The effect of certain dietary factors on the apparent absorption of magnesium by the rat

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(*Received* 18 *June* 1962—*Revised* 4 *October* 1962)

Numerous workers have established a connexion between the metabolism of magnesium and various dietary factors. Thus Colby & Frye (1951 *b*) working with young rats on a low-Mg, low-calcium diet observed reduction in blood Mg levels and increase in mortality rate when the casein content of the diet was increased from 24 to 50%. Increased intake of Ca salts or of phosphates has been reported to affect Mg metabolism in rats (Tufts & Greenberg, 1937–8; Colby & Frye, 1951 *a*; Hegsted, Vitale & McGrath, 1956; O'Dell, Morris & Regan, 1960; McAleese & Forbes, 1961), guinea-pigs (O'Dell *et al.* 1960) and young dogs (Bunce, Chiemchaisri & Phillips, 1962) but no information is available from these experiments about the absorption of Mg. The results of Heller & Haddad (1936) indicate that the addition of calcium chloride to the drinking water of rats increased faecal Mg, and recently Alcock & MacIntyre (1960) found a decrease in absorption when rats receiving normal amounts of Mg and insufficient Ca had their Ca intake brought to normal. The experimental data of Malcolm (1905) are insufficient for any conclusions to be drawn concerning the effect of increased dietary Ca on Mg absorption in the dog. An increase in dietary phosphorus level reduced Mg absorption in the guinea-pig (O'Dell, Morris, Pickett & Hogan, 1957) and chick (Nugara & Edwards, 1961). Thus, although it is evident that Ca and phosphate impair Mg absorption in certain animals, the relative and combined effects of these two factors have not been studied to any great extent.

Mg absorption in healthy human subjects given a mixed diet low in protein and moderately low in Ca and supplemented with vegetables has been shown to be improved when protein replaced an equi-caloric amount of sugar or fat (McCance, Widdowson & Lehmann, 1942). The effect of increased dietary Ca is less clear; thus De & Basu (1949) found that it impaired Mg absorption, but no definite effect is apparent from the results of Leichsenring, Norris & Lamison (1951) either with increased dietary Ca alone, or with increased Ca and phosphate.

In the ruminant, Head & Rook (1955, 1957) have suggested that the high levels of ammonia that occur in the rumen of cows grazing spring grass may interfere with Mg absorption from the small intestine or may reflect a reduction in the concentration there of amino acids, which could also result in poorer absorption.

In the experiments now reported the effect was studied of protein level, ammonium salts, Ca carbonate and sodium orthophosphate on the apparent absorption of Mg. The main object was to study factors affecting the absorption of Mg, and retention

https://doi.org/10.1079/BJN19630013 Published online by Cambridge University Press

was determined in only one experiment. Rats were given diets containing about 20 mg Mg/100 g diet, a level reported adequate for growth and maintenance of normal levels of blood Mg in young rats (Kunkel & Pearson, 1948).

EXPERIMENTAL

Diets and general technique

The composition of, and content of some inorganic constituents in, the low-Mg basal diets and the experimental diets are given in Tables 1 and 2. Male hooded Norwegian rats were housed individually in metabolic cages of the Hopkins type (Ackroyd & Hopkins, 1916) and fed to appetite. Diets were moistened with distilled water to prevent scattering. In addition, a vitamin mixture was given by pipette that provided a weekly dose of 1·4 mg DL-α-tocopherol, 360 i.u. vitamin A and 26 i.u. vitamin D. Distilled water was given in glass bulbs. Faeces and urines were collected daily and, at the end of the collection period, the separator, urine container and glass funnel were rinsed with hot, about 0·6N-HCl, and the rinsings added to the urine.

Table 1. *Percentage composition of the low-magnesium basal diets and their mean content of Mg and water*

Component	Basal diet no. 795	Basal diet no. 796
Unextracted casein (Glaxo Laboratories Ltd)	8·0	16·0
Salt mixture*	3·6	3·6
Margarine fat	10·0	10·0
Maize starch	68·0	60·0
Sugar	10·0	10·0
Choline chloride	0·4	0·4
Vitamin mixture†	0·05	0·05
Mg	0·0035	0·0034
Water	9·96	9·39

* Contained (parts): sodium chloride 22, calcium carbonate 76, potassium dihydrogen orthophosphate 103, tripotassium citrate (monohydrate) 125, iron citrate (trihydrate) 5, trace-element mixture (de Loureiro, 1931) 0·7.

† Contained (parts): inositol 15·5, nicotinic acid 10, calcium pantothenate 10, *p*-aminobenzoic acid 7·5, thiamine hydrochloride 3, riboflavin 3, pyridoxine hydrochloride 0·8, biotin 0·05, folic acid 0·1, menaphthone (2-methyl-1:4-naphthaquinone) 0·05.

Analytical methods

Diets were ashed at 550° and digested with hot, about 6N-HCl for determination of Ca and Mg. For determination of P, 10% (w/v) sodium acetate solution was added before ashing and the ash digested with hot, about 8N-HNO_3. Faeces were ashed at 550° and digested with hot, about 6N-HCl; in Expt 4 sodium acetate solution was added before ashing. The chloride concentration of these solutions of faeces was not sufficient to interfere with the P determinations. The combined urines and washings were evaporated to dryness, ashed at 520° and digested with hot, about 3·4N-HCl. Ca was determined by McCrudden's (1911) volumetric method and P by the molybdivanadate method (Kitson & Mellon, 1944; Hanson, 1950). Mg was determined by the method described by Godden (1937, p. 39) with the following modifications:

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Table 2. *Composition of, and content of some inorganic constituents in, the experimental diets*

	Composition			Inorganic constituents				
		Salt added/kg basal diet						
Experimental diet no.	Basal diet no.	Description	Amount (g)	Magnesium (mg/100 g)	Calcium (mg/100 g)	Phosphorus (mg/100 g)	Ammonium ion (mg/100 g)	Water (%)
795A	795	$MgSO_4.7H_2O$	1·80	21·5	342	318	●	9·08
796A†	796	$MgSO_4.7H_2O$	1·80	21·2	337	385	27	9·09
796A–C	796A	NH_4HCO_3	13·0	19·5	●	●	250	9·71
796A–D	796A	$C_6H_5O_7(NH_4)_3$	20·0	20·2	●	●	385	8·71
842	796	$NH_4MgPO_4.6H_2O$	1·82	20·3	●	●	●	8·15
843	796	$MgHPO_4.3H_2O$ (approx.)	1·29	20·6	●	●	●	8·40
847	796	{$MgSO_4.7H_2O$ {$Na_2HPO_4.12H_2O$	1·88 46·0	20·7	323	791	●	10·81
849	796A	$CaCO_3$	8·50	20·6	682	389	●	8·98
850	847	$CaCO_3$	8·50	20·1	652	784	●	10·74

● No determination made.

† Different batches of diet were prepared and in consequence the Ca, P and Mg content varied to some extent. Mean values are quoted here, but appropriate values were used for calculations in different experiments.

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Ca was precipitated as the oxalate at pH 4–5 (Smith, 1957) and the tube and precipitate were washed once with about 1 ml distilled water; sodium citrate was used to prevent the co-precipitation of iron during the precipitation of the ammonium magnesium phosphate, and the 33 % (v/v) ammonia solution quoted by Godden (1937, p. 40) for washing the Mg precipitate was replaced by one of 10 % (v/v) strength. Two precipitations of the Mg were found necessary for satisfactory results. The precipitate was finally dissolved in a small volume of about 2N-HNO_3, the solution transferred to a volumetric flask and P determined as above. In order to check for manganese contamination, additional ammonium magnesium phosphate precipitates were prepared from faeces in Expts 1 and 4 and from the low-Mg basal diets 795 and 796, and Mn was either estimated colorimetrically by oxidation to permanganate (Godden, 1937, p. 19) or removed from the precipitate by conversion into the insoluble hydrated oxide with bromine water (Duckworth & Godden, 1938; Davidson, 1952). In all these instances Mn contamination was negligible, but was not checked in other Mg determinations. Ammonia was determined by addition of magnesium oxide to a suspension of diet in water and distillation (Association of Official Agricultural Chemists, 1955). The value for ammonium content quoted in Table 2 is the mean of determinations made on the freshly prepared diet, and on samples that had been moistened with water and left to stand overnight to simulate feeding conditions. Only very slight losses of ammonia occurred in the latter. To reduce the loss of ammonia during storage, the diets were kept in air-tight containers.

Procedure

Until they received the experimental diets, all animals had been on the laboratory stock diet (McKinlay, 1951) from weaning. In Expts 1 and 2, litter-mates of similar body-weight were selected directly from animals on the stock diet. In Expts 3 and 4, litter-mates were chosen from rats that had received the experimental diets in ordinary cages for 1 week; by this method of selection it was hoped to have animals of a more uniform size at the beginning of the metabolic period. Collection of excreta was begun when all the animals had become accustomed to the new surroundings, and were accepting their diets and growing satisfactorily.

Expt 1. Absorption and retention of Mg at two levels of protein intake. Six pairs of 46-day-old litter-mates were given diets containing 8 or 16 % casein (diets 795A and 796A, Table 2) in ordinary cages for 6 days. The animals were then transferred to metabolic cages. Collection of excreta was begun 1 week later and continued for 10 days.

Expt 2. Effect of ammonium bicarbonate (diet 796A–C) *and tri-ammonium citrate (diet* 796A–D) *on Mg absorption.* Six groups of three 14½-week-old litter-mates were given diet 796A, 796A–C or 796A–D (Table 2) in the metabolic cages. Collection of faeces was begun 11 days later and continued for 1 week.

Expt 3. Absorption of Mg from ammonium magnesium orthophosphate (diet 842) *and magnesium hydrogen orthophosphate (diet* 843). To investigate the effect of ammonia without the possibly complicating effects of the acid radicals of the ammonium salts used in Expt 2, the absorption of Mg from diets 842 and 843 (Table 2) was compared.

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Litters, in excess of the number required for the metabolic period, of 8-week-old rats were transferred to diet 842 or 843 so that litter-mates were distributed between the two diets. After 1 week, six pairs of litter-mates of similar body-weight were selected, one litter-mate from each diet, and placed in metabolic cages. Collection of faeces was begun 1 week later and continued for 1 week.

Expt 4. Effect of calcium carbonate and sodium orthophosphate level on the absorption of Mg. Six groups of four litter-mate rats were selected, as for Expt 3, from 10-week-old rats that had already been given the experimental diets 796A, 847 (high-P), 849 (high-Ca) or 850 (high-Ca, high-P) (Table 2) for 1 week. They were placed in metabolic cages and collection of faeces was begun 9 days later and continued for 1 week.

RESULTS AND DISCUSSION

The results are given in Tables 3–6.

Absorption and retention of Mg at two levels of protein intake

Table 3 shows that an increase in the dietary protein at the expense of the carbohydrate increased the percentage absorption of Mg from 71·7 to 73·8, but that the difference just failed to reach the 5 % level of significance. Because of the much greater gain in weight of the rats in the higher-protein group during the pre-experimental period, the initial body-weights of the animals in the two groups were very different at the beginning of the metabolic period and those having more protein consumed more food and continued to gain weight more rapidly. The endogenous loss of Mg by these animals is not known, but if such losses were proportionately less in the higher-protein group the small difference in apparent absorption could be accounted for. McCance *et al.* (1942), using peptone, gelatin, gluten or egg white as supplements, found that Mg absorption in man was increased from 32 to 41 % when the protein content of the diet was almost trebled. This difference in absorption was much greater than that now observed with rats when dietary protein was doubled (Table 3). The diet used by McCance *et al.* (1942) had as a component '92 % wheatmeal bread' which contains phytate. The amount of bread in this diet was not mentioned, but, in view of the statement of McCance *et al.* (1942) that commercial 'phytin' is more soluble in solutions of α-amino acids than in water, it is possible that the effect of protein on Mg absorption may be more marked with diets containing phytate.

Table 3 shows that, although the percentage of Mg retained by animals in the higher-protein group was significantly greater ($0·01 > P > 0·001$), the quantity of Mg retained per unit gain in weight was slightly less ($0·05 > P > 0·01$) than in those in the lower-protein group. Thus, although more Mg was retained by the rats in the higher-protein group, dietary Mg was apparently not sufficient to meet their requirements. The reduced blood Mg levels and increased mortality rate observed by Colby & Frye (1951*b*) in rats given a diet containing 50 % casein was not, however, due to a higher Mg requirement because of increased growth, since these animals grew less than those given the lower-protein diet.

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Table 3. *Expt 1. Mean values in a 10-day metabolic test, for weight gain and magnesium intake, excretion, apparent absorption and retention of six pairs of litter-mate rats given diets of different casein content*

Diet* no.	Casein content (%)	Initial weight (g)	Weight gain (g)	Mg intake (mg)	Mg excreted (mg)			Mg absorbed		Mg retained		
					Urine	Faeces	Total	mg	%	mg	%	mg/g wt gain
795 A	8	181	26·7	30·4	12·2	8·6	20·8	21·8	71·7	9·6	31·6	0·36
796 A	16	230	44·3	35·1	12·7	9·2	21·9	25·9	73·8	13·2	37·6	0·30
Standard error of the mean (5 df)		—	±1·97	±0·92	—	—	—	—	±0·62	—	±0·76	±0·015

* See Table 2.

Table 4. *Expt 2. Mean values in a 7-day metabolic test, for weight gain and magnesium intake and apparent absorption of six groups of three litter-mate rats given diets of different ammonia content*

Diet* no.	Ammonium ion (mg/100 g)	Initial weight (g)	Weight gain (g)	Mg intake (mg)	Mg in faeces (mg)	Mg absorbed	
						mg	%
796 A	27	358	21·2	25·5	11·5	14·0	54·9
796 A–C	250	352	20·5	22·7	10·3	12·4	54·6
796 A–D	385	367	21·5	24·0	10·1	13·9	57·9
Standard error of the mean (10 df)		—	—	±0·31	—	—	±1·83

* See Table 2.

https://doi.org/10.1079/BJN19630013 Published online by Cambridge University Press

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Effect of the ammonium ion on Mg absorption

The mechanism by which ammonia interferes with Mg metabolism in the ruminant is as yet unknown. It is possible that the amount of Mg available for absorption can be reduced by the formation of ammonium magnesium phosphate or that ammonia alters the electric potential difference between the blood and gut contents, thus affecting the passage of ions through the gut wall. The results in Table 4 show, however, that the addition to the diet of ammonium bicarbonate or tri-ammonium citrate had no significant effect ($P > 0.05$) on the apparent absorption by rats of Mg from the basal diet (796A). Mg was also absorbed to the same extent from magnesium hydrogen orthophosphate and ammonium magnesium orthophosphate (Table 5). Ross (1961), using a preparation of isolated small intestine from rats, was also unable to detect any interference by ammonium chloride with the transport of Mg.

Table 5. *Expt* 3. *Mean values, in a 7-day metabolic test, for weight gain and magnesium intake, excretion and apparent absorption of six pairs of litter-mate rats given diets containing ammonium magnesium orthophosphate* (*diet* 842) *or magnesium hydrogen orthophosphate* (*diet* 843)

Diet* no.	Initial weight (g)	Weight gain (g)	Mg intake (mg)	Mg in faeces (mg)	Mg absorbed mg	Mg absorbed %†
842	285	29·8	26·2	8·4	17·8	67·9 ± 2·34
843	286	31·3	25·1	8·3	16·8	66·9 ± 1·66

* See Table 2.
† Value with its standard error.

Effect of level of calcium carbonate and sodium orthophosphate on the absorption of Mg

The results for the apparent absorption of Mg, Ca and P from diets varying in Ca and phosphate content are given in Table 6. The absorption of Mg was significantly reduced ($P < 0.001$) by an increase in the level of phosphate or Ca in the diet, and was further reduced ($0.01 > P > 0.001$) when both were simultaneously increased. Increase in dietary phosphate had no significant effect on percentage absorption of Ca at either level of dietary Ca. Increase in dietary Ca decreased significantly ($P < 0.001$) the percentage of P absorbed at each level of phosphate. The finding that absorption of Mg was reduced with increase in dietary Ca is in agreement with the work of Alcock & MacIntyre (1960), and Tufts & Greenberg (1937–8) also found that the severity of the signs of Mg deficiency in rats was increased when the Ca and P levels of a diet containing 5 mg Mg/100 g, 0·87 % Ca and 0·80 % P were changed to 1·16 % Ca and 0·75 % P. When the levels of Ca and P were 1·66 and 1·00 %, respectively, the minimum Mg content of the diet necessary for normal growth was increased to about 13 mg/100 g diet. However, O'Dell *et al.* (1960), using a diet containing 0·4 % P and 5 mg Mg/100 g, did not find that Mg deficiency signs were accentuated when the Ca level was increased from 0·9 to 1·7 %. The effect of increased phosphate (Table 6) was similar to that found by O'Dell *et al.* (1957), who reported that the absorption of Mg by the guinea-pig was impaired when dietary phosphate was substantially increased, but in contrast to their findings Ca absorption was not affected in the experiment described here. With rats, O'Dell *et al.* (1960) also found that the severity of the signs of Mg deficiency was increased when the level of dietary P was increased. No marked effect of phosphate on Mg absorption is apparent from the results of Meintzer & Steenbock (1955) who gave rats a diet containing 0·12 % Mg and 0·14 or 0·34 % P, but these levels of P were lower than those used by O'Dell *et al.* (1957, 1960). The further reduction in Mg absorption when the dietary Ca and phosphate levels were simultaneously increased (Table 6) is of interest in connexion with the observations of O'Dell *et al.* (1960) that in the absence of adequate Mg guinea-pigs receiving a diet containing a high level of Ca and P gained less weight

Table 6. *Expt 4. Mean values, in a 7-day metabolic test, for weight gain and magnesium, calcium and phosphorus intake and apparent absorption of six groups of four litter-mate rats given diets of different Ca and P content*

Diet* no.	Ca (mg/100 g)	P (mg/100 g)	Initial weight (g)	Weight gain (g)	Mg intake (mg)	Mg in faeces (mg)	Mg absorbed	
							mg	%
796A	335	392	317	23·2	25·8	8·6	17·2	66·7
847	323	791	310	23·0	26·5	14·1	12·4	46·8
849	682	389	315	19·3	26·9	15·3	11·6	43·1
850	652	784	307	19·5	24·5	17·5	7·0	28·6
Standard error of the mean (15 df)			—	—	±1·18	—	—	±3·29

Diet* no.	Ca intake (mg)	Ca in faeces (mg)	Ca absorbed		P intake (mg)	P in faeces (mg)	P absorbed	
			mg	%			mg	%
796A	420	194	226	53·8	492	59	433	88·0
847	413	193	220	53·3	1010	94	916	90·7
849	893	657	236	26·4	509	224	285	56·0
850	797	546	251	31·5	959	254	705	73·5
Standard error of the mean (15 df)			±13·4	±2·13	—	—	±29·4	±1·11

* See Table 2.

and died sooner than animals given diets containing less Ca and P. Similarly, Forbes (1961), studying the effect of two levels of dietary Ca and P, found that the visible signs of Mg deficiency in rats were produced most readily on diets containing the higher levels of Ca and P. Forbes (1961) increased the content of Ca of a basal diet containing 14 mg Mg/100 g from 0·4 to 0·8 % and that of P from 0·2 to 0·6 %. Tufts & Greenberg (1937–8), however, did not apparently observe any marked accentuation of external signs of Mg deficiency in rats given a diet containing only 5 mg Mg/100 g when dietary Ca was increased from 0·39 to 0·87 % and dietary P from 0·45 to 0·80 %.

It is evident from the results presented that dietary Ca and phosphate have a marked effect on the absorption of Mg and should be considered when assessing the adequacy of a diet with respect to Mg. Protein (casein) appeared to have only a slight effect on Mg absorption at the levels studied, but by increasing the growth rate it probably increased the Mg requirement of the animal.

https://doi.org/10.1079/BJN19630013 Published online by Cambridge University Press

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SUMMARY

1. The effect of the dietary level of casein on the apparent absorption and retention of magnesium, and of the ammonium ion on its apparent absorption, has been studied in rats aged between 2 and 4 months. The apparent absorption of Mg, calcium and phosphorus at two levels of dietary Ca and orthophosphate has also been investigated. In all the experiments the Mg level of the diets was about 20 mg/100 g diet.

2. An increase in dietary casein level from 8 to 16 % caused a slight but insignificant increase in the apparent percentage absorption of Mg. The gains in body-weight and the percentage of Mg retained by the animals given the higher-protein diet were significantly greater than those of the animals on the lower-protein diet.

3. The apparent absorption of Mg was not affected when the ammonium ion as ammonium bicarbonate, tri-ammonium citrate or ammonium magnesium phosphate was added to the diet.

4. The apparent absorption of Mg was significantly reduced by an increase in the dietary level of Ca from 0·34 to 0·68 % or of P from 0·39 to 0·79 % and was further reduced when Ca and P were simultaneously increased to levels of 0·65 and 0·78 %, respectively. The increase in dietary phosphate had no significant effect on apparent percentage absorption of Ca at either level of Ca intake, but the increase in dietary Ca decreased significantly the apparent percentage absorption of P at each level of phosphate intake.

I am grateful to Dr K. M. Henry and Dr J. A. F. Rook for advice, to Mrs M. R. Hussey for carrying out the ammonia analyses, and to Miss M. V. Chapman for help in feeding the animals.

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Printed in Great Britain

https://doi.org/10.1079/BJN19630013 Published online by Cambridge University Press