Response to Vitamin D of Magnesium Deficient Rats.* (32123)

FIMA LIFSHITZ,† HELEN C. HARRISON, AND HAROLD E. HARRISON
Departments of Pediatrics, Johns Hopkins University School of Medicine and Baltimore City Hospital, Baltimore, Md.

The changes of plasma calcium concentration during magnesium deficiency have been extensively studied in ruminants. Blaxter and Sharman(1) fed calves a low magnesium diet and found that plasma magnesium concentrations fell progressively from 2.0 mg/100 ml to about 1.0 mg/100 ml without change in the plasma calcium concentrations. When plasma magnesium levels fell below 0.8 mg/100 ml plasma calcium concentrations decreased rapidly. McAleese and Forbes(2) obtained similar findings in lambs that were kept indoors and fed a low magnesium diet. They also showed that addition of calcium and vit. D to the ration had no influence on the hypomagnesemia and hypocalcemia. L'Estrange and Axford(3) in 3 separate experiments in which lactating ewes were fed a low magnesium, normal calcium diet found that hypocalcemia developed when the plasma magnesium concentrations decreased below 1 mg/100 ml. Supplementary dietary magnesium increased the concentrations of both calcium and magnesium in plasma to normal within a few days. Smith(4) reported that efficiency of calcium utilization and plasma calcium concentrations in hypomagnesemic calves were restored to normal by the addition to the diet of large amounts of vit D (70,000 i.u./day) whereas physiologic amounts were ineffective.

The above observations suggest that magnesium deficiency in calves and sheep interferes with calcium homeostasis and that the vit D requirement in the magnesium deficient animal is greater than in the normal (4). In order to test whether this is also true in the rat, measurements of concentrative calcium transport by the rat small intestine *in vitro* were made in preparations from vit D deficient and vit D fed magnesium deficient and control rats. The effect of vit. D treatment on serum calcium and citrate concentrations of hypomagnesemic and control rats were also measured.

Material and methods. Male Sprague-Dawley rats at weaning were divided into groups at random and given a vit. D free diet‡ that differed only in the magnesium content as described previously(5).

The following analytical methods were used: Serum magnesium, Schachter(6) serum calcium, Harrison and Harrison(7), inorganic phosphate, Fiske and Subarrow(8) and serum citrate, Natelson, Pincus and Lugovoy (9), statistical treatment, Natrella(10).

The concentrative calcium transport studies were made on everted intestinal loops as previously described(11). Duodenal loops from magnesium deficient and control animals both vit. D deficient and vit. D treated were studied. The intestinal loop was fiilled with 0.6 ml of modified magnesium-free Krebs-Henseleit bicarbonate buffer(12) containing 0.02 M glucose. The loop was immersed in 5 ml of the same buffer solution. The initial

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† Fellow, Training Program in Pediatric Research, Johns Hopkins Univ. School of Med. USNPHS TI-HD-91.

‡ The basal diet consisted of casein (180 g), dextrose (231 g), corn starch (450 g), peanut oil (80 g), vit. D free vitamin mixture (20 g), choline chloride (0.5 g), magnesium free salt mixture (8 g), and $CaHPO_4$ (16 g). A magnesium salt mixture (2.0 g/kilo) was added to the diets of the control groups.

The vit. D free vitamin mixture consisted of thiamine HCl (4 mg), riboflavin (5 mg), pyridoxine (5 mg), nicotinamide (10 mg), calcium pantothenate (28 mg), p-amino benzoic acid (200 mg), inositol (200 mg), alpha tocopherol (110 mg), glucose (19.438 g). In addition a supplement of beta carotene (10 mg) and 2-methyl,1-4-napthoquinone (25 mg) were added in the peanut oil.

The "magnesium free salt mixture" consisted of NaCl (138 g), KCl (224 g), $FePO_4:H_2O$ (41 g), KI (0.16 g), $MnSO_4$ (0.70 g), and $CuSO_4$ (0.18 g). The magnesium salt mixture consisted of $MgCO_3$ (50 gm) + $MgSO_4$ (32 g).

TABLE I. Effect of a Single Dose of Vitamin D on Concentrations of Calcium, Phosphorus and Magnesium in Serum of Magnesium Deficient and Control Rats Previously Depleted of Vitamin D. In this experiment they were also fed a low-calcium, low-phosphorus, diet 72 hr before they were bled. The vitamin D treated animals received 500 i.u. of vitamin D_2 P.O. 18 hr before they were bled.

	Vitamin D deficient			Vitamin D treated		
Serum (mg/100 ml)	Mg deficient (11)		Control (9)	Mg deficient (6)		Control (6)
Ca	5.5 ± .41		5.8 ± .69	7.3 ± .70	*	8.3 ± .42
Mg	.33 ± .04	‡	1.99 ± .45	.44 ± .02	‡	1.86 ± .14
P	7.5 ± .58	*	8.9 ± 1.22	7.0 ± 1.60	†	9.1 ± .90

Code:

Data are means ± 95% confidence intervals. ( ) indicates no. of animals.
* Differences significant at $P<.05$ level.
† " " " $P<.01$ "
‡ " " " $P<.005$ "
The statistical comparison is between magnesium deficient and control rats of similar vitamin D status.

concentrations of stable Ca and Ca^{45} in the solution within the loops (serosal fluid) and outside bathing the mucosal surface (mucosal fluid) were the same *i.e.*, 0.2 mM Ca/l and 0.05 uc Ca^{45}/ml. Concentrative transport of calcium was measured as the ratio of the concentration of Ca^{45} in serosal fluid to that in mucosal fluid (Cs/Cm) at the end of a 90 minute incubation at 37°C.

Results. Table I depicts the results of the first experiment. The magnesium depleted and control rats were studied after 18 days on a diet deficient in vit. D. In this experiment, they were placed on a low calcium, low-phosphorus diet (basal diet without $CaHPO_4$) for 72 hours and half of the animals were given 500 i.u. vit. D_2§ orally 18 hours before all were bled. Magnesium deficient animals showed a diminished response to vit. D as measured by the degree of elevation of the serum calcium concentration ($P<0.05$).

The effect of a small dose of vitamin D on concentrative transport of calcium by everted duodenal loops *in vitro* was measured in magnesium deficient and control rats that had been on a vit. D deficient diet for two weeks. The mean body weight (± 95% confidence intervals) for the control animals was 90 ± 6.1 g and for the magnesium deficient rats 70 ± 4.2 g. Fig. 1 depicts the response to 3 i.u. of vit. D_2§ given by stomach tube 72 hours before the animals were killed. The concentration ratios between the serosal and mucosal fluid (Cs/Cm) achieved by the everted duodenal loops were the same for the magnesium deficient and control rats when vitamin D deficient. Following treatment with vit. D the intestinal preparations from control rats showed a significantly greater increase in concentrative calcium transport than the preparations from the magnesium deficient rats ($P<0.005$). The rise of serum calcium concentration following this small dose of vit. D was also greater in control rats than in magnesium depleted animals ($P<0.005$).

Since severe magnesium deprivation might have caused changes in the intestine which allowed less vit. D to be absorbed, thus accounting for the diminished response seen in the magnesium deficient animals, a group of rats was studied after the intramuscular administrations of the same dose (3 i.u.) of vit. D_2 72 hours before they were killed. This experiment demonstrated the same lessened response to vit. D of the magnesium deficient rats as measured by increase in serum calcium and by the *in vitro* transport of calcium against a concentration gradient by everted duodenal loops.

The effect of vit. D on serum citrate is shown in Fig. 2. Serum citrate was measured 72 hours after vit. D_2 was given intramuscularly. The vitamin D deficient animals are grouped together since we have shown that the serum citrate concentration of magnesium deficient vitamin D deficient rats is the same

§ Vitamin D given as crystalline calciferol (Nutritional Biochemicals Co.) dissolved in propylene glycol.

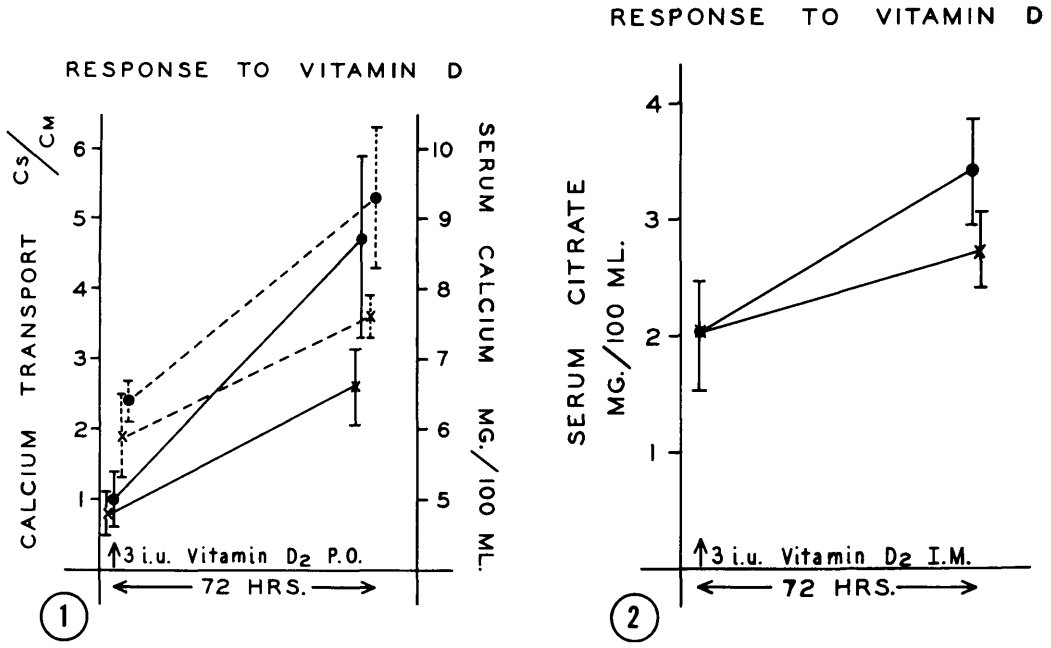

FIG. 1. Effects of 3 i.u. vit. D on concentrative transport of calcium *in vitro* and on serum calcium concentrations of magnesium deficient and control rats. Differences in concentration ratios between the serosal and mucosal fluid (Cs/Cm) after vit. D treatment are significant (P. <0.005). Differences in serum calcium concentration following vitamin D are also significant (P. <0.005). Code: Data are means from 6 observations in each group. ×, Magnesium deficient; ●, Control; ————, Concentrative transport of calcium; — — — —, Serum calcium; ⊥ ⊥ 95% confidence intervals.

FIG. 2. Serum citrate concentrations before and after intramuscular administration of 3 i.u. of vit. D. Differences in serum citrate levels after vit. D treatment are significant (P<0.005) in control animals only. Code: Data are means from 6 observations in each group. ×, Magnesium deficient; ●, Control; ————, Serum citrate; ⊥ 95% confidence intervals.

as that of vitamin D deficient rats with normal magnesium content(5). An effect of a small dose of vit. D (3 i.u.) on the serum citrate concentration was evident in the control animals (P<0.005) but the magnesium deficient rats showed no significant response.

Discussion. The degree of response to vit. D was measured in these experiments by increase of serum calcium and citrate concentrations and of concentrative transport of calcium *in vitro* of intestinal loops prepared from control rats and rats given vit. D. The effective dose of vit. D differs with time between administration of vitamin and the determination of response. When a moderately large dose of vit. D_2, 500 i.u., is given and the serum calcium response tested 18 hours later the effect is much less than maximum and is analogous to the results obtained 72 hours after a small dose of vit. D_2, 3 i.u. In both types of experiments the magnesium deficient rats show significantly less effect of vit. D than the control rats. In the second series of experiments all of the physiologic effects of vit. D measured including augmentation of concentrative transport of calcium *in vitro* by the small intestine and increase of serum calcium and citrate concentrations were less in the magnesium depleted animals. Magnesium deficiency did not alter intestinal transport *in vitro* in the vitamin D-deficient rats indicating that the action is on the effect of vit. D in enhancing calcium transport rather than on the transport system itself. The effect of vit. D in increasing serum calcium in the vitamin D deficient rats was probably not due entirely to increased intestinal uptake of calcium since the animals had been placed on a low calcium diet during the 72 hours between administration of the

vit. D and the taking of the blood samples. The poor response of the serum calcium concentration of the magnesium deficient rats suggests that the effect of vit. D in increasing calcium mobilization from the skeleton is also reduced by magnesium depletion.

The partial refractoriness of magnesium deficient rats to small doses of vit. D as measured by intestinal transport of calcium *in vitro* was previously observed in rats given 100 i.u. per week during the 3 week period of magnesium depletion(13). No difference between magnesium deficient and control rats was noted, however, when a larger dose of vit. D was fed, 1000 i.u. per week. The diminished response to vit. D. of magnesium deficient rats can, therefore, be overcome by increased vit. D dosage. It has been shown in hypomagnesemic calves that pharmacologic doses of vit. D restored calcium utilization and serum calcium concentrations to normal whereas physiologic amounts were ineffective (4). Previously we have also shown that the serum citrate concentration of magnesium deficient rats did not rise 72 hours after oral administration of a single dose of 5,000 i.u., but a significant increase resulted in rats given 20,000 i.u. of vit. D(5).

This dose response can explain the failure of demonstration of hypocalcemia in magnesium deficient rats in the great number of studies which have been done, although in ruminants(2,14) and man(15,16) an association between hypomagnesemia and hypocalcemia has been found. The serum calcium concentrations of hypocalcemic and hypomagnesemic calves did not rise when calcium salts and vit. D were given but increased to normal values following magnesium administration(14). Similar findings have been reported in patients with malabsorption secondary to resection of portions of the small intestine(17). In addition there have been reports of patients with hypomagnesemia associated with hypoparathyroidism who were refractory to treatment until given magnesium salts(18,19). The concept of partial vit. D unresponsiveness in magnesium depleted subjects may explain these findings in animals and patients. In the many studies of magnesium deficiency in rats hypercalcemia has been a significant finding(20). The magnesium deficient rat shows many of the findings of hyperparathyroidism including hypercitricemia(21,5) increased calcium absorption (22) and hypophosphatemia with increased phosphate clearances(5). The rats have in all of these studies been given sufficient quantities of vit. D to obscure the partial insensitivity to vit. D which in this animal can only be shown by measurement of the response to small doses of vitamin D.

The mechanism whereby magnesium deficiency reduces the response to low concentrations of vit. D is unknown. There is a lag period between vit. D administration to an animal and detection of a physiologic effect. This lag period is decreased when large doses of vit. D are given(23). This time interval may be the period necessary for incorporation of vit. D into the cell structure, for the metabolic transformation of the steroid to an active molecule or for induction of a specific protein molecule through activation of DNA directed synthesis of mRNA. Whatever the role of magnesium may be in facilitating the physiologic response to vit. D the effects of magnesium deficiency on this response in the rat are only evident at low concentrations of vit. D.

Summary. Measurements of concentrative calcium transport by the rat small intestine *in vitro* were made in preparations from vitamin D deficient and vitamin D fed magnesium deficient and control rats. The effects of vitamin D treatment on serum calcium and citrate concentrations of hypomagnesemic and control rats were also measured. These studies indicate that magnesium deficient rats are less responsive to physiologic amounts of vitamin D than control rats.

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Turnover of Structural Protein Fractions in Denervated Muscle.* (32124)

JOSEPH L. PATER AND ROBERT R. KOHN
Institute of Pathology, Western Reserve University, Cleveland

Very little is known about mechanisms of protein degradation in degenerative processes, or in normal turnover. Studies utilizing denervated muscle as a model have indicated that the rapid loss of some of the structural protein is due to accelerated degradation rather than retarded synthesis(1,2). The interpretation of these results, as well as the planning of future investigations of this system, require the resolution of certain questions. First, the possibility must be considered that structural proteins such as myosin occur in different pools which have different rates of turnover, and that myosin extracted by the usual methods may not be representative of the other structural proteins nor of the less soluble myosin. If this were the case, a given protein in fractions of varying solubility could be related on a precursor-product basis, as in the case of collagen where the soluble protein is transformed into the insoluble(3). Furthermore, only some of the fractions, for example, ones representing final products, might be degraded in atrophy. In this case only certain fractions would be suitable substrates for studying the mechanism of degradation. Finally, there is the difficulty of distinguishing between changes in protein turnover in atrophying muscle which are due to the cellular content of factors required for protein synthesis and degradation, and those which are related to functional status of muscle in the animal.

To attempt to answer some of these questions and to provide preliminary data which might suggest possibilities for further investigation of this system the following experiments were performed: 1) The turnover of a radioactive amino acid was compared between control and atrophying muscles in 4 differently soluble fractions of skeletal muscle structural protein; 2) Myosin was extracted from similar fractions, and turnover was compared among the 4 fractions themselves in control and atrophying muscles, as well as between the control and atrophic representatives of each fraction; 3) Incorporation into structural protein was followed

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