a total of 24 h, from four wethers that had been on the same dietary régime as sheep A and B for the previous 15 days. No evidence for a diurnal variation in the concentration of Mg in faeces was obtained.

I gratefully thank Dr J. T. Stamp and Dr E. J. Butler for helpful discussions, Dr D. I. Nisbet and Mr N. J. L. Gilmour who helped with the dissection, Mr P. Tothill, Department of Medical Physics, University of Edinburgh, for the use of a scintillation counter and Mrs A. Smart and Miss N. Hutchison for technical assistance.

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Printed in Great Britain