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Mechanisms of Glucocorticoid-Induced Osteopenia: Role of Parathyroid Glands[1] (39396)

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Excessive glucocorticoids can cause osteopenia in animals (1) and man (2). However, the mechanisms by which they produce this effect are not entirely clear. *In vitro* studies have shown that glucocorticoids inhibit vitamin A or parathyroid hormone (PTH)-induced bone resorption (3). *In vivo* studies have shown that glucocorticoids inhibit bone formation (4, 5). However, the bone resorption studies *in vivo* have given conflicting results (5, 6). In studies which showed increased bone resorption due to glucocorticoids, excessive PTH has been postulated as one of the mechanisms of this increase. Excessive PTH secretion has been shown to occur in rats (7) and in man (8) after acute or chronic cortisol administration.

Recent studies have indicated that the glucocorticoids' effect on bone resorption may be dose-dependent, low doses causing increased resorption and high doses causing decreased or normal resorption (9, 10). The increased bone resorption was again postulated to be the result of excessive PTH secretion.

The present studies were conducted to evaluate the role of parathyroid glands in the production of glucocorticoid-induced osteopenia.

Materials and Methods. Sprague-Dawley rats initially weighing 260-290 g were maintained on a diet containing 1.7% calcium and 1.5% phosphorus. All rats were injected with 45 μCi of ^{45}Ca ip in three divided doses at 3-day intervals. Ten days after the last dose of ^{45}Ca, half the rats were parathyroidectomized (PTX) and the other half were left intact. Parathyroidectomy was confirmed by serum calcium levels of less than 7.0 mg/100 ml and undetectable serum PTH levels.

The animals were divided into four groups 1 week after the parathyroidectomy, at which time injections were begun and continued daily for 17 weeks. Group I: Intact rats were given normal saline sc daily to serve as control (n = 6). Group II: Intact rats were given cortisol (Cortef, Upjohn Company, Kalamazoo, Mich.), 5 mg/kg/day sc (n = 6). Group III: PTX rats were given normal saline sc daily (n = 5). Group IV: PTX rats were given cortisol, 5 mg/kg/day sc (n = 4).

The rats were kept in individual metabolic cages. The animals were bled by orbital sinus puncture at 2 weeks, 12 weeks, and at the end of the study at 17 weeks. The serum was separated and analyzed immediately for ^{45}Ca activity and frozen for subsequent determinations of calcium, phosphorus, and PTH.

Two weeks after beginning the injections, the feces were collected for two successive 72-h periods and then ashed. The ash was dissolved in hydrochloric acid for analysis of ^{45}Ca activity.

At the end of 17 weeks the rats were killed by ether anesthesia and the right femur was removed. The bone was quickly freed of soft tissue and weighed. The length was determined by verneir calipers. The bones were then bisected with a fine saw and freed of the marrow tissue with a jet of water. The bones were dried in crucibles overnight in an oven at 100°, and the dry weight obtained. The bones were then ashed in a muffle furnace at 800° overnight and the ash weight was obtained. The bone ash was dissolved in hydrochloric acid for deter-

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mination of calcium content and ^{45}Ca activity.

The calcium and phosphorus determinations were made by the autoanalyzer method of Kessler and Wolfman (11). The ^{45}Ca-activity determinations were made by dissolving the samples in Bray's scintillation solution (12) and counting them in a Packard liquid scintillation counter. Sufficient counts were accumulated to reduce the counting error to 2% or less in all samples. Serum PTH determinations were made by the radioimmunoassay method of Hargis *et al.* (13). The data were analyzed by Student's *t* test. The statement of significant difference denotes a *P* value of less than 0.05.

Results. There were no group differences in the initial body weights of the rats. Saline-injected PTX and intact rats progressively gained weight and had similar final weights (Fig. 1). The cortisol-treated intact and PTX rats lost minimal weight initially and then remained stable between 90 to 95% of the initial body weight (Fig. 1).

Figure 2 shows the serum PTH values of the intact rats. The cortisol-injected group had significantly greater serum PTH levels at all three time periods studied.

Serum calcium and phosphorus determinations made at the time of bleeding at 17 weeks are shown in Table I. There was no significant difference between the serum calcium of the saline-treated and cortisol-treated intact animals. Cortisol tended to decrease the serum calcium in the PTX rats although the decrease did not reach statistical significance. As expected, the serum phosphorus was significantly greater in the PTX animals than in the intact animals.

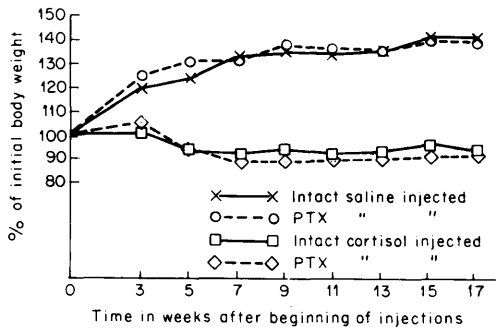

FIG. 1. Effect of cortisol injections or of normal saline injections on the body weight of intact and PTX rats. The results are expressed as a percentage of the initial body weight. Each point represents the mean weight for the group at each time shown on the abscissa.

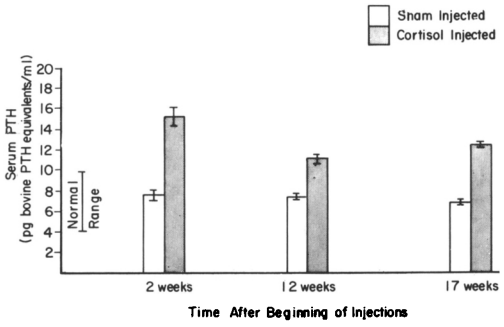

FIG. 2. Effect of cortisol injections or of normal saline injections on the serum PTH concentration in intact rats at 2, 12, and 17 weeks after the beginning of the injections. Each bar represents the mean ± SE for the group.

The results of serum ^{45}Ca expressed as total counts per minute per milliliter of serum are shown in Fig. 3. Cortisol treatment significantly reduced the serum ^{45}Ca activity in both the intact and the PTX rats except at 17 weeks in the intact group.

The fecal ^{45}Ca activity expressed as counts per minute per 24 hours is shown in Fig. 4. The PTX saline-injected rats had significantly less fecal ^{45}Ca as compared to intact saline-injected rats. Cortisol treatment significantly decreased the radioactivity in the feces of both intact and PTX rats.

The data pertaining to bone are portrayed in Table II. The PTX saline-injected rats had higher ash weight and percentage ash content (ash weight × 100)/(dry weight) as compared to intact saline-treated rats. Cortisol treatment reduced the percentage ash content in both intact and PTX rats (Table II). The ash weight of bone when expressed as per centimeter length of bone (ash weight)/(length in cm) was significantly greater in PTX saline-injected rats as compared to intact saline-injected rats and was reduced significantly by cortisol treatment in both intact and PTX rats.

The total ^{45}Ca activity in the bone was significantly greater in PTX saline-injected rats as compared to intact saline-injected rats. An effect of cortisol treatment on total bone ^{45}Ca could not be detected in either the intact or PTX groups despite the decrease in the serum and fecal ^{45}Ca (presumably because the great amount of radioactiv-

TABLE I. Effect of Cortisol Injections or of Normal Saline Injections on Serum Calcium and Phosphorus at 17 Weeks after Beginning of Injections in Intact and PTX Rats.[a]

Group	Serum calcium (mg/100 ml)	Serum phosphorus (mg/100 ml)
I. Intact saline injected	9.66 ± 0.05	4.9 ± 0.31
II. Intact cortisol injected	9.67 ± 0.14	4.71 ± 0.31
III. PTX saline injected	7.10 ± 0.20	8.76 ± 0.52
IV. PTX cortisol injected	6.67 ± 0.20	9.96 ± 0.84

[a] All values are expressed as the mean ± SE.

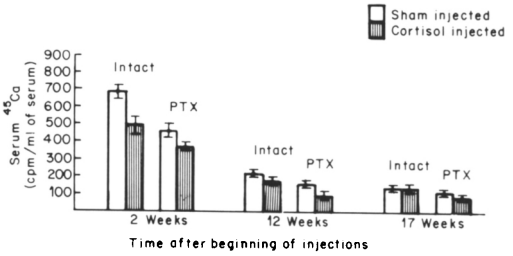

Fig. 3. Effect of cortisol injections or of normal saline injections on serum ^{45}Ca in intact and PTX rats at 2, 12, and 17 weeks after the beginning of the injections. Each bar represents the mean ± SE for the group.

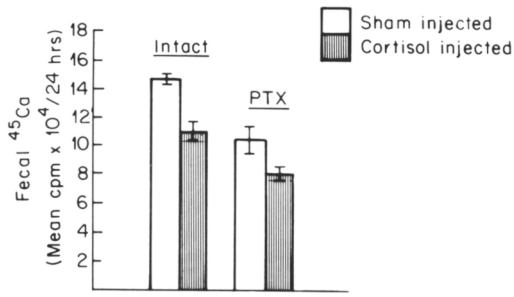

Fig. 4. Effect of cortisol injections or of normal saline injections on the fecal ^{45}Ca in intact and PTX rats at 2 weeks after the beginning of the injections. Each bar represents the mean ± SE.

ity in bone precluded detecting smaller changes secondary to decrease in fecal ^{45}Ca). Bone specific activity (^{45}Ca cpm/mg of Ca) was significantly increased by cortisol treatment in PTX rats. The intact rats showed a similar trend, although the results were not statistically significant.

Discussion. The observation of increased serum PTH induced by cortisol in the present study is in agreement with the previous studies in rats (7) and in man (8). Glucocorticoids decrease the intestinal absorption (14) and increase the renal excretion of calcium (15), and therefore can secondarily increase PTH secretion. The other mechanism by which serum PTH could be increased by cortisol is by inhibition of bone resorption with a tendency toward hypocalcemia causing compensatory increase in PTH. *In vitro* studies have shown that cortisol inhibits vitamin A or PTH-induced bone resorption (3). In this study a decrease in serum and fecal ^{45}Ca and an increase in bone ^{45}Ca specific activity were interpreted as evidence of decreased bone resorption *in vivo*. By these criteria, bone resorption was inhibited by cortisol treatment in the PTX rats. In the intact animals injected with cortisol, decreased bone resorption was suggested by decreased serum and fecal ^{45}Ca activity at 2 weeks and decreased serum ^{45}Ca activity at 12 weeks. However, the serum ^{45}Ca activity was not decreased by cortisol at 17 weeks, and the bone ^{45}Ca specific activity was not significantly increased in these intact animals. These observations suggest that the effect of cortisol to decrease bone resorption had been overcome by the compensatory increase in PTH secretion.

In the present study the bone mass was determined by dry and ash weights of the bone. It was significantly increased in the PTX saline-injected rats as compared to intact saline-injected rats. This was probably on the basis of decreased bone resorption in the former group, in the absence of PTH. Cortisol-treated animals were significantly smaller in size as compared to their respective saline-injected controls, and therefore the ash and dry weights of the bones cannot be directly compared. To obviate this, the dry and ash weights were compared on the basis of per centimeter length of the bone. Dry and ash weights expressed in this manner were significantly reduced by cortisol treatment in both the intact and the PTX rats as compared to their respective controls. Percentage content has been shown to decrease in osteoporosis (16). In the present study cortisol treatment significantly re-

TABLE II. EFFECT OF CORTISOL INJECTIONS OR OF NORMAL SALINE INJECTIONS ON VARIOUS PARAMETERS OF THE RIGHT FEMUR IN INTACT AND PTX RATS.[a]

Group	Dry weight (mg)	Ash weight (mg)	Ash (%) [(Ash weight/ Dry weight) × 100]	Dry wt in mg/Length in cm	Ash wt in mg/Length in cm	Total calcium (mg)	Total ^{45}Ca (cpm × 10^3)	Specific activity (Total ^{45}Ca cpm/Calcium in mg)
I. Intact saline injected	658.8 ± 10.7	436.7 ± 6.1	66.29 ± 0.32	169.8 ± 2.1	113.2 ± 1.2	162.2 ± 2.3	1017.5 ± 12.4	6280 ± 127
II. Intact cortisol injected	579.8 ± 10.5*	371.3 ± 8.9*	64.02 ± 0.53*	159.7 ± 2.0*	102.3 ± 1.9*	137.8 ± 3.8*	940.4 ± 61.4	6820 ± 411
III. PTX saline injected	712.7 ± 14.3**	495.7 ± 10.6**	69.54 ± 0.20**	183.5 ± 3.0**	127.6 ± 2.3**	181.6 ± 6.8**	1105.9 ± 29.7**	6131 ± 326
IV. PTX cortisol injected	597.8 ±'8.3***	391.3 ± 8.8***	65.43 ± 0.56***	167.5 ± 2.3***	109.7 ± 2.4***	145.5 ± 2.6***	1091.1 ± 64.7	7480 ± 321***

[a] All values are expressed as the mean ± SE.

* Denotes that Group II values are significantly different ($P < 0.05$) from Group I.

** Denotes that Group III values are significantly different ($P < 0.05$) from Group I.

*** Denotes that Group IV values are significantly different ($P < 0.05$) from Group III.

duced the percentage ash content in both intact and PTX rats.

As indicated above, cortisol injections reduced the bone resorption in PTX rats and caused a tendency toward decreased resorption in the intact rats. In spite of this, the bone mass was reduced by cortisol injections in both these groups. These studies would therefore indicate that in rats (i) cortisol, 5 mg/kg/day, induces osteopenia by inhibition of bone formation, and (ii) the presence of parathyroid glands is not essential for production of cortisol-induced osteopenia.

The observations in the present study are compatible with the hypothesis by Jee (9) and Rasmussen (10) that high doses of cortisol, i.e, 5 mg/kg/day, or greater, induce osteopenia predominantly by inhibition of bone formation.

Summary. Intact and PTX rats previously injected with ^{45}Ca received cortisol, 5 mg/kg/day, for 17 weeks. Bone resorption as determined by serum and fecal ^{45}Ca and bone ^{45}Ca specific activity were reduced by cortisol in the PTX rats and showed a similar tendency in intact rats. In spite of this, the bone mass, as determined by ash content, was reduced by cortisol in both the intact and PTX animals. The data show that (i) cortisol, 5 mg/kg/day, produces osteopenia by inhibition of bone formation, and (ii) the presence of parathyroid glands is not essential for the production of this osteopenia.

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