Interrelationships of Thyroxine, Citrate, and Renal Calcification in the Magnesium-Deficient Rat[1] (35736)

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The close association of magnesium deficiency and renal calcinosis has been known for a number of years (1). However, the mechanism by which calcium phosphate crystals deposit in the renal tissue still remains unresolved. Magnesium deficiency in its earliest stages has been shown to induce hypercalcemia, hypocalciuria, and hyperphosphaturia (2). All these changes also accompany hyperparathyroidism. Indeed, for calcium phosphate to deposit in kidney tissue of the magnesium-deficient rat, the animals must have intact parathyroid glands (3, 4) and an adequate supply of vitamin D (5, 6).

It was observed that parathyroid hormone (PTH) (7) and vitamin D (5) increased the urinary excretion of citrate in normal animals, while Lifshitz *et al.* (5) observed that the urinary citrate concentration was reduced in magnesium-deficient vitamin D-treated rats with functional parathyroid glands and that increasing citrate excretion by alkalinization of the diet prevented renal calcification of magnesium deficiency. They proposed that citrate could play a major role in the prevention of calcium deposits by virtue of its strong chelating effect towards calcium. It is possible that calcium could form a soluble complex with citrate thus reducing the chance for the formation of insoluble calcium phosphate.

Recent observations have shown that renal calcification of magnesium-deficient rats can be reduced by prior administration of L-thyroxine (T_4) (4, 8). Jacob and Forbes (6) found increased urinary citrate excretion in magnesium-deficient rats treated with vitamin D and T_4. However, vitamin D-treated animals developed calcified kidneys while those given T_4 did not. They concluded that urine citrate was not correlated with renal calcification which was in contrast to the suggestion of Lifshitz *et al.* (5).

The following experiments were conducted to study in more detail the interrelationships of T_4, citrate, and renal calcification in the magnesium-deficient rat.

Methods. In the first experiment sixty-five 100-g male albino rats of the Sprague-Dawley strain were preconditioned to the basal diet (Table I), supplemented to contain 500 ppm magnesium, for a period of 5 days. They were then divided into four groups of 15 rats each and one group of 5. The day before the experiment was to begin a 24-hr urine sample was collected from the group of five animals receiving the magnesium-adequate diet. Blood was obtained from the abdominal aorta after an anesthetic dose of ether, and the kidneys were saved for analysis. The tissue and urine samples were subsequently analyzed for citrate, calcium, and phosphorus content. In addition serum was analyzed for magnesium content. Two groups of 15 rats continued receiving the adequate diet while two groups of 15 were fed the basal, 50 ppm magnesium, diet. Both diets contained 0.6% phosphorus and 0.9% calcium. Each day of the experimental period half of the animals in each dietary regimen was treated with in-

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TABLE I. Composition of the Basal Diet.

Ingredient	Per cent
Casein[a]	15.0
DL-Methionine	0.5
Corn oil	8.00
Cellulose[b]	3.0
Cod liver oil	0.5
Vitamin mix[c]	5.0
Mineral mix[d]	4.0
Choline chloride	0.2
Glucose[e]	63.8

[a] Vitamin-Free Test Casein, General Biochemicals Inc., Chagrin Falls, Ohio.

[b] Solka Floc, Brown Company, Boston, Mass.

[c] Contained: (in mg/kg mixture) thiamine–NCl, 200; riboflavin, 120; pyridoxine–HCl, 80; Ca pantathenate, 320; biotin, 4; nicatinic acid, 500; folic acid, 10; B_{12}-mannitol, 400 (B_{12} present at 0.1%); menodione, 7; glucose to make 1 kg.

[d] Contained (in per cent of mixture) MgO, 0.167; NaCl, 5.090; K_2CO_3, 3.302; $FeSO_4 \cdot 7H_2O$, 0.314; $MnSO_4 \cdot H_2O$, 0.388; $CuSO_4 \cdot 5H_2O$, 0.050; $ZnCO_3$, 0.103; $CaHPO_4$, 51.431; $CaCO_3$, 18.918; KI, 0.001; K_2HPO_4, 7.100; and glucose, 13.136.

[e] Cerelose, Corn Products Refining Company, New York.

traperitoneal (ip) injections of 10 μg T_4/100 g body wt/day. After 1, 3, and 5 days on experiment and prior to killing, 24-hr urine collections were made on five animals from each of the four treatment groups. Blood, kidney, and urine samples were collected and analyzed in the same manner as were samples from the initial five rats.

The second experiment was designed to overcome the large variation experienced in the concentration of a number of urine components in the 24-hr collection. In this experiment 100-g rats were preconditioned to the magnesium-supplemented basal diet for 5 days at which time they were divided into four groups of 10 animals each. Two of these groups continued to receive the adequate diet while two groups received the basal diet containing 50 ppm magnesium. Ten rats from each dietary treatment received intraperitoneal injections of 10 μg T_4/100 g body wt/day throughout the 5-day experimental period. After having consumed the magnesium-deficient diet and having received one injection of T_4, urine collections were made during the last 4 days. At the end of this collection period all animals were killed and blood and kidneys removed for analysis of mineral and citrate content.

Mineral concentration in tissue and urine was determined by standard methods of atomic absorption spectrophotometry. Phosphorus was determined by the method of Fiske and SubbaRow (9). Citrate concentration of serum, kidney, and urine was determined by the method of Ettinger (10). All data were analyzed by analysis of variance appropriate for a factorial design (11).

Results. Experiment 1. Induction of magnesium deficiency did not affect weight gain during this 5-day experiment but T_4 reduced average weight gain by about 20%, or to 5 g daily. The data from experiment 1 are presented in Table II. Serum magnesium decreased as magnesium deficiency progressed. This was significant as early as 1 day after initiation of the magnesium-deficient diet. Thyroxine had no significant effect on the concentration of this mineral in serum.

Calcium concentration of serum was erratic throughout the experiment. After 1 day on the magnesium-deficient diet serum calcium was elevated whether T_4 was present or not, this effect being most obvious in the T_4-treated group. By the end of the experiment this T_4-treated group showed lower serum calcium than the nontreated magnesium-deficient group. Thyroxine had no effect on serum calcium in groups receiving adequate magnesium.

As a result of T_4 treatment, serum phosphorus was increased significantly after the first day regardless of magnesium status of the animal. On the other hand, it was reduced by day 5 in the magnesium-deficient group not receiving T_4.

By the third day on experiment magnesium-deficient rats receiving T_4 had a very high concentration of citrate in the serum but this decreased markedly by the fifth day. Throxine treatment of magnesium-adequate animals had a different effect on serum citrate, producing a constant increase after the first day. Rats receiving an adequate supply of magnesium maintained a constant level of citrate throughout the experiment.

TABLE II. Serum and Kidney Minerals and Citrate as Affected by Treatment and Time (Experiment 1).

		Treatment			
	Mg:	+	+	−	−
	Day T_4:	−	+	−	+
Serum (mg/100 ml)					
Mg	1	2.0[a]	1.9[a]	1.5[b]	1.4[b]
	3	2.0[a]	2.2[a]	1.0[b]	1.0[b]
	5	2.1[a]	2.3[a]	.8[b]	.9[b]
Ca	1	9.3[a]	9.6[a]	10.1[b]	10.6[c]
	3	9.4[a]	9.5[a,b]	10.1[a]	9.9[b]
	5	9.7[a]	9.6[b]	10.6[c]	9.9[a,b]
P	1	9.4[a]	9.8[a]	9.0[a]	10.4[b]
	3	9.4[a]	10.7[b]	9.1[a]	10.4[b]
	5	10.3[a]	11.7[b]	9.2[c]	10.7[a,b]
Citrate	1	8.1[a]	8.0[a]	7.4[a]	9.1[b]
	3	7.9[a]	9.9[b]	8.3[a,b]	12.2[c]
	5	8.6[a]	10.9[b]	8.4[a]	9.0[a]
Kidney Ca/mg/g dry tissue	1	.49[a]	.48[a]	.43[b]	.42[b]
	3	.42[a]	.49[a]	3.58[b]	.46[b]
	5	.51[a]	.64[a]	20.90[b]	.54[b]
Kidney citrate μg/g wet tissue	1	44[a]	53[b]	46[a]	61[b]
	3	54[a]	64[b]	55[a]	64[b]
	5	53[a]	66[b]	140[c]	54[a]

[a] Means with different superscripts within lines differ significantly ($p < .05$).

After 1 day on the deficient diet, calcium concentration in renal tissue increased markedly and continuously for the duration of the experiment. Those deficient animals receiving T_4 had no more calcium in their kidneys than those animals receiving an adequate dietary supply of magnesium.

Kidney citrate was increased by T_4 treatment regardless of magnesium status up to the third day of the experiment. However, by the fifth day kidney citrate of the magnesium-deficient group receiving T_4 had returned to the concentration maintained by the magnesium-adequate group without T_4. Between the third and fifth day there was a dramatic increase in kidney citrate to almost 3 times normal in rats receiving the magnesium-deficient diet but no T_4.

Values for calcium and phosphorus in 24-hr urine samples were extremely variable for the three collection periods. Thus, no significant difference between treatments could be calculated for either ion. Citrate concentration was also variable during the initial phase of the deficiency but was stabilized by the fifth day. Table III shows the results of that collection. Citrate excretion was reduced by magnesium deficiency but was not affected by treatment with T_4.

Experiment 2. The data of experiment 2 shown in Table III. Urine values for this experiment represent the average of a 4-day collection and are expressed as units of a component excreted per 24 hr/100 g body wt at the time of killing. When expressed in this manner, results comparable to the first experiment were obtained for urine citrate. Magnesium deficiency significantly reduced urine citrate in both T_4-treated and non-treated groups. This experiment differed from experiment 1 by showing a significant increase in urine citrate in magnesium-adequate T_4-treated rats.

Urine calcium and phosphorus were significantly affected by each treatment. Urine calcium was decreased by magnesium depriva-

TABLE III. Serum, Kidney, and Urine Minerals and Citrate as Affected by Treatment (Experiment 2).

		Treatment			
	Mg:	+	+	−	−
	T_4:	−	+	−	+
Urine citrate (mg/24 hr/100 g body wt)					
Experiment 1	Day 5	.60[a,a]	.70[a]	.16[b]	.27[b]
Experiment 2	Days 2–5	.72[a]	.98[b]	.17[c]	.29[c]
Urine Ca (mg/100 hr/100 g body wt)		1.03[a]	.53[b]	.34[b]	.51[b]
Urine P (mg/24 hr/100 g body wt)		6.2[a]	3.7[b]	8.2[c]	6.9[a]
Kidney Ca (mg/g wet tissue)		.11[a]	.10[a]	1.1[b]	.17[a]
Kidney citrate (μg/g wet tissue)		68[a,b]	76[a,c]	85[c]	57[b]
Serum Ca (mg/100 ml)		10.4[a]	9.6[c]	10.8[b]	11.0[b]
Serum P (mg/100 ml)		11.2[a]	12.4[c]	10.1[b]	11.3[a]

[a] Means with different superscripts within lines differ significantly ($p < .05$).

tion and by T_4 in the magnesium-adequate group, but the T_4 and magnesium effects were not additive. Phosphorus concentration in urine of magnesium-fed rats was lowered by approximately one-half by T_4. Depriving the animals of a normal magnesium intake increased urine phosphorus. This increase over the control was prevented by administering T_4 but was not decreased to the same extent as in the magnesium-fed group.

Kidney calcium concentration in this experiment followed the same pattern as day 5 of experiment 1. Magnesium deficiency increased calcium levels while T_4 treatment inhibited this increase. Kidney citrate showed a different pattern when compared to the results of experiment 1. Thyroxine treatment prevented the increased kidney citrate exhibited by animals fed magnesium-deficient diets. Thyroxine did not cause a significant increase in kidney citrate of the magnesium-adequate animals as it did in experiment 1.

The main effect of magnesium deprivation was to increase serum calcium and decrease serum phosphorus. Thyroxine, on the other hand, had varied effects on both ions. A significant interaction between magnesium and T_4 was calculated showing that in magnesium-adequate animals T_4 lowered serum calcium, whereas, in the deficient group no change by T_4 was observed. Phosphorus was increased by T_4 at both levels of magnesium.

Discussion. The purpose of this study was to determine if there was an association between thyrotoxicosis and kidney calcification that could be correlated with the urine or tissue concentration of citrate. It was observed by Jacob and Forbes (6) that no correlation could be found between the calcification syndrome and urinary citrate excretion of magnesium-deficient rats treated with T_4. The present series of experiments likewise showed no correlation between these two parameters. The data in these experiments are somewhat at variance with those of Jacob and Forbes (6). Their research showed a significant increase in urine citrate when magnesium-deficient rats were injected with T_4. The maximum increase, however, was much lower than in the magnesium-adequate animals. This increase in citrate might still have been sufficient to prevent the accumulation of calcium phosphate in the renal tubules. In contrast, the present experiments showed no significant increase in urine citrate when magnesium-deficient rats were injected with T_4. Nonetheless, the effect T_4 had in reducing calcification of magnesium deficiency was not associated with its effect on citrate metabolism.

Experiments with dogs have shown that only a small amount of citrate in the urine is derived from blood citrate (12), and therefore, would not necessarily reflect the concentration of this metabolite in blood. Jacob and Forbes (6) suggested that urine citrate may depend largely on kidney tissue synthesis. This study showed that thyroxine effectively

increased citrate levels in blood and kidney of both magnesium-deficient and magnesium-fed rats during the administration of three daily doses of the hormone. However, by the fifth day T_4 no longer caused an increased citrate in blood and kidney of magnesium-deficient rats but continued to do so in magnesium-adequate animals. If urine citrate concentration is dependent on blood or kidney tissue levels then the 4-day pooled urine samples from T_4-treated animals in experiment 2 should have been elevated. The data in Table III indicate that this was not the case. T_4 administration to magnesium-deficient rats had no significant effect on the citrate concentration of pooled urine samples just as it had no effect on the concentration in a single day's collection of urine.

Blood and tissue citrate levels depend on its rate of synthesis and degradation in tissue. One might observe an increased concentration of citrate in kidney tissue if the isocitric dehydrogenase enzyme were inhibited in some way, or, if there were an increased rate of synthesis with no concomitant increase in degradation. One or both of these conditions were prevalent in rats fed either level of magnesium and injected with T_4. As previously mentioned magnesium-deficient rats treated with T_4 for 3 days had increased levels of blood and kidney citrate. At 5 days the level was reduced to that of the control while the concentration in magnesium-adequate T_4-treated rats continued to rise throughout the 5-day experiment. We are unable to explain the decrease in kidney and serum citrate found on day 5 in the magnesium-deficient T_4-treated animals. In magnesium-deficient rats not treated with T_4 there was a highly significant increase in kidney citrate but no similar elevation of blood citrate. This could have been caused by the extremely high levels of calcium in these kidneys resulting in inhibition of isocitric dehydrogenase and the accumulation of citrate in the tissue. It is known that calcium is a strong inhibitor of this enzyme in the kidney (13). Another possibility is that citrate was bound nonspecifically to the calcium phosphate deposits during their formation, and therefore was not free to initiate an increase in blood or urine concentrations.

The effects of magnesium deficiency on magnesium, calcium, and phosphorus concentrations in blood and urine are directionally similar to those noted many times by others (14, 15). Changes in these parameters are suggestive of an alteration in parathyroid hormone (PTH) output (3, 4). Indeed, it has been demonstrated by radioimmunoassay technique that plasma PTH concentration increases in sheep when their intact parathyroid glands are perfused with a medium containing low magnesium (16). However, the present study has shown that changes in some of these PTH-induced parameters can be reversed by administration of thyroxine. Daily administration of T_4 to magnesium-deficient rats reversed the effect of the deficiency on blood and urine phosphorus. Serum phosphorus was lower in magnesium-deficient rats than in the controls but increased significantly when T_4 was administered. Animals receiving magnesium-deficient diets excreted more phosphorus in the urine than their controls. However, if T_4 was given, excretion was reduced to concentrations equal to that of rats fed magnesium but given no T_4. This reduction in phosphorus excretion might have been enough to lessen the chances of exceeding the solubility constant of calcium phosphate, therefore, preventing the deposition of calcium phosphate crystals in kidney tissue. Gabbani *et al.* (17) have shown a reversal of the PTH effect on calcium and phosphorus concentrations in serum by T_4. They observed that pretreatment of rats with T_4 prior to an overdose of PTH prevented kidney calcification, inhibited hypercalcemia, and stimulated an increase in serum phosphorus. Our data are consistent with this finding.

Summary. Two experiments were conducted with young male albino rats to investigate the interrelationships between thyroxine, citrate metabolism, and renal calcification in magnesium deficiency. Magnesium deficiency decreased urinary citrate markedly, increased kidney calcification, had no effect on serum citrate, and raised kidney citrate. Thyroxine administration did not materially affect citrate excretion but did offset accumulation of calcium and citrate in the kidney

tissue of magnesium-deficient animals. It is concluded that urinary citrate does not play a role in prevention of kidney calcification.

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