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Amelioration of Nutritional Secondary Hyperparathyroidism in Rats by Adrenalectomy (40210)

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Although parathyroid hormone is the principal regulator of calcium homeostasis, adrenal cortical hormones also influence the metabolism of this ion. As far back as 1911, it was observed that removal of the adrenal glands ameliorates tetany in parathyroidectomized rats (1). This suggested that the adrenal glands secreted a calcium lowering hormone and more recent studies have confirmed that glucocorticoids may antagonize many actions of exogenous parathyroid hormone (2).

Recently, we have shown that adrenalectomy prevented the fall in serum calcium and rise in serum phosphate that occurs in secondary hyperparathyroidism induced by a high phosphate diet (3). The present study was initiated to determine whether skeletal and renal changes of secondary hyperparathyroidism also could be inhibited by adrenalectomy. The results show that adrenalectomy prevented to a large extent the renal lesions and completely prevented the skeletal lesions.

Materials and methods. Male adult Holtzman rats weighing 270–300 g were used in all experiments. Unless otherwise noted, all rats were fed a complete semisynthetic diet which contained 0.8% calcium, 0.4% phosphorus and 0.25% magnesium as previously described (4). Bilateral adrenalectomy was performed under light ether anesthesia at least seven days before the balance study. The sham-operated rats had similar incisions and their kidneys and adrenal glands were handled briefly. Adrenalectomized and sham-operated control rats were given distilled water containing 1% NaCl while kept in metabolism cages designed to separate urine and feces. Steroids were administered subcutaneously after dissolution in 30% ethanol (Table III). Control animals received the same amount of 30% ethanol. Preparation of kidneys for histologic and chemical evaluation was carried out as previously described (5). Histologic grading of nephrocalcinosis was done by a double-blind design and based on counts of the number of calcified deposits seen on 10 microfrontal sections of kidney (1–10 deposits = +, 11–30 deposits = ++, 31–60 = +++, etc.). Femora were fixed in neutral-buffered formalin, demineralized using citrate and embedded in paraffin. Thin sections were stained with hematoxylin-eosin. The lesions consisted of innumerable resorption cavities containing many osteoclasts. Severity of *osteitis fibrosa cystica* was based on numbers of such lesions per unit area with 5+ being most severe. + Indicates a slight increase in number of resorption cavities. Calcium and magnesium were determined by atomic absorption spectrophotometry and phosphate by a modification of the Fiske-Subbarow procedure (6). Analyses for serum calcium, magnesium and phosphate were made on trichloroacetic acid filtrates. Urinary analyses were made directly on diluted samples. Stool and tissue analyses were made after ashing the specimens in a muffle furnace at 650° for 18 hr, dissolving the residue in dilute HCl and diluting to suitable volumes. Student's *t* test or analysis of variance (7) was performed on the data.

Results. Effects of a high phosphate diet in adrenalectomized rats. In Fig. 1 it may be seen that adrenalectomy prevented the fall in serum calcium and rise in serum phosphate that high phosphate diets (Ca/P = 1/8) induce in intact rats (5, 8). These results confirm our earlier findings (3). Also, the severe *osteitis fibrosa cystica* found in intact rats fed high phosphate diet (8) was completely prevented by adrenalectomy, although occasional resorption cavities were present (Table I). The kidney lesions of the adrenalectomized rats were considerably reduced when evaluated histologically. When expressed as percent of dry weight, calcium, magnesium and phosphate contents of the kidneys were significantly less than those of the intact rats.

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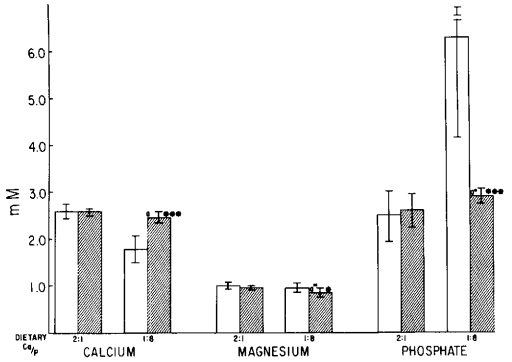

FIG. 1. Effect of high phosphate diet on serum Ca, Mg and PO_4. Six rats in each group were fed the high phosphate diet for approximately 60 days. The height of each bar represents the serum value at the end of the experiment. The clear bars refer to the intact rats and the cross-hatched bars refer to the adrenalectomized animals. The brackets indicate the standard deviations from the mean. ***Significantly different from intact rats fed the same diet, $P < 0.001$, *means $P < 0.05$. $^{a'''}$Significantly different from adrenalectomized rats fed diet with Ca/P = 2, $P < 0.001$, $^{a''}P < 0.01$, $^{a}P < 0.05$.

Thus, the chemical analysis paralleled the histologic evaluation of calcification. Of interest is the observation that the dry weight of the kidneys of the adrenalectomized rats fed either the control diet or high phosphate diet were significantly greater than those of intact rats fed the same diets. The significance of this finding is not known.

Effect of prednisolone and aldosterone on absorption and excretion of calcium, magnesium and phosphate by adrenalectomized rats fed a normal diet.

In Table II it may be seen that adrenalectomy *per se* had no significant effect on the serum level of calcium but the administration of prednisolone to adrenalectomized rats lowered it significantly ($P < 0.01$) when compared to the intact rats. Neither adrenalectomy nor the administration of prednisolone or aldosterone altered net absorption or excretion of calcium, phosphate or magnesium in a 12-day balance study. In these studies, net absorption is defined as intake minus fecal output.

Effect of prednisolone on absorption and excretion of calcium, magnesium and phosphate by intact rats fed a normal diet.

In a 12-day balance study in which intact animals were allowed to eat *ad libitum*, pred-

TABLE I.[a]

	Bone lesions		Kidney lesions		Kidney Values: Dry weight (mg)		H_2O		Calcium (% dry wt)		Phosphate (% dry wt)		Magnesium (% dry wt)	
Dietary Ca/P	2:1	1:8	2:1	1:8	2:1	1:8	2:1	1:8	2:1	1:8	2:1	1:8	2:1	1:8
Intact [6]	0	+++++	0	+++++	279	250[a]	78.2	83.2[a''']	0.083	1.94[a''']	3.46	7.26[a''']	0.094	0.200[a''']
					(27)	(11)	(1.6)	(1.2)	(0.023)	(0.35)	(0.21)	(0.35)	(0.013)	(0.013)
Adrex [6]	0	+	0	++	346**	355**	77.5	80.5[a'']**	0.128	1.09[a'']**	3.47	5.43[a'']***	0.086	0.140[a'']***
					(41)	(62)	(1.2)	(1.2)	(0.076)	(0.35)	(0.20)	(0.56)	(0.005)	(0.013)

[a] Kidney and Skeletal Data: The high phosphate diet (Ca/P = 1/8) contained 0.4% Ca, 3.2% P and 0.25% Mg, and the control diet (Ca/P – 2/1) contained 0.8% Ca, 0.4% P and 0.25% Mg. The rats were fed their diets for 2 months. *** Significantly different from intact rats fed same diet, $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. $^{a'''}$ significantly different from similar rats fed diet with Ca/P = 2/1, $P < 0.001$, $^{a''}$ $P < 0.01$, $^{a}P < 0.05$. The numbers in brackets are the number of rats in each group. The numbers in parentheses are the standard deviations from the means which are directly above. Grading of kidney and bone lesions is described under Materials and Methods.

TABLE II.[a]

Treatment	Serum (m*M*)	Intake (mmol)	Absorbed (mmol)	Urinary (mmol)
		Calcium values		
Control [7]	2.71 (0.09)	30.9 (0.9)	11.9 (2.6)	0.65 (0.16)
Adrex [7]	2.57 (0.20)	31.9 (1.1)	12.4 (1.9)	0.59 (0.11)
Adrex + prednisolone [6]	2.51** (0.07)	32.1 (1.1)	13.2 (0.7)	0.45 (0.12)
Adrex + aldosterone [6]	2.59 (0.20)	31.8 (4.1)	13.1 (2.5)	0.59 (0.24)
		Phosphate values		
Control	3.06 (0.18)	23.6 (0.7)	10.5 (1.5)	4.23 (1.13)
Adrex	2.76 (0.30)	24.4 (0.08)	10.1 (1.9)	4.0 (0.4)
Adrex + prednisolone	3.08 (0.39)	24.5 (0.8)	9.6 (0.8)	3.9 (0.7)
Adrex + aldosterone	2.77 (0.28)	24.3 (3.1)	8.7 (1.8)	4.23 (1.0)
		Magnesium values		
Control	1.17 (0.07)	18.8 (0.6)	8.9 (1.2)	2.9 (0.8)
Adrex	1.12 (0.03)	19.4 (0.6)	8.9 (1.0)	2.7 (0.4)
Adrex + prednisolone	1.11 (0.06)	19.4 (0.7)	9.1 (0.8)	2.5 (0.3)
Adrex + aldosterone	1.14 (0.09)	19.2 (2.5)	9.1 (1.3)	3.0 (0.5)

[a] Effect of prednisolone and aldosterone on absorption, excretion and serum values of calcium, phosphate and magnesium by adrenalectomized rats. Five days after adrenalectomy, (Adrex) rats were put in individual cages and habituated to the diet for 6 days prior to the 12-day balance study. Prednisolone was administered subcutaneously daily at a level of 0.5 mg/100 g body wt and aldosterone at a level of 2.5 μg/animal. The numbers in parentheses are the SD from the means which are directly above. The numbers in brackets are the number of rats used in each group. ** Significantly different from sham $P < 0.01$.

nisolone decreased food intake and caused a significant weight loss (Table III). Despite decreased intake of calcium and phosphate by steroid treated rats, no significant changes were found in their serum levels or excretion. The only significant change was a fall in the serum level of magnesium following prednisolone treatment.

Discussion. It is clear that the absence of all adrenal hormones had a beneficial effect on the skeletal and renal lesions of secondary hyperparathyroidism induced by the feeding of diet with high phosphate. The amelioration of the lesions following adrenalectomy implies that the adrenal glands secrete a hormone(s) which is involved in the pathogenesis of the renal and skeletal lesions. Although the mechanism involved in the amelioration of the lesions by adrenalectomy is not known, two possible explanations may be: (a) De-

creased secretion of parathyroid hormone and, (b) blunted responses of the target organs. There are studies which support either possibility. Glucocorticoids have been shown to increase PTH secretion *in vitro* (9) and *in vivo* (10). In their absence, it is quite conceivable that insufficient hormone may be elaborated to cause the renal and skeletal lesions. The higher level of calcium in the serum of the adrenalectomized rats fed high phosphate diet in the present study (Fig. 1) further supports the idea of decreased parathyroid hormone secretion in the absence of the adrenal glands. Parathyroid hormone would appear to be of greater importance in causing renal deposits than the elevated level of serum phosphate since nephrocalcinosis does not occur in parathyroidectomized rats which have markedly elevated levels of serum phosphate (5, 8). However, definitive proof must await the measurement of circulating immunoreactive PTH. It also has been shown that adrenalectomy diminished the hypercalcemic response of parathyroid hormone (11) suggesting a permissive action of the adrenal hormones with respect to PTH activity.

It is doubtful that increased calcium absorption or decreased calcium excretion by adrenalectomized rats fed the high phosphate diet is responsible for the higher levels of calcium in the serum, since balance studies indicated no significant differences between intact and adrenalectomized rats fed a normal diet. However, the possibility that adrenalectomized rats may absorb more calcium from high phosphate diets than intact rats cannot be ruled out. Also, the administration of prednisolone, in amounts sufficient to affect body weight, or aldosterone, in amounts that alter sodium and potassium excretion, had no significant effect on the absorption or excretion of these ions at least during the 12-day balance period. With respect to calcium metabolism, similar findings as in these studies have been found in adrenalectomized dogs (12), for an Addisonian patient (13) and for the adrenalectomized rats (14).

The lack of effect of glucocorticoids on calcium absorption *in vivo* is in contrast to studies with the everted rat gut where they inhibit active transport of calcium (15–17). In studies on the effect of magnesium ions on calcium absorption, striking differences between net calcium absorption measured *in vivo* (18, 19) and calcium transport measured *in vitro* have also been noted (20). Unless studies *in vivo* confirm results obtained *in vitro,* active transport should not be equated with absorption by the whole animal.

It has been suggested that adrenal corticoids may alter the metabolism of vitamin D (21, 22) although there is not complete accord on this point (23–25). Even if steroid treatment altered vitamin D metabolism in this study, the alterations had no effect on overall calcium absorption. This is not surprising since the portion of the intestine distal to the duodenum contains over 90% of the surface

TABLE III.[a]

Treatment	Initial weight (g)	Weight change (g)	Food intake (g)
Control [6]	270 (4)	+88 (2)	222 (5)
Prednisolone	270 (5)	−43 (11)	163** (16)

	Intake (mmol)	Absorbed (mmol)	Urine (mmol)	Serum (mM)
	Calcium values			
Control	44.2 (1.0)	15.0 (4.0)	0.22 (0.03)	2.65 (0.03)
Prednisolone	32.6** (3.3)	12.5 (1.8)	0.33 (0.13)	2.63 (0.06)
	Phosphate values			
Control	28.8 (0.6)	10.6 (2.1)	3.4 (1.0)	3.15 (0.27)
Prednisolone	21.2** (2.1)	5.5** (0.5)	3.7 (1.0)	2.73 (0.31)
	Magnesium values			
Control	20.1 (0.5)	4.0 (1.5)	0.85 (0.7)	1.06 (0.05)
Prednisolone	14.8** (1.5)	4.5 (1.7)	0.38 (0.25)	0.91** (0.05)

[a] Effect of prednisolone on absorption and excretion of calcium, magnesium and phosphate by intact rats: The rats were habituated to the diets and kept in individual metabolism cages for 6 days prior to the balance study which lasted 12 days. The steroid treated rats received 0.75 mg/100 g body wt subcutaneously in 30% ethanol daily. The numbers in parentheses are the standard deviations from the means which are directly above. The numbers in brackets are the number of rats used in each group. ** Different from respective controls at $P < 0.01$.

area of the small intestine and is intimately involved in calcium absorption via passive diffusion while the area involved in active transport is much smaller. In man, the distal portion of the intestine also seems to be more important quantitatively for calcium absorption than the area involved in active transport (26).

Summary. Removal of the adrenal glands prevented *osteitis fibrosa cystica* that occurs when intact rats are fed diets containing high phosphate. It also decreased the incidence of calcific deposits in the kidney. It is suggested that decreased secretion of PTH may be one of the causative factors in the beneficial effects of adrenalectomy. Balance studies showed that intestinal absorption and renal excretion of calcium and phosphate by rats fed a normal diet were unaffected by adrenalectomy or the administration of prednisolone or aldosterone.

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