VITAMIN D AND MAGNESIUM ABSORPTION [1]

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(Received for publication December 17, 1954)

The effect of vitamin D on the absorption of Mg is of more than passing interest, for while Day and McCollum ('39) found that 85% of ingested Mg was absorbed by rats on a low P ration, it has been assumed generally that it is poorly absorbed. (Mendel and Benedict, '09; Schmidt and Greenberg, '35; Tibbetts and Aub, '37; Leichsenring et al., '51.) This conclusion was based on results obtained with other animals than the rat and with other than purified diets. The role of vitamin D cannot be assessed from the published data. In the experiments of Day and McCollum vitamin D was fed to all of the rats used in their experiments. Outside of the data obtained in metabolic trials the published data on the Mg content of tissues also are not helpful in determining the effect of vitamin D. While the Mg content of rat bones has been found to be increased in rickets (Gassmann, '10; von Euler and Rydbom, '31) the amount in the blood serum apparently is reduced (Bomskov and Kruger, '31).

Our interest in the effect of vitamin D on Mg absorption originated through our concern over the possible modifying effect of changes in Mg absorption on Ca and P balances when vitamin D was given. An ionic antagonistic effect of Ca and Mg is well recognized and as some of our observed effects of vitamin D were not those ordinarily attributed to this vitamin

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison. This research was supported by funds provided by the Wisconsin Alumni Research Foundation.

we have sought to ascertain if the Mg content of the diet could be a factor. On the supposition that differences in the published reports of the availability of Mg might have been arrived at with rations differing in P content or in the nature of the P in the rations some experiments were carried out with semi-synthetic rations in which P was added as inorganic phosphate or as phytic acid under acidic as well as basic conditions.

EXPERIMENTAL METHODS

Ninety-gram rats of the Sprague-Dawley strain were placed in metabolism cages for a 12-day period, during the last 9 days of which urine and feces were collected. Daily, for three or more half-hour periods, the rats were transferred for feeding to similar cages provided with sub-bottoms of filter paper to absorb any urine voided and to catch feces. At the end of the balance period the feces excreted in these cages were added to those collected in the metabolism cages and the filter papers, after brushing off any spilled food, were eluted with hot normal hydrochloric acid and rinsed twice with hot water. These elutions and rinses were added to the urine collections.

The food consumption was limited to 20 gm per rat for each three-day period in such a manner that this allotment lasted throughout the period and yet was consumed completely. This amount supported only very limited growth but this equalization of food intake eliminated difficulties due to variations in appetite and in the resultant mineral intake.

The basal ration was a modification of the semi-synthetic diet of Steenbock et al. ('51). It was a low P (0.016%), low Ca (0.012%), low Mg (0.02%) ration consisting of Cerelose (D-glucose monohydrate) 67, cooked egg white 18, roughage [2] 3, cottonseed oil [3] 10, and Ca-P-Mg-free salts 2. In this ration the level of magnesium has been reported to supply 4 times

[2] Ruffex.

[3] Wesson.

the minimum needs of the rat for growth, reproduction and lactation (Schmidt and Greenberg, '35).

Crystalline vitamins were added to the ration in a mixture with glucose. One gram of the mixture per kilogram of ration provided the following number of milligrams of the respective vitamins; thiamine hydrochloride, 4; riboflavin, 5; pyridoxine, 5; calcium pantothenate, 28; nicotinamide, 10; p-aminobenzoic acid, 200; and inositol, 200. Choline chloride was added to the ration at a level of 500 mg per kilogram of ration as an alcoholic solution sprayed on the Cerelose before the ration was mixed.

A supplement of 70 μg of β-carotene, 105 μg of 2-methyl-1,4-naphthoquinone, and 875 μg of α-tocopherol in cottonseed oil was supplied each week in three doses. Vitamin D, when given, was administered orally at the rate of 75 I.U. per three-day period as crystalline calciferol dissolved in cottonseed oil.

The salt mixture used by Steenbock et al., ('51) was modified by the omission of the Mg ingredient. Mineral additions to the basal diet were made at the expense of the Cerelose. Magnesium phosphate of the desired Mg/P ratio was prepared by dissolving $MgCO_3$ in standardized phosphoric acid, drying and grinding. The Mg phytate was prepared from a technical calcium phytate preparation[4] by removal of the Ca and precipitation with $MgCO_3$. Two preparations of varying Mg/P ratio were made by precipitating them respectively at a pH of 5 and 8. The former, later referred to as acidic Mg phytate, contained 8.3% Mg and 12.7% P while the latter, referred to as basic Mg phytate, contained 15.2% Mg and 8.0% P. The basic preparation contained an excess of $MgCO_3$ over that which could react with the phytic acid in the solution. The amount of Mg salts which could be fed was limited by their cathartic effect. Since a level of 0.24% Mg was the maximum which could be fed, the amount included in the diet was limited to 0.12% to avoid all danger of the incidence of diarrhea. Water was supplied for ad libitum consumption.

[4] Staley.

The urines after concentration and the feces as obtained were wet-ashed with nitric acid followed by perchloric acid. Inorganic P was determined essentially according to the method of Fiske and Subba Row ('25) while phytic acid was determined by the method of Pringle and Moran ('42). Mg was determined by an analysis for P in the magnesium ammonium phosphate precipitated after the removal of Ca as the oxalate.

RESULTS

The data in table 1 reveal that vitamin D increased Mg absorption. While the increase is small, never exceeding 19% of the intake which perforce had to be limited to 7 to 8 mgs daily, it was obtained consistently in 12 separate trials entailing the use of 170 rats. There is no evidence that this increase was the result of an increase in Mg requirements due to a stimulation of growth, as growth was increased inconsistently and, when it did occur, the increase was small, undoubtedly because of the limitation in food intake. Assessed on the basis of normal Mg requirements (Schmidt and Greenberg, '35) the intake of our animals exceeded this by a multiple of 20. Furthermore, as shown in table 2, the decrease in fecal excretion was more than compensated for by an increase in the urine so that the retention of Mg was actually decreased when vitamin D was given.

It is apparent from our data that Mg was absorbed readily even when given as the phosphate or phytate or under very basic conditions such as were provided by additions of 1.0% $CaCO_3$ and 2.6% $NaHCO_3$. Obviously the retention was limited with the carbonate as the phosphorus content of the basal ration was too low to meet the requirements of tissue growth but when P was provided either as Mg phosphate or as Mg phytate retention became possible. The greatest retention occurred at the highest level of P intake, namely, at 0.34% of the ration when Mg was furnished as the phosphate.

TABLE 1

The effect of vitamin D on magnesium absorption

ADDITIONS TO THE BASAL RATION [1]	INCREASE IN WEIGHT OF RATS IN 9 DAYS		Mg ABSORBED [2]		DIFFERENCE
	− D	+ D	− D	+ D	
	gm	*gm*	%	%	%
None	13	19	67.6	69.7	2.1
$MgCO_3$	19	15	60.2 (± 2.4)	68.3 (± 2.6)	8.1
$MgCO_3$ + 1.0% $CaCO_3$ + 2.6% $NaHCO_3$	9	11	71.3 (± 3.2)	76.7 (± 2.9)	5.4
Mg phosphate	7	7	58.5 (± 4.0)	66.5 (± 3.6)	8.0
Acidic Mg phytate	7	8	52.9 (± 3.4)	62.4 (± 2.9)	9.5
Acidic Mg phytate + 1.0% $CaCO_3$	11	8	58.0 (± 9.5)	61.2 (± 1.7)	3.2
Acidic Mg phytate + 0.42% $MgCO_3$	7	12	63.4 (± 2.1)	66.4 (± 2.9)	3.0
Basic Mg phytate	12	11	59.3 (± 2.8)	68.1 (± 2.3)	8.8

[1] Basal ration was low in P (0.016%), Ca (0.012%), and Mg (0.02%). Magnesium additions were all made at a level equivalent to 0.42% $MgCO_3$.

[2] Figures in parentheses give standard error of the mean calculated as follows: $\frac{\sqrt{\frac{\Sigma d^2}{n}}}{\sqrt{n}}$ where d is the deviation from the mean and n is the number of observations. Each group consisted of 6 rats except those fed the basic Mg phytate ration which had 12.

TABLE 2

The effect of inorganic and phytic acid phosphorus on the absorption and excretion of magnesium

ADDITIONS TO BASAL RATION	ADDITION OF VITAMIN D	NO. OF RATS	DAILY INTAKE	DAILY EXCRETION [1]			DAILY BALANCE
				Feces	Urine	Total	
	25 i.u./day		*mg*	*mg*	*mg*	*mg*	*mg*
$MgCO_3$ (0.016% P)	No	3	7.58	2.92	4.27	7.19	+ 0.39
	Yes	3	7.72	1.98	5.81	7.79	— 0.07
Mg phosphate (0.34% P)	No	3	7.32	2.46	1.36	3.82	+ 3.50
	Yes	3	7.41	2.09	2.30	4.39	+ 3.02
Mg phosphate (0.14% P)	No	6	7.08	2.94 (± 0.23)	3.07 (± 0.23)	6.01 (± 0.37)	+ 1.07
	Yes	6	7.20	2.41 (± 0.25)	4.62 (± 0.15)	7.03 (± 0.07)	+ 0.17
Mg phytate (0.14% P)	No	6	8.13	3.83 (± 0.16)	3.59 (± 0.16)	7.42 (± 0.11)	+ 0.71
	Yes	6	8.20	3.08 (± 0.14)	5.02 (± 0.10)	8.10 (± 0.07)	+ 0.10
Mg phytate + 1.0% $CaCO_3$ (0.14% P)	No	6	8.42	3.64 (± 0.57)	3.96 (± 0.51)	7.60 (± 0.44)	+ 0.82
	Yes	6	8.06	3.13 (± 0.15)	4.68 (± 0.12)	7.81 (± 0.26)	+ 0.25

[1] The standard error of the mean is given in parentheses except where pooled samples were used.

TABLE 3

The effect of magnesium, calcium, and phytic acid on phosphorus balances

ADDITIONS TO BASAL RATION	ADDITION OF VITAMIN D	DAILY INTAKE	DAILY EXCRETION [1]			DAILY BALANCE
			Feces	Urine	Total	
	25 i.u./day	*mg*	*mg*	*mg*	*mg*	*mg*
Mg phosphate	No	9.05	2.82 (± 0.16)	2.96 (± 0.40)	5.78 (± 0.65)	+ 3.27
(0.12% Mg, 0.14% P)	Yes	9.20	1.86 (± 0.14)	4.36 (± 0.33)	6.22 (± 0.24)	+ 2.98
Mg phytate	No	9.44	4.65 (± 0.10)	2.00 (± 0.30)	6.65 (± 0.35)	+ 2.79
(0.12% Mg, 0.14% P)	Yes	9.53	3.03 (± 0.21)	3.05 (± 0.33)	6.08 (± 0.38)	+ 3.45
Mg phytate + 1.0% $CaCO_3$	No	9.60	7.41 (± 1.35)	0.09 (± 0.02)	7.50 (± 1.33)	+ 2.10
(0.12% Mg, 0.14% P)	Yes	9.19	5.06 (± 0.26)	0.20 (± 0.06)	5.26 (± 0.21)	+ 3.93
Mg phytate	No	6.58	3.64 (± 0.35)	1.11 (± 0.27)	4.75 (± 0.12)	+ 1.83
(0.12% Mg, 0.11% P)	Yes	6.89	1.47 (± 0.38)	0.59 (± 0.20)	2.06 (± 0.59)	+ 4.83
Mg phytate + $MgCO_3$	No	6.70	3.89 (± 0.27)	0.79 (± 0.23)	4.68 (± 0.17)	+ 2.02
(0.20% Mg, 0.11% P)	Yes	7.22	1.88 (± 0.42)	1.26 (± 0.27)	3.14 (± 0.34)	+ 4.08

[1] The standard error of the mean is given in parentheses. All groups consisted of 6 rats.

TABLE 4

The effect of magnesium and calcium on the hydrolysis of phytic acid

ADDITIONS TO BASAL RATION	ADDITION OF VITAMIN D	DAILY PHYTATE P INTAKE	DAILY FECAL EXCRETION [1]			PHYTATE HYDROLYSIS
			Phytate	Inorganic	Other	
	25 i.u./day	*mg*	*mg*	*mg*	*mg*	%
Mg phytate	No	9.44	1.25	2.50	0.51	86.7
(0.12% Mg, 0.14% P)	Yes	9.53	0.89	1.32	0.52	90.7
Mg phytate + 1.0% $CaCO_3$	No	9.60	4.82	2.39	0.57	49.8
(0.12% Mg, 0.14% P)	Yes	9.19	2.28	1.66	0.72	75.2
Mg phytate	No	6.58	0.93 (± 0.12)	1.40 (± 0.15)	1.08 (± 0.31)	85.9
(0.12% Mg, 0.11% P)	Yes	6.89	0.21 (± 0.08)	0.69 (± 0.16)	0.60 (± 0.26)	96.9
Mg phytate + $MgCO_3$	No	6.70	0.89 (± 0.11)	1.77 (± 0.14)	1.35 (± 0.34)	86.7
(0.20% Mg, 0.11% P)	Yes	7.23	0.37 (± 0.12)	0.91 (± 0.15)	0.68 (± 0.23)	94.9

[1] The standard error of the mean is given in parentheses except where pooled samples were used. All groups consisted of 6 rats.

The phosphate balances in 4 out of 5 experiments (table 3) reveal a very definite increase in P retention when vitamin D was given, the main import of which, together with the evidence on phytic acid hydrolysis (table 4) is: that phytic acid given as Mg phytate was available as a source of P in the absence of vitamin D even when its hydrolysis was depressed to 49.8% of the total intake by the addition of 1% $CaCO_3$. When fed without $CaCO_3$ the hydrolysis of added phytate was almost as great in the absence of vitamin D as when it was given. The retention of P also was essentially unchanged.

We have no explanation either for the mechanism of the increase in Mg absorption or for the decrease in its retention when vitamin D is given. Specifically the former may be attributed to an increase in the acidity of the tract or to an as yet unidentified improvement in metabolic activity in the intestinal tract as well as in other tissues. The decrease in the retention of Mg may be due to the correction of the increase in Mg content of tissue when Mg is substituted for Ca in a diet free from vitamin D. This would be in harmony with the observations of Gassmann ('10) and von Euler and Rydbom ('31) who found that the Mg content of bone is increased in rickets.

SUMMARY

A series of experiments with 90-gm rats revealed that Mg was absorbed equally well when added at a level of 0.12% of the ration as the carbonate, phosphate, or phytate to a low-P, low-Ca semi-synthetic diet. The amount absorbed in the absence of vitamin D ranged from 50 to 71% of that ingested to a range of 53 to 77% when vitamin D was given. This increase was obtained consistently with all rations and in the absence of a large need for the Mg available since most of that ingested appeared in the urine. Dietary changes, such as the addition of calcium carbonate and sodium bicarbonate, or inorganic phosphate did not affect this absorption but the addition of phytic acid reduced it slightly. Similarly, Mg

limited to the levels which could be fed without inducing catharsis was without effect on the absorption of P, or on the hydrolysis of phytic acid.

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