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THE ENDOGENOUS EXCRETION OF MAGNESIUM IN CATTLE[1])

By

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The incidence of hypomagnesaemia in ruminants has promoted interest in the absorption of dietary magnesium. This absorption is far from complete, but as with calcium and phosphorus, the fecal magnesium does not comprise only unabsorbed magnesium from the feed; there is also a substantial endogenous excretion of magnesium via the gastro-intestinal tract. Therefore, the simple difference between the dietary intake and fecal excretion is not an index of the "true digestibility" of dietary magnesium.

Furthermore, the endogenous excretion presumably varies with the type and quantity of the food intake, so that the endogenous excretion in the fasted animal cannot be used to calculate that proportion of the magnesium in various feeds which is unabsorbed. The essence of the problem lies in the "partitioning" of fecal magnesium into that unabsorbed from the diet and that secreted into the gut (and not subsequently reabsorbed) by the fed animal.

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Kleiber et al. (1951) have studied absorbability (or "true" digestibility) of dietary phosphate, using radioactive phosphate. They achieved a near-constant level of specific activity of plasma phosphate by repeated small injections of the isotope, and in this state it was assumed that the labile tissue phosphate of the animal was uniformly labelled.

Since the feed contained no radioactive phosphate, all the radioactive phosphate in the feces must come from gastro-intestinal secretions. If the radioactivity of tissue phosphate is known, the endogenous excretion of phosphate can be calculated from that fraction of the fecal phosphate which is radioactive, namely, from its specific activity. Thus: —

$$S = f \cdot \frac{\varphi}{\beta}$$

where S = rate of endogenous phosphate excretion
f = rate of total fecal phosphate excretion
φ = Specific activity of fecal phosphate
β = Specific activity of plasma(tissue) phosphate

The absorbability of dietary phosphate can then be calculated according to the equation, substituting for *S*,

$$a/i = 1 - \frac{f}{i}\left(1 - \frac{\varphi}{\beta}\right) \qquad (\textit{Kleiber}, 1961)$$

where *a* and *i* are the rates of phosphate absorption and total dietary intake respectively.

In a later modification of the method, *Luick et al.* (1957) injected P^{32} phosphate subcutaneously and intraperitoneally; the consequently slow absorption gave a near-constant level of plasma activity using only a single injection.

Visek and his co-workers (1953) have also successfully applied the isotope method for determining the absorbability of calcium. *Comar et al.* (1953) have compared values obtained for endogenous fecal calcium by this, the "isotope dilution" method, and a "comparative balance" method. It was shown that the isotope dilution method offered advantages over the comparative balance method, especially because of its independence of the availability of the dietary calcium.

Comparable studies of magnesium absorbability have been mainly with sheep and have used only small numbers of animals, probably because of the high cost and short half-life of Mg^{28} (*Field,* 1959, *MacDonald et al.,* 1959, *Care,* 1960). In a single

sheep, *Care* (1960) tested the isotope dilution method against the comparative balance technique and found close agreement between results from the two methods.

The present report is concerned with the absorption of the magnesium content of milk fed to calves and with that of the magnesium content of hay and grain fed to mature cows.

A comparison of the endogenous excretion of magnesium in calves and cows is of particular interest, as *Smith* (1959) has shown that the endogenous fecal excretion rises in calves with increasing age. The increase shown, however, did not completely account for the decreased utilization of magnesium observed as milk-fed calves get older.

METHOD AND CALCULATIONS

Magnesium^{28} is an isotope which decays with a half-life of 21.3 hrs. to Aluminium^{28}, emitting both γ and β rays. The Aluminium^{28} decays with a half-life of 2.3 minutes to stable Silicon^{28}, also emitting both γ and β rays. The isotope used in these experiments was supplied as $Mg^{28}Cl_2$ (with carrier) by the Brookhaven National Laboratory. The solution was neutralized and diluted appropriately prior to injection.

The short half-life of Magnesium^{28} presents considerable difficulties in maintaining a constant level of specific activity, as was achieved by *Kleiber et al.* (1951). Therefore a single intravenous injection was used for each animal.

Two calves and two cows were used in these experiments; Table 1 gives the relevant vital statistics of the animals. The calves were fed solely on whole milk, and the cows were given good-quality alfalfa hay and grain. All the animals were stabilized on their diets for 10 days before injection.

After injection of the isotope, blood samples were drawn at intervals, centrifuged, and the radioactivity of the plasma determined by counting in a well-type scintillation counter. Feces and urine were also collected throughout the experiment and an aliquot was taken from each sample for counting. The cows were milked at 3—4 hour intervals and the radioactivity of each milk sample was determined.

After counting, milk and fecal samples were wet-ashed with perchloric and nitric acids, evaporated to dryness, and dissolved in 0.5 N hydrochloric acid. Trichloracetic acid was added to the urine and plasma samples, which were then centrifuged and the

supernatant diluted to known volume. The solutions thus obtained were analyzed for magnesium, using the Beckman D-U spectrophotometer adapted for flame photometry and with a photo-multiplier modification of *Brown et al.* (1952). The magnesium content of the milk, hay, and grain fed to the animals was similarly determined by wet-ashing and flame photometry.

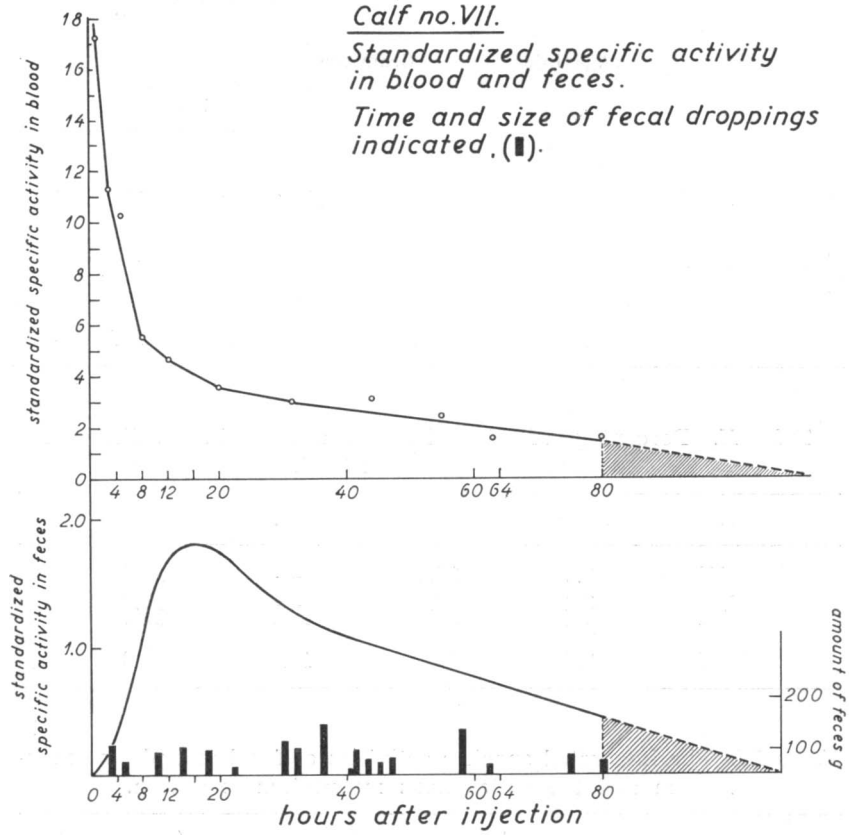
The observed counting data were corrected for the decay of the isotope and the specific activity of the magnesium in each sample was calculated. The specific activities were standardized according to the injected dose per kilogram body weight.

For each animal, the standardized specific activity (ϱs) of plasma magnesium was plotted against time, and the integral of this obtained. This integral, $\int_0^t \varrho_s \, dt$, is comparable to β in Kleiber's equation above.

The standardized specific activity of the fecal magnesium (F_s) in each sample was multipled by the time interval since the previous sample, and the sum of these products is the integral of the fecal magnesium standardized specific activity, $\sum_0^t F_s \Delta t$. This compares with φ in Kleiber's equation. It should be noted that specific activity of the fecal magnesium in each sample is the mean of all that added to the feces and stored in the rectum since the previous sample was taken; it is not the specific activity at the instant of sampling, as is true of plasma magnesium activity, for example. Therefore, the integral of the fecal magnesium specific activity, as calculated above, is fairly exact, and it is not the rough approximation that its form would at first suggest. It will be noted, however, that in Figure 1, a smooth curve has been fitted to both the plasma and fecal activity plots. This permitted the extrapolation of these curves to 120 hours, since the samples taken at 80 hours still had slight activity. In consequence, the integrals used are larger (by the shaded areas) than those strictly justifiable from sample collection.

RESULTS AND DISCUSSION

Table I presents the description of the animals, their injected doses of isotope and details of their magnesium balance. Table II shows the percentage of the injected isotope recovered in feces, urine, and where applicable, in the milk. The fraction of the injected isotope recovered in the feces is calculated from the



cpm/gm and total weight of each fecal sample actually collected (see Table II). The same recovery can be calculated from the total fecal magnesium excretion in 5 days and the integral of the extrapolated fecal magnesium specific activity plot. For the adult cows, it was found that the two calculations gave essentially the same result, but in the calves, the latter gave recovery fraction of about 4.0 percent instead of the 3.0 percent from the more direct calculation.

Table III lists the integrated specific activities of fecal and plasma magnesium, and the endogenous magnesium excretion as calculated from the ratio of these integrals multiplied by the total fecal magnesium excretion. It will be noted that when corrected for the metabolic body size of the animals ($W^{¾}$), the rate of endogenous magnesium excretion is closely similar in all four animals. For comparative purposes, our data are listed with those of other workers in Table IV. The mean endogenous excretion

Table I. Description of animals and their Mg-balance.

Trial No.	Animal	Weight kg.	Injected dose Counts per min.	Gram Magnesium daily in the				Mg Groos Balance (gms)
				Food	Feces	Urine	Milk	
VI	Jersey calf 2 months old	25.5	2.7×10^7	0.300	0.256	0.083	—	—0.039
VII	Jersey calf 2 months old	27.3	2.7×10^7	0.375	0.251	0.134	—	—0.010
IV	Holstein cow	487	1.6×10^8	31.33	20.34	8.48	1.85	+0.656
V	Holstein cow	558	1.6×10^8	29.74	25.59	7.84	1.87	—5.560

Table II. Percentage of injected dose recovered in the different excretions and in total.

Trial	Feces	Urine	Milk	Total
VI	3.0	18.0	—	21.0
VII	3.2	16.1	—	19.3
IV	4.6	50.4	13.4	68.4
V	3.5	49.8	10.5	63.8

Table III. The integrated specific activities of fecal and plasma magnesium and the endogenous magnesium excretion.

Trial	φ $\Sigma_o^t \Delta t$ Fs	β $\int_o^t \varrho s \, dt$	S Endogenous Mg $f \cdot \frac{\varphi}{\beta}$	S / $K^{3/4}$	a/i Absorbability
VI	86.57	248.4	89.3 mg.	7.90	0.445
VII	113.42	298.0	95.2 mg.	8.00	0.586
IV	16.92	504.7	696 mg.	6.76	0.373
V	20.68	604.7	876 mg.	7.62	0.169

for our two calves is about 3.5 mg./kg. on a simple body weight basis, which is consistent with the results of *Blaxter* and *McGill* (1956). *Smith* (1959) on the other hand, reported values as low as 1 mg./kg. in calves of similar age, but his animals were fed a synthetic, low magnesium milk diet. The endogenous excretion for our two mature cows was 1.5 mg./kg. body weight, which is considerably lower than the 3—5 mg./kg. reported by *Blaxter* and *McGill* (1956).

Table IV. Estimation of endogenous fecal magnesium in sheep and cattle.

Animal	Author	S mg.	Weight	S/kg.	$S/kg.^{3/4}$	Method
Sheep	*Field, 1959*	205 mg.	—	—	—	Comparative balance technique
Sheep	*Field et al.*, 1958	100 mg. 250 mg.	50 kg. 69 kg.	2.0 3.6	5.3 10.4	Balance exp.
Sheep	*MacDonald et al.* 1959	227 mg.	45 kg.	5.0	13.1	Isotope dilution method
Sheep	*Care*, 1960	227 mg. 156 mg.	44.5 kg. 75 kg.	5.1 2.1	13.2 6.1	Isotope dilution method
Calf	*Smith*, 1959	23 mg. 50 mg. 282 mg.	46 kg. 50 kg. 128 kg.	0.5 1.0 2.2	1.3 2.7 7.4	Balance exp.
Calf	*Blaxter & McGill*, 1956	—	—	3—4	—	Balance studies
Calf	*Simesen et al.*	92 mg.	26 kg.	3.5	8.0	Isotope dilution method
Cow	*Blaxter & McGill* 1956	—	—	3—5	—	Balance studies
Cow	*Simesen et al.*	786 mg.	523 kg.	1.5	7.2	Isotope dilution method

(S = endogenous, fecal magnesium).

The disappearance of Mg^{28} from the blood and its appearance in the feces are plotted for one calf in Figure I. Similar curves were obtained for all four animals except that the peak fecal activity in the cows occurred at about 12 hours after injection, as compared with 16—20 hours in the calves. The difference in delay is opposite to that to be expected from the generally faster turnover of body constituents in young animals.

Since the Mg^{28} solution was not carrier-free, its injection caused an immediate 10—20 percent increase in the plasma magnesium concentration which lasted about an hour. This is a departure from the ideal conditions of a tracer experiment, but *Smith* (1959) concluded that subcutaneous injection of magnesium sulfate had no effect on the endogenous fecal excretion in calves.

More serious criticisms applicable to this experiment are those raised by *Field* (1960, 1961), who considers that the em-

ployment of the isotope dilution method for estimating the endogenous fecal magnesium excretion involves some erroneous assumptions. Chiefly, he criticizes the assumption that, "the specific activity of the eventually reabsorbed intestinal secretions is the same as that of the remainder of the secretions". Such an assumption would indeed be wrong, but the point is irrelevant since the validity of the isotope dilution method does not depend on this assumption.

We feel that an important source of confusion is the term "reabsorption". The chyme contains a mixture made up of both dietary magnesium and secreted magnesium; some of this mixture is *absorbed* and enters the tissue fluids and some remains in the gut. Some of the originally secreted magnesium is present in the mixture which is actually absorbed, but, in the present context, it is unnecessary to speculate on *how much*. That portion of the originally secreted magnesium which is not absorbed and which finally appears in the feces is defined as the endogenous fecal magnesium excretion.

If the secreted magnesium includes Mg^{28}, then all of the radioactivity in the feces is from unabsorbed secreted magnesium. The ratio of the specific activity of the fecal magnesium and that of the originally secreted magnesium describes the proportion of secreted to dietary magnesium in the feces. The absolute quantities of each may then be calculated from the total magnesium content of the feces. The only necessary assumption is that the specific activity of the secreted magnesium is the same as that of the plasma, since the former is inaccessible for direct assay in the intact animal.

It will be noted that this method does not tell us anything about the completeness of the mixing of secreted Mg^{28} with the chyme, or the extent to which it is bound and rendered unabsorbable. These are of physiological interest, but irrelevant to the determination of endogenous fecal magnesium excretion *per se*. If the secreted magnesium is more absorbable than dietary magnesium, it merely shows that this is true of secreted magnesium at any time under comparable conditions, with or without the isotope.

It might be expected, of course, that most of the secreted magnesium remains in the more absorbable phase, and equilibrates only slowly with the bound magnesium of dietary origin. If the absorbable phase has a higher specific activity then, the

removal of some of this magnesium by absorption will leave a progressively larger proportion of bound (and relatively non-radioactive) magnesium in the gut. This would lower the overall specific activity (absorbable plus unabsorbable) as the chyme moves down the gut. *Field* (1961) found such a decrement in both the solid and liquid components of the chyme taken from successive portions of the small intestine of a sheep. It should be noted that the liquid component (separated by centrifugation) can be expected to contain "bound" magnesium, just as plasma does.

We conclude that these experiments demonstrate the feasibility of using Mg^{28} for measuring the availability of dietary magnesium. In particular, we propose that the determination of endogenous fecal magnesium losses in hypomagnesemic animals will show whether changes in absorption and secretion are important in the etiology of the condition.

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SUMMARY

The endogenous fecal magnesium excretion has been determined in two 8-week old Jersey calves and in two mature Holstein cows. The average values were 3.5 mg./kg. body weight for the calves, and 1.5 mg./kg. for the cows. The average Mg absorption was 52 percent for the milk-fed calves, and 27 percent for the cows, which received hay and grain.

ZUSAMMENFASSUNG

Die endogene Magniumausscheidung beim Rind.

Mit Hilfe von Mg^{28} wurde die endogene Magniumausscheidung in die Faeces bei zwei 8 Wochen alten Jerseykälbern sowie zwei erwachsenen, schwarz-weissen „Holstein"-Kühen bestimmt. Als durchschnittliche Werte wurden bei den Kälbern 3,5 mg pro kg Lebendgewicht und bei den Kühen 1,5 mg pro kg Lebendgewicht gefunden.

Die Kälber, die mit Milch gefüttert wurden, absorbierten durchschnittlich 52 % des zugeführten Magniums, während die Kühe, deren Futter in Luzernheu und Getreide bestand, durchschnittlich 27 % absorbierten. Diese Resultate werden diskutiert und mit denjenigen früherer Untersuchungen verglichen.

RESUMÉ

Den endogene magniumudskillelse hos kvæg.

Ved hjælp af Mg^{28} er den endogene magniumudskillelse til fæces blevet bestemt hos to 8 uger gamle Jerseykalve, samt hos to voksne, sortbrogede „Holstein" køer. De gennemsnitlige værdier blev for kalvene fundet at være 3,5 mg/kg legemsvægt, og for køerne 1,5 mg/kg legemsvægt.

Kalvene, som fodredes med mælk, absorberede gennemsnitligt 52 % af den tilførte magnium, medens køerne, som fodredes med lucernehø og kærne, gennemsnitligt absorberede 27 %. Disse resultater diskuteres og sammenlignes med tidligere undersøgelser.

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