Evidence that Increased Circulating 1_{α},25-Dihydroxyvitamin D is the Probable Cause for Abnormal Calcium Metabolism in Sarcoidosis

NORMAN H. BELL, PAULA H. STERN, ELIZABETH PANTZER, TUSHAR K. SINHA, and HECTOR F. DELUCA, *Departments of Medicine and Pharmacology, Indiana University Medical School and Veterans Administration Medical Center, Indianapolis, Indiana 46202; Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611; and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706*

ABSTRACT Mean plasma 1_{α},25-dihydroxyvitamin D [1_{α},25$(OH)_2$D] was significantly increased and serum parathyroid hormone was suppressed in three patients with sarcoidosis and hypercalcemia. Prednisone lowered the mean plasma 1_{α},25$(OH)_2$D to normal range and corrected the hypercalcemia. To elucidate the mechanism for the increased sensitivity to vitamin D in this disorder, the effects of orally-administered vitamin D_2 were determined in seven normal subjects, four patients with sarcoidosis and normal calcium metabolism and three patients with sarcoidosis and a history of hypercalcemia who were normocalcemic when studied. Serum and urinary calcium, serum 25-hydroxyvitamin D (25-OHD), plasma 1_{α},25$(OH)_2$D and, in some studies, calcium balance were measured. Vitamin D_2, 250 μg a day for 12 d, produced little, if any, change in mean plasma 1_{α},25$(OH)_2$D and in urinary calcium in the normals and in the patients with normal calcium metabolism. In contrast, vitamin D_2 produced increases in plasma 1_{α},25$(OH)_2$D from concentrations which were within the normal range (20–55 pg/ml) to abnormal values and increased urinary calcium in two patients with abnormal calcium metabolism. In an abbreviated study in the third patient, vitamin D_2, 250 μg a day for 4 d, also increased plasma 1_{α},25$(OH)_2$D abnormally from a normal value. There was a highly significant correlation between plasma 1_{α},25$(OH)_2$D and urinary calcium. Serum 25-OHD and serum calcium remained within the normal range in all subjects and patients. These findings provide evidence that the defect in calcium metabolism in sarcoidosis probably results from impaired regulation of the production and(or) degradation of 1_{α},25$(OH)_2$D. Prednisone may act to correct the abnormal calcium metabolism by reducing circulating 1_{α},25$(OH)_2$D.

Received for publication 17 July 1978 and in revised form 28 February 1979.

INTRODUCTION

Abnormal calcium metabolism in sarcoidosis is characterized by enhanced intestinal absorption of the ion and hypercalciuria with or without hypercalcemia, which may be associated with renal stones, nephrocalcinosis, and impaired renal function (1–8). Patients with sarcoidosis exhibit increased sensitivity to small doses of vitamin D, which are ineffective in normal subjects (1, 4–6). The observation that serum antirachitic activity has been shown to be within the normal range (3, 5) has led to the conclusion that the abnormal calcium metabolism in sarcoidosis results not from hypervitaminosis D but from increased sensitivity to vitamin D (3, 5).

It is known that vitamin D is converted by the liver to 25-hydroxyvitamin D (25-OHD)[1] (9), which in turn undergoes 1_{α}-hydroxylation by the kidney to form 1_{α},25-dihydroxyvitamin D (1_{α},25$(OH)_2$D) (10, 11), the most potent metabolite of the vitamin that augments the intestinal absorption of calcium (11) and stimulates bone resorption (12).

The mechanism for the abnormal sensitivity to vitamin D in sarcoidosis has not been determined. In the present work evidence is presented that the defect in calcium metabolism results from increases in circulating 1_{α},25$(OH)_2$D.

[1] *Abbreviations used in this paper*: PTH, parathyroid hormone; 25-OHD, 25-hydroxyvitamin D; 1_{α},25$(OH)_2$D, 1_{α},25-dihydroxyvitamin D.

METHODS

Eight normal adult men ranging in age from 20 to 70 yr and eight patients with sarcoidosis, four men and four women ranging in age from 24 to 63 yr, were studied. They were hospitalized on the Clinical Research Center of the Indiana University Medical School and were given a constant daily diet and fluid intake. Fasting serum samples were collected at intervals of 4 d and were analyzed for calcium (13), 25-OHD and parathyroid hormone (PTH). Plasma was also obtained for $1_{\alpha},25(OH)_2D$. 24-h urine collections and, in some studies, 4-d fecal pools were obtained. Diet, urine, and stools were analyzed for calcium (13). Vitamin D_2 (Winthrop Laboratories, New York) in propylene glycol was given daily as a single oral dose. $1_{\alpha},25(OH)_2D_3$, synthesized by methods previously reported (14), was made up as a sterile solution in propylene glycol. It was given as a single intravenous or oral morning dose. Three patients were studied both while hospitalized at the Indiana University Hospital and as outpatients. In these individuals, fasting samples were obtained for creatinine as well as for calcium, PTH, 25-OHD, and $1_{\alpha},25(OH)_2D$. Serum creatinine was measured by AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.).

Serum 25-OHD was measured by a competitive serum protein-binding method (15); in most instances, values were obtained after chromatography on Sephadex LH-20 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) (16). In our laboratory the normal range without prior chromatography is from 10 to 80 ng/ml and with chromatography is from 8 to 61 ng/ml. Results obtained without chromatography are so noted in the text.

Plasma $1_{\alpha},25(OH)_2D$ was measured by bioassay as previously described (17, 18). Samples were either extracted with dichloromethane and chromatographed on Sephadex LH-20 in the solvent system Skellysolve B (Skelly Oil Co., Tulsa, Okla.):chloroform:methanol (9:1:1) or with benzene, which was then washed with 0.1 M phosphate buffer, pH 10.5 (17). Most of the samples were processed by the latter method. The extracts were then further fractionated by high pressure liquid chromatography on μPorosil (Waters Associates, Inc., Milford, Mass.) with the solvent system 13% isopropanol in *n*-hexane. In this sytem $1_{\alpha},25(OH)_2D_2$ elutes with $1_{\alpha},25(OH)_2D_3$ (17–19). The fractions that contained both metabolites were collected. Recovery was determined by addition to the serum of [26,27-^{3}H]$1_{\alpha},25(OH)_2D_3$. $1_{\alpha},25(OH)_2D$ was then measured by release of ^{45}Ca from fetal rat bones in organ culture (17, 18). Each serum sample was assayed at two dilutions and the value for each sample and standard was the mean of four or six replicate samples (18). Because in this system $1_{\alpha},25(OH)_2D_2$ and $1_{\alpha},25(OH)_2D_3$ are equipotent in causing release of ^{45}Ca (20), and because fractions that contained both metabolites were collected, the assay measures the total amount of $1_{\alpha},25(OH)_2D$. The mean value in normal subjects is 33±2 pg/ml and range is 20 to 55 pg/ml (n = 16). The 95% confidence values (mean±2 SD) are 18 to 48 pg/ml. The interassay variation is 9.1% (n = 17).

In several studies plasma was assayed for $1_{\alpha},25(OH)_2D$ in patients during treatment with prednisone. Samples were obtained 8 or more h after the last dose of the steroid. In view of the known effects of steroids to inhibit bone resorption in this assay system (21) and the demonstration that prednisone is rapidly converted to prednisolone in man (22, 23), additional studies were carried out. Both steroids were clearly separated from $1_{\alpha},25(OH)_2D$ during high pressure liquid chromatography in the system described above. It was also demonstrated that an extract of plasma from one of the patients given prednisone did not inhibit the release of ^{45}Ca produced by added $1_{\alpha},25(OH)_2D$ in cultured rat bones.

Serum PTH was measured by radioimmunoassay as previously described (24, 25) with two antisera, which predominately measure the carboxy-terminal portion of the molecule. In the first assay, antiserum from chicken No. 9 (kindly supplied by Dr. E. Slatopolsky, Washington University School of Medicine, St. Louis, Mo.) was used at a final concentration of 1:20,000 and highly purified bovine PTH (Inolex Corp., Biomedical Div., Glenwood, Ill.; lot 155258, 971 U/mg) was the standard. Serum PTH is detectable in 85% of normal subjects and the normal range is from undetectable (<150 pg/ml) to 550 pg/ml (24, 25). In the second assay, antiserum from chicken 77125 developed in this laboratory was employed at a final concentration of 1:10,000 and highly purified bovine PTH (Inolex Corp.; lot 1508 D003, 867 U/mg) was the standard. Serum PTH is detectable in over 98% of normal subjects and the normal range is from undetectable (<100 pg/ml) to 540 pg/ml (n = 66).[2] The intraassay and interassay variations are 12 and 16.7%, respectively.

Student's t test was used to determine the significance of differences of paired or unpaired samples. Correlation coefficient and Student's t test were carried out with a Hewlett-Packard calculator (model 9810A, Hewlett-Packard Co., Palo Alto, Calif.).

RESULTS

Eight of the nine patients had the diagnosis of sarcoidosis confirmed histologically (Table I). Patient B, who had never had a biopsy, had a history of hypercalcemia, granulomatous disease on chest x ray, and a negative skin test for tuberculosis. Five of the other patients also had a history of hypercalcemia and one of these had had kidney stones.

Three patients had hypercalcemia, one of them on two separate occasions (Table II). Serum PTH was either undetectable (patients G and H) or in the low normal range (patient I). Plasma $1_{\alpha},25(OH)_2D$, determined in two samples in each patient, was either elevated or in the upper range of normal. Mean plasma $1_{\alpha},25(OH)_2D$ was 62±6 pg/ml in these patients, a value significantly higher ($P < 0.001$) than that of 33±2 pg/ml obtained in normal adult subjects (n =16) and was reduced significantly by prednisone to 26±4 pg/ml (P

[2] Bell, N. H. Unpublished observations.

TABLE I
Clinical Findings in Patients with Sarcoidosis

Patient	Age	Sex	Biopsy	Hypercalcemia*	Renal stones*
A	26	M	+	−	−
B	64	F	−	+	−
C	63	M	+	−	−
D	57	F	+	−	−
E	49	M	+	+	+
F	48	F	+	+	−
G	24	M	+	+	−
H	59	F	+	+	−
I	22	M	+	+	−

* By history.

TABLE II

Effects of Prednisone on Serum Calcium, PTH, 25-OHD, and Creatinine, and Plasma $1_{\alpha},25(OH)_2D$ in Three Patients with Sarcoidosis and Hypercalcemia

Patient	Serum calcium	Serum PTH	Serum 25-OHD	Plasma $1_{\alpha},25(OH)_2D$	Serum creatinine	Prednisone	Duration
	mg/dl	*pg/ml*	*ng/ml*	*pg/ml*	*mg/dl*	*mg/d*	*wk*
G	13.6	—	129*	66	1.5		
	9.8	—	106*	12	1.0	60	17
	11.1	<100‡	—	84	—		
	9.8	216	—	29	—	7.5	1.3
	9.8	206	—	34	—	7.5	6
H	12.7	<150‖	9	73	9.0		
	15.0	—	—	45	9.3		
	9.8	407	—	32	5.2	15	6
I	13.8	156‡	8	43	2.0		
	13.2	169	5	61	1.9		
	9.2	334	—	21	1.5	40	2

* Determined without preparative chromatography (14).
‡ Normal range, <100–542 pg/ml (n = 66).
‖ Normal range, <150–550 pg/ml (23).

<0.02). Prednisone lowered the mean serum calcium from 13.3±0.5 to 9.7±0.1 mg/dl ($P < 0.01$). Serum 25-OHD was abnormally increased in patient G who had had exposure to sunlight while playing golf during the summer (he denied having taken vitamin D) and it remained elevated after treatment with prednisone.

Serum creatinine was increased with hypercalcemia in each of the patients and was lowered after correction of the hypercalcemia by prednisone (Table II). In patient H, renal function was severely impaired and improved substantially with treatment.

In view of the known abnormal sensitivity to vitamin D of patients with sarcoidosis (1, 4–6), the effects of vitamin D_2, 250 μg(10,000 IU)/d for 12 d, were compared in seven normal subjects and in seven patients with sarcoid and a normal serum calcium.

In the normal subjects and patients with normal calcium metabolism, vitamin D_2 produced very little change in mean plasma $1_{\alpha},25(OH)_2D$, which remained within the normal range (Table III), or mean serum and urinary calcium (Table IV). Mean serum 25-OHD remained within the normal range in both the normals and patients (Table III). Whereas the increases in mean plasma $1_{\alpha},25(OH)_2D$ and mean urinary calcium were statistically significant ($P < 0.05$) in the four patients, the actual differences were quite modest, averaging 14 and 21%, respectively.

Three patients (including patient G) previously had had hypercalcemia and had been treated with prednisone so that at the time of study their serum calcium was normal. The interval after the last dose of steroid ranged from 3 d in patient F (who had received a 4-d course) to over 1 yr in patient E. Patient G had received prednisone, 5 mg every other day for 6 mo, at the time of evaluation. The drug was not given during the study.

In patient E (Fig. 1), vitamin D_2 increased serum 25-

TABLE III

Effects of Vitamin D_2 on Serum 25-OHD and $1_{\alpha},25(OH)_2D$ in Normal Subjects and Patients with Normal Calcium Metabolism

	Serum 25-OHD				Plasma $1_{\alpha},25(OH)_2D$	
Days*.....	1	5	9	13	1	13
	ng/ml				*pg/ml*	
Normals						
A	24	32	41	51	31	33
B	24	31	33	31	34	34
C	13	24	19	12	42	39
D	61	47		28	28	20
E	15	21	29	11	20	25
F	14	12	41	28	28	31
G	35‡	50‡	60‡	43‡	25	35
	27±6	31±5	37±6	29±6	30±3	31±2
Patients						
A	23‡	23‡	30‡	29‡	37	39
B	6	9	13	18	28	31
C	24	31	30	38	27	30
D	8	16	31	29	25	33
	15±5	20±5	26±4	28±4	29±3	33±2§

* Vitamin D_2, 250 μg/d, was given orally from days 1 through 12.
‡ Determined without preparative chromatography (13).
§ $P < 0.05$ day 1 vs. day 13.

TABLE IV

Effects of Vitamin D_2 on Serum and Urinary Calcium in Normal Subjects and Patients with Normal Calcium Metabolism

	Serum calcium		Urinary calcium*	
Days‡	1	13	1–4	9–12
	mg/dl		*mg/d*	
Normals				
A	9.3	9.3	179	182
B	10.1	9.3	163	150
C	9.1	9.6	272	269
D	9.5	9.2	152	154
E	8.7	10.0	265	201
F	10.0	10.1	150	169
G	9.7	9.7	271	248
	9.5±0.2	9.6±0.1	207±22	196±18
Patients				
A	9.0	9.5	190	240
B	9.7	10.2	131	146
C	9.4	10.1	112	134
D	9.0	8.6	116	144
	9.3±0.2	9.6±0.4	137±18	166±25§

* Represents the mean of 4 d.

‡ Vitamin D_2, 250 μg/d, was given orally from days 1–12.

§ $P < 0.05$.

OHD from 11 ng/ml (day 5) to as high as 24 ng/ml (day 17) and plasma 1_α,25(OH)$_2$D from 29 pg/ml (day 5) to 58 pg/ml (day 9) and 74 pg/ml (day 17). Mean urinary calcium increased from 190±22 (SE) mg/d (days 5 through 8) to 379±23 g/d (days 13 through 16) ($P < 0.02$). Fecal calcium decreased during vitamin D_2 and serum calcium remained within the normal range. In patient F (Fig. 2), vitamin D_2 increased plasma 1_α,25(OH)$_2$D from 23 pg/ml (day 1) to as high as 132 pg/ml (day 9). Serum 25-OHD remained within the normal range. Mean urinary calcium increased from 287±9 mg/d (days 1–4) to 406±31 mg/d (days 9 through 13, $P < 0.05$). Serum calcium remained normal and fecal calcium did not change. In patient G (Fig. 3), vitamin D_2 given for only 4 d increased plasma 1_α,25(OH)$_2$D$_3$ from 33 pg/ml (day 1) to 86 pg/ml (day 5). Serum 25-OHD was 26 ng/ml (day 1) and 21 ng/ml (day 5). Serum and urinary calcium stayed within the normal range. Thus, in three of the patients who had had hypercalcemia, vitamin D_2 increased plasma 1_α,25(OH)$_2$D from values that were within the normal range to values that were clearly abnormally elevated. Serum 25-OHD and serum calcium remained within the normal range. When given for a sufficient period of time in two patients, vitamin D increased urinary calcium significantly. The increases in plasma 1_α,25(OH)$_2$D had occurred after 4 d of treatment with vitamin D_2 in each

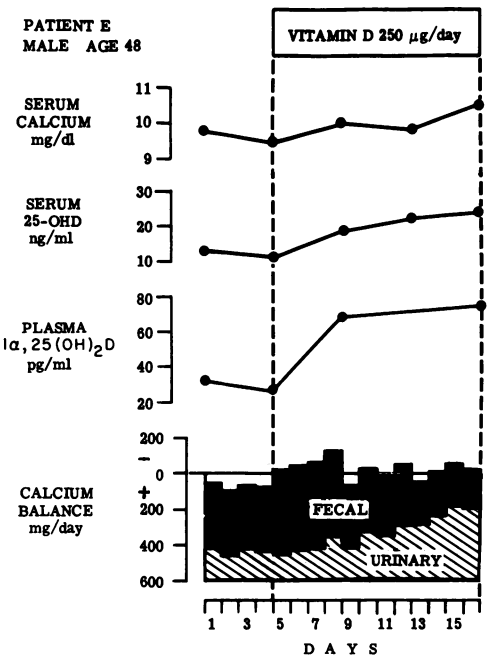

FIGURE 1 Effects of vitamin D_2 on serum calcium, serum 25-OHD, plasma 1_α,25(OH)$_2$D, and calcium balance in patient E with sarcoidosis. 25-OHD was determined without prior chromatography.

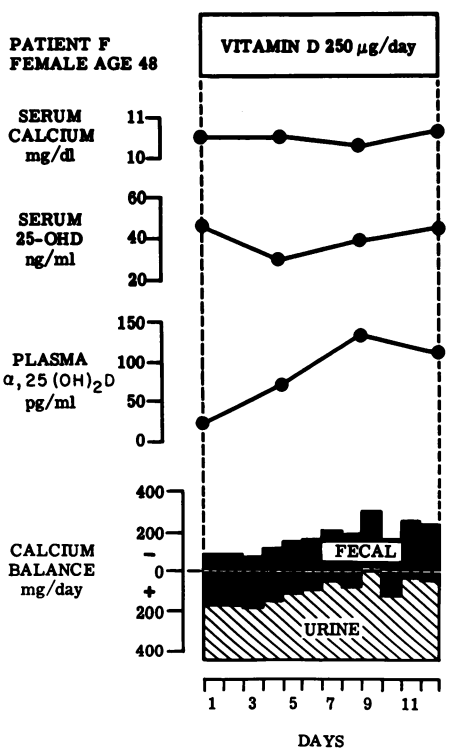

FIGURE 2 Effects of vitamin D_2 on serum calcium, serum 25-OHD, plasma 1_α,25(OH)$_2$D, and calcium balance in patient F with sarcoidosis.

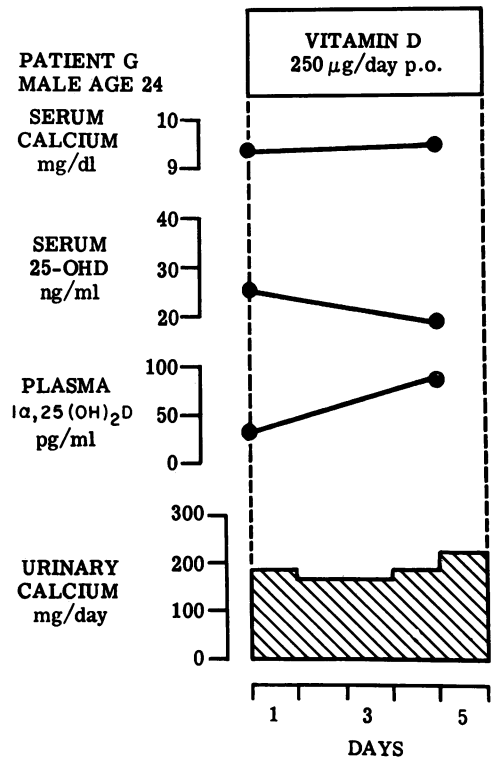

FIGURE 3 Effects of vitamin D_2 on serum calcium, serum 25-OHD, plasma $1_\alpha,25(OH)_2D$, and urinary calcium in patient G with sarcoidosis.

patient and always preceded the increases in urinary calcium.

There was no correlation between serum calcium and plasma $1_\alpha,25(OH)_2D$ in the patients either in the presence or absence of hypercalcemia. However, there was a highly significant correlation between plasma $1_\alpha,25(OH)_2D$ and urinary calcium in the normals and patients given vitamin D (Fig. 4). The correlation was also significant for the patients with or without abnormal calcium metabolism ($r = 0.713$, $P < 0.01$) as well as for the patients with abnormal calcium metabolism alone ($r = 0.772$, $P < 0.01$).

Studies were carried out to compare the effects of $1_\alpha,25(OH)_2D_3$ on serum and urinary calcium in normal subjects and patients to those produced in the patients by endogenous $1_\alpha,25(OH)_2D$ (Table V). $1_\alpha,25(OH)_2D_3$, given at doses of 1, 2, and 4 μg/d for 4 d at each dose, produced marked increases in urinary calcium in both the normal subjects and patients but did not increase the serum calcium abnormally. The changes were comparable whether the $1_\alpha,25(OH)_2D_3$ was given orally or intravenously. Thus, exogenously administered $1_\alpha,25(OH)_2D_3$ produced increases in urinary calcium that were comparable to those produced by $1_\alpha,25(OH)_2D$ in the patients from endogenous sources and in the doses used did not produce hypercalcemia.

DISCUSSION

Three patients were studied when hypercalcemic and showed suppression of serum PTH and significant increases in mean plasma $1_\alpha,25(OH)_2D$. Prednisone lowered the mean plasma $1_\alpha,25(OH)_2D$ significantly and corrected the hypercalcemia and serum PTH returned to the normal range. Cushard et al. (26) also observed suppression of serum PTH in six patients with sarcoidosis and hypercalcemia and the return of serum PTH to normal after correction of the hypercalcemia with prednisone.

PTH appears to be a major regulator of the renal production of $1_\alpha,25(OH)_2D$ in man; plasma values for the metabolite are increased in primary hyperparathyroidism and decreased in hypoparathyroidism (27–29). The findings of increased plasma $1_\alpha,25(OH)_2D$ despite functional hypoparathyroidism in the patients with hypercalcemia are all the more striking in view of these considerations. They suggest that plasma $1_\alpha,25(OH)_2D$ in patients with sarcoidosis and abnormal calcium metabolism is regulated by factors other than PTH.

Whereas vitamin D in modest doses did not alter the calcium metabolism or plasma $1_\alpha,25(OH)_2D$ in the normals or patients with normal calcium metabolism, it increased plasma $1_\alpha,25(OH)_2D$ and urinary calcium abnormally in the patients with a history of hypercalcemia without increasing their serum calcium. This lack of hypercalcemia is attributed to the low calcium

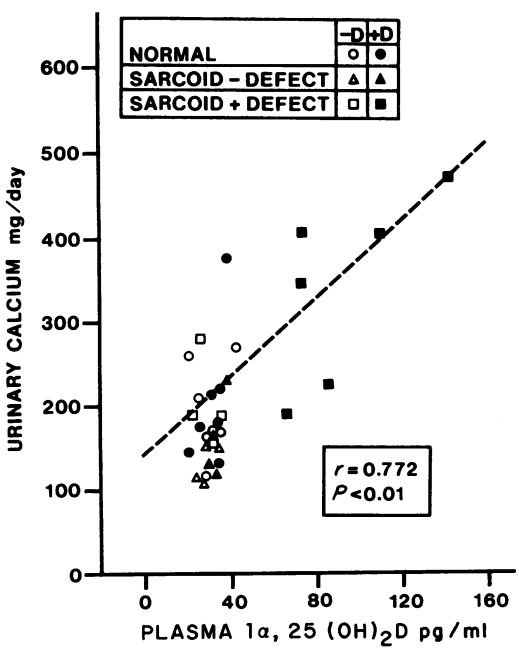

FIGURE 4 Relationship between plasma $1_\alpha,25(OH)_2D$ and urinary calcium in normal subjects and patients given vitamin D.

TABLE V

Effects of 1α,25(OH)$_2$D$_3$ on Serum and Urinary Calcium in Normal Subjects and Patients with Sarcoidosis

	Serum calcium					Urinary calcium*			
Days‡....	1	5	9	13	17	1–4	5–8	9–12	13–16
			mg/dl				*mg/d*		
Normals									
D	9.2	9.4	9.3	9.6	9.8	152	202	305	442
G	10.5	9.6	10.8	9.6	10.3	230	308	406	500
I§	10.4	9.5	10.3	10.2	10.2	188	207	360	510
	10.0	9.5	10.1	9.8	10.1	190	239	357	484
Patients									
C	9.9	9.6	9.6	9.9	10.6	167	281	317	363
E§	9.2	9.5	9.8	9.8	10.4	172	174	204	284
	9.6	9.6	9.7	9.8	10.5	170	228	260	323

* There was a highly significant correlation between the dose of 1α,25(OH)$_2$D$_3$ and mean 4-d urinary calcium ($r = 0.821$, $P < 0.01$).

‡ 1α,25(OH)$_2$D$_3$ was given daily as follows: 1 μg (days 5–8), 2 μg (days 9–12), and 4 μg (days 13–16).

§ 1α,25(OH)$_2$D$_3$ was given intravenously in these subjects and orally to the others.

intake (see below) in view of reports that show that serum calcium may vary with dietary intake of the ion in sarcoidosis (1, 4, 5). In similar studies, exogenously administered 1$_\alpha$,25(OH)$_2$D$_3$ produced comparable increases in urinary calcium without causing hypercalcemia. In fact, our studies were carried out with a low calcium diet to prevent possible hypercalcemia and its consequences on renal function.

Fractional intestinal absorption of calcium has been demonstrated to be increased in patients with sarcoid who have hypercalciuria and not hypercalcemia, and to correlate with urinary calcium (8). In our studies, plasma 1$_\alpha$,25(OH)$_2$D did not correlate with serum calcium either in the absence or presence of hypercalcemia but correlated strongly with urinary calcium. There are a number of possible reasons for this lack of correlation. In primary hyperparathyroidism, another disorder characterized by increases in plasma 1$_\alpha$,25(OH)$_2$D, intestinal absorption of calcium and urinary calcium, plasma 1$_\alpha$,25(OH)$_2$D also has been shown to correlate significantly with the fractional intestional absorption of calcium and with urinary calcium but not with serum calcium even when the patients are given the same calcium intake and a substantial number of individuals are evaluated (28). It is possible that variation in the ability of the kidneys of different patients to excrete an excess calcium load produced by increased circulating 1$_\alpha$,25(OH)$_2$D in these two disorders may obscure any net effect on serum calcium. In sarcoid, it is likely that hypercalcemia occurs when the compensatory ability of the kidneys to excrete the calcium load has been exceeded. As noted already, alteration of serum calcium in sarcoid by changes in dietary intake has been shown in a number of studies (1, 4, 5). The lack of control of calcium intake in our patients when they were hypercalcemic may be responsible in part for the lack of correlation between plasma 1$_\alpha$,25(OH)$_2$D and serum calcium. Also, two of the patients had diminished renal function, one severely, which would impair the compensatory ability of the kidneys to excrete calcium. Also, 1$_\alpha$,25(OH)$_2$D$_3$ was shown to produce dose-related increases in urinary calcium without causing hypercalcemia in normal subjects and patients. Thus, in these studies there was no correlation between the dose of 1$_\alpha$,25(OH)$_2$D$_3$ and the serum calcium.

Increases in circulating 25-OHD and not 1$_\alpha$,25-(OH)$_2$D are apparently responsible for hypercalcemia in vitamin D intoxication (30). Serum 25-OHD was modestly increased in only one individual (patient G) with sarcoid and hypercalcemia. However, it was far below the range (500 ng/ml or above) reported in patients (30) and rats (31) with vitamin D intoxication. Serum 25-OHD was not increased abnormally in any of the other patients even after they were given vitamin D. Thus, the abnormal calcium metabolism in sarcoid cannot be attributed to this metabolite.

Other investigators have found that prednisone lowers plasma 1$_\alpha$,25(OH)$_2$D. Chesney et al. (32) observed a significant reduction in mean plasma 1$_\alpha$,25(OH)$_2$D in children with glomerulonephritis who were treated with prednisone as compared to the mean value in children who were not given the steroid. Carre et al. (33) demonstrated that prednisone, the biologically active metabolite of prednisone (22, 23), does not alter

the rate of conversion of [^{3}H]25-hydroxyvitamin D_3 to [^{3}H]1_α,25$(OH)_2D_3$ but increases the rate of conversion of [^{3}H]1_α,25$(OH)_2D_3$ to a more polar, biologically inactive metabolite. We interpret our results to mean that prednisone acts in sarcoid by reducing the amount of 1_α,25$(OH)_2$D in the circulation. They do not exclude the possibility that prednisone may also inhibit the peripheral action of 1_α,25$(OH)_2$D (21).

Production of 1_α,25$(OH)_2$D is diminished in renal failure (27, 28). We reported studies in a patient with sarcoid, hypercalcemia, hypercalciuria, and an increased sensitivity to vitamin D who developed transient nephritis (34). Diminished intestinal absorption of calcium, hypocalcemia, and a lack of response to small doses of vitamin D occurred with the onset of renal disease. After recovery, there was recurrence of the changes in calcium metabolism characteristic of sarcoid. In this patient, the defect in vitamin D metabolism characteristic of renal failure was superimposed on the defect produced by sarcoid. The resulting changes in calcium metabolism may have resulted from changes in circulating 1_α,25$(OH)_2$D. The findings in this patient support the concept that renal production of 1_α,25$(OH)_2$D may be important in the pathogenesis of the abnormal calcium metabolism in sarcoid.

In summary, the data presented provide a strong argument that increases in circulating 1_α,25$(OH)_2$D account for the abnormal calcium metabolism in sarcoid. The evidence is as follows: (*a*) mean plasma 1_α,25$(OH)_2$D is significantly increased in patients with hypercalcemia who have suppression of serum PTH, (*b*) mean plasma 1_α,25$(OH)_2$D is brought into the normal range and hypercalcemia is corrected by prednisone, (*c*) vitamin D, in modest doses, markedly increases plasma 1_α,25$(OH)_2$D and urinary calcium (which correlate with each other) in patients with abnormal calcium metabolism but has no effect in normal subjects or in patients with normal calcium metabolism, and (*d*) comparable dose-related increases in urinary calcium are produced by 1_α,25$(OH)_2D_3$.

The modest but significant increases in the plasma 1_α,25$(OH)_2$D and urinary calcium produced by vitamin D in the patients with "normal" calcium metabolism raises the possibility that these individuals might also exhibit abnormalities if challenged with larger doses of the vitamin. Additional studies are warranted to clarify this issue and to determine the cause for the abnormal metabolism of vitamin D in this disorder.

Finally, a number of adult patients are being reported with (*a*) hypercalcemia, suppression of serum PTH, and elevation of plasma 1_α,25$(OH)_2$D, (*b*) no clinical evidence for sarcoidosis, and (*c*) reduction of plasma 1_α,25$(OH)_2$D and correction of hypercalcemia by prednisone (35).[3] It is possible that the mechanism for defective vitamin-D metabolism in these patients may be similar to that in sarcoid. In our view, these individuals may eventually be shown to fall into the category of an acquired form of idiopathic hypercalcemia, a disease more commonly found in infants and children and characterized by an abnormal sensitivity to vitamin D (36, 37).

[3] B. Frame. Personal communication.

ACKNOWLEDGMENTS

We thank Marilyn Quine for capable secretarial assistance.

Supported in part by grants AM 11262, 14881, and M01 RR 750 (Clinical Research Center) and research Career Development Award 4 KO4 AM 70210 from the U. S. Public Health Service.

REFERENCES

1. Anderson, J., E. E. Dent, C. Harper, and G. R. Philpott. 1954. Effect of cortisone on calcium metabolism in sarcoidosis and hypercalcemia, possible antagonistic actions of cortisone and vitamin D. *Lancet.* **II:** 720–724.
2. Henneman, P. H., E. F. Dempsey, E. L. Carroll, and F. Albright. 1956. The cause of hypercalciuria in sarcoid and its treatment with cortisone and sodium phytate. *J. Clin. Invest.* **35:** 1229–1242.
3. Thomas, W. C., H. G. Morgan, T. B. Connor, L. Haddock, C. E. Bills, and J. E. Howard. 1959. Studies of anti-rachitic activity in sera from patients with disorders of calcium metabolism and preliminary observations on the mode of transport of vitamin D in human serum. *J. Clin. Invest.* **38:** 1078–1085.
4. Jackson, W. P. U., and C. Dancaster. 1959. A consideration of the hypercalciuria in sarcoidosis, idiopathic hypercalciuria, and that produced by vitamin D. A new suggestion regarding calcium metabolism. *J. Clin. Endocrinol. Metab.* **19:** 658–680.
5. Bell, N. H., J. R. Gill, Jr., and F. C. Bartter. 1964. On the abnormal calcium metabolism in sarcoidosis: evidence for increased sensitivity to vitamin D. *Am. J. Med.* **36:** 500–513.
6. Hendrix, J. Z. 1966. Abnormal skeletal mineral metabolism in sarcoidosis. *Ann. Intern. Med.* **64:** 797–805.
7. Bell, N. H., and F. C. Bartter. 1967. Studies of 47Ca metabolism in sarcoidosis: evidence for increased sensitivity of bone to vitamin D. *Acta Endocrinol.* **54:** 173–180.
8. Reiner, M., G. Sigurdsson, V. Nunziata, M. A. Malik, G. W. Poole, and G. F. Joplin. 1976. Abnormal calcium metabolism in normocalcaemic sarcoidosis. *Br. Med. J.* **2:** 1473–1476.
9. Ponchon, G., A. L. Kennan, and H. F. DeLuca. 1969. "Activation" of vitamin D by the liver. *J. Clin. Invest.* **48:** 2032–2037.
10. Fraser, D. R., and E. Kodicek. 1970. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature (Lond.).* **228:** 764–766.
11. Holick, M. F., H. K. Schnoes, H. F. DeLuca, T. Suda, and R. J. Cousins. 1971. Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine. *Biochemistry.* **10:** 2799–2804.
12. Raisz, L. G., C. L. Trummel, M. F. Holick, and H. F. DeLuca. 1972. 1,25 dihydroxycholecalciferol: a potent stimulator of bone resorption in tissue culture. *Science (Wash. D. C.).* **175:** 768–769.
13. Connerty, H. V., and A. R. Briggs. 1966. Determination of serum calcium by means of orthocresolphthalein complexone. *Am. J. Clin. Pathol.* **45:** 290–296.

14. Semmler, E. J., M. F. Holick, H. K. Schnoes, and H. F. DeLuca. 1972. The synthesis of 1_{α},25-dihydroxycholecalciferol—a metabolically active form of vitamin D. *Tetrahedron Lett.* **40:** 4147–4150.
15. Belsey, R. E., H. F. DeLuca, and J. T. Potts, Jr. 1974. A rapid assay for 25-OH-vitamin D_3 without preparative chromatography. *J. Clin. Endocrinol. Metab.* **38:** 1046–1051.
16. Dorantes, L. M., S. B. Arnaud, and C. D. Arnaud. 1978. Importance of the isolation of 25-hydroxyvitamin D before assay. *J. Lab. Clin. Med.* **91:** 791–796.
17. Stern, P. H., T. E. Phillips, S. V. Lucas, A. J. Hamstra, H. F. DeLuca, and N. H. Bell. 1977. Bone organ culture bioassay for determination of $1,25(OH)_2D$. *In* Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism. A. W. Norman, K. Schaefer, J. W. Coburn, H. F. DeLuca, D. Fraser, H. G. Grigoleit, and D. V. Herrath, editors. Walter de Gruyter, New York. 531–540.
18. Stern, P. H., A. J. Hamstra, H. F. DeLuca, and N. H. Bell. 1978. A bioassay capable of measuring 1 picogram of 1,25-dihydroxyvitamin D_3. *J. Clin. Endocrinol. Metab.* **46:** 891–901.
19. 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.
20. Stern, P. H., T. Mavreas, C. L. Trummel, H. K. Schnoes, and H. F. DeLuca. 1976. Bone resorbing activity of analogues of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol: effects of side chain modification and stereoisomerization on responses of fetal rat bones *in vitro*. *Mol. Pharmacol.* **12:** 879–886.
21. Stern, P. H. 1969. Inhibition by steroids of parathyroid hormone-induced Ca^{45} release from embryonic rat bone *in vitro*. *J. Pharmacol. Exp. Ther.* **168:** 211–217.
22. Powell, L. W., and E. Axelsen. 1972. Corticosteroids in liver disease: studies on the biological conversion of prednisone to prednisolone and plasma protein binding. *Gut.* **13:** 690–696.
23. Disanto, A. R., and K. A. DeSante. 1975. Bioavailability and pharmakokinetics of prednisone in humans. *J. Pharm. Sci.* **64:** 109–112.
24. Sinha, T. K., S. Miller, J. Fleming, R. Khairi, J. Edmondson, C. C. Johnston, Jr., and N. H. Bell. 1975. Demonstration of a diurnal variation in serum parathyroid hormone in primary and secondary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **41:** 1009–1013.
25. Sinha, T. K., H. F. DeLuca, and N. H. Bell. 1977. Evidence for a defect in the formation of 1_{α},25-dihydroxyvitamin D in pseudohypoparathyroidism. *Metab. Clin. Exp.* **26:** 731–738.
26. Cushard, W. G., Jr., A. B. Simon, J. M. Canterbury, and E. Reiss. 1972. Parathyroid function in sarcoidosis. *N. Engl. J. Med.* **286:** 395–398.
27. Haussler, M. R., D. J. Baylink, M. R. Hughes, P. F. Brumbaugh, J. E. Wergedal, F. H. Shen, R. L. Nielsen, S. J. Counts, K. M. Bursac, and T. A. McCain. 1976. The assay of 1_{α},25-dihydroxyvitamin D_3: physiologic and pathologic modulation of circulating hormone levels. *Clin. Endocrinol.* **5:** 151s–165s.
28. Kream, B. E., J. A. Eisman, and H. F. DeLuca. 1977. Intestinal cytosol binders for 1,25-dihydroxyvitamin D_3: use in competitive binding protein assay. *In* Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism. A. W. Norman, K. Schaefer, J. W. Coburn, H. F. DeLuca, D. Fraser, H. G. Grigoleit, and D. V. Herrath, editors. Walter de Gruyter, New York. 501–510.
29. Kaplan, R. A., M. R. Haussler, L. J. Deftos, H. Bone, and C. Y. C. Pak. 1977. The role of 1_{α},25-dihydroxyvitamin D in the mediation of intestinal hyperabsorption of calcium in primary hyperparathyroidism and absorptive hypercalciuria. *J. Clin. Invest.* **59:** 756–760.
30. Hughes, M. R., D. J. Baylink, P. G. Jones, and M. R. Haussler. 1976. Radioligand receptor assay for 25-hydroxyvitamin D_2/D_3 and 1_{α},25-dihydroxyvitamin D_2/D_3: application to hypervitaminosis D. *J. Clin. Invest.* **58:** 61–70.
31. Queener, S. F., and N. H. Bell. 1976. Treatment of experimental vitamin D_3 intoxication in the rat with cholestyramine. *Clin. Res.* **24:** 583A. (Abstr.)
32. Chesney, R. W., R. B. Mazess, A. Hamstra, H. F. DeLuca, and S. O'Reagan. 1978. Reduction of serum-1,25-dihydroxyvitamin-D_3 in children receiving glucocorticoids. *Lancet.* **II:** 1123–1125.
33. Carre, M., O. Ayigbede, L. Miravet, and H. Rasmussen. 1974. The effect of prednisolone upon the metabolism and action of 25-hydroxy and 1,25-dihydroxyvitamin D_3. *Proc. Natl. Acad. Sci. U. S. A.* **71:** 2996–3000.
34. Bell, N. H., and F. C. Bartter. 1964. Transient reversal of hyperabsorption of calcium and of abnormal sensitivity to vitamin D in a patient with sarcoidosis during episode of nephritis. *Ann. Intern. Med.* **61:** 702–710.
35. Schaefer, P. C., M. D. Lifschitz, S. Z. Fadem, and R. S. Goldsmith. 1978. Radioimmunoassay of 1_{α},25-dihydroxycholecalciferol in patients with renal disease. *In* Program of the Endocrine Society. 320. (Abstr.)
36. Fellers, F. X., and R. Schwartz. 1958. Etiology of severe form of idiopathic hypercalcemia of infancy; a defect in vitamin D metabolism. *N. Engl. J. Med.* **259:** 1050–1058.
37. Smith, D. W., R. M. Blizzard, and H. E. Harrison. 1959. Idiopathic hypercalcemia: a case report with assays of vitamin D in the serum. *Pediatrics.* **24:** 258–269.