Studies on magnesium in ruminant nutrition

9. Effect of potassium and magnesium intakes on development of hypomagnesaemia in sheep

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1. In Expt 1, five groups of four dry non-pregant ewes were given 1 kg/day of a semi-purified diet containing either 0·05, 0·10 or 0·15 % magnesium with 0 or 4·44 % added potassium. Plasma Mg concentrations were reduced by increasing the K intake ($P < 0·001$) and by reducing the Mg intake ($P < 0·1$). The effects were additive and, at the lowest Mg intake, supplementary K induced two cases of hypomagnesaemic tetany.

2. Expt 2 was similar to Expt 1 except that the ewes were suckling single lambs, they were given 2 kg food/day and the lowest dietary Mg concentration was 0·075 %. K again lowered plasma Mg values and induced two cases of tetany but reductions in Mg intake had no effect.

3. After repletion on the basal diet, sixteen lactating ewes from Expt 2 were transferred to a diet containing 0·05 % Mg with 0, 2·22 or 4·44 % added K. Plasma Mg concentrations fell rapidly on each treatment; the effect was greatest in K-supplemented groups ($P < 0·05$) and two cases of hypomagnesaemic tetany were induced at the highest K intake.

4. In each experiment the K supplements caused a rapid increase in plasma K concentrations to a relatively constant level some 5 mg/100 ml above the resting value of 18·0 mg/100 ml.

5. Some sheep refused K-supplemented diets at first and there was a tendency for refusals to be greatest at the lowest Mg intakes.

6. The results are discussed in relation to the aetiology of hypomagnesaemic tetany.

The sudden increase in potassium intake which occurs when ruminants first graze spring grass may contribute to the development of hypomagnesaemic tetany. Suttle & Field (1967) found that the apparent availability of magnesium to sheep was markedly reduced when the K content of a hay and concentrate diet was raised to a level found in 'tetany-inducing' pastures. Hypomagnesaemia was not, however, observed in this or in many earlier studies with K supplements (Pearson, Gray & Reiser, 1949; Eaton & Avampato, 1952; Odell, Hatfield, Shrewsberry, Gibson & MacVicar, 1952; Blaxter, Cowlishaw & Rook, 1960; Hendriks, 1962). The lack of effect of K on serum Mg concentrations led Blaxter & McGill (1956) to conclude that K had no direct effect upon Mg metabolism in ruminants. In some instances, however, an effect of K may have been masked by the presence of high Mg intakes since Kemp, Deijs, Hemkes & Van Es (1960) have shown that in cattle serum Mg concentrations do not reflect changes in Mg intake when Mg is present in excess of requirement.

We have, therefore, re-examined the effect of K on plasma Mg using diets with Mg concentrations of 0·05, 0·10 and 0·15 %, a range which includes the concentration commonly found in 'tetany-inducing' pastures (Kemp *et al.* 1960; Butler *et al.* 1963) and which was calculated to provide Mg in amounts above and below the estimated requirements for sheep (Agricultural Research Council, 1965).

EXPERIMENTAL

Animals

Twenty Scottish Blackface ewes, 4–6 years old and weighing approximately 40 kg, were used.

Design of experiments

Expt 1. Dry non-pregnant ewes were given 1 kg/day of basal diet, containing 0·15 % Mg and 0·26 % K, for 13 days. On the 9th day of pretreatment they were stratified according to their plasma Mg concentrations and allocated at random within strata to form five groups of four sheep. The treatments applied to these groups are given in Table 1; they were designated, first, by their Mg and, secondly, by their K content as high (H), intermediate (I) and low (L) and the same scheme was used in subsequent experiments. With the exception of diet LH which was given for only 6 days, all experimental treatments lasted for 12 days.

Table 1. *Expts 1 and 2. Dietary concentration (% air-dry feed) and daily intakes (g/day) of magnesium and potassium, and the duration of the treatments (days)*

| | | Mg | | K | |
| | Duration of | Dietary | Daily | Dietary | Daily |
Group	treatment	concentration	intake	concentration	intake
		Expt 1*			
HH	12	0·15	1·5	4·70	47·0
IL	12	0·10	1·0	0·26	2·6
IH	12	0·10	1·0	4·70	47·0
LL	12	0·05	0·5	0·26	2·6
LH	6	0·05	0·5	4·70	47·0
		Expt 2†			
HH	10	0·15	3·0	4·70	94·0
IL	10	0·10	2·0	0·26	5·2
IH	10	0·10	2·0	4·70	94·0
LL	10	0·075	1·5	0·26	5·2
LH	16	0·075	1·5	4·70	94·0

* Food allowance 1 kg/day.   † Food allowance 2 kg/day.

Expt 2. Twenty ewes with their 1–2 weeks old lambs were re-allocated to one of five groups by the same procedure as that used in Expt 1. The treatments (Table 1) differed from those in Expt 1 in two respects; food intake was increased to 2 kg/day to meet the additional requirements of lactation, and the lowest Mg concentration was raised to 0·075 % in view of the drastic effects of 0·05 % Mg in Expt 1. The experimental diets were given for 10 days with the exception of diet LH which was given for 16 days.

Expt 3. Sixteen of the ewes were repleted on the basal diet for 6 days immediately after Expt 2 was completed and then allocated in the usual way to one of three groups given diets (2 kg/day) containing 0·05 % Mg with 0, 2·22 or 4·44 % supplementary K (groups LL, LI and LH, respectively) for 11 days; the daily intake of Mg was 1·0 g and of K 5·2, 49·6 and 94·0 g, respectively. Groups LL and LI contained five ewes and group LH six ewes.

Diets

A pelleted, semi-purified diet was used with the following ingredients (%): oat hulls 47, starch 15, sugar 15, blood meal 10, urea 2·5, arachis oil 3·0, sodium bicarbonate 1·5, sodium sulphate decahydrate 0·5, sodium chloride 0·5, dicalcium phosphate 1·5, calcined magnesite 0·19, and added water 3. A trace element supplement provided 50 mg manganese, 20 mg zinc, 8 mg iodine and 2 mg cobalt per kg diet and a vitamin supplement provided 1000 i.u. retinol, 140 i.u. cholecalciferol and 36 i.u. DL-α-tocopheryl acetate per kg diet. This basal diet contained 0·15% Mg, 0·26% K, 0·47% Ca, 0·65% Na and 0·31% P. To obtain the treatment diets the Mg concentration of the basal diet was lowered by removing some of the calcined magnesite present and that of K was increased by adding 3·8 or 7·6% potassium chloride (General Purposes Reagent grade); the daily allowances of K-supplemented diets were correspondingly increased by 3·8 or 7·6%.

Management

During the experiments the ewes were housed in individual wooden pens with slatted floors and given deionized water *ad lib*. When Expt 1 was completed they were put out to pasture and tupped by rams of the same breed. The ewes were lambed in the individual pens where they remained with their single lambs throughout Expts 2 and 3. Blood samples were taken from the jugular vein at frequent intervals, for plasma mineral analysis, from the ewes in each experiment; samples were also taken from lambs before and after treatments in Expts 2 and 3, and the lambs were also weighed at those times. The daily food allowance was given in two equal feeds at 09.00 h and 16.00 h and refusals were recorded daily.

Analytical methods

Ca and Mg were estimated by atomic absorption spectroscopy (using a Model AA_2, Hilger-Watts, London). Plasma samples were diluted 1:20 with an aqueous solution containing 5% (w/v) trichloroacetic acid and 0·5% (w/v) lanthanum chloride. After centrifugation, Ca and Mg were determined in the supernatant fraction. Diet samples, weighing approximately 1 g, were dry ashed at 450–500°. The ash was dissolved in 10 ml 25% (v/v) hydrochloric acid, made up to 100 ml with distilled water and finally diluted 1:10 with 0·5% (w/v) lanthanum chloride. The techniques used for determining K (Field, 1964), Na and P (Suttle & Field, 1967) in plasma were described in earlier papers in this series. Plasma ultrafiltrates were obtained in Expts 2 and 3 by centrifuging plasma for 1 h at 35000 *g*, in apparatus modified from an original design of Dr D. J. Bell, Physiology Department, Edinburgh University (personal communication). Ca and Mg in ultrafiltrates were determined by the above procedures.

RESULTS

Food refusals

Expt 1. Sheep in groups other than LH generally consumed the whole of their allowance. The exceptions were one sheep from group IL which left 1·0 and 0·86 kg on days 1 and 2, respectively, and two sheep from group LL which left 0·77 and 0·90 kg on the 12th day when their plasma Mg concentrations were 0·34 and 0·56 mg/ 100 ml. In group LH two sheep refused most of their allowance on the 1st day; thereafter, little food was refused until the 5th and 6th days of treatment, when each animal left about 50% of its allowance.

Expt 2. Refusals were confined to the groups given supplementary K and to the first 5–6 days of treatment. They tended to increase as the dietary Mg concentration decreased and the mean percentage refusals during the whole period for groups HH, IH and LH were 4·11, 7·02 and 11·73%, respectively. The corresponding ranges for each group were 0–12·8, 3·2–15·5, 0–27·8% and the differences between the means were not significant. The refusals were not sufficiently great to cause an overlap in Mg intakes between the treatments.

Expt 3. Food was refused consistently throughout the experiment by one sheep from each of groups LL and LI and by three from group LH, the amounts being 16·9, 53·4 and 22·0, 40·4 and 56·7% of the allowance in the respective groups.

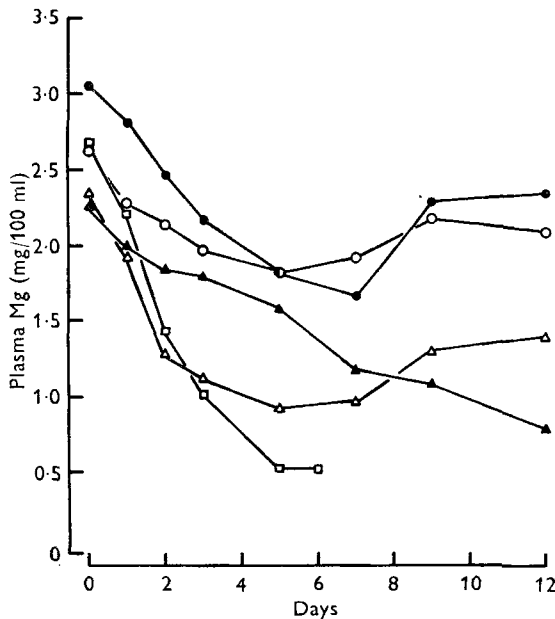During the pretreatment and repletion periods in Expts 1 and 2 no food refusals were recorded.

Fig. 1. Mean plasma magnesium concentrations in groups of dry ewes following changes in Mg and K intakes from 1·5 and 2·6 g/day to 1·5 and 47·0 g (O—O, group HH), 1·0 and 2·6 g (●—●, IL), 1·0 and 47·0 g (△—△, IH), 0·5 and 2·6 g (▲—▲, LL) or 0·5 and 47·0 g/day (□—□, LH).

Plasma Mg

Expt 1. The effects of the dietary treatments on the mean plasma Mg values for each group are shown in Fig. 1. During the pretreatment period, values were relatively constant with an overall mean of 2·54 mg/100 ml but the concentrations fell in each group during the treatment period. In groups HH, IL and IH, minimum values were found after 5–7 days, and they tended to increase during the latter half of the period.
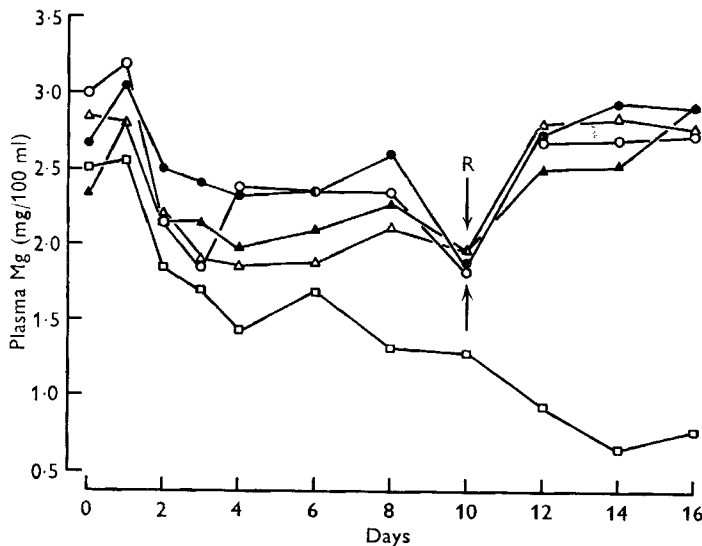

Fig. 2. Expt 2. Mean plasma Mg concentrations in groups of lactating ewes following changes in Mg and K intakes from 3·0 and 5·2 g/day to 3·0 and 94·0 g (O—O, HH), 2·0 and 5·2 g (●—●, IL), 2·0 and 94·0 g (△—△, IH), 1·5 and 5·2 g (▲—▲, LL) or 1·5 and 94·0 g/day (□—□, LH); R denotes commencement of repletion.

In group LL values continued to decline throughout the period reaching a final mean value of 0·80 mg/100 ml. When the results for groups IL, IH, LL and LH at the 5th day were analysed statistically the increase in K intake was found to have reduced plasma Mg levels by 1·0 mg/100 ml ($P < 0·001$); the decrease in Mg intake had reduced values by 0·33 ($P < 0·1$) and the effects were additive. Thus, minimum values were found in group LH, and two animals from this group developed hypomagnesae-mic tetany on day 6, having plasma Mg values of 0·14 and 0·30 mg/100 ml; the treat-ment was, therefore, discontinued. The addition of K to the basal diet (group HH) reduced the plasma Mg values in all sheep but the variation within the group prevented the effect from reaching statistical significance.

Expt 2. The changes in plasma Mg concentrations in the lactating ewes in response to the dietary treatments are shown in Fig. 2. As in Expt 1, values fell following the introduction of each treatment but the effects were smaller and, with the exception of group LH, were completed after 3–4 days. In group LH values fell steadily throughout the period of treatment and two cases of tetany occurred after 12 and 16 days in which the plasma Mg concentrations were 0·64 and 0·70 mg/100 ml. When the

treatment ceased after 16 days, the two remaining sheep had values of 0·60 and 1·04 mg/100 ml.

Covariance analysis revealed a significant correlation ($P < 0·05$; $R = 0·48$) between mean initial (last 3 days of pretreatment) and mean final (days 6, 8 and 10 of treatment) plasma Mg values. Analysis of the adjusted values for all groups except HH showed that the increase in K intake had significantly lowered plasma Mg concentrations on average by 0·63 mg/100 ml ($P < 0·05$); reducing the Mg intake had a small but non-significant effect and there was no significant interaction. K did, however, tend to reduce values more at the lower Mg intakes. Covariance analysis, of initial and final plasma Mg values in Expts 1 and 3 produced no significant relationships.
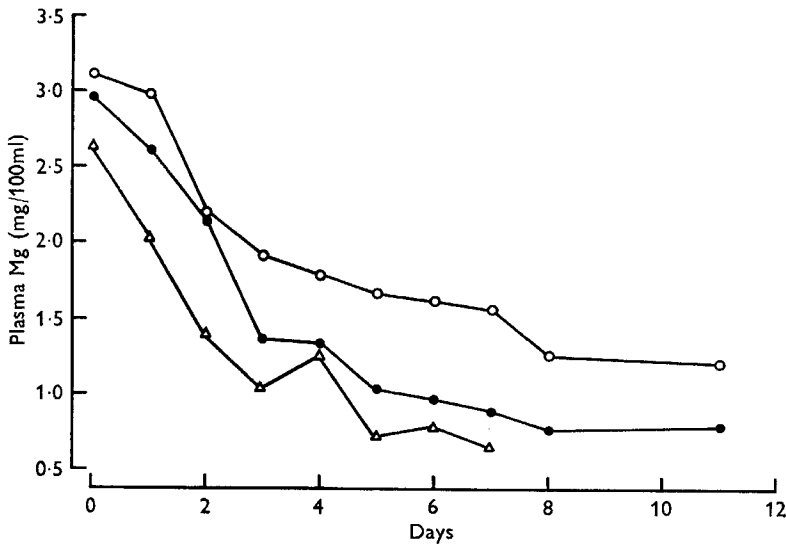

Fig. 3. Expt 3. Mean plasma Mg concentrations in groups of lactating ewes following changes in Mg and K intakes from 3·0 and 5·2 g/day to 1·0 and 5·2 g (○—○, LL), 1·0 and 49·6 g (●—●, LI) or 1·0 and 94·0 g/day (△—△, LH).

When the sheep, with the exception of group LH, were repleted with the basal diet after 10 days the mean plasma Mg values increased ($P < 0·001$) and were similar to the pretreatment values after 2 days (Fig. 2).

Expt 3. The mean initial plasma Mg value was $2·87 \pm 0·43$ (S.E.) mg/100 ml showing the adequacy of the repletive measures. Values fell in each group following the reduction in Mg intake to 1·0 g/day; the decrease was most rapid in the groups given a K supplement but there was little difference between groups LI and LH (Fig. 3). Two cases of hypomagnesaemic tetany with plasma Mg values of 0·26 and 0·34 mg/100 ml occurred at the highest K intake (group LH) on day 7. Plasma Mg levels continued to fall in group LL and in the survivors from group LH but there were no further cases of tetany. There was marked individual variation, however, particularly in the K-supplemented groups in which two animals showed only a small fall in plasma Mg values. The individual values on day 7 are given in Table 2 together with the average

Mg intakes over the entire experiment. There was evidently no definite relationship between these measurements, and variation in Mg intake within groups due to food refusals was clearly not the major factor causing plasma Mg values to vary; this also applied to Expts 1 and 2.

Table 2. *Expt 3. Relationship between variations in plasma magnesium concentrations and Mg intakes in lactating ewes given a low-Mg diet with or without a potassium supplement*

Group	K supplement (g/day)	Ewe no.	Mg intake (g/day)	Plasma Mg on day 7 (mg/100 ml)
LL	0	83	1·0	1·54
		94	1·0	1·90
		95	1·0	1·68
		86	1·0	1·68
		91	0·83	1·04
LI	44·4	99	1·0	1·00
		80	1·0	1·90
		72	1·0	0·54
		98	1·0	0·54
		88	0·47	0·40
LH	88·8	77	1·0	2·00
		97	1·0	0·36
		84	1·0	0·34
		90	0·78	0·64
		79	0·58	0·26
		85	0·43	0·40

Plasma K

In each experiment plasma K levels were increased on the 1st day of supplementation with K and remained elevated throughout the treatment period by some 5 mg/100 ml. There were no differences between the K-supplemented groups and there was no effect due to Mg intake. In Expt 1, for example, the overall mean value for the pretreatment period was $18·8 \pm 0·5$ (SE) mg/100 ml and that for all K-supplemented ewes during treatment was $23·3 \pm 0·5$ (SE) mg/100 ml ($P < 0·001$).

Plasma Ca, Na and inorganic P

The mean plasma concentrations of these elements were generally within the normal ranges throughout each experiment and the results are not, therefore, presented in detail. The two ewes which developed hypomagnesaemic tetany on day 6 in Expt 1 did have low plasma Ca values (4·4 and 4·3 mg/100 ml) at that time. Normal plasma Ca values were observed, however, in the four ewes with hypomagnesaemic tetany occurring in Expts 2 and 3.

Ultrafiltrable Ca and Mg

In Expt 2 there were no significant differences between the treatments in the proportions of the plasma Mg and Ca which were ultrafiltrable. The overall means of 100 observations were $57·2 \pm 1·3 \%$ for Mg and $49·7 \pm 1·7$ (SE) % for Ca. Values in

Expt 3 tended to decrease with time but there were no significant treatment effects. It is interesting to note that the two sheep from group LH with hypomagnesaemic tetany on day 7 of Expt 3 had exceedingly low ultrafiltrable Ca values of $21 \cdot 4$ and $17 \cdot 3 \%$.

Lambs

There were no differences between groups of lambs in either growth rate or plasma mineral concentrations in Expts 2 and 3. The results are not, therefore, presented in detail. The mean growth rate for the pure Blackface lambs was $0 \cdot 248$ kg/day.

DISCUSSION

It has been shown conclusively in these experiments that an increase in K intake can reduce plasma Mg concentrations in both dry and lactating ewes. Furthermore, when added to diets of low Mg concentration, K induced six cases of hypomagnesaemic tetany. These findings are in marked contrast to those of earlier workers who found no evidence of a fall in serum Mg concentrations in ruminants given high K intakes. In some instances this disagreement can be attributed to differences in Mg intake between experiments. In those of Eaton & Avampato (1952) and Pearson *et al.* (1949), the diet consisted of alfalfa hay and would have provided Mg greatly in excess of requirements. In these circumstances a factor interfering with Mg metabolism would be unlikely to lower serum Mg concentrations. In our studies a reduction in dietary Mg concentration from $0 \cdot 15$ to $0 \cdot 10 \%$ caused a slight fall in plasma Mg. This indicates that the highest Mg intakes barely met the ewes' requirements for Mg, and that conditions were ideal for demonstrating a hypomagnesaemic response to added K.

The failure of Blaxter *et al.* (1960) to lower plasma Mg concentrations in young calves cannot be explained in terms of a high Mg intake since they added K to diets either adequate or deficient in Mg. The Mg metabolism of their young calves would have differed from that of our mature sheep in two important respects, however. First, the younger animal possesses a large labile reserve of Mg in the skeleton which can counteract short-term dietary deficits. Secondly, the young calf absorbs large amounts of Mg from its large intestine (Smith, 1959); since K is absorbed higher up the alimentary tract an adverse effect of K on Mg absorption might thus be avoided. A further possibility is that the degree of Mg depletion in their low-Mg group was so severe that antagonistic effects of K would not be shown.

Both De Groot (1961) and Meyer & Steinbeck (1960) have observed a fall in serum Mg values in cattle given K supplements, but Kunkel, Burns & Camp (1953) are the only workers to have observed a similar response in sheep. In their studies the supplement, potassium bicarbonate providing 5% added K, caused a marked reduction of 35% in food intake. Since a reduction in food intake *per se* can rapidly lower serum Mg levels in the ruminant (Halse, 1960; Herd, 1966), this alone could have been responsible for the observed fall in serum Mg values. Although unpalatability was also encountered in our studies it was largely confined to group LH. The effect of K was evident at each level of Mg intake and, therefore, was not always confounded with lowered food intake. Furthermore, the effects of K within group LH in each experi-

ment were as pronounced in animals refusing little or no food as in those refusing the most food. A reduction of 50% in the K supplement in Expt 3 did not lessen the hypomagnesaemic response, and the effect of smaller variations in K intake within groups due to food refusals is probably negligible.

The fall in plasma Mg concentrations following an increase in K intake was probably due in part to a reduction in the uptake of Mg from the gut (Suttle & Field, 1967). The effect of added K was, however, greater than that of reducing Mg intakes by 50% in Expts 1 and 2, and it was largely independent of Mg intake. It is possible that K exerted an additional direct depressing effect on the circulating level of Mg.

The effect of increasing K intakes to levels experienced by sheep grazing spring pastures was sufficient to produce tetany when the dietary Mg concentration was reduced to 0·075%. Although this level is some 25–50% lower than those afforded by 'tetany-inducing' spring pastures (Kemp *et al.* 1960; Butler *et al.* 1963), the dry-matter intakes of lactating ewes on such pastures may be considerably less than the 1·8 kg/day which we provided; the Mg intakes in these two situations could, therefore, be comparable. Provided that the K in spring pasture acts in a manner similar to that of an inorganic supplement, our findings indicate that excessive intakes of K contribute to the development of hypomagnesaemia and tetany. The variability which was observed in the hypomagnesaemic response to added K can also be compared with that found in ruminants grazing spring pastures for the first time (Butler *et al.* 1963).

It is informative to examine the Mg intakes which were required in these studies to prevent mean plasma Mg values from falling below the lower limit of the normal range (i.e. 1·8 mg/100 ml; Allcroft, 1947). In the absence of added K, the Mg intake required was between 0·5 and 1·0 g/day for dry ewes and between 1·0 and 1·5 g/day for lactating ewes. In the presence of added K, however, the requirement increased to between 1·0 and 1·5 g for dry ewes and 1·5–2·0 g for lactating ewes. The Mg intakes required to prevent hypomagnesaemia in our studies are of a similar order to those predicted by a factorial method by the Agricultural Research Council (1965); they suggested requirements of 0·59 and 2·01 g/day, respectively, for dry and lactating ewes, assuming an apparent availability of 20%. The mean apparent availability of Mg in our basal diet to five wethers was found to be 26%.

Carr (1955) found that the degree of binding of Ca and Mg to plasma albumin in vitro was affected by the relative concentrations of those elements in the system, and Wilson (1964) has suggested that studies of the ultrafiltrable Mg in plasma might throw some light on the aetiology of hypomagnesaemic tetany. In our studies, considerable reductions in plasma Mg concentration were without effect on the proportions of bound Mg and Ca present in the plasma. The reduction in ultrafiltrable Ca in two cases of tetany in Expt 3 may have some significance, but further cases must be examined before an association is established. The mean values obtained for ultrafiltrable Mg and Ca, 57·2 and 49·7%, respectively, are similar to those recorded in man (Walser, 1961).

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