Studies on magnesium in ruminant nutrition

6*. Effect of intake of water on the metabolism of magnesium, calcium, sodium, potassium and phosphorus in sheep

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1. In Expt 1 balance studies were carried out with six wether sheep receiving chopped hay and concentrates and given, 0, 5, or 10 l. water/day by intraruminal infusion. Allocation to two 3 × 3 latin squares was arranged to allow calculation of main treatment and residual effects. 2. Infusing 10 l. water/day increased the total daily urinary output of magnesium, phosphorus and sodium by 31, 45 and 100% ($P < 0.05$). Faecal losses of Mg, and P tended to decrease correspondingly and with both elements retention remained positive but retention of Na became negative ($P < 0.05$). 3. Residual effects were not detected, and infusing 5 l. water/day generally produced intermediate and non-significant effects. 4. When pellets made from dried, ground, young spring pasture or concentrates rich in Mg were included in the diet of four wethers in Expts 2 and 3, infusions of 10 l. water/day produced much greater decreases in faecal losses and retentions of Na and K and greater increases in the urinary excretion of those elements than those found in Expt 1. Effects on Mg, P and Ca balance were similar to those in the main experiment. 5. The biological significance of these results and relationships between the observed effects and the aetiology of hypomagnesaemic tetany are discussed.

Hypomagnesaemia occurs frequently in sheep and cattle when they are turned out to graze lush pasture in spring (Sjollema, 1930*a*, *b*; Hemingway, Ritchie, Brown & Peart, 1965; Butler *et al.* 1963). Rook & Balch (1958) have shown that, when dairy cows were subjected to a sudden dietary change from a typical winter ration to cut spring herbage, the levels of magnesium in serum and urine and the apparent availability of dietary Mg were decreased. The change from winter foodstuffs to lush grass generally leads to an increase in the intake of water, protein and potassium by the grazing animal, any of which may contribute to the development of hypomagnesaemia. In the present study we have investigated the effect of raising the water intake of sheep, by means of prolonged infusions of water into the rumen, on the metabolism of magnesium, phosphorus, sodium, potassium and calcium. Total daily urinary outputs of Mg, P, Na and K were increased but the retentions of Na and K only were decreased during the period of infusion; apparent availability of each element was generally increased by the treatment.

METHODS

Expt 1. The direct and residual effects of water intake on the metabolism of Mg, P, Na, K and Ca were studied in six sheep which were allocated at random to one of three treatments, 0, 5 and 10 l./day supplementary water intake, in two 3 × 3 latin squares. Water intake was raised by infusing water into the rumen through fistulas. The

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sequence in which the treatments were applied to each sheep was that given by Cochran & Cox (1957) to determine residual as well as main treatment effects.

The sheep were given 600 g concentrates and 200 g chopped hay daily and this ration provided 1·80 g Mg, 3·17 g P, 1·09 g Na, 7·78 g K, 4·8 g Ca and 122 g crude protein/day. In the balance studies, urine was collected daily throughout a 10-day treatment period. Faeces were collected for the last 5 days of the period to allow time for faeces formed during the pretreatment period to be eliminated. Sheep were rested for 10 days between treatments and body-weights were recorded in the middle of the rest periods and at the beginning and end of the experiment.

Expt 2. The effect of water intake on the metabolism of Mg, P, Na, K and Ca was studied in four sheep given pellets made from ground dried grass prepared from lush spring pasture. The daily ration of 800 g pellets contained 1·03 g Mg, 2·28 g P, 3·16 g Na, 24·62 g K, 4·41 g Ca and 158 g crude protein. Balance studies were conducted for 10 days before and 10 days during the treatment period in which 10 l. water/day were infused into the rumen. The object of raising the water intake of sheep receiving grass pellets was to simulate the sudden increase in intake associated with a change of diet to lush spring herbage.

Expt 3. The effect of water intake on the urinary output of Mg, P, Na, K and Ca was studied further in four sheep on a high intake of Mg. Mg intake was raised to 2·94 g/day by giving daily 200 g Mg-rich concentrate together with 300 g chopped hay. This diet also provided 1·22 g P, 2·01 g Na, 6·24 g K, 3·07 g Ca and 44·4 g crude protein daily. Owing to a shortage of dietary materials, urine was collected for only 7 days before and 7 days during the treatment period, in which 10 l. water/day were infused into the rumen.

Animals

Sheep for Expts 1, 2 and 3 were taken from a pool of six Scottish Blackface wethers, 18 months old and weighing about 50 kg. They had been fitted with rumen fistulas 2 months previously.

Management

The sheep were housed individually in wooden pens with slatted floors and they had access to an unlimited supply of deionized drinking water. Voluntary water intake was measured daily. Weighed quantities of food were given twice daily throughout the experiment and for 1–2 weeks previously to achieve stable dietary conditions. Water intake was raised by infusing 5 or 10 l./day deionized water into the rumen. Infusion rates were controlled by micro-pumps (Distillers Company Ltd, Epsom, Surrey) which delivered water to a glass well sited above the pen (Fig. 1). Water dripped from the well through a side delivery arm and down rubber tubing to a 6 in length of $\frac{7}{32}$ in perforated polyvinyl chloride tubing, located within the neck of the rumen fistula. Urine was drawn from a collecting pouch under reduced pressure (Wainman & Paterson, 1963) and through a tube linked to the glass well by a glass sleeve gland (Quickfit and Quartz Ltd, Stone, Staffordshire). The whole unit rested in a ball race which allowed it to swivel with the movements of the sheep; the sheep was thus able to move about freely in its pen.

Collection of samples

Urine was collected daily in glass vessels containing 100 ml 50% (w/v) acetic acid. Portions of 20 ml were taken for analysis from the daily output and were stored at +1° under a layer of toluene. The faeces from five 24 h collection periods were pooled, dried at 100° and milled, and 50 g samples were retained for analysis. Serum was obtained from blood samples taken from the jugular vein at the beginning and end of each treatment period in Expt 1.

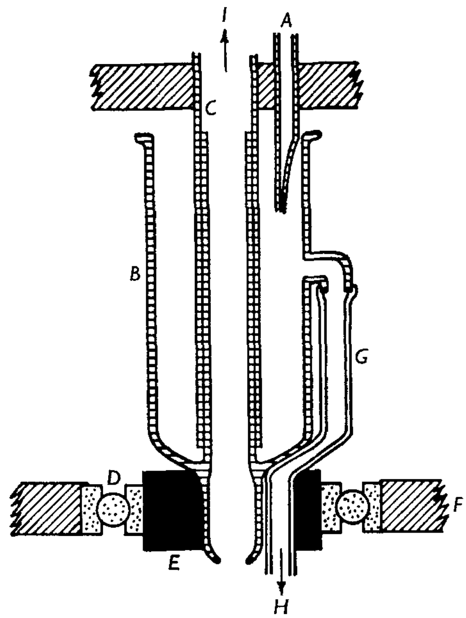

Fig. 1. Diagram of swivelling mechanism used in infusing water into and collecting urine from free-moving sheep. *A*, drip-feed from micro-pump; *B*, glass well; *C*, glass sleeve gland; *D*, steel ball race; *E*, rubber bung; *F*, fixture above animal pen; *G*, PVC tube linked to side delivery arm; *H*, direction of flow of infused water; *I*, direction of flow of urine under vacuum.

Analytical techniques

Details of the techniques used to estimate Mg, K and Ca in serum, urine, faeces and diets were given in an earlier paper in this series (Field, 1964). Na in ashed solutions or urine was estimated with an EEL (Evans Electro Selenium Ltd, Halstead, Essex) flame photometer after suitable dilution and P was estimated by the method of Fiske & Subbarow (1925).

Statistical analysis

The analysis of variance used for Expt 1 is illustrated by Cochran & Cox (1957). Residual and overall treatment effects were tested by the *F* variance ratio. Student's *t* test was used to assess the significance of between-treatment effects. In Expts 2 and 3 the paired *t* test was used to compare the results obtained in the periods before and during which water was infused.

RESULTS

Expt 1

Water intake and urine output. The infusion of water caused an immediate decrease in voluntary water intake and drinking ceased within 48 h of infusing 5 or 10 l. water/day. It can be seen from Table 1 that the total intakes of water in the three groups of sheep were 1·2, 5·0 and 10·0 l. The output of urine increased by about 3·2 and 7·0 l. when the water intake was increased by 3·8 and 8·8 l. respectively (Table 1).

Intake and digestibility of food. The daily food intake and the digestibility of ingested food (67%) were not affected by the treatments; this suggests that the infusions did not cause gross digestive disturbances.

Urinary excretion of Mg, P, Na, K and Ca. The urinary concentrations of each element studied fell rapidly as the output of urine increased, but the total daily output of each element was generally increased (Table 2). The outputs of Mg, P and Na were increased by 30·7, 45·1 and 101·5% respectively when 10 l. water/day were infused into the rumen. Infusion of 5 l./day also increased the excretion of Mg, P and Na but the effect was only significant ($P < 0·05$) for Mg. Infusion of 10 l. water/day slightly increased the urinary excretion of K and Ca, but the effects were not significant.

Table 1. *Expt* 1. *Voluntary water intake* (*l./day*) *and volume of urine excreted* (*l./day*) *by six sheep receiving hay and concentrates and given* 0, 5 *or* 10 *l. water/day by intra-ruminal infusion for* 10 *days*

(Mean values with their standard errors)

Water infused	Voluntary water intake	Urinary excretion
0	1·235 ± 0·258	0·827 ± 0·103
5	0·004 ± 0·003	4·068 ± 0·226
10	0·002 ± 0·002	7·817 ± 0·196

Faecal excretion of Mg, P, Na, K and Ca. Differences in the mean daily excretion of Mg, P, Na, K and Ca in the faeces between treatments were not significant (Table 2). There were marked differences between individuals in the amounts of Ca excreted in the faeces.

Retention of Mg, P, Na, K and Ca. Infusion of 5 or 10 l. water/day did not significantly affect the retentions of Mg and P; this was partly a reflection of the balance attained between the increased urinary excretion and increased apparent absorption of these elements (Table 2). The infusion of 10 l./day caused a significant ($P < 0·05$) decrease in the retention of Na, and probably decreased the retention of K ($0·05 < P < 0·1$). Sheep receiving 10 l. water/day showed a mean negative Ca retention of 382 mg/day, but the wide variation between periods and animals precluded the demonstration of a significant treatment effect.

Residual effects. There was no evidence that the effect of any treatment continued beyond the 10-day rest period and affected the responses to subsequent treatments.

Table 2. *Expt 1. Mean urinary and faecal outputs and retentions (mg/day) of Mg, P, Na, K and Ca by six sheep receiving chopped hay and concentrates and given 0, 5 or 10 l. water/day by intraruminal infusion for 10 days*

	Mg			P			Na			K			Ca		
Water infusion (l./day)	In urine	In faeces	Re-tained	In urine	In faeces	Re-tained	In urine	In faeces	Re-tained	In urine	In faeces	Re-tained	In urine	In faeces	Re-tained
0	436	1122	240	1542	1072	555	473	204	416	6057	701	1025	29	4929	−152
5	527	923	347	1951	1123	97	711	149	232	6060	581	1141	69	4787	−50
10	570	954	274	2237	723	210	953	217	−77	6634	650	498	71	5118	−382
SE of difference between means	±27*	±81 NS	±97 NS	±195*	±197 NS	±363 NS	±174*	±23 NS	±177*	±420 NS	±153 NS	±300 NS	±30 NS	±309 NS	±335 NS

NS, not significant.
* $P < 0{\cdot}05$.

Table 3. *Expt 2. Mean urinary and faecal outputs and retentions (mg/day) of Mg, P, Na, K and Ca for periods of 10 days before and during the infusion of 10 l. water/day into the rumen of four sheep receiving spring grass pellets*

	Mg			P			Na			K			Ca		
	In urine	In faeces	Re-tained	In urine	In faeces	Re-tained	In urine	In faeces	Re-tained	In urine	In faeces	Re-tained	In urine	In faeces	Re-tained
Before infusion	151	1008	−130	924	1490	−135	2077	569	515	17272	4318	3033	1	3768	641
During infusion	236	705	88	1162	987	130	3652	283	−774	25373	1359	−2109	5	3797	608
Effect of infusion	85	−304	219	238	−503	265	1574	−286	−1289	8101	−2959	−5142	4	−29	33
SE of mean difference between treatments	±14	±96	±107	±119	±154	±150	±379	±123	±382	±484	±681	±1054	±1	±435	±418
Significance of paired *t* test	$P < 0{\cdot}05$	NS, but $P < 0{\cdot}1$	NS	NS	$P < 0{\cdot}05$	NS	$P < 0{\cdot}01$	NS	$P < 0{\cdot}05$	$P < 0{\cdot}001$	$P < 0{\cdot}05$	$P < 0{\cdot}05$	$P < 0{\cdot}05$	NS	NS

NS, not significant.

Serum concentrations of Mg, P, Na, K and Ca. Concentrations of Mg, P, Na, K and Ca in the serum were not affected by raising the intake of water and the results are not, therefore, presented.

Expt 2

Faecal and urinary outputs and retentions of Mg, P, Na, K and Ca. Values obtained for the mean urinary and faecal outputs and retentions of Mg, P, Na, K and Ca, before and during the infusion of water into the rumen of four sheep on a diet of spring grass pellets, are given in Table 3. It can be seen that the increase in water intake caused an increase in the urinary excretion and a decrease in the faecal excretion of the elements studied, but the extent of the effect varied between the elements.

The mean daily excretion of Mg and P in urine increased by 56 and 26%, respectively during the infusion period. The increase in urinary P excretion ranged from 28 to 569 mg/day, but the effect was not significant. Amounts of Mg and P excreted in the faeces were decreased by 30 and 34%, respectively when the water intake was raised. The decrease in P was significant and that in Mg almost reached significance, both at the 5% level of probability. Quantitatively, the decrease in faecal losses was greater than the increase in urinary losses, so that retention of both Mg and P tended to increase.

Losses of Na and K in urine were increased respectively by 76 and 47% when water was infused into the rumen. Although faecal losses of Na and K were reduced by 50 and 69%, the additional uptake of these elements was quantitatively insufficient to compensate for the higher urinary losses, with the result that retentions of both Na and K were significantly ($P < 0.05$) reduced during the period of infusion.

Although there was a significant ($P < 0.05$) increase in the small amount of Ca excreted in the urine, the infusion of water had no effect on the faecal losses or retention of Ca.

Rate of change in urinary excretion of Mg, P, Na and K. The design of this experiment enabled us to assess the rate at which an increase in the water intake affected the excretion in the urine of Mg, P, Na and K.

Fig. 2 shows that the output of Mg and K was increased after only 24 h and continued to increase gradually for 4–8 days. The effect on urinary excretion of P and Na was more rapid, but with Na the effect diminished as the infusion continued from the 2nd to the 10th day. When the infusion ceased, the urinary output of each element returned to the pre-infusion level within 2–3 days. Results for Ca are not presented in Fig. 2 because the wide day-to-day variation in excretion makes it impossible to discern trends in the urinary output of Ca over short periods.

Expt 3

Urinary outputs of Mg, P, Na, K and Ca. The effects of infusing 10 l. water/day on the output in the urine of Mg, P, Na, K and Ca by four sheep given a Mg-rich concentrate were similar to those recorded in Expt 2, and the results are given in Table 4. There was a tendency for the urinary excretion of each element studied to be increased. The increases were significant for Mg, Na and K. Urinary excretion of P was increased in each animal, but the range of values, 198–1262 mg/day, again prevented the difference from being statistically significant. Infusion had no effect on urinary losses of Ca. Although a complete balance study was not conducted, it can be seen by comparing the urinary output of Na with intake (Table 4) that the sheep were in negative Na balance before the infusion began. The additional losses of

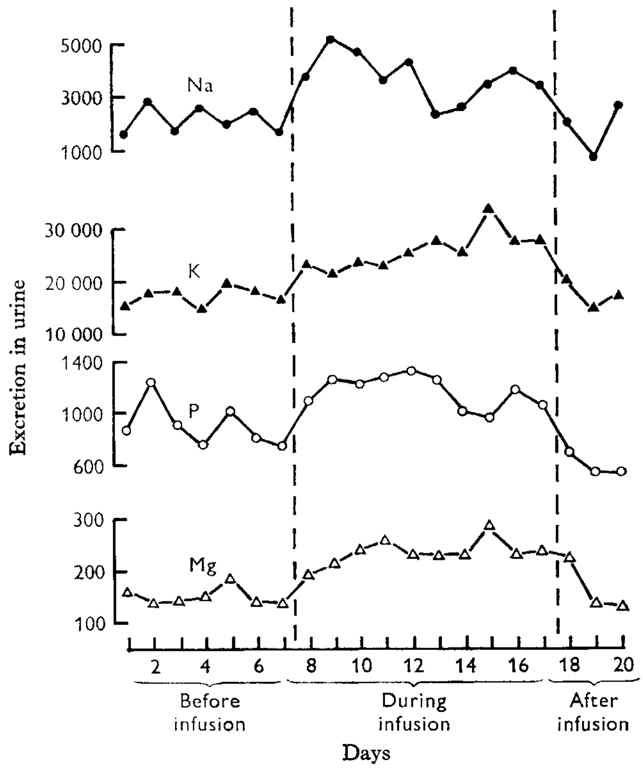

Fig. 2. Expt 2. Mean urinary excretion (mg/day) of Mg, △–△, P, ○–○, K, ▲–▲, and Na, ●–●, before, during and after infusion of 10 l. water/day into the rumen of four sheep receiving spring grass pellets.

Table 4. *Expt 3. Mean daily intakes and urinary outputs (mg/day) of Mg, P, Na, K and Ca for periods of 7 days before and during the infusion of 10 l. water/day into the rumen of four sheep receiving a Mg-rich concentrate and hay*

	Intake	Urinary output: Before infusion	Urinary output: After infusion	Change in output	Standard error of mean difference between treatments	Significance of paired *t* test
Mg	2942	697	1095	+398	±119	$P < 0.05$
P	1220	667	1177	+510	±253	NS
Na	2006	2908	10815	+7907	±599	$P < 0.001$
K	6238	7821	15168	+7347	±1523	$P < 0.05$
Ca	3067	7	10	+3	±1	NS

NS, not significant.

7·9 g/day Na and 7·3 g/day K which occurred during the infusion period could not have been corrected by increased absorption of Na and K from the gut, with the result that the retentions of both elements were decreased.

DISCUSSION

Before discussing the biological significance of the marked effects of water intake on mineral metabolism recorded in our experiments it is necessary to discuss the possibilities that the intake of water was raised to unphysiological levels and that experimental errors could have contributed to or produced the observed effects. The technique of infusing 5–10 l. water/day into the rumen was used in order to simulate, in part, the dietary change experienced by sheep at the onset of grazing in spring. A lactating ewe consuming 2 kg dry matter/day provided initially by hay and concentrates containing 85% DM and then by lush pasture containing 20% DM would increase its consumption of water from 0·3 to 8·0 l./day. Total water intake would probably increase some fourfold in these circumstances and the increases in water intake obtained in our experiments were of this order.

The changes in general metabolism (Annison, Lewis & Lindsay, 1959*a*, *b*) and in Mg metabolism (Rook & Balch, 1958) following a sudden change of diet are rapid. We, therefore, studied the urinary outputs of Mg, P, Na, K and Ca immediately after commencing the infusion treatments. Faeces were collected for the latter half of the 10-day infusion period to allow time for faeces formed during the pre-infusion period to be excreted. Although this delay may have been insufficient to eliminate a carry-over effect completely, faeces formed in the pre-infusion period would have contained higher concentrations of Mg, P, Na and K than faeces formed during the infusion period, when the apparent absorption of these elements was increased. The effect of carry-over of faeces would, therefore, be to nullify rather than produce the observed increases in apparent availability. Increases in the amounts of gut contents would cause increases in the apparent absorption of dietary constituents, but such changes were probably small, since the throughput of dry matter was not altered during treatment and body-weights remained steady between treatments. Furthermore, the daily output of urine returned to pre-infusion levels within 2 days of the end of the infusion, which suggests that little of the infused water remained in the gut to be excreted. Differences between treatments in the amounts of Mg, P, Na and K secreted in sweat were considered not to have been large enough to affect the interpretation of our results.

The changes in apparent availability of Mg and P in Expt 1 and Mg, P, Na and K in Expt 2 following an increase in water intake are given in Table 5. They could have arisen through reductions in the endogenous losses or increases in the true absorption of those elements, but the latter seems more likely. Daily endogenous losses of 100–250 mg Mg (Field, McCallum & Butler, 1958; MacDonald, Care & Nolan, 1959) and 250 mg Na (from Devlin & Roberts, 1963) would be expected in our sheep. By comparing these losses with the observed decreases in faecal excretion of Mg (Tables 2 and 3) and of Na (Table 3) it can be seen that the complete elimination of endogenous losses would be required to produce the observed increases in apparent absorption. The decreases in faecal excretion of Mg were significant ($P < 0{\cdot}001$) when the results from Expts 1 and 2 were pooled. Endogenous losses of P in mature wethers consuming 2–3 g P daily would probably amount to 1–1·5 g/day (Lueker & Lofgreen, 1961; Gueguen, 1962). Reductions of 20–50% in endogenous losses of P would be required to account for the increase in apparent availability of dietary P.

The effect of water infusions in increasing the urinary outputs of P, Na and K is in agreement with some observations by other workers. Sellers & Roepke (1951) reported increases in the urinary output of P, Na and also Ca in the 2 h following the sudden administration of 10 gal water into the rumen of pregnant cows. Oyaerts (1962) found increases in the urinary output of Na but not of Mg by sheep given 6 l. water/day by ruminal infusion. Urinary losses of Na and K in rats (Kellogg, Burack & Isselbacher, 1954) and of Na, K and P in fasting human subjects (Urbach, Phelps, Steiger & Bellet, 1953) are also increased when additional water is given. An effect of water intake on the excretion of Mg in urine has not been recorded hitherto.

Table 5. *Effect of infusing* 10 *l. water/day on the apparent availability* (%) *of dietary Mg, P, Na and K in sheep given hay and concentrates* (*Expt* 1) *or grass nuts* (*Expt* 2)

	Expt 1*		Expt 2†	
	Before infusion	During infusion	Before infusion	During infusion
Mg	37·5	46·9	2·0	31·5
P	66·2	77·3	34·6	56·7
Na	81·3	90·0	82·0	91·2
K	91·1	91·7	82·5	94·5

* Mean result for six sheep.
† Mean result for four sheep

There were differences in the effect of raising the water intake on the balance between the respective faecal and urinary excretions of each element studied. Differences between Na and K on the one hand and Mg and P on the other could occur in several ways. In the first instance raising the water intake increases urinary losses of Na and K more than of Mg and P. The body contains a large labile pool of both Na (Forbes, 1962) and K (Wilde, 1962) which could be drawn on to compensate for and indeed prolong the excessive urinary losses of Na and K. With Mg and to a lesser extent P, the labile pool in the body is much smaller and could not provide for large and continued losses in the urine. Since the apparent availability of dietary Mg and P is relatively low (Table 5), the infusion of water can and indeed does increase considerably the absorption of these elements from the diet. It can be seen in Table 5 that only 10–20% of the dietary Na and K is normally unavailable, and there is little opportunity for uptake from the diet to increase during infusion and compensate for the increased urinary excretion of Na and K. Raising the water intake, therefore, caused large decreases in the retention of Na and K but had little influence on the retentions of Mg and P.

Ca metabolism was not apparently affected by water intake. The absence of an effect on the excretion of Ca in urine was probably due to the effectiveness of reabsorptive mechanisms which normally allow little Ca to enter the urine. There is no reason to suppose that the true absorption of Ca would not be increased along with the other elements during the infusion of water. In the absence of storage of Ca, increased absorption would give rise to increased endogenous losses of Ca, with the result that faecal losses of Ca would remain constant when the water intake was raised.

The effects of raising the water intake on Mg and P metabolism in the three experiments described were fairly consistent despite the use of three rations in which the amounts of Mg, K and protein provided varied three- to four-fold. Urinary losses of K were, however, increased more by infusing water in Expts 2 and 3 than in Expt 1 and the urinary excretion of Na was eight- to ten-fold higher during infusion in Expt 3 than in Expts 1 and 2. It is difficult to relate these differences to variations in ration composition. The low dry-mater intake and the higher ratio of Na to K in the diet of the sheep in Expt 3 may have contributed in some way to the excessive urinary losses of Na. It is possible that the increased losses of Na and K in the later experiments resulted, from a decreasing tolerance to water loading. Residual effects were not, however, found in Expt 1, and in Expts 1 and 2 the total daily excretion of water, Na and K returned to normal within 2–3 days of reducing the intake of water. The excessive daily loss of Na in Expt 3 was equivalent to about 8–9% of the 90 g Na which a mature sheep carcass contains, and the losses of K were of a similar order (Suttle & Field, unpublished results). The sheep obviously could not withstand such high losses if water intake were increased for long periods. The negative balances of 1·3 g Na and 5·1 g K obtained during infusion in Expt 2 also represent a considerable drain on reserves of Na and K in the body.

The effects of infusing water on Mg metabolism were completely opposite to those found when the diet of ruminants is changed from typical winter foodstuffs to lush pasture. Introduction to lush pasture causes a decrease in the apparent avilability and the urinary excretion of Mg, whether the change is accompanied by a decrease (Rook & Balch, 1958) or an increase in the intake of Mg (Field, unpublished results). The apparent availability of Mg in the spring grass pellets was increased from 2·0 to 32·4% and daily excretion of Mg in the urine increased by 56% in Expt 2. If the high water intake from lush pasture has effects similar to those of infused water then the effectiveness of those factors causing a fall in the apparent availability of dietary Mg and in the Mg content of serum and urine when animals are turned out to grass assumes added significance.

Although the direct effects of water intake on Mg metabolism do not suggest relationships to the development of hypomagnesaemic tetany, other effects may be related. Thus the large decreases in retention of Na and K resulting from the infusion of water may be important since there are indications that uptake of both K (De Groot, 1962) and Na (Paterson & Crichton, 1960; Ross, 1961) may effect the development of hypomagnesaemia and the associated tetany.

Sheep in late pregnancy can consume over 6 l. water/day (Forbes & Boaz, 1965). In view of the large effects which increases in water intake exerted on mineral metabolism in our experiments, it would seem desirable to extend the investigation to the pregnant animal. The extent to which results from mineral absorption studies using isolated loops of intestine filled entirely with aqueous media apply to absorption under normal dietary conditions also requires further study in the light of our findings.

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