Studies on magnesium in ruminant nutrition

4.* Balance trials on sheep given different amounts of grass nuts

By A. C. FIELD

Moredun Institute, Gilmerton, Edinburgh

(*Received* 27 *June* 1961)

In Part 1 of this series (Field, McCallum & Butler, 1958), an account was given of balance experiments in which sheep were given spring herbage collected from a variety of pastures. A rectilinear relationship was found between urinary excretion and dietary intake of magnesium and the regression coefficient varied considerably from sheep to sheep. From these values tentative figures were calculated for the availability of dietary Mg.

In view of the importance of these findings in relation to the aetiology of hypomagnesaemic tetany in sheep and the development of methods for the determination of availability of Mg, these investigations were extended. A series of Mg and calcium balance trials was carried out in which each of four wethers was given three different amounts of the sample of grass nuts for successive 15-day periods. The trials were repeated at an interval of 1 year.

EXPERIMENTAL

The experiments were carried out with four apparently healthy North Country Cheviot wethers (referred to as sheep A, B, C and D), aged about 4 years at the beginning of the experiments and weighing 59, 75, 66 and 68 kg, respectively. Sheep B had been used in a previous experiment (Field *et al.* 1958). The sheep were harnessed for the separate quantitative collection of urine and faeces and housed in individual cages.

Two experiments were conducted at an interval of about 1 year. Each comprised a series of balance trials in which the sheep were given 900, 1200 and 1500 g artificially dried grass in the form of nuts each day over successive periods of 15 days. The sequence in which these amounts were given was the same for each sheep; the sequence being chosen at random in series 1 and in series 2. Each trial was made up of a 9-day preliminary and a 6-day collection period. A replicate balance trial at the 1200 g level of intake was carried out in the first experiment to obtain an estimate of reproducibility. The sheep were fed twice daily with equal portions at 10 a.m. and 4 p.m. and distilled water was always available.

The grass nuts were purchased in one batch from a local merchant, thoroughly mixed and stored in galvanized steel bins until required. Their composition is given in Table 1.

* Paper no. 3: *Brit. J. Nutr.* (1961), **15**, 349.

Table 1. *Percentage composition of nuts made from artificially dried grass*

Moisture	11·3	Nitrogen-free extractives	37·7
Crude protein	8·6	Ash	10·2
Ether extract	3·1	Magnesium	0·128
Crude fibre	29·1	Calcium	0·644

Faeces and urine were collected over 24 h periods, the faeces into Polythene bags and the urine into a Polythene bottle containing 100 ml of 50% (v/v) glacial acetic acid (AR). After the volume of urine and acid had been measured, about 250 ml were filtered through nylon cloth and one-tenth of the total volume was taken for chemical analysis. At the end of the collection period, the daily samples were pooled, and duplicate samples were taken for Ca and Mg estimation. The faeces were weighed daily and transferred to a Polythene bowl. After thorough mixing, one-fifth was weighed out into a Polythene bag which was closed with a rubber band and stored at 4°. Samples taken over the whole period were pooled in this bag. The composite sample was then divided into three approximately equal portions which were dried separately in an electric oven at 100–105° and ground in a Christy Norris mill with a 1 mm sieve.

At 11 a.m. on the last day of each trial, samples of serum were taken for Ca and Mg estimation.

The methods for ashing and estimating the Ca and Mg in the serum, urine and faeces were those used in previous experiments (Field *et al.* 1958).

RESULTS

Urinary Mg excretion. The mean daily values for urinary Mg excretion (UMg) are given in Table 2, together with the daily intake (DMg) to which they are clearly related. The regression coefficients of UMg on DMg differed significantly between sheep ($P < 0·01$), but not between experiments, whereas the constant terms in the regression equations differed both between sheep ($P < 0·005$) and between experiments ($P < 0·005$). Values for the regression coefficients and constant terms are given in Table 3.

Table 2. *Intake and excretion of magnesium by the sheep*

(Mean daily values for each collection period)

Expt no.	Intake (g)	Sheep A		Sheep B		Sheep C		Sheep D	
		U(g)	F(g)	U(g)	F(g)	U(g)	F(g)	U(g)	F(g)
1	1·02	0·097	0·94	0·024	1·04	0·052	1·04	0·106	0·98
	1·36	0·112	1·28	0·036	1·37	0·074	1·49	0·152	1·19
	1·70	0·147	1·49	0·053	1·73	0·126	1·65	0·190	1·44
2	1·02	0·077	0·87	0·032	0·86	0·032	0·98	0·096	0·91
	1·36	0·103	1·11	0·036	1·23	0·062	1·20	0·135	1·16
	1·70	0·127	1·50	0·047	1·61	0·072	1·60	0·166	1·50

Standard deviations (6 df) of urinary and faecal excretion are ±0·007 and ±0·056 g/day, respectively.
U, urinary excretion; F, faecal excretion.

The second-order interactions (sheep × experiment × level of feeding) have been used as the estimates of error for carrying out significance tests for each of the measurements analysed.

Faecal Mg excretion. The mean daily values for faecal Mg excretion (FMg) are also given in Table 2 and they too are clearly related to daily intake (DMg). There were some indications in the first experiment of a non-linear relationship, but the effect was small and of no great practical significance; if it is ignored, the regression coefficient of FMg on DMg was 0·882 and did not differ significantly between sheep or between experiments. There were significant differences in the constant terms of the regression equations, and these constants are given in Table 4.

Table 3. *Values for the constants b and c in the regression equation* $UMg = bDMg + c(g)$

	Sheep A	Sheep B	Sheep C	Sheep D
b (both experiments)	0·074	0·032	0·084	0·113
c (Expt 1)	0·017	0·000	−0·038	−0·006
c (Expt 2)	0·004	−0·012	−0·050	−0·019

The standard error of *b* is ±0·010 and that of *c* is ±0·014.
UMg, mean daily urinary excretion of Mg; DMg, mean daily intake of Mg.

Table 4. *Values for the constant c in the regression equation* $FMg = 0{\cdot}882DMg + c(g)$

Expt no.	Sheep A	Sheep B	Sheep C	Sheep D
1	0·060	0·160	0·180	0·090
2	−0·060	0·040	0·060	−0·030

The standard error of the constant term is ±0·080.
FMg, mean daily faecal excretion of Mg; DMg, mean daily intake of Mg.

The error due to sampling and chemical analysis, estimated as the variance between the triplicate samples, formed only a small part of the total error.

Mg balance and net absorption. The estimates of the mean daily balance and net absorption are given in Table 5, which shows that the net absorption was generally lower in the first than in the second experiment, and that differences in net absorption occurred between sheep when the Mg intake was the same. These differences were significant ($P < 0{\cdot}01$) but there were no significant differences between the mean net absorption at different levels of intake. Further, net absorption and urinary excretion were not correlated.

The net absorption is the arithmetical difference between the estimates of intake and faecal excretion of Mg. It is, therefore, subject to the same errors as both, but since the absorbed fraction is much less than the faecal fraction, the coefficient of variation for estimates of net absorption becomes much greater than that for faecal estimates. The standard error for each value of net absorption, calculated from the variation between the triplicate samples of faeces, was 0·0319 g/day and 95 % confidence limits were ±0·137 g/day.

The mean balances for the first and second experiments were significantly different ($P < 0{\cdot}01$) at −0·040 and +0·064 g/day, respectively.

Table 5. *Mean daily values (g) for magnesium intake, net absorption and balance of the sheep in each collection period*

		Sheep A		Sheep B		Sheep C		Sheep D	
Expt no.	Intake	Net absorption	Balance	Net absorption	Balance	Net absorption	Balance	Net absorption	Balance
1	1·02	+0·08	−0·02	−0·02	−0·04	−0·02	−0·07	+0·04	−0·07
	1·36	+0·08	−0·03	−0·01	−0·05	−0·13	−0·20	+0·17	+0·02
	1·70	+0·21	+0·06	−0·03	−0·08	+0·05	−0·07	+0·26	+0·07
2	1·02	+0·15	+0·07	+0·16	+0·12	+0·04	0·00	+0·11	+0·01
	1·36	+0·25	+0·15	+0·13	+0·09	+0·16	+0·09	+0·20	+0·07
	1·70	+0·20	+0·07	+0·09	+0·04	+0·10	+0·03	+0·20	+0·03

Standard deviations (6 df) of net absorption and balance are ±0·083 and ±0·044 g/day, respectively.

Table 6. *Concentration (mg/100 ml) of magnesium in the serum of the sheep*

Expt no.	Mg intake (g/day)	Sheep A	Sheep B	Sheep C	Sheep D
1	1·02	2·47	2·13	2·73	2·40
	1·36	2·67	2·55	3·13	3·13
	1·70	2·73	2·73	2·55	2·40
2	1·02	2·35	2·13	2·35	3·07
	1·36	2·40	2·50	2·67	3·07
	1·70	2·27	2·05	2·08	2·25

Standard deviation (6 df) is ±0·155 mg/100 ml.

Serum Mg. The serum Mg values are given in Table 6. Significant differences were found between sheep; at the intermediate level of Mg intake the values were significantly higher than at the extreme levels and the mean for the first experiment was significantly higher than that for the second, the values being 2·63 and 2·53 mg/100 ml, respectively.

The means for the sheep were significantly correlated ($r = 0{\cdot}984$, $P < 0{\cdot}05$) with the coefficients for the regression of UMg on DMg.

Urinary Ca excretion. No systematic differences were found between the respective values for the mean daily urinary excretion of Ca in the two experiments and they may be considered as replicates. The results are given in Table 7.

The observed increase in urinary Ca with increasing Ca intake was curvilinear ($P < 0{\cdot}005$) for sheep A, B and C. There was no correlation between the urinary excretions of Ca and Mg.

Faecal Ca excretion. The estimated mean daily values for faecal Ca excretion are given in Table 8, which shows that the values in the first experiment were generally lower than the corresponding ones in the second; differences existed between the sheep when the intake was the same and, as expected, the faecal excretion varied with intake. The differences were significant ($P < 0.01$) and independent. The mean values for the first and second experiments were respectively 7·42 and 6·79 g/day, a mean difference of 0·63 g or 9·2% of the mean Ca intake.

Table 7. *Mean daily urinary calcium excretion (g) of the sheep*

Expt no.	Ca intake (g/day)	Sheep A	Sheep B	Sheep C	Sheep D
1	5·14	0·035	0·024	0·035	0·014
	6·86	0·087	0·055	0·085	0·027
	8·58	0·159	0·097	0·171	0·030
2	5·14	0·029	0·052	0·053	0·018
	6·86	0·050	0·041	0·070	0·022
	8·58	0·126	0·095	0·145	0·048

Table 8. *Mean values (g) for daily intake, faecal excretion (F), net absorption (A) and balance (B) of calcium in the sheep*

Expt no.	Intake	Sheep A			Sheep B			Sheep C			Sheep D		
		F	A	B	F	A	B	F	A	B	F	A	B
1	5·14	5·48	−0·34	−0·37	5·57	−0·43	−0·45	6·22	−1·08	−1·11	6·49	−1·35	−1·36
	6·86	7·05	−0·19	−0·28	7·37	−0·51	−0·56	7·64	−0·78	−0·86	7·42	−0·56	−0·59
	8·58	8·76	−0·18	−0·34	9·04	−0·46	−0·56	9·44	−0·86	−1·03	8·54	+0·04	+0·01
2	5·14	4·46	+0·68	+0·65	4·20	+0·94	+0·89	5·87	−0·73	−0·78	5·90	−0·76	−0·78
	6·86	6·23	+0·63	+0·58	6·95	−0·09	−0·13	6·52	+0·34	+0·27	7·14	−0·28	−0·30
	8·58	8·18	+0·40	+0·27	8·87	−0·29	−0·39	8·37	+0·21	+0·07	8·75	−0·17	−0·22

Standard deviations (6 df) of faecal excretion, net absorption and balance are ±0·319, ±0·318 and ±0·276 g/day, respectively.

The relationship between faecal excretion and intake was investigated with the pooled values from the two experiments and found to be rectilinear ($P < 0.005$) with no evidence of curvilinearity. The regression coefficient for all sheep was 0·935.

Ca balance and net absorption. The results obtained for the mean daily Ca balance and net absorption are given in Table 8. A marked feature of the values for the net absorption was the large sampling and analytical error associated with them; the standard error was ±0·216 g/day, for each value, calculated from the variation between the triplicate samples of faeces, and the 95% confidence limits were ±0·933 g/day. With two exceptions, none of the net absorption figures were significantly different from zero, but they showed non-random variation between sheep and between experiments. These differences were significant (sheep, $P < 0.05$; experiments, $P < 0.01$). The values for the net absorption of the sheep were correlated with neither the urinary Ca excretion nor the net absorption of Mg.

On statistical analysis the results for the balance gave similar results to those for net absorption, but the difference between experiments was slightly greater at 0·74 g/day or 10·8% of the mean Ca intake.

Serum Ca. The values obtained for the serum Ca concentrations are not recorded here since the only noteworthy feature was that in the first experiment the mean serum Ca value for the sheep decreased with increasing intake, whereas the opposite occurred in the second experiment.

DISCUSSION

DMg depends upon the dry-matter consumption and the concentration of Mg in the dry matter of the diet. In their study of the relationship between UMg and DMg, Field *et al.* (1958) varied DMg by using different amounts of spring herbages of differing Mg concentration, whereas in the experiment now described DMg was changed by varying only the dry-matter consumption.

The two experiments gave essentially the same results in that UMg was rectilinearly related to DMg, and the regression coefficients for the individual sheep on the same experiment differed significantly. The fit of the data to a regression was significantly better for the grass nuts than for the spring herbages, which suggests that factors existed in the different samples of spring herbage which modified slightly the relationship between UMg and DMg. These could act by changing the availability, endogenous faecal excretion or balance of Mg.

The regression coefficients for the sheep (0·032, 0·074, 0·084 and 0·113) were generally small. The individual differences, although small, were reproducible in so far as they were the same on the two occasions on which they were measured when the sheep were 4 and 5 years of age. Although the regression coefficients for the two sheep in the experiment of Field *et al.* (1958) were larger and the individual differences greater (0·126 and 0·263), they were not significantly different from those in the experiment now described. One sheep was used in both series of experiments, and its regression coefficient was significantly lower ($P < 0·05$) in this experiment (0·032) than in the previous experiment (0·126) when the sheep was only 18 months old. This difference may be attributed to the difference in either the age of the sheep or in the diet, but further work is required to elucidate which one of the two factors is responsible.

The linear relationship between UMg and DMg could only arise if the balance and endogenous faecal excretion of Mg were constant or linearly related to DMg. No evidence of a linear relationship between the balance and DMg was obtained in this investigation or in that of Field *et al.* (1958), and the balance may be considered a constant in each series of experiments. There is no direct evidence in the literature on the variability of endogenous FMg because of the difficulty of determining it. Such information as is available on this point comes partly from studies on the fate of intravenously administered Mg salts to dogs, sheep and man (Mendel & Benedict, 1909; Dryerre, 1936; McCance & Widdowson, 1939). In these experiments all the injected Mg was rapidly excreted in the urine, indicating that the endogenous Mg excretion may be independent of the amount of Mg absorbed from the gut by the animal. Since endogenous FMg is secreted Mg which has escaped reabsorption, any factor that alters intestinal secretions or reabsorption may change endogenous FMg. Because saliva is a major source of secreted Mg and because the volume of saliva secreted during feeding depends upon the amount and coarseness of the food (Denton, 1957; Balch, 1958), it is possible that endogenous faecal excretion of Mg, like that of nitrogen, is linearly related to dry-matter consumption and, if so, there must be a partial regression between UMg and dry-matter consumption. No evidence for such a relationship was found in the values for UMg and dry-matter consumption given by Field *et al.* (1958) and, consequently, for a linear relationship between endogenous FMg and dry-matter intake. The range of dry-matter consumption (790–1330 g/day) was almost identical with the one used in the experiment now described (798–1330 g/day). A further possibility is that the reabsorption of secreted Mg is dependent upon the amount of Mg in the ingesta and hence on the DMg. Studies on the absorption of Mg with ^{28}Mg by Field (1961) have shown that the reabsorption of Mg is a very efficient process and is probably controlled by other factors than those that limit the absorption of DMg.

From the above consideration it is highly probable that the endogenous faecal excretion of Mg is a constant under any given set of conditions and it follows that the regression coefficient, expressed as a percentage, is the availability of DMg (Field *et al.* 1958). Although the availability of Mg in feedingstuffs is usually small, the individual values (3·2, 7·4, 8·4 and 11·3%) obtained for grass nuts are the smallest yet recorded for Mg.

It follows from the relationship between UMg and DMg that if the balance remains constant FMg should be linearly related to DMg with a regression coefficient equal to $1-b$, where b is the regression coefficient of UMg on DMg. This relationship is independent of the variability of endogenous FMg. The relationship was found to be linear but the regression coefficient was the same for each sheep. This value, 0·88, i.e. $(1-b)$, corresponds to a value of 0·12 for b which is of the same order as the mean for the four sheep (0·076) found from the relationship between UMg and DMg. The failure to detect differences in availability (b expressed as a percentage) between individual sheep by means of the method based on FMg clearly demonstrates the difference in sensitivity in the two methods which is due entirely to the greater sampling error in the estimation of FMg.

In theory the negative constant term in the regression equations of UMg and DMg is equal to the sum of the endogenous faecal excretion and the balance. Similarly, the endogenous faecal excretion is equal to the constant term in the relationship between FMg and DMg. In practice these terms cannot be estimated with sufficient accuracy to give reliable figures for the endogenous faecal excretion. They are subject to errors associated with the sample mean as well as the regression coefficient. The latter's contribution to the error predominates and is responsible for the large 95% confidence interval of the constant terms. For this reason it was not possible to draw any conclusions on the effect of age on endogenous faecal loss.

The net absorption value may be taken as a measure of the amount of Mg available to the animal for growth and milk production, but the accuracy with which this figure can be determined is low. In the experiments described above, even when the error associated with the determination of DMg was ignored, 95% confidence limits attached to the estimates of net absorption were $\pm 0·137$ g/day. Thus any method for determining the utilization of DMg based on net absorption will be inaccurate.

The factors which control the concentration of Mg in the serum are not completely understood, but one important factor is the balance between the Mg entering and leaving the body, since the skeletal reserves of Mg are less readily available in the adult animal (Blaxter & McGill, 1956). If the urinary excretion of Mg can be described in terms of filtration–reabsorption mechanism (Barker, Clark & Elkinton, 1959) the serum Mg level should be related to UMg. Such a correlation has been found for cows (Rook, Balch & Line, 1958), but neither previous experiments with sheep (Field *et al.* 1958) nor those described here have revealed this relationship in sheep.

Since Visek, Monroe, Swanson & Comar (1953) have found in cattle that the endogenous faecal Ca excretion is a constant, it can be shown that the proportion of Ca in grass nuts unavailable to the animal was a constant over the range of Ca intake investigated and equal to the regression coefficient of faecal Ca on dietary Ca. This value, 0·936, was the same for all sheep and represents an availability of 6·4%.

Contrary to many reports in the literature, there was no evidence of an interrelationship between Ca and Mg metabolism over the range of intake investigated.

SUMMARY

1. The results of two experiments carried out at an interval of 1 year are reported. Each experiment comprised a series of magnesium and calcium balance trials in which each of four Cheviot wethers was given 900, 1200 or 1500 g/day of the same batch of grass nuts for 15-day periods.

2. For all sheep there was a highly significant rectilinear relationship between the amount of Mg in the urine and the Mg intake, but there were significant differences between the regression coefficients for the individual sheep. From these equations estimates of the percentage absorption of the Mg in grass nuts by the individual sheep were obtained and ranged from 3·2 to 11·3%.

3. The relationship between faecal Mg output and Mg intake differed in the two experiments; in the first it was slightly curvilinear and in the second rectilinear. These relationships gave an estimate of 12% for the availability of the Mg in grass nuts to all the sheep.

4. The estimation of the availability of dietary Mg and endogenous faecal loss of Mg by the methods based on the regression of urinary Mg and of faecal Mg on Mg intake are discussed, and it is concluded that for the former the urine method is more sensitive and for the latter neither method is accurate enough to give reliable estimates.

5. No correlation between serum Mg concentration and the amount of Mg in the urine was detected but the mean values for the former were correlated with the animals' ability to absorb dietary Mg.

6. For all sheep the increase in age from 4 to 5 years had no effect on the efficiency of absorption of dietary Mg, but the concentration of Mg in the serum and urinary Mg excretion were higher at 4 than at 5 years of age.

7. No interrelationships between Mg and Ca metabolism at the level of the dietary intakes investigated could be detected. Urinary and faecal Ca excretion were correlated with intake; the former relationship was curvilinear and the latter rectilinear.

From the latter an estimate of 6·4% for the availability of the Ca in grass nuts was obtained.

I am grateful to my colleague Dr E. J. Butler for his helpful criticisms, to Dr J. F. D. Greenhalgh of the Edinburgh School of Agriculture for the chemical analysis of the grass nuts and to Mrs A. Smart, Miss N. Hutchison and the technical staff of the Chemistry Department, Moredun Institute, for their assistance.

REFERENCES

Balch, C. C. (1958). *Brit. J. Nutr.* **12**, 330.
Barker, E. S., Clark, J. K. & Elkinton, J. R. (1959). *J. clin. Invest.* **38**, 1733.
Blaxter, K. L. & McGill, R. F. (1956). *Vet. Rev. Annot.* **2**, 35.
Denton, D. A. (1957). *Quart. J. exp. Physiol.* **42**, 72.
Dryerre, H. (1936). *Vet. J.* **92**, 163.
Field, A. C. (1961). *Brit. J. Nutr.* **15**, 349.
Field, A. C., McCallum, J. W. & Butler, E. J. (1958). *Brit. J. Nutr.* **12**, 433.
McCance, R. A. & Widdowson, E. M. (1939). *Biochem. J.* **33**, 523.
Mendel, L. B. & Benedict, S. R. (1909). *Amer. J. Physiol.* **25**, 1.
Rook, J. A. F., Balch, C. C. & Line, C. (1958). *J. agric. Sci.* **51**, 189.
Visek, W. J., Monroe, R. A., Swanson, E. W. & Comar, C. L. (1953). *J. Nutr.* **50**, 23.

Printed in Great Britain