Prevention of Phosphate-induced Nephrocalcinosis by Parathyroidectomy[1] (36241)

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In 1930, MacKay and Oliver (1) produced nephrocalcinosis in young rats by feeding diets that contained high levels of phosphate regardless of whether the phosphate salts were acidic or alkaline (2). Since that time, this particular form of nephrocalcinosis has been extensively studied in man and animals; but, as yet, the basic mechanism involved in the deposition of mineral in the kidney has not been elucidated. It has been suggested that the cause of the calcific deposits was supersaturation of the kidney fluid as a result of excessive excretion of phosphate (2). Paradoxically, the oral ingestion of large amounts of phosphate has been shown to prevent the recurrence of renal stones (3). Hypercalcemia has also been invoked as a causative agent (4). Others have postulated the precipitation of an organic matrix, which initiates precipitation as the primary event (5). The following studies were undertaken to study the effects of varying dietary ratios of calcium to phosphate on the production of nephrocalcinosis in intact and parathyroidectomized adult rats in an attempt to ascertain which of these ions was principally involved in the production of nephrocalcinosis, and also to determine whether parathyroid hormone was involved in this particular form of nephrocalcinosis.

Materials and Methods. Adult male Holtzman rats (weighing about 300 g) were used in these studies. The experimental groups were fed a semisynthetic diet and alterations in calcium or phosphate content were made as described previously (6). The parathyroidectomized rats had their parathyroid glands removed by surgery at least 1 week before the rats were fed the experimental diets. To prevent a large disparity in weight gains between intact and parathyroidectomized rats, during the period following surgery and prior to the feeding of the experimental diets, the intact rats had their food intake restricted such that their body weight changes were similar to those of the operated animals. During the period of feeding the experimental diets, the control rats were pair-fed to the parathyroidectomized animals to insure the same mineral intake by both groups. The experimental period lasted 17 days; at which time, the rats were sacrificed, blood was collected; and the kidneys were removed. Ash content, calcium, magnesium, and phosphate were determined on the kidneys. Calcium and magnesium were determined by atomic absorption spectrophotometry and phosphate by a modification of the Fiske-Subbarow procedure (7).

Results and Discussion. Table I shows the experimental design and the kidneys showing nephrocalcinosis. Group I was fed our normal control diet which has a Ca/P ratio of 2, and which produces excellent growth in young, and maintenance in adult rats. Group II was fed a diet with a Ca/P ratio of 1:8. The calcium content is adequate for growth and maintenance, while the phosphorus content is excessive. Group III was fed a diet with a Ca/P ratio of 8:1; the calcium content is excessive, while that of the phosphorus is adequate for growth and maintenance.

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TABLE I. Dietary Calcium:Phosphorus Ratios and Nephrocalcinosis.

Group	Dietary conditions		Nephrocalcinosis[a]			
			Gross		Microscopic	
	Ca:P ratio	% Ca / % P	Control	Parex	Control	Parex
I	2:1	0.8 / 0.4	0	0	0	0
II	1:8	0.4 / 3.2	++++	0	++++	0
III	8:1	3.2 / 0.4	0	0	0	0
IV	1:1	3.2 / 3.2	0	0	++	0

[a] ++++ = severe nephrocalcinosis; ++ = moderate nephrocalcinosis.

The diet of Group IV had excessive amounts of calcium and phosphorus and had a Ca/P ratio of about 1. Only the intact rats of Group II showed gross signs of nephrocalcinosis. Their kidneys were swollen, grey, and covered with patchy deposits of granular mineral as described previously (2). None of the kidneys of rats without parathyroid glands showed any signs of mineral deposits. Microscopic examination of kidney sections stained by the von Kossa method, revealed that the intact rats of Group IV also had nephrocalcinosis. Its distribution appeared different from Group I in that mineral deposits were also found in the distal portion of the nephron.

Table II shows the percentage ash of the kidneys of intact rats fed a diet with Ca/P ratio of 1:8 (Group II) and 1:1 (Group IV), but both containing high P, was significantly higher than that of intact rats fed the control diet (Group I), while that of kidneys of rats fed 3.2% Ca and 0.4% P (Group III) was lower. There were *no* significant differences in percentage ash between any of the kidneys of aparathyroid rats. The calcium, phosphate, and magnesium contents of the kidneys of intact rats in Groups II and IV also were significantly higher than those of intact rats fed the control diet (Group I) and this correlated well with the microscopic evidence of nephrocalcinosis. The data also show that the calcific deposits contain magnesium and phosphate. The mineral content, *i.e.*, Ca, Mg or PO_4, of the kidneys of parathyroidectomized rats was less than that of their pair-fed controls although not always statistically different. The results from intact rats show that when endogenous parathyroid secretion is suppressed, *i.e.*, by feeding a diet with a high dietary Ca/P ration (Group III), that excessive dietary calcium alone cannot produce nephrocalcinosis. However, excessive dietary phosphate, which produces nutritional secondary hyperparathyroidism in adult intact rats, does cause nephrocalcinosis (Group II, intact rats). Also, if dietary calcium and phosphate both are excessive, nephrocalcinosis does occur in intact rats (Group IV). Calcific deposits in the kidneys of this group are very likely in part the result of a slight hyperactivity of the parathyroid glands since the dietary Ca/P ratio was only about 1, and in part by overloading the kidney tubules by both elements simultaneously. These studies clearly indicate that in the absence of parathyroid secretion, diets high in calcium, phosphate, or in both elements will not cause nephrocalcinosis in adult rats. It has also been demonstrated that parathyroid hormone increases the tubular reabsorption of calcium (8, 9) and magnesium (10). If the concentrations of calcium and magnesium within the kidney cell are raised by this mecha-

TABLE II. Effect of Various Calcium and Phosphate Intakes on the Mineral Content of Rat Kidney.[a]

	Ash (%)		Calcium (mEq)		Magnesium (mEq)		Phosphate (mEq)	
Group	Control	Parex	Control	Parex	Control	Parex	Control	Parex
I	4.4	4.2	0.010	0.010	0.021	0.019	0.339	0.325
	(0.4)	(0.8)	(0.002)	(0.002)	(0.001)	(0.001)	(0.018)	(0.009)
II	5.2	3.9[c]	0.064	0.006[b]	0.030	0.016[b]	0.452	0.262[b]
	(0.3)	(0.7)	(0.020)	(0.002)	(0.002)	(0.001)	(0.038)	(0.008)
III	3.2	4.2	0.013	0.010[d]	0.023	0.019[c]	0.343	0.325
	(0.5)	(0.8)	(0.004)	(0.003)	(0.002)	(0.002)	(0.014)	(0.022)
IV	5.1	4.1[d]	0.029	0.007[b]	0.025	0.020[c]	0.398	0.309[c]
	(0.4)	(0.9)	(0.002)	(0.002)	(0.002)	(0.001)	(0.037)	(0.021)

[a] Values in parentheses refer to the standard deviation from the means which are directly above.

[b] Significantly different from pair-fed control: $p < .001$; [c] $p < .01$; [d] $p < .05$.

nism, the presence of excessive amounts of phosphate very likely would result in the precipitation of mineral in the kidney.

Summary. Intact rats, fed diets high in phosphate, develop nephrocalcinosis; but parathyroidectomized rats do not. Neither intact nor parathyroidectomized rats, fed diets high in calcium, have mineral deposits in their kidney. Intact rats, but not parathyroidectomized rats, fed diets high in calcium and phosphate, develop kidney calcification. The mineral deposits consist of at least calcium, magnesium, and phosphate.

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