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Magnesium Deficiency
II. Isoenzymes of Serum Alkaline Phosphatase in Acute Magnesium Deficiency[1] (37854)

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A characteristic sign of magnesium deficiency is a fall in serum alkaline phosphatase. Heaton (1) found that after 15 days, the activity of serum alkaline phosphatase in Mg deficient rats had decreased to 50% of the control value. Restoring the plasma Mg levels to normal values by adding Mg^{2+} stimulated a slight increase in alkaline phosphatase (AP) activity, but not to the normal level. Pimstone, Eisenberg and Stallone (2) found no evidence of a circulating inhibitory substance in magnesium deficient serum to explain the decrease in alkaline phosphatase activity. This paper reports the results of an investigation of the early changes in the activity of the isoenzymes of serum alkaline phosphatase and their correlation with the initial decrease in serum magnesium concentration.

Materials and Methods. Fifty male albino rats were equilibrated on normal rat chow for 2 days. The rats (mean wt 124 ± 5.8 g) were then fed Heggtveit low magnesium test diet (General Biochemicals) and triple distilled deionized water. Two to three rats were killed at 12 hr intervals up to 6.5 days, at which time the characteristic red ears of magnesium deficiency began to appear in the remaining animals. Rats were then killed on a 24 hr basis up to Day 12. The animals to be killed were anesthetized with ethyl ether and exsanguinated via heart puncture. The drawn blood was allowed to clot, and the serum was collected after centrifugation.

A control group consisting of 11 rats was placed on the Heggtveit diet supplemented with 500 ppm Mg ($MgSO_4$, A. R. Grade).

Serum calcium and magnesium were determined by atomic absorption spectrophotometry utilizing a Unicam 1900 instrument (4). Inorganic phosphorous was determined by the Fiske–Subbarow method (5). Total serum alkaline phosphatase was determined using Phosphatabs (Scientific Products).

The isoenzymes of serum alkaline phosphatase were determined by a modification of Righetti and Kaplan's (3) method. One milliliter of serum was acidified with 1 drop of 0.25 *M* acetic acid. Then 0.05 ml of neuraminidase (Calbiochem) was added to the serum, and the mixture was incubated for 24 hr at 37.5°. After incubation the pH of the serum was checked and if necessary neutralized to pH 7.0. Enzyme-digested serum (20 μl) was electrophoresed on 7.5% polyacrylamide disk electrophoresis gels using a Tris–glycine buffer 0.2 *M* pH 8.4. The gels were stained according to the method of Kaplan and Rogers (6) with the following variation. The gels were incubated in α-naphthyl phosphate for 1.75 hr at 37.5°.

Results. The rats developed bright red ears within 6.5 days on the deficient diet. The ears had completely blanched in the remaining animals by Day 11.

The control serum values were (mean ±

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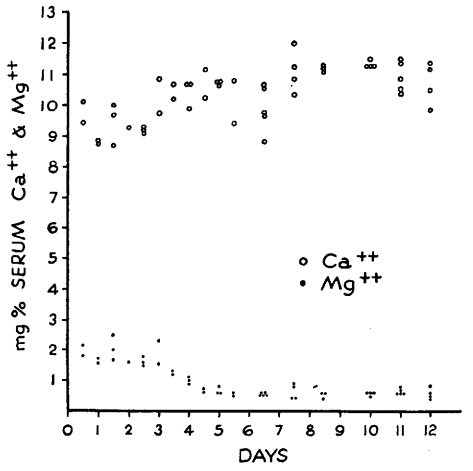

FIG. 1. Changes in serum levels of Ca^{2+} and Mg^{2+} during acute magnesium deficiency.

SEM): calcium, 10.1 ± 1.0 mg%; magnesium, 2.52 ± 0.70 mg%; inorganic phosphorous, 10.1 ± 0.8 mg%; and alkaline phosphatase, 20.4 ± 5.4 Klein-Babsen-Reed units times 3.

Serum magnesium began to decrease after about 3.5 days and plateaued at around 0.6 mg% at 4.5 days (Fig. 1). Calcium exhibited an elevation to above 11 mg% at 7.5 days. Serum alkaline phosphatase decreased between 6.5 and 7.5 days (Fig. 2). Serum inorganic phosphorous levels also began to fall at 6.5 days.

Electrophoresis of the individual sera indicated that bone and intestinal alkaline phosphatase isoenzymes were approximately equal in their reactions on the acrylamide gels (Fig. 3). The level of the neuraminidase-sensitive bone isoenzyme began to decrease after 6.5 days (Fig. 3, gels 39 through 49), whereas there was no apparent change in the activities of the intestinal and general isoenzyme of alkaline phosphatase.

Discussion. The initial fall in serum magnesium concentration occurred several days before any other changes had taken place. The rate of decrease was close to that reported by Tufts and Greenberg (7) in their animals fed a diet containing less than 1 mg Mg/100 g diet. However, there was no indication of a "back swing toward normal levels" during our experiment. The difference could be attributed to the difference in the diets; the Heggtweit diet used in this study has a much lower Mg content (0.2 mg/100 g).

The hypercalcemia along with the decrease in inorganic phosphorous has been previously reported (2). The abrupt changes in concentrations of these ions occurring at the time of the red ears suggests that the fast exchange pool of magnesium had been depleted.

The isoenzyme patterns of rat sera indicated the presence of three separate isoenzymes of alkaline phosphatase. The decrease in the neuraminidase-sensitive component, which is mainly of bony origin, can be attributed to the inhibition of bone

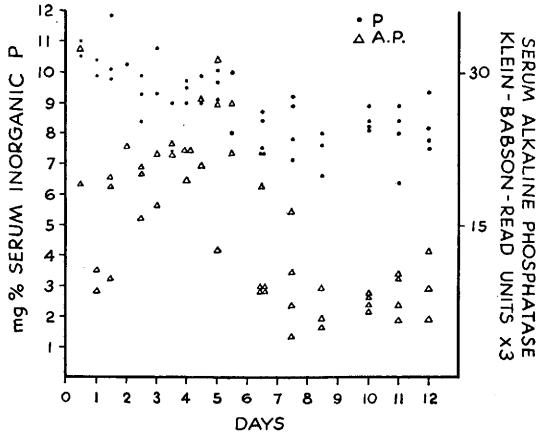

FIG. 2. Changes in serum levels of inorganic phosphate and alkaline phosphatase during acute magnesium deficiency.

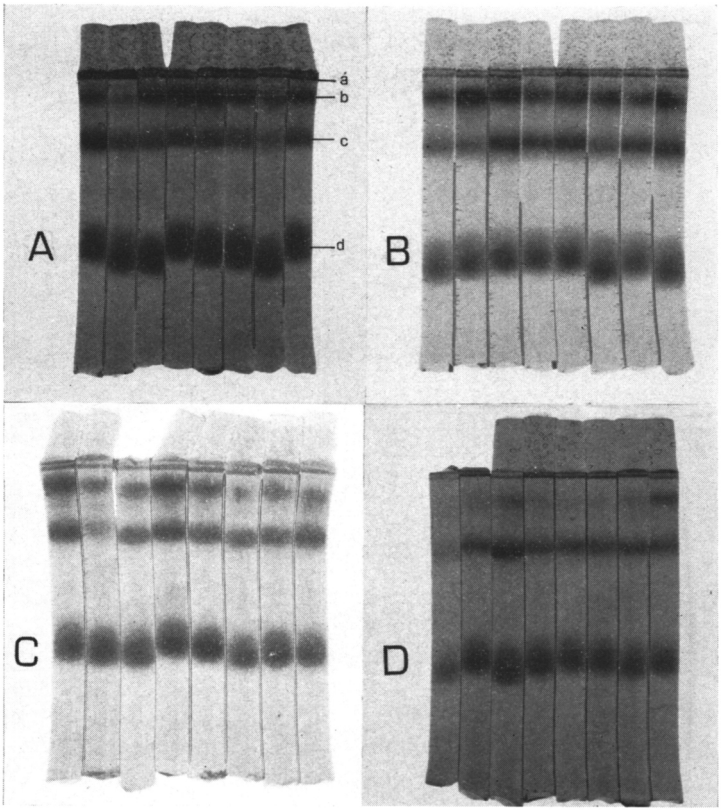

FIG. 3. Photographs of electrophoretic patterns for neuraminidase digested serum during various stages of magnesium deficiency. Bands are for (a) generalized isoenzyme of alkaline phosphatase (AP), (b) bone AP, (c) intestinal AP and (d) serum albumin. Groups (A) sera 1–8, normal serum Mg and AP levels; (B) sera 17–24, low Mg and normal AP; (C) sera 34–41, low Mg and beginning decrease in AP; and (D) sera 42–49, low Mg and low AP.

formation during Mg deficiency, which has been well established (8–10). The presence of the intestinal isoenzyme and the general isoenzyme of alkaline phosphatase in the electrophoresis patterns of Mg deficient sera indicates that Mg deficiency does not affect either the synthesis or release of these two isoenzymes. The decrease in serum alkaline phosphatase in Mg deficiency can be attributed mainly to the loss of the bone alkaline phosphatase and not to a generalized decrease in all alkaline phosphatase isoenzymes. The loss of a single isoenzyme suggests the response to Mg deficiency is due to a cell specific sensitivity to the deficiency.

Summary. The activity of serum alkaline phosphatase was investigated during the early stages of acute dietary magnesium deficiency in the rat. Electrophoretic patterns of the serum showed the presence of three isoenzymes for alkaline phosphatase. Only the neuraminidase-sensitive isoenzyme, which is of bony origin, was decreased during Mg deficiency. This decrease in a single isoenzyme suggests a cell specific sensitivity to the deficiency.

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