SUMMARY

1. Free glutamic acid in a concentration of about 1 g./100 ml. has been chromatographically identified in the seminal plasma of the domestic cock. Small amounts of alanine, aspartic acid, glycine, serine and an unidentified ninhydrin-reacting substance have also been detected. About 100 mg. of creatine/100 ml. is also present.

2. About 80 % of the seminal plasma non-protein nitrogen is accounted for by glutamic acid plus creatine.

3. It is probable that these substances are mainly of testicular origin and that the glutamic acid plays a major part in maintaining the osmotic pressure and pH.

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Calcium and Magnesium Metabolism in Calves

3. ENDOGENOUS FAECAL EXCRETION AND ABSORPTION OF MAGNESIUM*

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It has previously been shown (Smith, 1957, 1958) that the proportion of dietary magnesium excreted in the faeces by milk-fed calves increases as the calves get older. It is not clear whether this increase is due to decreased absorption of magnesium, increased endogenous faecal excretion or in part to both. The values for endogenous faecal excretion reported by Blaxter & Rook (1954) do not give any information on this question since they refer only to young calves.

The present work provides evidence on this matter and on the effect of the level of magnesium in the diet on the efficiency of magnesium absorption.

* Part 2: Smith (1958).

EXPERIMENTAL

General

The calves were all males and all were Friesians except for calf 7A, which was an Angus × Ayrshire crossbred, calf 11A which was an Angus × Shorthorn/Friesian crossbred and calf 18B which was a Hereford × Ayrshire crossbred.

The experiments consisted essentially in the determination of faecal and urinary excretion of magnesium while the calves were receiving low-magnesium synthetic milk and, in some cases, whole milk containing different amounts of magnesium. The calves were reared on whole milk until the experimental periods were started and those kept longer than 9 weeks (2A, 4A, 7A and 11A) were given supplements of iron, copper and manganese from 9 weeks of age and 70 000 i.u. of vitamin D₃/day from 18 to 23 weeks of

age. Balance experiments carried out on these four calves during the initial period when they were receiving whole milk have been reported previously (Smith, 1958), and further details of the treatment of the calves during this period are given in that paper. For at least 8 days before balance experiments on low-magnesium synthetic milk were carried out, the calves were kept in pens without bedding to ensure that no foreign matter was eaten. Milk-fed calves, particularly after they are a few weeks old, possess a great craving for solid food. Although our calves were muzzled they still contrived to ingest an appreciable amount of their bedding even though wood shavings were used for this purpose. The amounts of magnesium involved are not very important (less than about 4% of the total ingested magnesium) when a normal milk diet is given but might become relatively large when the low-magnesium synthetic milk is fed. In general, when balance experiments were carried out the calves were fed with the appropriate diet for 8 days before faeces and urine collections were started. These collections were made while the calves were kept in metabolism cages as described previously (Smith, 1957).

Determinations of magnesium in plasma, milk, faeces and urine were carried out as previously described (Smith, 1957). Determinations of polyethylene glycol in samples of faeces were carried out according to Smith (1959).

Synthetic milk

This was prepared essentially according to the procedure of Dr J. W. G. Porter (unpublished data), which was itself a modification of the method of Flipse, Huffman, Webster & Duncan (1950). The main difference in our procedure, apart from the omission of magnesium chloride, was in the replacement of calcium caseinate by Soluble Light White Casein (British Drug Houses Ltd.), which is essentially sodium caseinate (about 1.25% of sodium) and had a lower magnesium content than other products tested. The resulting calcium deficit was made up by adding extra calcium chloride. The net result of these changes was to replace magnesium by an approximately equivalent amount of sodium. Apart from this our synthetic milk contained the major inorganic elements and radicals in approximately the proportions occurring in cows' milk. The constituents of the milk are shown in Table 1. The casein was dissolved by adding it in successive small amounts to a volume of water equivalent to 10% of the final volume at about 80° with continuous mechanical agitation of the mixture. Glucose and the inorganic salts were dissolved in separate further amounts of water and added to the casein solution. The margarine was heated to about 60–70° and mixed with an approximately equal volume of water at about the same temperature and containing Lubrol W (Imperial Chemical Industries Ltd.) [1.4% (w/w) of the margarine]. The mixture was homogenized in a Waring Blender and the resulting cream added to the rest of the constituents. The vitamins were added in suitable solutions or suspensions to the cold mixture and the whole was made up to volume. The milk was usually prepared in batches of about 70 l. and stored at a temperature of 4° for not more than 2 weeks before use.

Calves at about 3–5 weeks of age given 4.40 l. of synthetic milk/day gained in weight an average of 1.1 kg./week, compared with about 2.1 kg./week for calves fed with a similar amount of whole milk. Corresponding

Table 1. *Composition of synthetic milk*

The milk was made up with distilled water.

Component	Amount/l.
Casein (Soluble Light White, British Drug Houses Ltd.)	33.4 g.
Glucose	76.8 g.
Margarine	13.4 g.
Na ₂ HPO ₄ ·12H ₂ O	3.82 g.
K ₂ HPO ₄	2.94 g.
Ca(OH) ₂	1.21 g.
CaCl ₂ ·6H ₂ O	3.40 g.
Ferric citrate	0.18 g.
MnSO ₄ ·7H ₂ O	0.04 g.
CuSO ₄ ·5H ₂ O	0.04 g.
CoSO ₄ ·7H ₂ O	0.04 g.
Citric acid	1.18 g.
Thiamine	1.1 mg.
Nicotinic acid	2.2 mg.
Calcium pantothenate	2.2 mg.
Pyridoxine hydrochloride	1.1 mg.
Riboflavin	2.2 mg.
p-Aminobenzoic acid	2.2 mg.
α-Tocopherol	1.1 mg.
Choline chloride	220 mg.
Inositol	22 mg.
Menaphthone	0.2 mg.
Biotin	0.01 mg.
Folic acid	0.05 mg.
Vitamin A	1000 i.u.
Vitamin D ₃	2000 or 8000 i.u.*

* 2000 i.u. for calves 2B, 4B, 18B and 12, 8000 i.u. for calves 2A, 4A, 7A and 11A.

figures for calves at about 26–32 weeks given 8.80 l./day were 1.4 kg./week on synthetic milk and 2.9 kg./week on whole milk.

For determination of magnesium, a 20 ml. sample of the milk was shaken with 5 ml. of 20% (w/v) trichloroacetic acid. After allowing to stand at 37° overnight the mixture was filtered and 5 ml. of the filtrate added to a 15 ml. conical centrifuge tube. Subsequently the determination was carried out as described previously for the determination of magnesium in whole milk (Smith, 1957). The synthetic milk contained about 0.6–0.8 mg. of magnesium/100 ml.

RESULTS

Endogenous faecal magnesium

The cumulative excretion of magnesium in the faeces of three calves (2B, 4B and 18B) receiving a diet of low-magnesium synthetic milk is shown in Fig. 1. The calves were 2–3 weeks old when the collections were started. Two of these calves were given daily subcutaneous injections of magnesium sulphate solution (equivalent to 1 g. of magnesium/day) during part of the time. It was found that after about 3 weeks on the synthetic-milk diet (the calves had received this diet for 8 days before the collections started) the excretion of magnesium tended to increase. Calf 12, treated in a similar way to calf 4B between 9 and 13 weeks of age,

behaved in a similar manner in this respect. The change occurred irrespective of whether magnesium injections were given during the first (calf 18B) or during the last (calves 4B and 12) half of the collection. Some increase of this type might be expected as a result of the previously reported (Smith, 1957) decrease in the intrinsic ability of calves to utilize magnesium as they get older. In these experiments, however, this effect was probably reinforced by a general digestive impairment since the total bulk of the faeces increased considerably after about 3 weeks on the synthetic-milk diet. Thus for calves 4B and 18B respectively the average daily excretion of faeces was 49 g. (30% dry matter) and 53 g. (33% dry matter) during the first 12 days of the collection and

89 g. (24% dry matter) and 121 g. (31% dry matter) during the second 12 days. This was not due simply to uneven evacuation of faeces since polyethylene glycol, which was added to the diets of these two calves throughout the collections (2 and 5 g./day respectively) was recovered to approximately the same extent (83–93%) in the first and last halves respectively of the collections.

There was no indication that injection of magnesium led to any marked change in the endogenous faecal excretion although urine excretion was enormously increased and plasma magnesium was maintained at or above normal levels (Table 2).

In view of the possibility of digestive disturbances occurring after about 3 weeks on the synthetic-milk diet it appears that a more reliable estimate of endogenous faecal excretion can be obtained from our results by accepting only values for samples collected before that time. Such samples were used in deriving the results presented in Table 3. Since magnesium injections did not appear to affect endogenous faecal excretion markedly, results from two calves which received

Fig. 1. Cumulative excretion of magnesium in the faeces by calves receiving a diet of low-magnesium synthetic milk providing about 0.030 g. of magnesium/day. The calves were 2–3 weeks old when the collections started. During the periods shown by a solid line the calves were given daily subcutaneous injections of 1 g. of magnesium (as the sulphate). No injections were given during the periods shown by a broken line.

Table 2. Plasma magnesium and urine excretion of magnesium in young calves receiving low-magnesium synthetic milk with, in some cases, subcutaneous injections of magnesium

Mean results ($\pm$ S.D.) are presented for calves 2B, 4B, 18B and 12; no magnesium injections were given to calf 2B. Two determinations (or during magnesium injection two pairs of determinations) of plasma magnesium were made for each calf during each period.

	Urine excretion of magnesium (mg./day)	Plasma magnesium (mg./100 ml.)
No injection	10 $\pm$ 3	1.65 $\pm$ 0.17
Injection (1 g. of magnesium/day)	720 $\pm$ 30	2.16 $\pm$ 0.09–3.59 $\pm$ 0.21*

* The lower value was for samples taken 24 hr. after the injection; the higher value was for samples taken 1 hr. after the injection.

Table 3. Faecal excretion of magnesium in calves receiving low-magnesium synthetic milk

Mean values are presented for the calves shown. The daily excretion figures for each of the calves were averages ($\pm$ S.D.) derived from 8- or 12-day collections.

Age range (weeks)	Calves	Average body wt. (kg.)	Milk fed (l./day)	Average magnesium intake (mg./day)	Faecal excretion of magnesium (mg./day)	Endogenous faecal excretion of magnesium (mg./kg. body wt./day)	
						Maximum estimate*	Minimum estimate†
2–5	2B, 4B, 18B	46	4.40	30	29 $\pm$ 4	0.63 $\pm$ 0.10	0
9–11	12	50	4.40	33	61	1.2	0.56
26–32	2A, 4A, 7A, 11A	128	8.80	55	315 $\pm$ 62	2.5 $\pm$ 0.5	2.0 $\pm$ 0.5

* Assuming 100% absorption of dietary magnesium.

† Assuming no absorption of dietary magnesium (except for the youngest group of calves where a negative figure results from making this assumption).

such injections have been included in Table 3. One was calf 18B (see Fig. 1) in the younger group and the other was calf 2A in the older group. The latter calf suffered from convulsions soon after being transferred to the metabolism cage when it was receiving synthetic milk (plasma magnesium 0.5 mg./100 ml.), and magnesium injections (daily subcutaneous injection of magnesium sulphate solution containing 1 g. of magnesium) had to be given as a therapeutic measure. With the exception of calf 18B (the plasma magnesium of calf 2A remained below normal at between 1.1 and 1.9 mg./100 ml. despite the injections) all the animals were hypomagnesaemic during the faeces collections shown in Table 3, and they excreted very little magnesium in the urine (see Tables 2 and 4).

Since the synthetic-milk diet unavoidably contained a small amount of magnesium, which may have appeared to a greater or lesser extent in the faeces as unabsorbed material, the results in Table 3 can only give with certainty maximum and minimum values for endogenous magnesium excretion, depending upon whether no absorption or complete absorption of dietary magnesium is assumed. However, it will be shown later that there is reason to suppose that the proportion of dietary magnesium absorbed by the older calves (mean about 40%) is, within the limits examined, largely independent of the level of magnesium intake. It seems reasonable therefore to assume that about 60% (considerable variation in this assumed figure would not affect the calculation very greatly) of the small amount of dietary magnesium would appear in the faeces. On this basis the mean endogenous faecal excretion of the four calves examined at 26–32 weeks of age was 2.2 (s.d. ± 0.5 , range 1.4–3.4) mg./kg. body wt./day. No similar information on the effect of the level of dietary magnesium on magnesium utilization has been obtained for the younger calves. In the absence of digestive disturbances, however, it seems probable for these calves that a low dietary level of magnesium would be absorbed at least as efficiently as a higher level. It will be shown later

(see Fig. 3) that calves of this age (2–5 weeks) absorb about 75% of the magnesium in a whole-milk diet. Assuming this absorption efficiency for the calves on synthetic milk it can be calculated that the mean endogenous faecal excretion of magnesium by these calves was 0.48 (s.d. ± 0.10 , range 0.30–0.63) mg./kg. body wt./day. In this case differences in the assumed figure for absorption would have a considerable effect on the calculated result and this figure is therefore less reliable than that for the older calves.

Effect of dietary intake of magnesium on efficiency of magnesium absorption

When the experiment began the calves (2A, 4A, 7A and 11A at 26, 25, 21 and 21 weeks of age respectively) had been receiving 8.80 l. of whole milk/day with additions of copper, iron and manganese and 70 000 i.u. of vitamin D₃/day for at least 2 weeks (for details see Smith, 1958). Their plasma-magnesium levels were between 0.9 and 1.8 mg./100 ml. Collections of faeces and urine were made for 8 days on this diet (period 1) and the

Fig. 2. Relationship between intake and faecal excretion of magnesium in calves 26–32 weeks of age. Mean values for four calves are given with standard deviations shown as vertical bars. The numbers shown by the experimental points correspond to the numbers of the periods described in the text, where details of diets are given.

Table 4. *Plasma magnesium and urine excretion of magnesium in calves receiving diets containing different levels of magnesium*

Mean results ($\pm$ s.d.) are presented for calves 2A, 4A, 7A and 11A; values for 2A on low-magnesium synthetic milk are omitted since this calf was given magnesium injections at that time. Two plasma-magnesium determinations were made for each calf during each period. All the calves received 8.80 l. of milk/day and, where appropriate, supplementary magnesium was given as the chloride. Other mineral and vitamin supplements were given as described in the text.

Period	Diet	Magnesium intake (g./day)	Urine excretion of magnesium (mg./day)	Plasma magnesium (mg./100 ml.)
1	Whole milk	1.16	13 $\pm$ 2	1.33 $\pm$ 0.15
2	Whole milk + supplementary magnesium	3.10	210 $\pm$ 110	1.88 $\pm$ 0.11
3	Low-magnesium synthetic milk	0.055	6 $\pm$ 2	0.95 $\pm$ 0.13
4	Whole milk	1.07	11 $\pm$ 6	0.92 $\pm$ 0.10

calves were then given the same diet with the addition of 2 g. of magnesium/day (as the chloride). After 8 days another 8-day collection of faeces and urine was started (period 2) and the calves were then transferred to the low-magnesium synthetic-milk diet. After a further 8 days the collection of faeces and urine was started, from which the previously recorded values on endogenous faecal excretion were calculated (period 3). Finally the calves were returned to the whole-milk diet without supplementary magnesium and 8 days later a further 8-day balance period started (period 4). Mean figures for plasma magnesium and urine excretion of magnesium during these periods are given in Table 4. Mean values for faecal excretion at the different levels of intake are shown in Fig. 2. In comparing the effects of these different levels on the efficiency of magnesium utilization it is necessary to consider the possibility of changes occurring during the experiment in the intrinsic ability of the individual calves to utilize magnesium. For three of the calves (2A, 4A, 7A) there was little difference in the proportion of dietary magnesium appearing in the faeces between periods 1 and 4. The ability of these calves to utilize magnesium had by this time apparently reached a fairly steady state [after the initial fall when the calves were younger (Smith, 1958)] and it is reasonable to conclude therefore that a similar intrinsic ability to utilize magnesium existed during periods 2 and 3. Calf 11A showed an appreciable fall in its ability to utilize magnesium between periods 1 and 4 (57 and 85% respectively of the intake appeared in the faeces) and the difference apparent in the means for these periods in Fig. 2 was principally due to this calf. However, even for this calf the overall mean value for periods 1 and 4 is probably close to the true value obtaining between these two periods and can be compared with the results obtained for periods 2 and 3. Assuming therefore that the endogenous faecal excretion is constant for the different intake levels, the approximately linear relationship existing between magnesium intake and faecal excretion of magnesium revealed in Fig. 2 suggests that the proportion of dietary magnesium absorbed is largely independent of the level of intake. The mean calculated figure for efficiency of magnesium absorption by our calves was about 40%.

DISCUSSION

An increase on a unit body weight basis in the amount of endogenous faecal magnesium between calves aged 2-5 weeks and calves aged 26-32 weeks is apparent from our results whatever assumptions are made about the fate of the small amount of magnesium ingested on the synthetic-milk diet. Equally, however, the increase is insufficient to

account for more than a part of the decreased utilization of magnesium observed as milk-fed calves get older (Smith, 1957, 1958). This is shown in Fig. 3. Thus it seems that the main primary cause of this decrease in magnesium utilization and the concomitant hypomagnesaemia is decreased absorption of magnesium. It is probable that at least part of the increase in endogenous faecal excretion is secondary to this change in absorption and is brought about by decreased reabsorption of magnesium secreted high up in the alimentary tract. Previous results (Smith, 1959) suggest that about 0.1 g. of magnesium would be secreted daily in the saliva of a 30 weeks milk-fed calf, so that this alone could contribute about 20% of the total endogenous excretion if only about 40% were reabsorbed.

The question remains whether the poor magnesium-absorption efficiency developing in older milk-fed calves and the apparent inability of such calves to increase this efficiency on low dietary intakes is a particular property of the milk diet or a normal physiological quality of the calf. Since there did not appear to be any data in the literature on the proportion of dietary magnesium excreted in the faeces by normally fed calves at about 6-9 months of age, we have analysed feed and faeces

Fig. 3. Diagrammatic representation of the fate of magnesium ingested by milk-fed calves of different ages. The total heights of the columns represent the magnesium intake and the divisions shown are mean results based on balance data reported previously (Smith, 1957, 1958) and on estimates of endogenous faecal excretion reported in the present paper. The younger calves were receiving 4-40 l. of milk/day and the older calves 8-80 l. of milk/day.

samples obtained in 7-day balance experiments carried out on steers of this age by Dr J. C. Murdoch for another purpose. For animals receiving hay (10 balance periods with four calves) and silage + flaked maize (eight balance periods with four animals) the mean proportions of dietary magnesium in the faeces were $88.3 \pm 1.5\%$ and $78.5 \pm 1.3\%$ respectively. Making an allowance of 2.2 mg./kg. body wt./day for endogenous faecal excretion these figures correspond to absorption efficiencies of about 21 and 27% respectively. It appears therefore that these normally fed calves did not absorb magnesium more efficiently than our milk-fed calves of a similar age (see Fig. 3). It should be remarked, however, that normally fed calves receive some five times as much magnesium as calves given milk, so that in them this absorption provides an excess of magnesium which is excreted in the urine. There is little information on whether normally fed calves can become adapted to lower magnesium intakes by greatly increasing absorption efficiency, although the fact that Ray (1942) has reported the occurrence of hypomagnesaemia in calves receiving a diet of wheat straw and cereals (providing a magnesium intake of the order of a half of that which would be provided by hay and cereals) suggests that they cannot. Results of most balance experiments on adult bovines (Blaxter & McGill, 1956) suggest that a poor ability to absorb magnesium is usually maintained to a similar degree in older animals.

Our results for endogenous faecal excretion for older calves are compatible with the results for adult bovines (3–5 mg./kg. body wt./day) quoted by Blaxter & McGill (1956). Our figures for calves of about 2–5 weeks of age are, however, considerably lower than the figures quoted by these authors for young calves [3–4 mg./kg. body wt./day—data presumably derived from the results of Blaxter & Rook (1954)]. It seems possible that the magnesium-absorption efficiency in the calves used in deriving the latter figures had already fallen considerably at the time of measurement. Our values for young calves are similar on a unit body weight basis to the values for endogenous faecal excretion

of magnesium in dogs (about 0.9 mg./kg. body wt./day) obtained by Nicolaysen (1936). This author also found that magnesium injection did not markedly affect endogenous faecal excretion.

SUMMARY

1. Determinations of the faecal excretion of magnesium in calves fed with low-magnesium synthetic milk have been used to estimate endogenous faecal excretion. With certain assumptions, this excretion has been calculated to be about 0.5 mg./kg. body wt./day at 2–5 weeks of age and to increase to about 2.2 mg./kg. body wt./day at 26–32 weeks. This increase is not sufficient to account for the decrease which occurs in the utilization of magnesium with age in milk-fed calves. Both phenomena are probably secondary to a decrease in absorption efficiency.

2. Subcutaneous injection of magnesium sulphate had no apparent effect on the endogenous faecal excretion.

3. The average efficiency of magnesium absorption in milk-fed calves aged 21–34 weeks was estimated to be about 40%. This appeared to be largely independent of dietary magnesium at least up to an intake of about 3 g./day.

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