Production, Degradation, and Circulating Levels of 1,25-Dihydroxyvitamin D in Health and in Chronic Glucocorticoid Excess

E. SEEMAN, R. KUMAR, G. G. HUNDER, M. SCOTT, H. HEATH III, and B. L. RIGGS,
Endocrine Research Unit, Department of Medicine, Department of Rheumatology, Cancer Centre Statistics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

ABSTRACT The decreased intestinal absorption of calcium and accelerated bone loss associated with chronic glucocorticoid excess may be mediated by changes in vitamin D metabolism, leading to decreased availability of circulating 1,25-dihydroxyvitamin D. This hypothesis was examined in 14 patients with either endogenous or exogenous glucocorticoid excess. Analysis of paired serum samples (mean±SE) in 13 patients during euglucocorticoidism and during hyperglucocorticoidism showed that glucocorticoid excess resulted in small decreases of plasma 25-hydroxyvitamin D concentrations (22±2–18±2 ng/ml; $P < 0.05$) but no significant changes in plasma 1,25-dihydroxyvitamin D (32±8–23±6 pg/ml) or serum immunoreactive parathyroid hormone (21±2–18±2 μleq/ml). Additionally, we studied plasma kinetics of [^{3}H]1,25-dihydroxyvitamin D_3 after intravenous bolus administration in 10 hyperglucocorticoid patients and in 14 normal controls. Assessment with a three-compartment model showed no significant abnormalities in production rates (hyperglucocorticoid patients 1.2±0.3 μg/d, controls 1.5±0.2 μg/d) or metabolic clearance rates (hyperglucocorticoid patients, 32±3 ml/min; controls, 31±3 ml/min). Moreover, there were no significant differences in cumulative excretion of radioactivity in urine (hyperglucocorticoid patients, 18±2%; controls, 14±2%) or feces (hyperglucocorticoid patients, 60±9%, controls, 54±6%). We conclude that glucocorticoid excess does not effect plasma levels, production, or degradation of $1,25(OH)_2D$ in humans. Thus, other mechanisms must be postulated to explain satisfactorily the abnormalities of bone structure and intestinal calcium absorption that may occur after chronic glucocorticoid therapy.

Received for publication 10 February 1980 and in revised form 4 June 1980.

INTRODUCTION

Decreased intestinal absorption of calcium is found consistently in patients with chronic glucocorticoid excess (1–6) and may contribute to bone loss. Because the vitamin D-endocrine system is the major regulator of intestinal calcium absorption, it has been postulated that glucocorticoids may adversely affect vitamin D metabolism (7, 8).[1] Studies attempting to demonstrate an effect of glucocorticoids on vitamin D metabolism, however, have yielded conflicting results. Investigators have variously reported that chronic glucocorticoid excess increases the metabolic turnover of vitamin D (9), decreases plasma 25-hydroxyvitamin D (25-OH-D) (6, 10, 11), decreases plasma 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (12), or increases metabolic degradation of $1,25(OH)_2D$ at tissue sites (13). Moreover, serum 25-OH-D has been reported to be normal (14, 15), and serum $1,25(OH)_2D$ has even been reported to be increased by glucocorticoid excess in certain experimental models (4, 16). Others (16–20) have postulated a direct effect of supraphysiologic doses of glucocorticoids on gut that is independent of vitamin D metabolism.

The present study was designed to determine whether chronic glucocorticoid excess affects plasma levels, production, and degradation of $1,25(OH)_2D$ in human beings. To control for the possibility that variability between subjects might obscure small changes in plasma vitamin D metabolites within the normal range, we used individual patients as their own controls by measuring paired samples in euglucocorticoid and hyperglucocorticoid states. Also, possible

[1] *Abbreviations used in this paper*: i, immunoreactive; PTH, parathyroid hormone; 25-OH-D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D. The term "vitamin D" refers to both vitamin D_2 and vitamin D_3.

J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/80/10/0664/06* $1.00
Volume 66 October 1980 664–669

subtle changes in production or degradation of $1,25(OH)_2D$ were studied by making kinetic measurements after intravenous administration of a dose of high-specific activity $[^3H]1,25(OH)_2D_3$.

METHODS

Protocol. We studied 14 patients with chronic glucocorticoid excess. The nature of the study was explained to the patients, and written consent was obtained. Six patients had endogenous Cushing's syndrome; eight were receiving supraphysiologic doses of glucocorticoids for connective tissue disorders. All subjects had normal renal function, had no other recognizable disorders, and were receiving no drug known to be associated with altered calcium metabolism. All of the patients with endogenous Cushing's syndrome were women; their mean age was 42 yr (range, 32–59 yr). Urinary excretion of 17-ketogenic steroids for this latter group was 22.7±2.8 (mean ± SD) mg/d (range, 19–26 mg/d; normal range, 2–12 mg/d). The estimated duration of spontaneous Cushing's syndrome was 3 yr (range, 0.5–7 yr). All patients were surgically treated: five by transphenoidal microadenomectomy of the pituitary, the sixth by removal of an adrenal adenoma. Of the patients receiving exogenous glucocorticoids, six were women (mean age, 62 yr; range, 48–73 yr) and two were men (ages 32 and 70 yr). The underlying connective tissue disorders were temporal arteritis (five patients), polymyalgia rheumatica (two patients), and systemic lupus erythematosus (one patient). All had received prednisone (mean dose, 50 mg/d; range, 30–60 mg/d) for 1 mo at the time of the second study.

We obtained blood samples in both the hyperglucocorticoid and euglucocorticoid states in 13 patients. For patients with endogenous Cushing's syndrome, the euglucocorticoid state was assumed to exist by 2–3 mo after surgical correction of Cushing's syndrome. This was ascertained by measurements of plasma and urinary corticosteroids. For patients receiving exogenous glucocorticoids, the euglucocorticoid state was the period immediately before institution of glucocorticoid therapy. The blood samples were analyzed for serum or plasma 25-OH-D, $1,25(OH)_2D$, immunoreactive parathyroid hormone (iPTH), calcium, phosphate, creatinine, alkaline phosphatase, and glutamic-oxaloacetictransaminase.

Four of the six patients with endogenous Cushing's syndrome and six of the eight patients receiving prednisone also had $[^3H]1,25(OH)_2D_3$ kinetic studies done during the hyperglucocorticoid state. Results were compared with those obtained in 14 healthy control subjects (13 women, mean age age 56 yr, range 33–77 yr; and 1 man, age 33 yr). We performed all studies in a metabolic ward from March through August 1979. During the study, the patients were maintained on a diet calculated by a dietitian to simulate the habitual diet with respect to calcium, phosphorus, calories, and vitamin D. For the kinetic studies, all patients received 1–2 μCi of $[^3H\text{-}23,24]1,25(OH)_2D_3$, (110 Ci/mmol) or $[^3H\text{-}26,27]1,25(OH)_2D_3$ (160 Ci/mmol) in 2 ml propylene glycol by intravenous bolus injection. Blood samples were withdrawn from an indwelling catheter in the opposite arm at 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 720, and 1,440 min. After $[^3H]1,25(OH)_2D_3$ administration, stool and urine were collected for 6 d.

Laboratory methods. We measured plasma 25-OH-D by the radioligand method of Haddad and Chyu (21) as modified by Horst et al. (22), plasma $1,25(OH)_2D$ by a modification (23) of the method of Eisman et al. (24), and serum iPTH by a radioimmunoassay system as described by Arnaud et al. (25). The PTH antiserum employed, GP1M, reacts primarily with determinants in the 44–68 region of the PTH molecule (26) and is particularly sensitive for detection of increased parathyroid gland function (27). All analyses were carried out in single assays to avoid interassay variation. The intraassay coefficient of variation for the $1,25(OH)_2D$ was 4%. Plasma calcium was determined by atomic absorption spectrophotometry (normal range, 8.9–10.1 mg/dl). Other biochemical measurements were made by standard auto analyzer techniques (Technicon Instruments Corp., Tarrytown, N. Y.).

$[^3H\text{-}23,24]1,25(OH)_2D_3$ was prepared from $[^3H\text{-}23,24]\text{-}25(OH)D_3$, (110 Ci/mmol) (Amersham Corp., Arlington Heights, Ill.), with use of rachitic chick mitochondria (28). $[^3H\text{-}26,27]1,25(OH)_2D_3$ (160 Ci/mmol) was purchased from New England Nuclear (Boston, Mass.). We confirmed radiochemical purity of the product by co-chromatography with standard $1,25(OH)_2D_3$ using a high-performance liquid chromatographic technique (29). Radioactivity in 2-ml plasma samples was determined by direct scintillation counting. Aliquots of urine (4 ml) and stool (2 g) were combusted in a Packard 306A tissue oxidizer (Packard Instrument Company, Inc., Downer's Grove, Ill.). Polyethylene glycol stool marker (500 mg) was administered orally 3 times/d to permit estimation of the recovery of radioactivity in feces (30).

We pooled aliquots of plasma from the first 45 min after injection and from 45–240 min to determine the nature of the vitamin D metabolite(s) in the plasma. The pooled samples were extracted as previously reported (31). The chloroform phase was evaporated to dryness on a rotary evaporator. The residue was redissolved in 1 ml of chromatography solvent by using a 16-g, 2 × 18-cm, Sephadex LH-20 column developed and eluted with 500 ml of chloroform/hexane (75:25, vol/vol). After this procedure, the column was eluted with 500 ml of chloroform/hexane/methanol (75:25:2).

Statistical methods. Compartmental analyses of isotope disappearance from plasma were carried out as previously described (32). Data were analyzed by computer with the program developed by the Statistical Analysis Systems Institute (33). The metabolic clearance rate was calculated by dividing the administered dose by the time integral of the area under the $[^3H]1,25(OH)_2D_3$ plasma disappearance curve extrapolated to infinity.[2] Assuming steady-state conditions, the production rate was calculated as the product of the metabolic clearance rate and the plasma concentration of $1,25(OH)_2D$. Differences in hormone concentrations, plasma calcium, phosphate, and alkaline phosphatase were analyzed by paired *t* test.

RESULTS

Plasma measurements. Values for plasma hormonal measurements, calcium, phosphate, and alkaline phosphatase are given in Table I. Plasma 25-OH-D showed a small but statistically significant decrease in the hyperglucocorticoid state as compared with the euglucocorticoid state. Plasma $1,25(OH)_2D$ concentrations were lower in four of six patients with active endogenous Cushing's syndrome than after surgical intervention, and in five of seven patients receiving prednisone, but these changes were not statistically significant. There was no significant change in serum iPTH. Serum calcium, phosphate, alkaline phosphatase,

[2] Using the exponential function $y = H_1e^{-\alpha_1 t} + H_2e^{-\alpha_2 t} + H_3e^{-\alpha_3 t}$, the area under the disappearance curve = $H_1/\alpha_1 + H_2/\alpha_2 + H_3/\alpha_3$, where H_n and α_n are the y-intercept and slope, respectively, of the nth exponential function.

TABLE I
Relevant Plasma and Serum Measurements during Euglucocorticoid and Hyperglucocorticoid States

Measurements	Euglucocorticoid	Hyperglucocorticoid	P*
Calcium, *mg/100 ml*	9.5±0.1	9.4±0.1	NS
Phosphate, *mg/100 ml*	3.9±0.2	3.5±0.2	NS
Alkaline phosphatase, *U/liter*	140±10	135±8	NS
25-OH-D, *ng/ml*	22±2	18±2	<0.05
$1,25(OH)_2D$, *pg/ml*	32±8	23±6	NS
iPTH, *μleq/ml*	21±2	18±2	NS

* Significance of difference.

glutamicoxaloacetictransaminase, and creatine were in the normal range in the euglucocorticoid state and did not change significantly in the hyperglucocorticoid state.

Kinetic measurements. Disappearance of radioactivity in plasma at all time points was similar in both groups (Fig. 1). Nonetheless, only points up to 240 min were used for kinetic analyses because of relatively low levels of radioactivity at subsequent time points, and because of concern that at longer intervals [^{3}H]-$1,25(OH)_2D_3$ might be converted into other forms. A three-component open system was used for compartmental analyses. In control and hyperglucocorticoid subjects, the metabolic clearance rate, the production rate, and other kinetically derived parameters did not differ significantly (Table II).

Chromatography of pooled plasma samples at time points 0–45 and 45–240 min showed only a single peak of radioactivity that co-chromatographed with the $1,25(OH)_2D_3$ standard. 95% of the radioactivity was chloroform-extractable, and recovery from the columns averaged 95%.

Cumulative radioactivity excreted from the body during the 6 d of collection, expressed as a percentage of the dose administered, was 54±6% (mean±SE) in the stool and 14±2% in the urine of the control subjects, and 60±9% in stool and 18±2% in the urine for the hyperglucocorticoid patients (not significantly different) (Fig. 2).

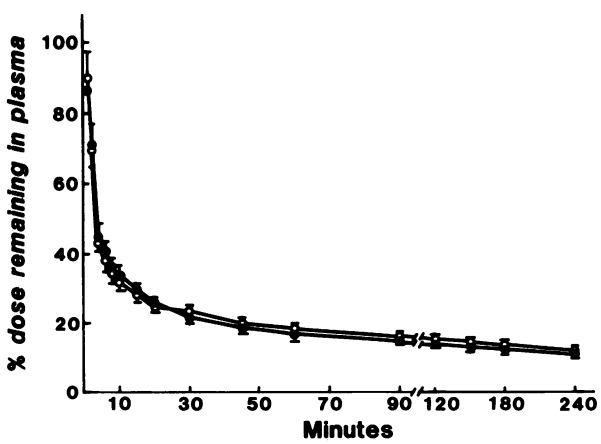

FIGURE 1 ^{3}H-$1,25(OH)_2D_3$ plasma disappearance. Percentage of administered [^{3}H]1,25-dihydroxyvitamin D_3 (mean ±SE) remaining in plasma in 14 controls (○) and 10 hyperglucocorticoid (●) subjects.

DISCUSSION

Although $1,25(OH)_2D$ is the hormonal, physiologically active form of vitamin D, only minimal information is available on rates of production or degradation of the compound. Mawer et al. (34) studied plasma disappearance after intravenous injection of 1 μg of [^{3}H]$1,25(OH)_2D_3$ in four normal subjects and in three patients with hypoparathyroidism. The half-times for the rapid and slow phases of disappearance of 14.0 and 81.2 h, respectively, are considerably longer than those found by us and Gray et al. (32). We believe that the most likely explanations for these differences are that their doses were pharmacologic (100 times greater than the tracer doses we employed) and that their serum half-times were calculated from plasma values from 4 h to 14 d (ours were calculated from 1.0–240 min). Using a three-compartmental model, Gray et al. (32)

TABLE II
Plasma Metabolic Clearance Rate and Production Rate of 1,25-Dihydroxyvitamin D in Control and Hyperglucocorticoid Subjects

Plasma $1,25(OH)_2D$	Control *n = 14*	Hyperglucocorticoid *n = 10*	P*
Metabolic clearance rate, *ml/min*	31±4	32±3	NS
Production rate, *μg/d*	1.5±0.2	1.2±0.3	NS

* Significance of difference.

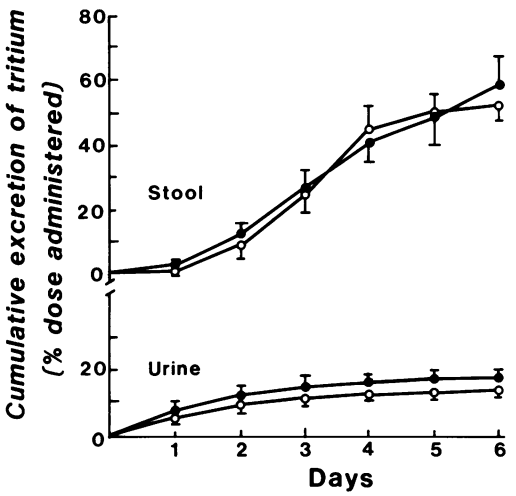

FIGURE 2 Cumulative excretion of tritium (mean±SE) in stool and urine in eight normal (○) and 8 hyperglucocorticoid (●) subjects.

made kinetic analyses on plasma data from two normal volunteers who had received tracer doses of [^{3}H]-1,25$(OH)_2D_3$. They reported that production rates were 0.34 and 1.02 μg/d, respectively, for a 26 yr-old woman and a 25-yr-old man. We also found that disappearance of [^{3}H]1,25$(OH)_2D_3$ from plasma was resolved by a three-compartmental model, although we are reluctant to assign anatomic or physiologic identities to the mathematically derived compartments and rate constants. Production rates for 1,25$(OH)_2D_3$ in our healthy control subjects (13 women and one man, ages 33–77 yr) were similar to those for the two subjects studied by Gray et al. (32). We recognize that kinetically determined parameters are model-dependent (35). Thus, in this study, compartmental analysis was used to compare kinetic parameters in normal subjects and patients with glucocorticoid excess; no difference was found in the two groups. Whereas the determination of production rates of 1,25$(OH)_2D$ is subject to the uncertainties alluded to above, it is of interest that the calculated production rate of 1,25$(OH)_2D$ (mean±SE 1.5±0.2 μg/d, median 1.25 μg/d) is consistent with the replacement dose required in states of 1,25$(OH)_2D$ deficiency (36, 37).

Many investigators have attempted to demonstrate an effect of supraphysiologic doses of glucocorticoids on metabolism of vitamin D to its physiologically active form, 1,25$(OH)_2D$. Chesney et al. (12) reported decreases in plasma concentrations of this metabolite in a group of children receiving glucocorticoids for a variety of chronic glomerular diseases; however, the presence of chronic renal disease with proteinuria makes these observations difficult to interpret. By contrast, Hahn et al. (4) reported elevated serum 1,25-$(OH)_2D$ concentrations in normal subjects receiving 20 mg of prednisone daily for 14 d, and Lukert et al. (16) reported elevated values in rats after 7 d of prednisolone administration. Lukert et al. (38) were able to detect radiolabeled 1,25$(OH)_2D_3$ in the plasma of human subjects receiving glucocoticoids for pulmonary disease after administration of [^{3}H]vitamin D, whereas they were unable to do so in normal subjects. From these data, they inferred that plasma levels of 1,25$(OH)_2D_3$ were elevated.

Before our study, kinetic measurements of 1,25-$(OH)_2D$ metabolism in states of glucocorticoid excess have been made only in experimental animals. Kimberg, Favus, and co-workers (17, 18) failed to find any significant effect of hydrocortisone therapy on the metabolism of either [^{3}H]vitamin D_3 or [^{3}H]25-OH-D_3 in the rat. Carre et al. (13) studied metabolism of [^{3}H]1,25$(OH)_2D_3$ in the intestinal wall of prednisone-treated rats but provided no data on serum values; they reported no effect on rate of conversion of [^{3}H]25-OH-D_3 to [^{3}H]1,25$(OH)_2D_3$ but, rather, found increased conversion to a more polar, biologically inactive intestinal metabolite.

Under the conditions of our study, we found no significant alterations in metabolism of 1,25$(OH)_2D$ during chonic glucocorticoid excess. Concentrations of the hormone in paired plasma samples showed no significant difference between euglucocorticoid and hyperglucocorticoid states. After intravenous administration of [^{3}H]1,25$(OH)_2D_3$, the kinetic parameters and the cumulative whole-body excretion of radioactivity remained unchanged. These findings suggest that chronic glucocorticoid excess does not have major effects on production and degradation of 1,25$(OH)_2D$.

We found a small but statistically significant decrease in mean plasma levels of 25-OH-D during glucocoticoid excess. This finding is consonant with the results of an earlier study by Avioli et al. (9), who found that prednisone administration reduced the plasma half-life of [^{3}H]vitamin D in normal humans, and with the more recent studies by Klein et al. (6), Bressot et al. (10), and Baynard et al. (11), which documented lower concentrations of serum 25-OH-D using radioligand assays. The decrease of 25-OH-D levels was more substantial in patients with endogenous Cushing's syndrome, possibly because of the much longer duration of exposure to chronic glucocorticoid excess. In contrast, Aloia et al. (15) reported normal 25-OH-D concentrations in patients with exogenous hypercorticoidism; however, the doses of prednisone administered were not stated, and their four patients with endogenous Cushing's syndrome had disease of apparently shorter duration than those in our study. Hahn et al. (14) reported normal serum 25-OH-D levels in patients receiving chronic supraphysiologic doses of prednisone (mean, 22 mg/d) when compared with age-matched controls. In another study, Hahn et al. (4)

found no change in serum 25-OH-D concentrations after the administration of a daily dose of 20 mg of prednisone for 2 wk. These discrepant findings may be related to differences in dose, inasmuch as Klein et al. (6) have reported that glucocorticoid dose and plasma 25-OH-D concentrations are inversely related.

The effect of glucocorticoid excess on PTH secretion is also controversial. Elevated iPTH values in rats (39) and humans (5, 40, 41) after administration of supraphysiologic doses of glucocorticoids have been reported. In the present study, we found no increase in serum iPTH when euglucocorticoid and hyperglucocorticoid values were compared using a radioimmunoassay system that is highly sensitive to increases in parathyroid gland function (26).[3] Inasmuch as PTH regulates 25-OH-D 1-hydroxylase activity (42), the absence of secondary hyperparathyroidism is further evidence against a major reduction in availability of $1,25(OH)_2D$ in hyperglucocorticoidism.

Many variables appear relevant to the effect of glucocorticoids on vitamin D metabolism, especially the dose and duration of treatment and the nature of the underlying disease. In the present study of hyperglucocorticoid patients, we found a small but significant decrease in plasma 25-OH-D concentrations, a small but nonsignificant decrease in plasma $1,25(OH)_2D$ concentrations, and no changes in rates of production or degradation of $1,25(OH)_2D$. Thus, effects of glucocorticoid excess on vitamin D metabolism, if present, must be minor. Effects at the level of target tissues were not studied and, thus, cannot be excluded. We conclude that mechanisms other than impaired production or increased degradation of $1,25(OH)_2D$ must be postulated to explain satisfactorily the abnormalities of bone structure and intestinal calcium absorption that commonly occur after chronic glucocorticoid therapy.

ACKNOWLEDGMENTS

This investigation was supported in part by National Institutes of Health grants AM25409, AM19607, and RR585 from the U. S. Public Health Service, and by grants from the Minnesota Arthritis Foundation and the R. K. Mellon Foundation.

REFERENCES

1. Caniggia, A., and C. Gennari. 1973–74. Effect of 25-hydroxycholecalciferol (25-HCC) on intestinal absorption of ^{47}Ca in four cases of iatrogenic Cushing's syndrome. *Helv. Med. Acta.* **37:** 227–234.
2. Wajchenberg, B. L., V. G. Pereira, J. Kieffer, and S. Ursic. 1969. Effect of dexamethasone on calcium metabolism and ^{47}Ca kinetics in normal subjects. *Acta Endocrinol.* **61:** 173–192.
3. Gallagher, J. C., J. Aaron, A. Horsman, R. Wilkinson, and B. E. C. Nordin. 1973. Corticosteroid osteoporosis. *Clin. Endocrinol. Metab.* **2:** 355–368.
4. Hahn, T. J., D. T. Baran, and L. R. Halstead. 1979. Alterations in mineral and vitamin D metabolism produced by subacute prednisone (Pr) administration in man. 61st Annual Meeting of the Endocrine Society, Abstract 492. p. 195.
5. Hahn, T. J., L. R. Halstead, S. L. Teitelbaum, and B. H. Hahn. 1979. Allered mineral metabolism in glucocorticoid-induced osteopenia: effect of 25-hydroxyvitamin D administration. *J. Clin. Invest.* **64:** 655–665.
6. Klein, R. G., S. B. Arnaud, J. C. Gallagher, H. F. DeLuca, and B. L. Riggs. 1977. Intestinal calcium absorption in exogenous hypercortisolism: role of 25-hydroxyvitamin D and corticosteroid dose. *J. Clin. Invest.* **60:** 253–259.
7. Harrison, H. E., and H. C. Harrison. 1960. Transfer of Ca^{45} across intestinal wall in vitro in relation to action of vitamin D and cortisol. *Am. J. Physiol.* **199:** 265–271.
8. Williams, G. A., E. N. Bowser, W. J. Henderson, and V. Uzgiries. 1961. Effects of vitamin D and cortisone on intestinal absorption of calcium in the rat. *Proc. Soc. Exp. Biol. Med.* **106:** 664–666.
9. Avioli, L. V., S. J. Birge, and S. W. Lee. 1968. Effects of prednisone on vitamin D metabolism in man. *J. Clin. Endocrinol. Metab.* **28:** 1341–1346.
10. Bressot, C., P. J. Meunier, M. C. Chapuy, E. Lejeune, C. Edouard, and A. J. Darby. 1970. Histomorphometric profile, pathophysiology and reversibility of corticosteroid-induced osteoporosis. *Metab. Bone Dis. Rel. Res.* **1:** 303–311.
11. Baynard, F., P. Bee, J. P. Louvet, and C. Boulard. 1972. 25-Hydroxycholecalciferol dynamics in human plasma. Proceedings of the Fourth International Congress on Endocrinology. Excerpta Medica International Congress Series 256, Washington, D. C., Abstract 597. p. 238.
12. Chesney, R. W., A. J. Hamstra, R. B. Mazeso, and H. F. DeLuca. 1978. Reduction of serum 1,25-dihydroxyvitamin D_3 in children receiving glucocorticoids. *Lancet.* **II:** 1123–1125.
13. Carre, M., O. Ayigbede, L. Miravet, and H. Rasmussen. 1974. The effect of prednisone upon the metabolism and action of 25-hydroxy- and 1,25-dihydroxyvitamin D_3. *Proc. Natl. Acad. Sci. U. S. A.* **71:** 2996–3000.
14. Hahn, T. J., L. R. Halstead, and J. G. Haddad, Jr. 1977. Serum 25-hydroxyvitamin D concentrations in patients receiving chronic corticosteroid therapy. *J. Lab. Clin. Med.* **90:** 399–404.
15. Aloia, J. F., M. Roginsky, K. Ellis, K. Shukla, and S. Cohen. 1974. Skeletal metabolism and body composition in Cushing's syndrome. *J. Clin. Endocrinol. Metab.* **39:** 981–985.
16. Lukert, B. P., S. W. Stanbury, and E. B. Mawer. 1973. Vitamin D and intestinal transport of calcium: effects of prednisolone. *Endocrinology.* **93:** 718–722.
17. Kimberg, D. V., R. D. Baerg, E. Gershon, and R. T. Graudusius. 1971. Effect of cortisone treatment on the active transport of calcium by the small intestine. *J. Clin. Invest.* **50:** 1309–1321.
18. Favus, M. J., D. V. Kimberg, G. N. Millar, and E. Gershon. 1973. Effects of cortisone administration on the metabolism and localization of 25-hydroxycholecalciferol in the rat. *J. Clin. Invest.* **52:** 1328–1335.
19. Favus, M. J., M. W. Walling, and D. V. Kimberg. 1973. Effects of 1,25-dihydroxycholecalciferol on intestinal calcium transport in cortisone-treated rats. *J. Clin. Invest.* **52:** 1680–1685.
20. Kimberg, D. V. 1969. Effects of vitamin D and steroid hormones on the active transport of calcium by the intestine. *N. Engl. J. Med.* **280:** 1396–1405.

[3] Similarly, we have found no increase of iPTH in rats treated with massive doses of cortisol (Heath, H., III, and Fox, J. Unpublished observations).

21. Haddad, J. G., and K. J. Chyu. 1971. Competitive protein binding radioassay for 25-hydroxycholecalciferol. *J. Clin. Endocrinol. Metab.* **33:** 992–995.
22. Horst, R. L., R. M. Shepard, N. A. Jorgensen, and H. F. DeLuca. 1979. The determination of the vitamin D metabolites on a single plasma sample: changes during partuition in dairy cows. *Arch. Biochem. Biophys.* **176:** 235–243.
23. Kumar, R., W. R. Cohen, P. Silva, and F. H. Epstein. 1979. Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. *J. Clin. Invest.* **63:** 342–344.
24. Eisman, J. A., A. J. Hamstra, B. E. Kream, and H. F. DeLuca. 1976. A sensitive, precise and convenient method for determination of 1,25-dihydroxyvitamin D in human plasma. *Arch. Biochem. Biophys.* **176:** 235–243.
25. Arnaud, C. D., H. S. Tsao, and T. Littledike. 1971. Radioimmunoassay of human parathyroid hormone in serum. *J. Clin. Invest.* **50:** 21–34.
26. Gallagher, J. C., B. L. Riggs, C. M. Jerpbak, and C. D. Arnaud. 1980. The effect of age on serum immunoreactive parathyroid hormone in normal and osteoporotic women. *J. Lab. Clin. Med.* **95:** 373–385.
27. Arnaud, C. D., R. S. Goldsmith, P. J. Bordier, and G. W. Sizemore. 1974. Influence of immunoheterogeneity of circulating parathyroid hormone on results of radioimmunoassays of serum in man. *Am. J. Med.* **56:** 785–793.
28. Gray, R. W., J. L. Omdahl, J. G. Ghazarian, and H. F. DeLuca. 1972. 25-hydroxycholecalciferol-1-hydroxylase. *J. Biol. Chem.* **247:** 7528–7532.
29. Kumar, R., S. Nagubandi, V. R. Mattox, and J. M. Londowski. 1980. Enterohepatic physiology of 1,25-dihydroxyvitamin D_3. *J. Clin. Invest.* **65:** 277–284.
30. Wilkinson, R. 1971. Polyethylene glycol 4000 as a continuously administered nonabsorbable faecal marker for metabolic balance studies in human subjects. *Gut.* **12:** 654–660.
31. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
32. Gray, R. W., A. E. Caldar, D. R. Wilz, J. Leman, Jr., G. A. Smith, and H. F. DeLuca. 1978. Metabolism and excretion of ^{3}H-1,25$(OH)_2$ vitamin D_3 in healthy adults. *J. Clin. Endocrinol. Metab.* **46:** 756–765.
33. Barr, A. J., J. H. Goodnight, J. P. Sall, and J. T. Helwig. 1976. A Users Guide to S. A. S. Statistical Analysis Systems Institute, Inc., Raleigh, N. C.
34. Mawer, E. B., J. Backhouse, M. Davies, L. F. Hill, and C. M. Taylor. 1976. Metabolic fate of administered 1,25-dihydroxycholecalciferol in controls and in patients with hypoparathyroidism. *Lancet.* **I:** 1203–1206.
35. Brown, R. F., E. R. Carson, L. Finkelstein, K. R. Godfrey, and R. P. Jones. 1979. Adequacy of measurements in compartmental modelling of metabolic systems. *J. Clin. Invest.* **50:** 2159–2167.
36. Peacock, M., P. J. Heyburn, J. E. Aaron, G. A. Taylor, W. B. Brown, and R. Speed. 1979. Osteomalacia: treated with 1α-hydroxy or 1,25-dihydroxyvitamin D. *In* Vitamin D, Basic Research and its Clinical Application. A. W. Norman, K. Shaefer, D. V. Herrath, H. G. Grigoleit, J. W. Coburn, H. F. DeLuca, E. B. Mawer, and T. Suda, editors. Walter de Gruyter & Co. New York. 1177–1183.
37. Kooh, S. W., D. Fraser, H. F. DeLuca, M. F. Holick, R. E. Belsey, M. G. Clark, and T. M. Murray. 1975. Treatment of hypoparathyroidism and pseudohypoparathyroidism with metabolites of vitamin D: evidence for impaired conversion of 25-hydroxyvitamin D to 1α,25-dihydroxyvitamin D. *N. Engl. J. Med.* **293**(17): 840–844.
38. Lukert, B. P., and J. S. Adams. 1976. Calcium and phosphorus homeostasis in man. *Arch. Intern. Med.* **136:** 1241–1248.
39. Williams, G. A., W. C. Peterson, E. N. Bowser, W. J. Henderson, G. K. Hargis, and N. J. Martinez. 1974. Interrelationship of parathyroid and adrenocortical function in calcium homeostasis in the rat. *Endocrinology.* **95:** 707–712.
40. Lukert, B. P., and J. S. Adams. 1976. Calcium and phosphorus homeostasis in man. *Arch. Intern. Med.* **136:** 1249–1253.
41. Fucik, R. F., J. C. Kukreja, G. K. Hargis, E. N. Bowser, W. J. Henderson, and G. A. Williams. 1975. Effect of glucocorticoids on function of the parathyroid glands in man. *J. Clin. Endocrinol. Metab.* **40:** 152–155.
42. DeLuca, H. F. 1976. Recent advances in our understanding of the vitamin D endocrine system. *J. Lab. Clin. Med.* **87:** 7–26.