Enzyme, Adenosine Triphosphate, and Blood Cellular Changes in Magnesium Deficient and Control Rats[1] (35637)

RONALD J. ELIN, W. D. ARMSTRONG, AND LEON SINGER

Department of Biochemistry, Medical School, University of Minnesota, Minneapolis, Minnesota 55455

In 1960, Hanna *et al.* (1) reported low plasma alkaline phosphatase levels in patients with hypomagnesemia. This was also observed by Heaton (2), Pimstone *et al.* (3), and Smith and Nesbit (4) in magnesium deficient animals. Pyrophosphatase is present within the erythrocyte and has an absolute requirement of magnesium for enzymic activity (5). Based on the above observations, it was decided to examine the activity of a magnesium dependent extracellular enzyme, plasma alkaline phosphatase; a magnesium dependent intracellular enzyme, erythrocyte pyrophosphatase; and, since it is well known that magnesium is a cofactor for reactions involving ATP (6, 7), the whole blood ATP concentration at 4 consecutive weekly intervals in magnesium deficient and control rats.

Other studies were performed to determine whether alterations in enzyme activity were permanent or would return to normal if the magnesium deficient animals were given a diet of normal magnesium content. The enzyme activities were also evaluated with pair-fed magnesium deficient and control animals since it was apparent that the magnesium deficient animals become progressively anorexic.

Materials and Methods. Twenty-seven Holtzman male white rats, weighing about 110 g, were individually caged and provided a magnesium deficient test diet (8) and deionized water *ad libitum*. The magnesium content of the diet by analysis was 0.11 ± 0.01 mmoles/kg. A similar group of rats was given the control diet prepared by adding magnesium sulfate to the diet to raise the magnesium content to 16.5 mmoles/kg. Each animal was weighed daily.

At 1-week intervals for 4 consecutive weeks, six animals were removed from each group, anesthetized with ether, and bled by direct heart puncture. The plasma alkaline phosphatase activity (9), erythrocyte pyrophosphatase activity (10), whole blood ATP concentration (11), plasma magnesium concentration (12), and whole blood hemoglobin (Hb) concentration were determined. Hematocrit (HCT) values and erythrocyte counts were obtained and blood smears were prepared. The erythrocyte counts were obtained only during the third and fourth weeks of the experiment.

To study the effect of magnesium repletion on enzyme activity, 35 Holtzman male white rats, weighing about 130 g, were individually caged and provided the magnesium deficient diet and deionized water *ad libitum*. Eighteen similar rats were handled in a like manner except that they were given the control diet. After 3 weeks on these regimens, 6 rats from each group were sacrificed and 12 of the animals on the magnesium deficient diet were switched to the control diet. On the fourth and fifth weeks of the experiment, six animals from each of the three experimental treatment groups were sacrificed, and the plasma alkaline phosphatase activity (9), erythrocyte pyrophosphatase activity (10), plasma and erythrocyte magnesium concentrations (12), and whole blood hemoglobin concentration were determined on each animal.

For the pair-fed study, 10 Holtzman male white rats, weighing about 125 g, were individually caged and given 9 g of the magnesium deficient diet daily and deionized water *ad libitum*. A control group of 10 rats was

[1] This investigation was supported by Grant DE-01850 from the National Institute of Dental Research, Bethesda, Maryland.

TABLE I. Blood Factors and Experimental Weight Gains with Time in Magnesium Deficient Rats.

		Weeks			
		1	2	3	4
Wt gain (g)	Mg deficient (6)	32.62 ± 1.32	50.88 ± 4.72	54.13 ± 4.65	76.81 ± 7.35
	Control (6)	50.93 ± 4.74	104.96 ± 3.98	151.29 ± 4.42	196.33 ± 8.71
Plasma magnesium (mg/100 ml)	Mg deficient (6)	0.42 ± 0.06	0.30 ± 0.03	0.33 ± 0.03	0.27 ± 0.03
	Control (6)	1.91 ± 0.01	1.93 ± 0.02	1.94 ± 0.02	1.91 ± 0.02
Plasma alkaline phosphatase activity[a]	Mg deficient (6)	40.86 ± 2.47	37.44 ± 2.26	39.82 ± 2.01	40.40 ± 3.37
	Control (6)	81.45 ± 2.84	86.34 ± 4.77	85.67 ± 4.46	89.90 ± 4.80
Erythrocyte pyrophosphatase (mg of P/g of Hb)	Mg deficient (6)	113.17 ± 5.03	76.67 ± 2.85	69.33 ± 8.61	65.17 ± 7.71
	Control (6)	113.17 ± 3.87	108.00 ± 1.18	114.17 ± 3.82	108.33 ± 4.68
Whole blood ATP (mg/100 ml)	Mg deficient (6)	23.37 ± 0.98	20.94 ± 0.94	18.76 ± 1.47	18.22 ± 1.62
	Control (6)	24.80 ± 0.62	26.00 ± 0.81	25.60 ± 0.64	25.67 ± 0.47
Whole blood ATP corrected for HCT[b]	Mg deficient (6)	60.45 ± 0.61	51.15 ± 0.87	56.44 ± 2.84	53.15 ± 3.36
	Control (6)	61.01 ± 1.00	63.10 ± 1.27	65.63 ± 0.51	63.67 ± 0.69

[a] The units of plasma alkaline phosphatase activity are milligrams of *para*-nitrophenol liberated per 100 ml of plasma.

[b] Mg% ATP × (100/HCT).

handled in the same manner, but given 9 g of the control diet daily. On day 28 of the experiment, each animal was sacrificed and the plasma alkaline phosphatase activity (9), erythrocyte pyrophosphatase activity (10), and plasma magnesium concentration (12) were determined.

Results. All results are expressed as means with values of the SEM. The numbers in parentheses in Tables I–IV give the number of animals in the experiment.

A comparison of the weights of the animals during the experiment indicated that there was an abrupt decrease in the ability of animals to gain weight on a magnesium deficient diet between the fourth and fifth days on the diet. The weight gains at weekly intervals are given in Table I.

A large difference in the plasma magnesium concentrations was observed between the two groups at the end of the first week (Table I). A further decrease in plasma magnesium concentrations occurred in the magnesium deficient group at the end of the second week but not at a significant level ($p<0.10$). Plasma alkaline phosphatase activity (Table I) showed a significant difference ($p<0.001$) between the magnesium deficient and control groups after 1 week; and this difference was observed throughout the remainder of the experiment. Although no difference was found for the erythrocyte pyrophosphatase activity between the two groups (Table I) at 1 week, by the second week this activity in the magnesium deficient group was significantly reduced ($p<0.001$) and remained essentially constant for the next 2 weeks. The same pattern of change with time as observed for the erythrocyte pyrophosphatase activity was noted for the whole blood ATP concentration (Table I). When the whole blood ATP concentrations were corrected for the deviations in hematocrit between the two groups by multiplying each determined value by 100/HCT, the difference in ATP concentrations between the two groups remained, but after the second week became less significant with time. This correction was applied since ATP is present mainly within the erythrocytes (6).

The changes in the blood and erythrocyte indices are presented in Table II. At the 3- and 4-week periods, significant differences ($p<0.005$) were present for hemoglobin, hematocrit, erythrocyte count, mean corpuscular volume, and mean corpuscular hemoglobin. Microscopically, the magnesium deficient erythrocytes had lost their central lucency and appeared as spherocytes after the third week.

TABLE II. Blood Changes and Erythrocyte Indices with Time in Magnesium Deficient Rats.

		Weeks			
		1	2	3	4
Hb (g/100 ml)	Mg deficient (6)	12.95 ± 0.44	13.57 ± 0.29	11.43 ± 0.33	11.17 ± 0.26
	Control (6)	13.40 ± 0.30	13.68 ± 0.12	13.43 ± 0.16	13.63 ± 0.14
HCT (%)	Mg deficient (6)	38.70 ± 1.60	40.83 ± 1.20	33.00 ± 1.15	34.00 ± 0.93
	Control (6)	40.70 ± 1.00	41.17 ± 0.48	39.00 ± 0.82	40.33 ± 0.72
Erythrocyte count (millions/mm³)	Mg deficient (6)			3.91 ± 0.17	3.74 ± 0.16
	Control (6)			5.42 ± 0.13	5.44 ± 0.14
Mean corpuscular Hb conc (%)	Mg deficient (6)	33.56 ± 0.33	33.27 ± 0.37	34.73 ± 0.78	32.81 ± 0.37
	Control (6)	32.96 ± 0.22	33.25 ± 0.24	34.49 ± 0.50	33.81 ± 0.18
(ng)	Mg deficient (6)			29.40 ± 0.83	30.00 ± 0.67
	Control (6)			24.84 ± 0.52	25.10 ± 0.37
Mean corpuscular Vol (μ^3)	Mg deficient (6)			84.76 ± 2.23	91.42 ± 2.73
	Control (6)			72.00 ± 0.80	74.25 ± 1.07

The magnesium deficient animals that were switched to the control diet after 3 weeks of inadequate magnesium intake gained significantly more weight ($p<0.001$) than the control animals during the fourth and fifth weeks. The base line values for enzyme and blood elements at 3 weeks and the experimental value for the fourth and fifth week results (*i.e.*, first and second weeks of repletion) are shown in Table III. The plasma alkaline phosphatase activity of the magnesium repleted animals was greater than the control values at the first and second weeks of repletion. Paralleling the plasma alkaline phosphatase activity, the plasma magnesium concentration of the repleted group returned to approximately control values by the first week of repletion.

The erythrocyte pyrophosphatase activity of the magnesium repletion group returned to approximately control values by the second week of repletion, but a difference between

TABLE III. Enzyme and Blood Changes in Magnesium Repletion.

Group of animals (week of expt.)	Plasma		Erythrocyte		
	Mg (mg%)	Alkaline phosphatase[a] (mg of *p*-nitrophenol/100 ml)	Mg (mg/100 ml)	Pyrophosphatase (mg of P/g of Hb)	Hb (g/100 ml)
Control					
3 (6)	1.87 ± 0.02	80.69 ± 6.87	5.40 ± 0.14	110.67 ± 2.67	13.58 ± 0.24
4 (6)	1.91 ± 0.02	77.80 ± 5.90	5.52 ± 0.13	114.67 ± 6.02	14.07 ± 0.25
5 (6)	1.89 ± 0.02	69.98 ± 3.05	5.34 ± 0.12	111.33 ± 3.84	13.63 ± 0.21
Mg deficient					
3 (6)	0.32 ± 0.03	42.16 ± 2.19	2.86 ± 0.11	77.83 ± 2.77	11.23 ± 0.46
4 (6)	0.28 ± 0.02	44.86 ± 3.10	2.38 ± 0.32	55.17 ± 4.42	10.35 ± 0.66
5 (6)	0.27 ± 0.01	42.17 ± 1.70	2.28 ± 0.25	56.00 ± 2.92	10.18 ± 0.38
Mg repleted					
4 (6)	1.84 ± 0.04	90.51 ± 9.62	4.02 ± 0.17	80.33 ± 3.95	12.63 ± 0.44
5 (6)	1.85 ± 0.01	78.80 ± 3.15	4.40 ± 0.18	101.50 ± 2.78	12.73 ± 0.33

[a] The units of plasma alkaline phosphatase activity are milligrams of *para*-nitrophenol liberated per 100 ml of plasma.

TABLE IV. Pair-Fed Animals (28 days).

	Plasma alkaline phosphatase[a] (mg of *p*-nitro phenol/100 ml)	Erythrocyte pyro phosphatase (mg of P/g of Hb)	Plasma Mg (mg/100 ml)	Wt (g)		
				Start	End	Difference
Mg deficient (9)	39.97 ±2.37	47.67 ±2.54	0.32 ±0.01	126.0 ±1.1	189.0 ±2.6	63.0 ±2.4
Control (10)	61.49 ±2.23	104.10 ±2.57	1.90 ±0.02	127.0 ±1.0	212.0 ±3.8	85.0 ±3.9
p Value	0.001	0.001	0.001	0.40	0.001	0.001

[a] Same as footnote of Tables I and III.

the control and deficient groups ($p>0.001$) was present at 1 week (Table III). The erythrocyte magnesium and hemoglobin showed a similar pattern except the erythrocyte magnesium of the repletion group was still significantly below the control value ($p<0.005$) at the second week of repletion. A significant difference ($p<0.001$) existed between the magnesium deficient and control groups of animals of the pair-fed experiment for plasma alkaline phosphatase activity, erythrocyte pyrophosphatase activity, plasma magnesium concentration, and body weight gain at 28 days (Table IV).

Discussion. A significant change in the ability of the magnesium deficient animals to gain weight occurred between the fourth and fifth days on the diet, with a concomitant appearance of red ears. Bois (13) showed that the vasodilatation associated with the red ears is due to endogenous histamine liberation. It thus appears that the initial stress of a magnesium deficient condition in the rat occurs between the fourth and fifth days on this magnesium deficient diet, and this is manifest by the red ears and an abrupt impairment of ability to gain weight.

By the end of the first week in the magnesium deficient animal, the plasma magnesium concentration and the plasma alkaline phosphatase activity had already reached apparent base line values and changed minimally during the next 3 weeks. Chutkow (14) showed that the plasma magnesium concentration in the rat had fallen by 30% after the animal had been on a magnesium deficient diet for 16 hr. Other authors have shown that the urinary excretion of magnesium falls markedly when the animal is placed on a magnesium deficient diet, and may approach zero by the third day (14, 15). Consequently, the plasma magnesium concentration falls rapidly at first and apparently a new equilibrium between plasma and tissue magnesium levels is reached by 1 week (Table I). The values for plasma alkaline phosphatase activity in the control and deficient groups (Table I) were consistent with the findings of other investigators (2–4).

The values obtained for erythrocyte pyrophosphatase activity in control animals (Table I) were consistent with the results in the literature (16). Herz *et al.* (16) found erythrocyte pyrophosphatase activity to be directly related to the proportion of reticulocytes among the erythrocytes. It has been shown that erythrocyte magnesium falls slowly in magnesium deficiency (17, 18). Tufts and Greenberg (19) suggested that the magnesium content of erythrocytes reflects the plasma level of magnesium at the time the erythrocytes are produced. Ginsburg *et al.* (20), using ^{28}Mg *in vitro*, could find no appreciable exchange between intracellular erythrocyte magnesium and the ^{28}Mg in the medium. He concluded that the erythrocyte magnesium content after erythrocyte formation did not change appreciably with changes in plasma electrolytes.

The above observations lend credence to the present experimental results for erythrocyte pyrophosphatase activity in magnesium deficiency. Even though a sizable decrease in plasma magnesium was present by 1 week, a concomitant change would not have occurred in erythrocytes, and, consequently, the eryth-

rocyte pyrophosphatase activity for the deficient and control animals was the same. By the end of the second week, a significant decrease ($p<0.001$) in erythrocyte pyrophosphatase activity in magnesium deficient animals was present.

The values for the whole blood ATP concentration (Table I) became progressively smaller during the experiment. Since previous studies have shown a decrease in erythrocyte membrane ATPase activity directly proportional to the concentration of magnesium in the incubation medium (21), it is possible that all reactions utilizing ATP are depressed in magnesium deficiency. This would probably result in diminished ATP synthesis, as was observed in this experiment.

The decrease in number of erythrocytes (Table II) in the magnesium deficient rat suggested that they were unable to produce erythrocytes at a normal rate and/or there was an increase in the rate of destruction of erythrocytes after eating the magnesium deficient diet 3 weeks. The erythrocytes that were produced in the magnesium deficient animals had greater volumes than those of control cells as shown by the mean corpuscular volumes (Table II), and supported microscopically by the presence of spherocytes on blood smears. Nakao *et al.* (22) demonstrated that the depletion of erythrocyte ATP produced a reversible disc–sphere transformation of the erythrocytes. This observation was confirmed by Weed *et al.* (23) who proposed that a sol–gel change occurs at the interface between the erythrocyte membrane and the cell interior of the ATP depleted erythrocyte. They also noted a marked increase in calcium content of the ATP depleted erythrocyte and suggested that this triggers the sol–gel change which can be reversed by ATP, magnesium, and EDTA (ethylenediaminetetraacetate). These observations, plus the demonstrated reduction of whole blood ATP in magnesium deficiency in this study, suggest the mechanism for the observed spherocytic transformation of erythrocytes in magnesium deficiency.

The magnesium repletion animals were able to gain approximately 60% more weight than the control animals during the first and second weeks of repletion. This finding indicated that the stunted growth of the magnesium deficient animal was partially, if not completely, reversible when magnesium was again provided at normal levels.

The changes in the plasma components, namely, plasma alkaline phosphatase activity and plasma magnesium concentration, showed a prompt response to approximately control values after 1 week of magnesium repletion (Table III). This indicated that the plasma changes closely paralleled the dietary magnesium levels. The fact that more plasma alkaline phosphatase activity was present in the repletion animals than the normally fed animals suggested a "rebound phenomenon" in the synthesis of the enzyme.

The change in the erythrocyte variables, namely, erythrocyte pyrophosphatase activity, erythrocyte magnesium concentration, and hemoglobin, showed a slower progression toward the control values than did serum components. It has been postulated that the magnesium content of the erythrocyte closely reflects the serum magnesium concentration at the time of erythrocyte formation (19). The repletion study supports this view since a progressive change toward the control values occurred during the two weekly intervals for the three erythrocyte values cited above, but all three values were still below the control levels after 2 weeks on the normal magnesium diet.

The changes observed between the magnesium deficient and control pair-fed animals were essentially the same as those found for the animals receiving the respective diets *ad libitum* at 4 weeks (Table IV).

Summary. The magnesium deficient rats developed red ears and were unable to gain weight at a normal rate after 5 days on the magnesium deficient diet. The plasma magnesium concentration in animals on a magnesium deficient diet for 1 week was approximately 20% of control values and dropped further, although insignificantly, during the following 3 weeks.

The plasma alkaline phosphatase activity in magnesium deficient animals was depressed to approximately 50% of control values by 1 week and remained at this level over the next 3 weeks. A significant decline in the erythrocyte pyrophosphatase activity in

magnesium deficient rats did not occur until the second week and remained constant during the following 2 weeks. The slower change in erythrocyte pyrophosphatase activity, when compared with plasma alkaline phosphatase activity, was related to a slower decrease in erythrocyte magnesium content. The whole blood ATP content was significantly lower in the magnesium deficient animals after 2 weeks.

The number of erythrocytes per unit volume of whole blood progressively decreased with time in magnesium deficient animals when compared with control values. The magnesium deficient animals had a macrocytic, normochromic anemia. Microscopically, the erythrocytes were spherocytic in magnesium concentration in magnesium repleted animals returned to control values within 1 week. The erythrocyte factors, namely, erythrocyte pyrophosphatase activity, erythrocyte magnesium concentration, and hemoglobin, in magnesium repleted animals progressed toward the control values, but were still below control values at the end of 2 weeks. Pair-fed magnesium deficient and control animals exhibited essentially the same changes as animals provided the respective diets *ad libitum* at 28 days.

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Received Jan. 22, 1971. P.S.E.B.M., 1971, Vol. 137.