An Inverse Relationship Between Calcium and ATP in Renal Tissue of Magnesium-Deficient Rats (36020)

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It has been reported recently that dietary ethionine exaggerates the kidney calcification of the magnesium-deficient rat (1). One of the major metabolic effects of ethionine in liver is to decrease adenosine triphosphate (ATP) concentration (2), apparently via formation of *S*-adenosyl ethionine. This is a relatively stable compound and as a result of its accumulation, ATP synthesis decreases because of a lack of substrate.

This report presents the outcome of investigations into a possible relationship between the ATP-lowering effect of ethionine and its stimulation of calcification. It records the observation that magnesium-deficient kidney calcification is closely associated with lowered kidney ATP levels and that restoration of magnesium adequacy increases the kidney ATP to calcium ratio.

Experimental Procedure. All of the experiments, reported below, employed young male albino rats of the Sprague-Dawley strain, weighing initially 90–100 g. The basal diet, deficient in magnesium, contained 15% casein, 0.3% *dl*-methionine (except in Exp. 1), 8% corn oil, 3% woodfloc, 5% vitamin–glucose[1]; 3.8% mineral mix (1); and 64.9% glucose monohydrate. This diet contained about 0.65% of calcium and of phosphorus, and 80 ppm of magnesium. Feeding was *ad libitum* and distilled water was always available.

When each animal had spent his assigned time on experiment, he was anesthestized lightly with ether, blood was drawn from the abdominal aorta, and kidneys were immediately removed. One kidney from each rat was quickly weighed and then placed in 5–10 vol of boiling water to inactivate ATPase. The other kidney was dried, wet ashed, and subsequently analyzed for calcium by means of atomic absorption spectrophotometry. The kidneys used for ATP assay were boiled for 20 min, chilled in an ice bath, homogenized, and centrifuged. The supernatant solution was analyzed for ATP by the luciferin reaction according to Strehler and Totter (3), using internal standards and recording peak intensity of illumination detected with a Turner fluorometer. In Exp. 1, livers were also assayed for ATP. Serum magnesium was measured by atomic absorption.

Exp. 1. Thirty-two rats were used in a study with a 2^3 factorial design in which the variables were magnesium (80 and 600 ppm), methionine (0 and 0.3% added), and ethionine (0 and 0.15%). Two animals from each of the eight groups were killed on day 3 of the experiment and the remaining two were killed on day 6.

Exp. 2. Forty rats were used in a 2^2 factorially designed study, whose variables were magnesium (80 and 600 ppm), and L-thyroxine (0 and 10 ppm). Five of the 10 animals in each group were killed on day 7 and the remainder on day 14.

Exp. 3. Sixty rats were fed the magnesium-adequate (600 ppm) diet for 3 days, following which 30 of them received the magnesium-deficient diet. Five rats from each group were killed 2, 4, 6, and 9 days after initiating the deficient diet feeding. The remaining 10 deficient animals were replaced on the adequate diet and five were killed (along with 5 continuously adequate rats) after 2 and 8 days of repletion.

Results. Exp. 1. The treatment group averages are shown in Table I. The effects of time, 3 vs 6 days of deficiency, are not shown in Table I. These effects were a decrease in

[1] Complete mixture of Jacob and Forbes, J. Nutr. 100, 228 (1970).

TABLE I. Data of Expt. 1, Showing Effects of Magnesium Deficiency and Methionine and Ethionine Supplements on Serum Magnesium, Kidney Calcium, and Kidney and Liver ATP.

Treatment					Kidney			Liver ATP	
						ATP			
Mg	Me	Eth	Av daily gain (g)	Serum Mg (mg/100 ml)	Ca (mg/g of dry tissue)	(μg/g of wet tissue)	(% of control)	(μg/g of wet tissue)	(% of control)
—	—	—	4.3	1.29 ± 0.05[a]	1.67 ± 0.83	197 ± 8	76 ± 8	728 ± 97	134 ± 27
—	+	—	5.7	1.20 ± 0.11	2.57 ± 0.51	210 ± 16	88 ± 9	506 ± 64	88 ± 3
—	—	+	1.8	1.79 ± 0.11	3.04 ± 1.74	201 ± 28	71 ± 9	526 ± 82	93 ± 11
—	+	+	4.2	1.58 ± 0.31	29.6 ±23.3	128 ± 41	57 ±22	557 ± 35	99 ± 6
+	—	—	4.4	2.37 ± 0.35	0.37 ± 0.01	269 ± 32		593 ± 79	
+	+	—	6.2	2.02 ± 0.08	0.37 ± 0.01	239 ± 22		590 ± 65	
+	—	+	2.7	2.30 ± 0.02	0.59 ± 0.18	284 ± 11		656 ±110	
+	+	+	4.2	2.36 ± 0.03	0.38 ± 0.02	248 ± 29		442 ± 34	

[a] Mean ± SEM 4 animals/group.

serum magnesium and an increase in kidney calcium in the magnesium-deficient groups. The gain, serum Mg and kidney calcium data serve to corroborate our earlier findings (1) that ethionine exaggerates calcification in magnesium deficiency and corroborates unpublished data showing that a 0.3% methionine supplement to a 15% casein, magnesium-deficient diet also increases calcification. In these experiments, expression of tissue ATP as percentage of that in appropriate magnesium-adequate control animals serves to minimize variation in the ATP analysis between determinations made on different days. Although quite variable, the liver ATP values do not appear to be affected by magnesium deficiency. The kidney ATP levels were also quite variable, as were the kidney calcium

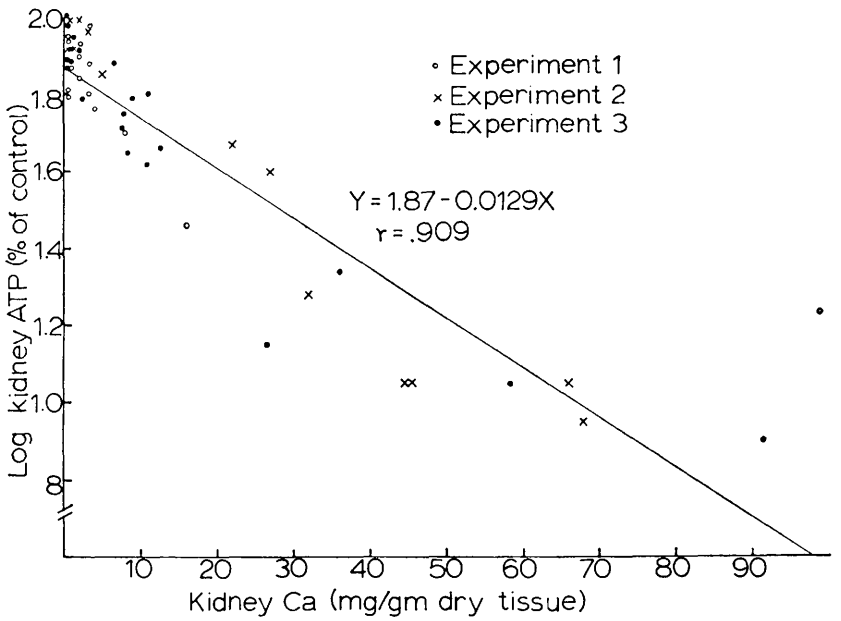

FIG. 1. Relationship between logarithm of kidney ATP (percent of control values) and milligrams of calcium per gram of dry kidney. Combined data from three experiments. Each point represents a single animal.

TABLE II. Data of Expt. 2, Showing Effects of Magnesium Deficiency and T_4 Supplementation on Serum Magnesium and Kidney Calcium and ATP.

Treatment			Av daily	Serum Mg	Kidney		
						ATP	
Mg	T_4	Days	gain (g)	(mg/100 ml)	Ca (mg/g of dry tissue)	(μg/g of wet tissue)	(% of control)
—	—	7	5.1	0.84 ± 0.04[a]	47.7 ± 13.5	67 ± 45	25 ± 17[b,c]
—	—	14	4.4	0.71 ± 0.03	43.0 ± 9.9	55 ± 16	25 ± 8[c]
—	+	7	4.6	0.90 ± 0.08	0.48 ± 0.06	283 ± 18	105 ± 7[d]
—	+	14	3.6	0.70 ± 0.03	1.68 ± 0.94	187 ± 11	86 ± 5[d]
+	—	7	7.7	2.05 ± 0.04	0.37 ± 0.01	282 ± 13	
+	—	14	7.4	2.15 ± 0.08	0.41 ± 0.01	224 ± 15	
+	+	7	5.5	2.48 ± 0.01	0.38 ± 0.02	241 ± 13	
+	+	14	5.2	2.27 ± 0.06	0.36 ± 0.01	225 ± 9	

[a] Mean ± SEM for 5 animals/group.

[b] Treatment means with different superscripts (c or d) are different ($p < .05$).

data, and showed a definite inverse relationship between ATP and calcium in the tissue. The relationship between these becomes more clear when individual values for ATP (log of percentage of control values) and calcium are considered as shown in Fig. 1. This preliminary experiment indicated that there was an association between these two parameters of kidney composition and that the amount of ethionine used did not directly affect tissue ATP levels.

Exp. 2 was designed to further examine the ATP–calcium relationship by again inducing magnesium deficiency and by investigating the effect of L-thyroxine (T_4), since this agent is known to prevent calcification of the magnesium-deficient kidney. The pertinent group data are shown in Table II and individual data for kidney calcium and ATP in Fig. 1. As in Exp. 1, the group average data for gain, serum magnesium, and kidney calcium are in agreement with previous findings concerning magnesium deficiency and T_4 treatment (4). The group means also show a correlation between kidney calcium and ATP levels. This relationship is again clarified in Fig. 1. It is apparent that magnesium deficiency does not lower kidney ATP unless kidney calcium is raised.

It occurred to us that this relationship might merely reflect an analytical artifact because of the large amount of calcium in the calcified kidneys. Analysis of the supernatant solutions of kidney homogenates used in the ATP assay showed that these added a maximum of 0.24 μg of calcium to the assay tube. We verified by direct addition of this amount of calcium to standard ATP solutions that no inhibition of activity resulted. We also found that with normal kidneys about 20–30%, but with highly calcified kidneys less than 1%, of the total calcium appeared in the supernatant solution.

Exp. 3 was conducted to study in more detail the time sequence existing between increase of calcium and decrease of ATP in the kidneys and to investigate the effect of restoring normal magnesium nutriture to deficient rats. Previous investigations have shown that magnesium-deficient rats do not lose kidney calcium when they are placed on an adequate diet even though their serum and bone magnesium are restored to normal (5). The group average data are shown in Table III and individual data for kidney calcium and ATP are plotted in Figs. 1 and 2.

Serum magnesium had decreased significantly by the second day of deficiency and continued to decrease during the 9 days of low-magnesium intake. By the second day of restoration of magnesium to deficient animals, the serum magnesium had returned to the normal range.

Kidney calcium was markedly elevated in the deficient animals. Because of extreme with-

TABLE III. Data of Expt. 3, Showing Effects of Magnesium Deficiency and Recovery on Serum Magnesium and Kidney Calcium and ATP.

Treatment (days); Mg				Kidney		
					ATP	
—	+	Wt gain (g/day)	Serum Mg (mg/100 ml)	Ca (mg/g of dry tissue)	(μg/g of wet tissue)	(% of control)
2		7.3	1.30 ± 0.06[a]	0.52 ± 0.11	191 ± 9.6	84 ± 4.3[bc]
4		6.7	1.17 ± 0.06	2.64 ± 0.83	186 ± 17.8	74 ± 7.1[cd]
6		6.1	0.90 ± 0.04	12.8 ± 3.5	78 ± 14.5	44 ± 8.1[d]
9		5.2	0.70 ± 0.02	34.8 ± 17.3	108 ± 37.7	42 ± 14.5[d]
9	2	5.3	1.92 ± 0.13	39.9 ± 23.0	94 ± 26.9	40 ± 11.5[d]
9	8	5.8	2.18 ± 0.06	6.2 ± 1.4	217 ± 16.4	82 ± 6.1[c]
	2	7.8	2.04 ± 0.08	0.38 ± 0.01	228 ± 7.4	
	4	7.5	1.92 ± 0.09	0.36 ± 0.01	251 ± 9.9	
	6	6.6	1.92 ± 0.05	0.36 ± 0.02	177 ± 7.0	
	9	6.4	1.92 ± 0.07	0.33 ± 0.01	252 ± 10.3	
	11	6.4	1.95 ± 0.07	0.54 ± 0.13	234 ± 4.2	
	17	6.3	2.16 ± 0.03	0.45 ± 0.07	266 ± 1.5	

[a] Mean ± SEM for 5 animals/group.

[b] Treatment means with different superscripts (c or d) are different ($p < .05$).

in-group variation the trend toward greater calcification as time progressed from day 6 to 9 is not significant, nor is the apparent decrease in kidney calcium in those deficient animals given normal magnesium intakes for 8 days. ATP concentration in the kidneys decreased during magnesium deficiency as the calcium increased. In those deficient animals receiving a magnesium-adequate diet for 8 days, the ATP level was significantly higher than in those without restoration of magnesium status. The data for the deficient animals are included in Fig. 1. Figure 2 presents kidney ATP and calcium data from deficient and from repleted animals in Exp. 3, showing less than 13 mg of Ca/g of dry tissue. The two equations presented have statistically similar slopes but the kidney ATP values,

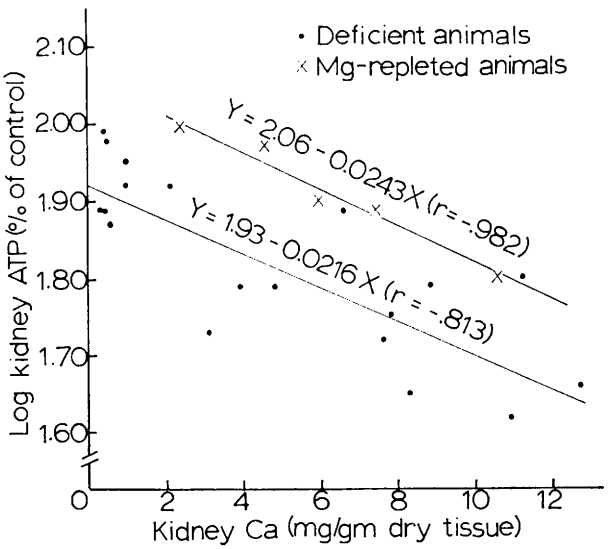

FIG. 2. Effect of magnesium repletion on relationship between kidney ATP and calcium.

expressed as a percentage of the controls are significantly higher for those animals with restored magnesium status for 8 days.

Discussion. With respect to the major objective of these experiments, a study of the relationship of kidney ATP and calcium levels in magnesium deficiency, several points deserve comment.

The correlation coefficient of —0.909 shown in Fig. 1 indicates that more than 80% of the variation observed in kidney ATP levels is accounted for by variation in kidney calcium levels. By inference, then, the length of time animals are exposed to magnesium deficiency is not an important determinant of kidney ATP except as calcium accumulates in the kidney. These data do not reveal clearly which phenomenon occurs first, decrease of ATP or increase of calcium, although at normal kidney calcium levels there is a tendency for ATP to be decreased in the magnesium-deficient animals.

Although the repletion experiment indicated an increase in ATP at constant kidney calcium level, the fact that the slopes of the regression lines shown in Fig. 2 are not different indicates that recovery of ATP is by no means complete in this time interval of 8 days.

The cause and the significance of the ATP depression are not clear. The assay medium used contained an excess of magnesium for the purpose of activating the luciferase reaction. Although not measured in these experiments, our previous experience indicates that kidney levels of magnesium are not materially altered in the degree of deficiency attained, although it is evident from the serum magnesium values that a lesser amount of magnesium is being processed by the kidney tubules.

In the event of ATP deficiency in a tissue one might expect a decreased activity of glycolytic or Krebs cycle enzymes. Although we have not investigated this, a report by Gunther (6) indicates that none of five glycolytic enzymes and two Krebs cycle enzymes tested showed altered activity in kidney tissue of magnesium-deficient rats.

Support for the concept of decreased tissue ATP in magnesium deficiency is found in the recent publication of Elin *et al.* (7) who found a 20% decrease in blood ATP in rats given a 2.6 ppm magnesium diet. In their work, however, the decrease was first noted only at the second week of deficiency and did not change during two subsequent weeks. In Exp. 2, reported here, kidney ATP had decreased by 75% at 7 days of magnesium deficiency.

The literature also provides a previous demonstration of an inverse relationship between tissue ATP and calcium. Weed *et al.* (8) found that human erythrocytes accumulated calcium to 4-fold normal values as their ATP concentration dropped during incubation. These data suggest that ATP is necessary for maintaining the normal gradient of calcium between cells and fluid by an active transport mechanism. Further experimentation is required to clarify these relationships.

Summary. A series of three experiments has demonstrated an inverse relationship between nephrocalcinosis induced by magnesium deficiency in the rat and the level of ATP determined in the kidney. Over 80% of the variation in kidney ATP was accounted for by variation in kidney calcium content.

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