Inverse Relationship Between Calcium and ATP in Renal Tissue of Magnesium-Deficient Rats: A Correction (37076)

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We reported in this journal (1) an inverse relationship between ATP and calcium concentrations in kidneys of magnesium-deficient rats. In a continuation of our research it has become apparent that the data obtained represents an analytical artifact. The following account is written to correct the record and to present a method of tissue preparation suitable for ATP analysis in the presence of calcium phosphate.

Methods. Procedures of handling animals and producing calcified kidneys were similar to those reported previously (1). A total of 45 male albino rats of Sprague–Dawley strain, weighing initially 100 g, were used. Numbers of rats and their treatment schedule are shown in Table I. The initial objective of this experiment was to clarify the effect of restoration of normal magnesium status on kidney ATP concentration. At termination of the feeding period rats were lightly anesthetized with ether, a kidney was rapidly exposed and frozen *in situ* with aluminum clamps precooled in liquid N_2. Following this, blood was obtained from the abdominal aorta and the other kidney was removed. The latter tissues were analyzed for calcium and magnesium by atomic absorption spectrophotometry. The quick-frozen kidney was pulverized in liquid N_2 and a weighed fraction of it was placed in 8–10 times its volume of 0.9 *N* $HClO_4$. Excess perchlorate was precipitated with K_2CO_3 and after centrifugation the solution brought to pH 7.4 with triethanolamine (TEA) buffer and used for ATP analysis by the luciferin reaction according to Strehler and Totter (2).

In the second experiment, the same analytical procedures were used except that 100 mg Na_2 EDTA was added to the $HClO_4$ extracts before neutralization. The justification for this alteration of procedure is presented in the "Results" section.

Results. Pertinent data derived from the tissue analyses are presented in Table I. Ten days of magnesium deficiency severely lowered serum Mg and increased kidney Ca in the usual fashion. Five days of restoration to a Mg-adequate state permitted serum Mg to be restored to control levels but failed to affect kidney calcium. This laboratory has reported

TABLE I. Effects of Magnesium Deficiency and Recovery Therefrom on Serum Magnesium and Kidney Calcium, and of Extraction Method on Kidney ATP Concentration.

Treatment groups		No. of	Serum Mg	Kidney Ca	Kidney ATP
Days 1–10	11–17	rats	(mg/100 ml)	(mg/g D.W.)	(μM/g W.W.)
		Expt 1. $HClO_4$ extraction of kidney			
+Mg		5	2.0 ± .06[a]	.36 ± .01	1.43 ± 0.6
−Mg		10	.97 ± .05	28.1 ± 4.5	.16 + .04
+Mg	+Mg	5	2.0 ± .06	.37 ± .03	1.86 ± .13
−Mg	+Mg	10	2.3 ± .06	24.4 ± 3.4	.34 ± .08
		Expt 2. $HClO_4$: EDTA extraction of kidney			
+Mg		5	2.3 ± .23	.29 ± .01	1.45 + .10
−Mg		10	.86 ± .04	66.6 ± 7.3	1.40 ± .06

[a] Mean ± S.E.

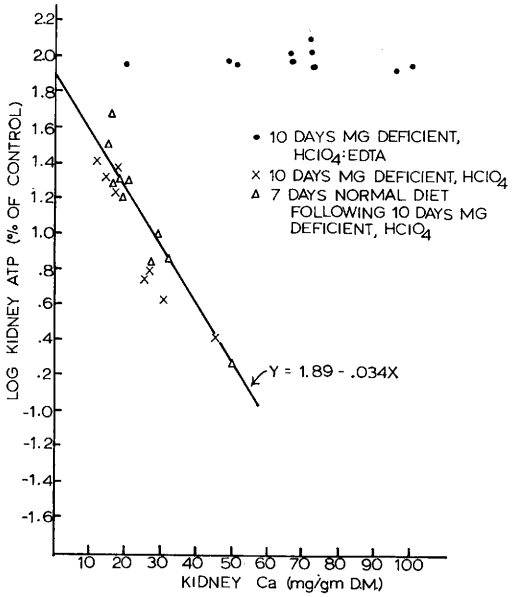

FIG. 1. Relationship between log kidney ATP (% of control values) and kidney calcium (mg/g dry matter) as affected by tissue extraction method. Addition of EDTA to the $HClO_4$ extraction medium prevents precipitation of ATP during subsequent neutralization.

previously that the calcification induced in kidneys by Mg deficiency is not materially changed by restoration of a normal Mg supply (3). ATP measured in kidney extracts was markedly affected by calcium level, but not by current magnesium status when $HClO_4$ was used as extractant and protein precipitant. Inclusion of EDTA in the extraction medium did not affect values obtained for ATP in normal kidneys but eliminated the previously observed decrease of ATP in calcified kidneys. This can be seen in the average values given in Table I and in the individual data shown in Fig. 1.

Discussion. In Expt 1 the freeze-clamping $HClO_4$ procedure was used in preparing kidneys instead of the boiling water method we employed in our previous work. In our hands the $HClO_4$ method gave about 3-fold higher values for ATP in normal kidneys than did the boiling water method and also provided a steeper slope to the line representing ATP decrease per unit of calcium increase (.034 vs .012). However, we did observe that when $HClO_4$ extracts of calcified kidneys were neutralized with TEA a precipitate formed. In a subsequent experiment we found that this precipitate could be dissolved with EDTA and that a major portion of the ATP "lost" as well as a significant portion of the kidney calcium could be found in the EDTA-dissolved precipitate. On this basis, EDTA was added to the centrifuged $HClO_4$ solutions in Expt 2 prior to neutralization. This prevented precipitate formation and the previously reported decrease of ATP calcified kidneys.

The major difference in composition between normal and calcified kidneys produced by magnesium deficiency involves an increase in both calcium and phosphorus in a 2:1 ratio. Since in our initial investigation kidneys were boiled we have now investigated the effect of boiling on assayable ATP when calcium and/or phosphate are added to standard solutions of ATP (Sigma Chemical Co.). When both were added, a precipitate formed, was centrifuged and eventually was redissolved with EDTA. The data obtained are presented in Table II and show that phosphorus alone does not affect ATP yield, that calcium alone will decrease the yield if samples are boiled and that the combination of calcium and phosphorus is quite active in depressing ATP yield, especially when the solutions are boiled. In a final test we measured the effect of graded levels of calcium in presence of a con-

TABLE II. Recovery of ATP[a] from Solutions Containing Calcium and Phosphate.

		Percent recovery of ATP			
		in solution		in precipitate	
m*M* Ca	m*M* PO_4	not boiled	boiled	not boiled	boiled
.1	.1	4.0	2.5	40	4.3
.1	0	97	56	no precipitate	
0	.1	97	83	no precipitate	

[a] Obtained from Sigma Chemical Co.

TABLE III. Effect of Varying Calcium Concentration on Percent ATP[a] Recovery in Boiled Solutions.

mM Ca[b]	% ATP recovered
.012	71
.025	60
.05	43
.075	26
.10	15
.15	6.1
.20	5.8

[a] Obtained from Sigma Chemical Co.
[b] All tubes contained 0.1 m*M* PO_4.

stant amount of phosphorus. The tubes were boiled 15 min, cooled, EDTA added to dissolve the precipitate and the solutions assayed for ATP. Results are shown in Table III. The log of percent ATP recovered bears a linear relationship to calcium present, just as found with calcified kidneys.

Summary. It has been shown that calcium phosphate present in extracts of rat kidneys and in standard solutions decreases the assayable ATP. This effect may be avoided by utilizing EDTA to prevent the formation of a calcium phosphate-ATP complex, and by avoidance of boiling the solutions containing calcium and phosphate.

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2. Strehler, B. L., and Totter, J. K., *in* "Methods of Biochemical Analysis" (D. Glick, ed.), p. 1, 341. Interscience, New York (1954).
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