Decrease in Serum Alkaline Phosphatase Activity Produced by Magnesium Depletion in Rats.* (31441)

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A patient with intestinal malabsorption who had developed osteomalacia was reported to have a paradoxically low serum alkaline phosphatase level(1). He had coincident hypomagnesemia and also excreted small amounts of phosphoethanolamine in the urine. This report and similar observations on 2 of our patients led us to investigate the effects of magnesium depletion on serum alkaline phosphatase activity and urinary phosphoethanolamine excretion in rats.

Materials and methods. Twenty Sprague-Dawley rats, 30 days old, were fed a magnesium-deficient diet.§ Mortality decreased the size of the group as the study progressed. The 3 animals that survived after receiving the low magnesium diet for 34 days were sacrificed. The same diet supplemented with 43 mmoles of magnesium chloride/kg was given to a comparable group of 20 control rats. The control rats were offered only the amount of food consumed by the experimental rats. A third group of 5 rats was given the magnesium-deficient diet for 8 days and then the magnesium-supplemented diet for 12 days. The rats were kept in individual metabolic cages for the entire study.

Blood and urine samples were collected on 8 occasions during the study period. Blood samples were obtained by cardiac puncture from animals under light ether anesthesia. Urine was collected from each animal during the nocturnal 12-hr period preceding each blood collection. During this period the rats were kept without food in metabolic cages containing screens to keep fecal material out of the collected urine.

Serum alkaline phosphatase was measured by the method of Shinowara *et al*(2), calcium by the method of Loken *et al*(3) and inorganic phosphorus by the method of Taussky and Shorr(4). The presence of urinary phosphoethanolamine was determined by the method of Efron(5).

Results. Magnesium deficiency produced a progressive and marked decrease in serum alkaline phosphatase activity in the experimental rats (Fig. 1). Concomitantly, the serum phosphorus level decreased and the serum calcium concentration increased (Table I). The hypercalcemia was slight and only lasted for a short period. In the group of rats fed the low magnesium diet for only 8 days, the decrease in serum alkaline phosphatase activity was reversed when the magnesium-supplemented diet was substituted (Fig. 1). Serum alkaline phosphatase activity in the control rats tended to rise until puberty was well established when it began to fall.

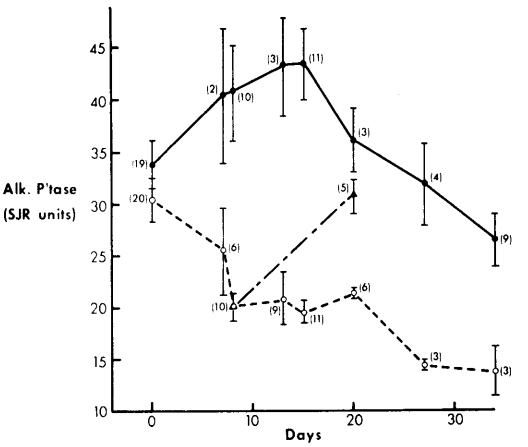

FIG. 1. Serum alkaline phosphatase activity in control and magnesium-deficient rats. ●, control group; ○, rats fed low magnesium diet; ▲, rats fed low magnesium diet for 8 days followed by magnesium-supplemented diet for 12 days. Vertical bars indicate 2 standard errors above and below each mean. Numbers in parentheses are numbers of rats tested at each point.

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TABLE I. Effect of Magnesium Deficiency on Levels of Calcium and Phosphorus in Serum.

Days on diet	Calcium (mEq/l)		Phosphorus (mg/100 ml)	
	Control	Exp	Control	Exp
0	4.95 ±.19	5.03 ±.21	11.1 ±.17	10.0 ±.36
7	5.17 ±.23	5.76 ±.08	10.4 ±.19	7.9 ±.14
8	5.06 ±.23	5.77 ±.27	10.0 ±.30	6.7 ±.22
13	4.80 ±.21	5.58 ±.37	9.2 ±.82	7.4 ±.50
15	5.10 ±.19	5.45 ±.06	9.8 ±.56	6.4 ±.25
20	5.43 ±.24	5.55 ±.15	9.3 ±.15	7.9 ±.51
27	5.33 ±.74	5.27 ±.17	10.0 ±.34	6.7 ±.32
34	4.78 ±.17	4.60 ±.08	8.8 ±.16	5.8 ±.43

Figures are mean ± standard error. Number of animals at each point is same as in Fig. 1.

To determine whether decreased serum magnesium level was directly responsible for the reduction in alkaline phosphatase activity, magnesium chloride, 2 mEq/l, was added *in vitro* to an equal volume of serum from control and magnesium-deficient rats. Raising the magnesium concentration increased phosphatase activity by 18 and 19% in sera from control and magnesium-deficient rats, respectively (Table II). When sera from control and magnesium-deficient rats were mixed in proportions of 3:1, 1:1, and 1:3 the enzyme activity reflected the proportions of the mixtures (Table III). Phosphoethanolamine was not detected in the urine of the control rats whereas it was detected sometime during the study in 30% of the magnesium-deficient rats. No correlation was found between the detection of urinary phosphoethanolamine and the levels of serum calcium or alkaline phosphatase or the duration of magnesium deficiency.

Discussion. It has long been known that magnesium is a co-factor for the alkaline phosphatases. Kay(6) found that the optional magnesium concentration for serum alkaline phosphatase activity ranged from 2-6 mEq/l. Because he found normal levels of magnesium in sera from many patients with a variety of diseases he concluded that the level of serum magnesium is of no importance in evaluating the level of serum alkaline phosphatase. Snyder and Tweedy(7) later reported a 25% decrease in serum alkaline phosphatase activity in rats on a magnesium-deficient diet but these results are difficult to interpret. The standard deviation of their observations was 14-46% of the mean values. The number of animals in each group was small and the ages when the experimental diet was initiated varied between 23 and 49 days. The control animals were not pair-fed with the experimental animals. Rats on the "control" diet had 20% less serum alkaline phosphatase activity than those on the standard laboratory diet. In the present study rats fed a magnesium-deficient diet had a statistically significant reduction in serum alkaline phosphatase activity when compared to healthy pair-fed control rats of the same age. Addition of only magnesium chloride to the diet reversed the decrease in alkaline phosphatase activity. These data clearly indicate that magnesium deficiency causes serum alkaline phosphatase activity to decrease. The reduction in enzyme activity is not a direct result of the reduced level of serum magnesium because addition of magnesium *in vitro* did not restore enzyme activity to normal levels. Mixing sera from normal and experimental rats revealed no evidence of a circulating inhibiting substance. Magnesium deficiency appears to inhibit synthesis or release of serum alkaline phosphatase.

In a genetically transmitted disorder, hypo-

TABLE II. Increase in Alkaline Phosphatase Level Resulting from Addition of Magnesium Chloride, 2.0 mEq/l, *in vitro* to Equal Volumes of Sera from Normal and Magnesium-Deficient Rats.

Group	No. of rats	Alkaline phosphatase level	
		Untreated serum	Serum + $MgCl_2$
Control	9	26.4 ± 2.2	31.2 ± 2.1
Magnesium-deficient	4	17.0 ± 1.0	20.2 ± .8

Values, expressed as Shinowara-Jones-Reinhart units, are means ± S.E.

TABLE III. Effect of Mixing Sera from Control and Magnesium-Deficient Rats in Various Proportions.

Proportions of serum mixed (%) Control : magnesium-deficient	Alkaline phosphatase activity (SJR units)
100 : 0	34.3
75 : 25	28.2
50 : 50	24.1
25 : 75	18.8
0 :100	14.3

phosphatasia, serum alkaline phosphatase activity is low and is associated with defective calcification of bone and excretion of phosphoethanolamine in the urine. The finding of phosphoethanolaminuria in patients with low serum alkaline phosphatase activity secondary to malabsorption syndrome confirms a close relationship between these two biochemical abnormalities. This association was seen in some of our magnesium-deficient rats but not in all. Because serum alkaline phosphatase can hydrolyze phosphoethanolamine it is tempting to speculate that the compound would appear in urine whenever alkaline phosphatase activity is low because it is a natural substrate for the enzyme. However, this association is not constant and the presence of phosphoethanolamine in urine could also be explained by a disorder of phospholipid metabolism.

Most reports on the effects of magnesium deficiency on calcium metabolism in animals agree that calcification of soft tissues is common and that deposition of new bone tissue often occurs. However, the levels of serum calcium were reported low(8), high(9,10) and normal or high(11). When serum calcium levels were reported "high," the degree of elevation was about ½ to 1 mEq/l. A similar elevation of serum calcium occurred in our magnesium-deficient rats but only during the first 2 weeks of the study. Increased intestinal and renal reabsorption of calcium is found in magnesium-deficient rats and may be partially responsible for the hypercalcemia (12). In our study, the subsequent decrease of serum calcium to levels observed in the control rats may have been a result of the diarrhea that developed in many of the animals.

It has been suggested that magnesium deficiency causes hypercalcemia by stimulating parathyroid activity(10). This is unlikely in view of the decline in alkaline phosphatase level that was observed in our magnesium-deficient rats.

The hypercalcemia that accompanies magnesium deficiency could also result from decreased deposition of calcium in bone. Hypercalcemia does occur in the infantile form of spontaneous hypophosphatasia. In this disease calcification of bone is impaired and phosphoethanolamine is excreted in the urine. It is unlikely that magnesium depletion is responsible for spontaneous hypophosphatasia in man, but it may induce a similar biochemical lesion as indicated by the low serum alkaline phosphatase activity and phosphoethanolaminuria found in a few magnesium-deficient patients and in our magnesium-deficient rats. Additional studies are in progress to determine whether or not other points of similarity exist between experimental magnesium depletion in rats and clinical hypophosphatasia.

Summary. Thirty-day-old rats were fed a magnesium-deficient diet for up to 34 days. There was a decrease in serum alkaline phosphatase activity which was reversed by adding magnesium chloride to the diet. The rats also developed hypophosphatemia and were mildly hypercalcemic for 14 days. Approximately one-third of the rats had phosphoethanolaminuria. These changes are similar to those seen in infantile hypophosphatasia in man. Magnesium deficiency in rats may induce a biochemical lesion similar to that of hypophosphatasia in man.

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