The Hypomagnesemia of Vitamin D Administration.* (29891)

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Little is known about the effects of vitamin D_2 on magnesium metabolism. Recently, in a short-term balance study in rats given large doses of vit D_2, Hanna(1) demonstrated hypomagnesemia accompanied by increased intestinal absorption and increased urinary excretion of magnesium. The occurrence of this hypomagnesemia has since been confirmed by others(2). During the period of vitamin administration, Hanna's experimental animals were in net positive magnesium balance, whereas their controls which received physiologic amounts of vit D_2 were in a slight negative balance. These data suggest the possibility that the hypomagnesemia induced by administration of vit D_2 followed as a consequence of a redisposition of this cation from the extracellular phase to bone or some cellular depot. The present experiments were undertaken to evaluate this problem in more detail. The experimental design was constructed so as to be most certain of the nature of the balance of magnesium.

Methods. Forty-eight 200 g male Sprague-Dawley rats were randomly placed into 2 groups. Both groups were pair-fed a basal diet previously described(3) that was deficient in magnesium, sodium, potassium, chloride and phosphate but contained calcium carbonate. By direct analysis the diet was found to contain 0.16 mEq of magnesium and 16.4 mEq of calcium per 100 g. All rats had free access to distilled water, and all were given by gavage once each day 5 ml of a solution containing 2 mMoles each of sodium and potassium, 2.5 mMoles of chloride and 0.75 mMoles of phosphate.

The animals were housed in metabolic cages which were placed over funnels containing mineral oil. Urine and feces were collected over intervals of 5 days just prior to and during the administration of vit D_2.

After 10 days on the special diets, just prior to initiation of the administration of vit D_2, 9 animals randomly chosen from the 2 identical groups were killed and the contents of their intestines, from stomach to rectum, immediately removed and analyzed for magnesium. The remaining animals labeled initially as the experimental group were then given 5 consecutive daily subcutaneous injections of 100,000 units of vit D_2 in sesame oil in the form of activated ergosterol (Whittier Labs—Ertron®). The animals initially labeled as controls received injections of sesame oil alone. At the end of the 15th day of the experiments, 24 hours after the fifth and last series of injections, all animals were anesthetized with hexobarbital, Na 5-(1-cyclohexen-1-YL)-1, 5-dimethylbarbiturate (Winthrop Labs—Evipal®) and exsanguinated from their abdominal aortas. Thigh and back muscles were removed and analyzed separately from the remainder of the carcass for their ion concentrations. Again, intestinal contents were examined for magnesium.

Serum and muscle were analyzed for sodium and potassium with an internal standard flame photometer, for chloride by the Cotlove chloridometer, for serum total CO_2 content by a manometric method, for urea nitrogen and phosphate with the autoanalyzer(4) and for serum total proteins by a temperature-compensated hand refractometer (American Optical—T.S. meter)(5). Serum calcium and magnesium, as well as urinary, fecal, muscle and carcass magnesium, were determined by flame photometry in a Zeiss spectrophotometer with double monochromator(6).

Results. When compared with their controls, the animals which received the large doses of vit D_2 demonstrated significant

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TABLE I.

	Serum								Muscle				Carcass
	Ca	Mg	Na	K	CO_2	Cl	Total prot	Urea N	Mg	Na	K	Cl	Mg
	(mEq/l)						(g%)	(mg%)	mEq/100 g FFDS†				mEq
Control(14)*	5.17	1.71	141	4.7	22.2	105	5.8	19.4	6.23	8.3	41.9	5.9	11.7
Vit D (15)	6.45	1.37	141	4.2	24.2	101	5.7	21.7	6.08	8.7	40.5	5.6	11.2
P	<.01	<.01		<.01		<.01		<.05					

* No. of animals in each experiment.
† Fat-free dry solids.

hypercalcemia and hypomagnesemia (Table I). The differences between the urinary magnesium excretions of the 2 groups, during the time of vitamin administration, were not significant (Table II). Neither were there significant differences between fecal magnesium excretions (Table II) or between the final intestinal magnesium contents (Table III).

The muscle magnesium concentrations per 100 g fat-free dry solids and total carcass magnesiums of the 2 groups were not significantly different (Table I).

The mean serum potassium and chloride levels of the vit D_2-treated group were slightly but significantly lower, and the mean blood urea nitrogen levels were somewhat higher than those of the controls. There were no significant differences in the concentrations of sodium, bicarbonate or total proteins.

The muscle potassium expressed as mEq per 100 g fat-free dry solids of both groups were similar (Table I). These values are lower than those ordinarily reported for rats and are consistent with the low values of muscle K previously observed in magnesium depletion(3).

TABLE II. Cumulative Magnesium Excretions During Administration of Vitamin D.

	Urine	Feces
	(μEq total)	
Control(14)	80	355
Vit D(15)	98	348

TABLE III. Gastrointestinal Lumenal Magnesium Content μEq Total.

Prior to admin of Vit D		After admin of Vit D	
Control	150	Control	127
		Vit D	118

Discussion. This experiment was done to study the hypomagnesemia consequent to administration to rats of large doses of vit D_2. Specifically scrutinized was the relative importance of acute changes in external magnesium balance as opposed to possible changes of the distribution of the cation within the body. Prior work had established the occurrence of this hypomagnesemia(1,2). The balance study of Hanna(1) done on rats fed normal amounts of magnesium and injected with large amounts of vit D_2 demonstrated hypomagnesemia, a net positive magnesium balance and hypermagnesuria. These findings in themselves suggest that the hypomagnesemia was primarily the result of a redistribution of the cation within the body.

In the experiment presented here, magnesium-depleted rats receiving an essentially magnesium-free diet were used to accentuate any possible differences in urinary or fecal magnesium excretion.

Definite hypomagnesemia was observed in the animals given the vit D_2 injections. Neither before nor during the period of administration of the vitamin was there any significant difference between the urinary or fecal magnesium excretions, or magnesium content of the intestines of the two groups. Clearly this would preclude any *necessary* action of vit D_2 on gastrointestinal absorption or secretion or urinary excretion of magnesium in producing the hypomagnesemia.

The lack of difference in total protein content of serum suggests that the hypomagnesemia is not readily ascribable to hemodilution. The possibility remains that there may have been some diminution in protein binding of magnesium induced by vit D_2 *per se*, or by the hypercalcemia consequent to its administration. Another possible explanation for the

vit D_2-induced hypomagnesemia would appear to be a redistribution of this cation from the extracellular phase to some tissue, presumably bone, independent of any specific influence on protein-binding of magnesium. The lack of difference between the muscle and carcass levels of magnesium in the two groups is compatible with this suggestion.

The slight but significantly depressed level of serum potassium of the vit D_2-treated group is interesting in view of the known adverse effect of this vitamin on renal conservation of potassium(1), but, unfortunately, urine potassium excretion was not determined.

Summary. The hypomagnesemia consequent to administration of large doses of vit D_2 to rats was studied. Two groups of animals were pair-fed a magnesium-deficient diet, and one was then given injections of vit D_2. Hypomagnesemia resulted in the vit D_2-treated group. During the period of vitamin administration there was no significant difference between the urinary or fecal magnesium excretion of the 2 groups. Nor were the differences significant between the levels of magnesium in the total carcass and muscle. The level of serum proteins also was not different in the 2 groups. It is suggested that the hypomagnesemia is due to a redistribution of magnesium from the extracellular phase to some other depot, which may well be bone. One mechanism might involve a reduced binding of magnesium by serum proteins due to some specific effect of vit D_2 (or perhaps to the resultant hypercalcemia). An alternative hypothesis is that vit D_2 has some other specific influence that promotes a deposition of magnesium in bone or some other depot.

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Enzymatic Degradation of Pentaerythritol Tetranitrate by Human Blood. (29892)

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After finding that the administration of pentaerythritol tetranitrate (PETN) leads to excretion of pentaerythritol (PE) by mice (1,2) and rats(3), it became of interest to learn whether blood alone possesses the capacity to degrade PETN. This point was investigated *in vitro* with human blood. Also evaluated were human blood plasma and erythrocytes. The study was conducted by incubating C^{14}-PETN with blood, plasma and red blood cells, sampling at various time intervals, and analyzing the C^{14} compounds by thin layer chromatography and radio-scanning(4). C^{14}-PETN in phosphate buffer was employed as a control system.

Materials and methods. C^{14}-PETN, labeled at carbon atoms 1 and 2, was synthesized from acetaldehyde *via* PE. The product (m. pt., 140-141.5°) was mixed with 7 parts by weight of C.P. lactose in order to minimize the explosion hazard. The activity of the lactose-C^{14}-PETN mixture was 0.59 millicurie/g.

Blood plasma prepared from human citrated whole blood was used without dilution. After repetitive washing with physiological saline, red cells from the same source were resuspended in saline to the original blood volume.

Aliquots (15.0 ml) of 0.4 M phosphate