JCI The Journal of Clinical Investigation

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J Clin Invest. 1968;47(10):2239-2252. https://doi.org/10.1172/JCI105909.

Research Article

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The Metabolic Fate of Vitamin D_3-^3H in Chronic Renal Failure

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ABSTRACT The absorption and metabolism of vitamin D_3-^3H was studied in eight patients with chronic renal failure. Although the intestinal absorption of vitamin D_3-^3H was normal, the metabolic fate of the vitamin was abnormal as characterized by a twofold increase in fractional turnover rate, an abnormal accumulation of biologically inactive lipid-soluble metabolites, and the urinary excretion of both vitamin D_3-^3H and biologically inactive metabolites. Neither alterations in water-soluble vitamin D_3 metabolites nor qualitative abnormalities in protein-binding of vitamin D_3 were observed in the uremic subjects. Although hemodialysis proved ineffectual in reversing the observed abnormalities in vitamin D_3 metabolism and excretion, renal homotransplantation was completely successful in this regard. These experiments support the conclusion that the resistance to therapeutic doses of vitamin D often seen in patients with chronic renal failure and renal osteodystrophy results from an acquired defect in the metabolism and excretion of vitamin D.

INTRODUCTION

The occurrence of skeletal abnormalities in patients with chronic renal disease (renal osteodystrophy) and the destructive effects of secondary hyperparathyroidism and chronic acidosis on bone in uremia have been well established (1–5). Recent observations by Dent, Harper, and Philpot (2) and Stanbury and Lumb (3, 4) suggest that renal osteodystrophy also represents a form of "acquired vitamin D–resistant" bone disease. It seems unlikely that secondary hyperparathyroidism accompanying chronic renal failure causes the vitamin D resistance since, in uremic patients, subtotal parathyroidectomy sufficiently complete to produce hypoparathyroidism fails to alter the resistance to vitamin D (6, 7). The nature of this proposed vitamin D resistance still remains one of the outstanding problems of uremic osteodystrophy. Since vitamin D and its metabolites act primarily by stimulating calcium and phosphate transport in bone, intestine, and kidney (8), any defect or alteration in its metabolic conversion may contribute to the abnormalities in mineral metabolism often cited in patients with renal osteodystrophy. The latter include: (*a*) defective intestinal absorption of calcium; (*b*) defective bone mineralization and metabolism; and (*c*) resistance to doses of vitamin D which are normally therapeutic. These observations suggest that chronic renal failure with its attendant prolonged uremic state may either result in the interference with the biological activity of vitamin D or alter its metabolism in such a way as to render it biologically inactive.

The recent availability of high specific activity preparations of radioactive vitamin D (8), as well as technical developments which facilitate the isolation of vitamin D and its metabolites from biological fluids of man (9, 10), now permit an analysis of vitamin D metabolism in chronic renal failure. Recent developments in hemodialysis and renal transplantation also offer an opportunity to evaluate the effect of temporary amelioration and

Received for publication 1 April 1968 and in revised form 22 May 1968.

(or) complete reversal of the uremic state on the metabolism of vitamin D in man. The present study with radioactive vitamin D_3 provides evidence that the metabolism and excretion of vitamin D is abnormal in patients with renal disease, and that these abnormalities can be reversed by renal homotransplantation.

METHODS

All subjects were studied on a Clinical Research Center after 14 days of adaptation to diets containing 800 U of vitamin D per day. Eight adults, 21–40 yr of age, with either chronic pyelonephritis or chronic glomerular nephritis (chronic renal disease, CRD) characterized by inulin clearances ranging from 1.7 to 11.4 ml/min, azotemia, hyperphosphatemia, proteinuria (Table I), and X-ray evidence of generalized skeletal demineralization and (or) osteitis fibrosa, and five normal, healthy adult volunteers of similar ages (29–32 yr) were used for these studies. None of the patients with CRD had previously received pharmacologic doses of vitamin D therapeutically. Two additional patients (J.P. and C.M., Table I) were restudied 1 month after renal homotransplantation. Another patient with CRD (T.D., Table I) was restudied after 5 consecutive days of continuous 10-hr periods of hemodialysis when blood urea nitrogen, calcium, inorganic phosphate, chloride, sodium, potassium, CO_2, and arterial pH had reverted toward normal. Finally, to evaluate the effect of proteinuria on the metabolism of vitamin D_3, D_3-^3H studies were also employed in a patient with severe proteinuria without uremia (G.S., Table I).

1 hr before breakfast and after a 12–15-hr overnight fast, 8–10 μc of vitamin D_3 1,2-^3H (D_3-^3H) with a specific activity of 110 μc/mg, prepared and purified by the method of Neville and DeLuca (11), was dissolved in 1.1 ml of absolute ethanol and delivered onto the tongue from a calibrated syringe. The D_3-^3H was then rinsed down with 250 ml of milk. Blood samples were withdrawn into heparinized containers at 5, 15, 30, and 45 min and at 1, 2, 4, 8, 12, 16, and 24 hr. Blood samples were then obtained at 24-hr intervals for the subsequent 5 days. Urine was collected in 24-hr pools for 3 consecutive days, refrigerated, and subsequently lyoph-

TABLE I

Clinical Data

Subjects	Age	Sex	Inulin clearance	Serum CO_2	Serum alkaline phosphatase	Arterial pH	Blood urea nitrogen	Serum calcium	Serum phosphorus	Urinary protein
			ml/min	*mEq/liter*	*King-Armstrong Units*		*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*g/24 hr*
Chronic renal disease										
J. T.	35	M	8.0	21	10		110	9.2	5.5	3.1
T. D.	21	M	2.3	16	22	7.27	196	8.2	9.5	2.2
J. P.	26	F	1.3	18	12	7.34	90	8.5	9.0	3.5
D. K.	40	M	11.4	17	17	7.33	142	9.0	5.3	2.5
S. S.	21	F	4.5	19	96	7.33	87	11.1	6.0	2.5
A. W.	21	F	8.0	20	4	7.32	55	10.3	5.7	3.3
C. M.	27	F	1.7	21	14	7.31	105	9.3	5.6	2.0
C. B.	27	M	6.0	16	96	7.36	122	9.4	8.6	1.2
Nephrotic syndrome										
G. S.	37	F	90.4	26	10	7.38	20	9.0	3.9	8.0
After renal transplantation										
J. P.	26	F	70.0	25	18	7.36	19	11.2	1.7	0.0
C. M.	27	F	43.6	24	14	7.34	28	10.9	2.2	0.0
After chronic dialysis										
T. D.	21	M	1.8	23	15	7.40	15	10.5	2.0	1.0
Normal subjects										
B. G.	31	M	120*	25	9		14	9.9	3.5	0.0
C. W.	29	M	114*	27	10		17	9.7	3.3	0.0
R. M.	32	M	130*	24	7		13	9.5	2.9	0.0
D. W.	29	F	110*	22	8		9	9.8	3.7	0.0
G. J.	31	F	126*	28	12		12	10.0	3.9	0.0

* Clearance of endogenous creatinine.

ilized. Cumulative fecal collections were made in 3-day pools for 6 days, homogenized with water in a Waring blender, and aliquots lyophilized. All fecal collections were initiated and terminated with enemata. Inulin clearance measurements were made according to methods previously described for this laboratory (12).

Determination of total ^3H in biological samples. Samples of whole plasma, lyophilized feces, and urine were combusted in a 2 liter Erlenmeyer flask by the procedures described previously (9, 10). After the resulting ^3H water vapor was frozen by immersing the flask in a dry ice–acetone bath, 20 ml of liquid scintillation counting solution A was added; 18 ml was then withdrawn and analyzed for radioactivity.

Extraction of radioactivity. Lipid-soluble radioactivity present in plasma, urine, and feces was extracted with methanol and chloroform by the procedure of Bligh and Dyer as previously described (9, 10). After separation of phases the water-methanol phase was reextracted twice with chloroform. Chloroform extracts were combined and concentrated by evaporation with nitrogen gas and 1–2-ml aliquots added to counting solution B and assayed for radioactivity. 2 ml of the aqueous extract was added to counting solution C and also assayed for radioactivity.

Chromatography. Silica gel G thin-layer chromatography of chloroform-soluble lipid extracts of plasma, urine, and feces were performed in a solvent of 10% acetone in *n*-hexane (v/v) as previously described (9, 10). After they were dryed, the plates were sprayed with 0.20% $KMnO_4$ in 1% Na_2CO_3 to locate the marker vitamin D. The radioactivity was then determined by scraping off successive 0.5 cm segments of silicic acid with a microscopic slide and the scrapings placed in a counting vial containing liquid scintillation counting solution B. The radioactivity migrating with the same R_f as the stable vitamin D markers was labeled "radioactive vitamin D_3." The radioactivity in the "vitamin D_3 band" was calculated either as a percentage of the administered dose or as a percentage of the total radioactivity in the combusted samples.

Concentrated chloroform extracts of 24-, 48-, and 72-hr plasma samples and of cumulative 3-day urine and 6-day fecal samples were redissolved in Skellysolve B [1] (a petroleum fraction boiling at 65–67°C) for application to multibore silicic acid columns. The columns and elution gradients used have been previously described (9, 10). 10-ml fractions from the columns were dried in a stream of air and redissolved in counting solution B for detection of radioactivity.

Electrophoresis. Electrophoresis of serum and urine was conducted according to a modification of previously published starch-block electrophoretic techniques (13). A 1:1 mixture of Geon [2] (polyvinyl chloride particles) and Pevicon [3] (polyvinyl chloride and polyvinyl acetate copolymers) was used in place of starch as the supporting medium. Electrophoresis of 10 ml of serum and 10-ml aliquots of lyophilized urine was conducted on the prepared polyvinyl blocks ($25 \times 38 \times 1.5$ cm) at 4°C with barbital buffer at pH 8.6. With a constant power source of 300 v and 50 ma, a 35-cm migration was usually obtained in 45–50 hr. The polyvinyl block was then divided into 1 cm strips which were placed in individual centrifuge tubes and extracted with 5 ml of cold 0.9% sodium chloride. The contents of each tube were centrifuged and the supernatant removed. The polyvinyl residue was then reextracted twice with 3-ml portions of cold 0.9% sodium chloride, the extracts pooled, and aliquots assayed for protein by measurements of optical density at 280 mμ and for radioactivity by liquid scintillation with counting solution C. In selected instances aliquots of the saline extracts of the polyvinyl residue were condensed by negative pressure ultrafiltration and the nature of the proteins migrating with the radioactive peak in the polyvinyl blocks examined by immunoelectrophoretic techniques according to conventional methodology (13).

Measurement of radioactivity. Counting solution A was used to measure the radioactivity of combusted samples, urine, and feces; the solution consisted of 40 mg of dimethyl-POPOP (1,4-bis [2,5-phenyloxazoly]) benzene, 4.0 g of PPO (2,5-diphenyloxazole), 200 ml of absolute ethanol, and 800 ml of toluene. Counting solution B, used to measure the tritium content of samples containing organic solvents, consisted only of 3.0 g of PPO and 100 mg of dimethyl-POPOP per liter of toluene. Counting solution C, used to measure tritium in aqueous extracts, was prepared according to the method of Bray (14). Radioactivity was detected with a Packard Tri-Carb liquid scintillation counter model 3000, equipped with external standardization for determination of counting efficiency.

Bioassay. Various pooled fractions obtained from silicic acid column chromatography of lipid extracts of plasma, fecal, and urine samples were concentrated to dryness in a flash evaporator or by a stream of nitrogen and then dissolved in diethyl ether. 10-ml samples of the ether solution were evaporated on the diet of rachitic rats used for the assay. The vitamin D antirachitic biological assays were subsequently performed according to official USP procedures (15). Silicic acid column fractions were also bioassayed for intestinal calcium transport activity with oxygen-dependent accumulation of ^{45}Ca by duodenal slices obtained from rachitic rats as described by Schachter, Dowdle, and Schenker (16). The oxygen-dependent accumulation of calcium (μmole/g slice) was calculated from the expression: [^{45}Ca concentration in slices under O_2 (count/min per g) − ^{45}Ca concentration in slices under N_2 (count/min per g)] × 1/[initial specific activity of ^{45}Ca in the medium (count/min per μmole of Ca)].

RESULTS

Disappearance and metabolic fate of vitamin D_3 in plasma. After the oral dose of D_3-^3H, plasma D_3-^3H rose gradually, attaining peak levels within 8–9 hr in all subjects and decreasing exponentially

[1] Skelly Oil Co., Tulsa, Okla.

[2] B. F. Goodrich Chemical Co., Akron, Ohio.

[3] Mercer Chemical Company, New York.

thereafter for the remainder of the 6 day study. As noted in Table II, peak plasma D_3-^3H levels were in each instance lower than normal in patients with CRD. The mean ± SE plasma D_3-^3H half-times for normal and CRD subjects were 27.8 ± 1.0 and 13.2 ± 1.1 hr respectively. This difference was highly significant with $P < 0.001$. The plasma D_3-^3H half-time in the patient with the nephrotic syndrome (G.S., Table I) was within normal limits at 29.0 hr. After renal homotransplantation, plasma D_3-^3H half-times reverted to normal in patients J.P. (15.5–28.0 hr) and C.M. (10.6–24.0 hr). No significant change in D_3-^3H disappearance was noted in T.D. after the 5 day period of hemodialysis (8.4–10.4 hr). Comparative analysis of total radioactivity in whole plasma and chloroform-soluble plasma extracts thereof in both normal and CRD subjects were similar with over 97% of the plasma radioactivity in the chloroform-soluble phase in each instance.

TABLE II

Plasma Half-Time (in Hours) of Vitamin D_3-^3H in Normal and Uremic Subjects*

Subjects	Half-time	Plasma D_3-^3H peak levels
		% dose/ liter plasma
Chronic renal disease		
J. T.	11.2	3.1
T. D.‡	8.4‡	1.5
J. P.§	15.5§	2.2
D. K.	12.3	1.7
S. S.	17.7	3.5
A. W.	14.1	2.9
C. M.‖	10.6‖	1.7
C. B.	16.0	3.5
Mean	13.2¶	2.5
±SE	±1.1	±0.3
Normal		
B. G.	26.4	5.3
C. W.	25.0	4.9
R. M.	30.5	6.1
D. W.	27.8	5.8
G. J.	29.2	6.4
Mean	27.8	5.7
±SE	±1.0	±0.3

* The radioactivity migrating with the same R_f as standard crystalline nonradioactive vitamin D_3 markers on thin-layer plates was labeled "radioactive vitamin D_3." Half-times were calculated by analysis of semilogarithmic plots of plasma "radioactive vitamin D_3" disappearance with time for the interval beginning at 8–9 hr (peak levels of D_3-^3H plasma concentration) and terminating 6 days after D_3-^3H administration.

‡ Half-time after chronic hemodialysis, 10.4 hr.

§ Half-time after renal transplantation, 28.0 hr.

‖ Half-time after renal transplantation, 24.0 hr.

¶ $P < 0.001$.

The results of silicic acid chromatography of chloroform extracts obtained from 48-hr plasma samples in normal and CRD subjects are summarized in Table III. In each instance four fractions were clearly separated. The material in peak 3 has been isolated previously from human plasma after D_3-^3H administration and identified as unaltered vitamin D_3 with potent antirachitic and intestinal biological activity (9). Peak 4, although unidentified structurally, has also been isolated from human plasma and shown to possess antirachitic activity (9), as well as a remarkable ability to restore active calcium transport mechanisms in the duodenal slices of rachitic rats (17). Since peak 4 is as biologically active as the parent vitamin in healing experimental rickets in rats and more potent than vitamin D_3 in stimulating the intestinal transport of calcium (17), this material may represent the metabolically active form of the vitamin. Peak 5 is still unidentified but, as shown in Table IV, it is biologically inert. Peak 2 has never been found in large amounts in animals or human subjects and is probably of limited biological significance. Its structure is presently unknown but, by means of saponification and cochromatography, it has been shown to be different from previtamin D and 5,6-*trans*-vitamin D (8). Although 57.7 ± 3.2% of the chromatographed lipid-soluble material in 48-hr plasma samples was normally present in peak 3 (Table III), only 21.6 ± 4.6% of the chloroform-soluble material could be identified as unaltered vitamin D in CRD subjects. At 48 hr, 38.3 ± 3.1 and 1.5 ± 0.6% of the chromatographed radioactivity was normally distributed between peaks 4 and 5 respectively. The metabolites which chromatographed as peak 4 and peak 5 were significantly increased in CRD subjects averaging 65.8 and 10.9% of the chromatographed chloroform-soluble radioactivity respectively. As illustrated in a normal subject in Fig. 1 and a CRD subject in Fig. 2, the abnormal distribution of plasma chloroform-soluble radioactivity noted at 48 hr was also present at 24 hr

TABLE III

*Distribution of Plasma Chloroform Extracts during Silicic Acid Column Chromatography**

Subject	Peak 2	Peak 3	Peak 4	Peak 5
		% total radioactivity		
Chronic renal disease				
J. T.	2.2	10.5	67.3	20.0
T. D.	2.0	13.5	71.5	13.0
J. P.	3.0	25.7	63.1	8.2
D. K.	3.1	14.4	75.0	7.5
S. S.	0.0	47.4	45.2	7.4
A. W.	0.2	32.3	63.7	3.8
C. M.	2.0	20.0	67.0	11.0
C. B.	1.6	8.7	73.4	16.3
Mean	1.8‡	21.6§	65.8§	10.9§
±SE	±0.4	±4.6	±3.3	±1.9
Nephrotic syndrome				
S. S.	5.0	55.7	36.0	3.3
After renal transplantation				
J. P.	1.5	57.0	38.5	3.0
C. M.	0.5	58.0	35.5	6.0
After chronic hemodialysis				
T. D.	6.4	18.5	67.1	8.0
Normal subjects				
B. G.	3.0	62.0	34.0	1.0
C. W.	2.4	53.2	44.4	0.0
R. M.	2.2	68.0	28.6	1.2
D. W.	4.0	54.5	39.5	2.0
G. J.	1.0	50.6	45.0	3.4
Mean	2.5	57.7	38.3	1.5
±SE	±0.5	±3.2	±3.1	±0.6

* The chloroform extracts of 10 ml of plasma obtained 48 hr after an oral dose of vitamin D_3-^3H. The extracts were chromatographed on multibore silicic acid columns with a gradient of diethyl ether in petroleum ether (9). 10 ml fractions were collected. Peak 2 represents fractions 10–12; peak 3, fractions 12–20; peak 4, fractions 38–47; peak 5, fractions 58–64.
‡ $P > 0.2 < 0.4$.
§ $P < 0.001$.

and intensified at 72 hr after the oral administration of D_3-^3H. As noted in Table IV and Fig. 3, unlike material which chromatographed as peak 4 in normal subjects, the peak 4 "metabolite" isolated from the plasma of CRD patients had limited biological activity on bone and intestine when assayed in identical concentrations as measured by radioactivity (disintegrations per minute)-vitamin D_3 equivalents.

The decreased biological potency of peak 4 substance extracted from the plasma of CRD subjects suggested that the substance was not homogeneous and contained more than one component with varied biological potencies. The material in peak 4 isolated from the uremic patients was subjected to acid and alkaline hydrolysis, β-glucuronidase hydrolysis, and saponification with 10% KOH in methanol. After these hydrolytic and saponification procedures the respective reaction mixtures were extracted with ether or chloroform and rechromatographed on multibore silicic acid columns as described above. Neither the hydrolytic nor the saponification procedures resulted in the liberation of free D_3-^3H, a finding which suggests that the biologically inactive components of peak 4 in uremic plasma were not merely conjugates of

TABLE IV

*Biological (Anti-Rachitic) Activity of Fractions from Normal and Uremic Plasma, Urine, and Feces**

Fraction	No. rats in assay	Calcification score
Plasma		
Peak 3 (normal)	9	7 ± 1.0
Peak 3 (uremic)	9	7 ± 0.5
Peak 4 (normal)	9	9 ± 0.5
Peak 4 (uremic)	9	2 ± 0.5
Peak 5 (normal)	9	0
Peak 5 (uremic)	9	0
Urine		
Peak 3 (uremic)	9	6 ± 1.0
Peak 4 (uremic)	8	3 ± 1.0
Peak 5 (normal)	9	0
Peak 5 (uremic)	9	0
Feces		
Peak 3 (normal)	7	6 ± 0.5
Peak 3 (uremic)	9	7 ± 1.0
Vitamin D standard (4.0 i.v./rat)	9	7 ± 1.0

* Fractions refer to those illustrated in Figs. 1 and 2. All fractions were bioassayed by the line test technique (15). The amount of each fraction applied to the diet of rachitic rats was estimated on the basis of the specific activity of the parent vitamin D_3-^3H: 242,000 dpm = 1 μg.

vitamin D_3. Ultrafiltrates [4] of sera obtained from normal and uremic patients 48 hr after oral D_3-^3H administration were also extracted according to the procedures of Bligh and Dyer as previously published (9). The chloroform extracts obtained were chromatographed on multibore silicic acid columns as outlined above. Plasma ultrafiltrates from both normal and uremic sera were found to be free of any substance migrating as peak 3 or peak 4, whereas peak 5 concentrations were appreciable in both instances.

When compared to normal subjects, the plasma of CRD patients contained, on the average, seven times the amount of material which chromatographed as peak 5 (Table III). As illustrated in Table IV, peak 5 obtained from the plasma of either normal or CRD subjects demonstrated no biological potency. Although renal transplantation resulted in a complete reversal of the deranged pattern of plasma chloroform–soluble vitamin D_3 metabolites (J.P. and C.M., Table III), hemodialysis was ineffectual in this regard (T.D., Table III).

Distribution of radioactivity and protein after electrophoresis. Electrophoretic patterns of the serum in normal and uremic subjects are shown in Fig. 4. In each instance four definite protein peaks were separated. During polyvinyl-block electrophoresis radioactivity migrated in two separate peaks which, in both uremic and normal plasma, coincided with the large rapidly moving peak D and a smaller less rapidly moving peak C (Fig. 4). Immunoelectrophoresis showed the presence of albumin in peak D and a combination of albumin, α1- and α2-globulins in peak C. As illustrated for D.K. in Fig. 5, radioactivity distribution patterns in the plasma and urine of patients with renal disease and proteinuria were remarkably similar. Saline extracts of polyvinyl strips containing the radioactivity of normal and uremic plasma and that of uremic urine were homogenized with methanol and chloroform by the Bligh and Dyer procedures described above. The chloroform-soluble radioactivity was applied to multibore silicic acid columns and chromatographed using the same elution gradient system as described above for whole plasma and urine. In each instance the chloroform-soluble radioactivity of the polyvinyl strips was composed of material which migrated as peak 3 and peak 4.

Urinary excretion. As noted in Table V, the mean cumulative urinary excretion of radioactivity for the 72-hr period after the oral D_3-^3H in five control subjects was 1.69% of the administered dose (range 1.25–2.00). As reported previously in studies describing the fate of an intravenous dose of D_3-^3H (9), urinary radioactivity in the five normal subjects was mainly water-soluble with an average of 8.7% of the radioactivity soluble in chloroform. In these same normal subjects free vitamin D_3 was also conspicuously absent from urine, and the chloroform-soluble radioactivity consisted entirely of the highly polar inactive peak 5 metabolite in each case (Table V). The total 72-hr urinary radioactivity in CRD subjects averaged 0.34% of the administered dose (range 0.09–0.62), and more than 70% of the

[4] Ultrafiltrates were obtained from serum by anerobic techniques utilizing an Araflo Ultrafiltrate Apparatus (Applied Research Associates, New York) with nitrogen filtration pressure approximating 300 lb.

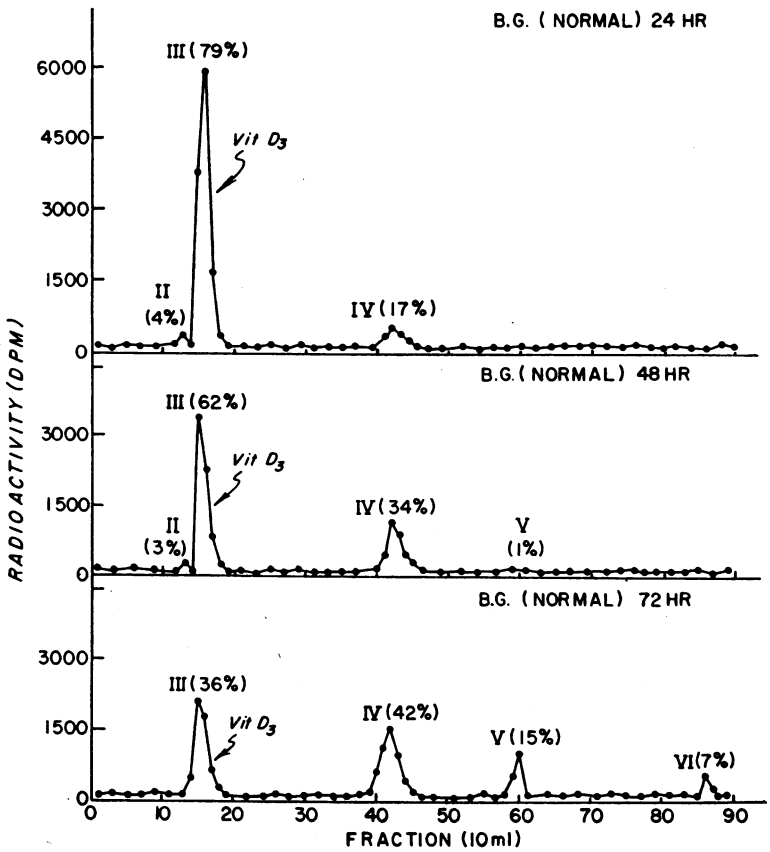

FIGURE 1 Column chromatographic profile of chloroform extracts of 10 ml of plasma obtained in a normal subject 24, 48, and 72 hr after an oral dose of D_3-^3H. The chloroform extracts were chromatographed on silicic acid columns as previously described, using gradient elution techniques (9, 10).

excreted radioactivity was chloroform-soluble (Table V). In each instance biologically active peak 3 (29.4 ± 7.4) and peak 4 (48.2 ± 8.7) constituted a significant portion of the chloroform-soluble radioactivity (Tables IV and V). 1.28% of the administered radioactivity was recovered in the urine of G.S. (nephrotic syndrome) with 24% lipid-soluble of which 54.8 and 36% represented peak 3 and peak 4 respectively. As illustrated in Table V, renal transplantation in patients J.P. and C.M. resulted in a disappearance of urinary peaks 3 and 4 with all the lipid-soluble radioactivity identified as biologically inactive peak 5. In contrast, no significant alteration in the abnormal D_3-^3H metabolite excretory pattern was observed in T.D. after a period of chronic hemodialysis.

Absorption and fecal excretion. D_3-^3H normally accounted for 71.6 ± 2.5 and 77.1 ± 2.2% of the chloroform-soluble fecal radioactivity in normal and CRD subjects respectively (Table VI). Silicic acid column chromatography of chloroform extracts was carried out in all subjects and resulted in a mean recovery of material which chromatographed as D_3-^3H of 9.8 ± 0.8% of the administered dose in normal subjects and 10.7 ± 0.7% in CRD subjects. In each case the material isolated in peak 3 proved to be biologically active (Table IV). The "net" absorption of D_3-^3H, calculated by assuming that the D_3-^3H not recovered in the feces had been absorbed, normally averaged 90.2% (range 87.3–91.7). The distribution of fecal radioactivity and "net" D_3-^3H absorption in CRD subjects did not significantly deviate from normal values (Table VI).

DISCUSSION

Many investigators have documented an impaired intestinal absorption of calcium in patients with chronic renal failure using either classical metabolic balance or isotopic marking techniques (1, 3, 18–20). A decreased intestinal absorption of calcium has also been noted in patients with chronic renal disease without manifest azotemia (21) or acidosis (3). Kessner and Epstein have

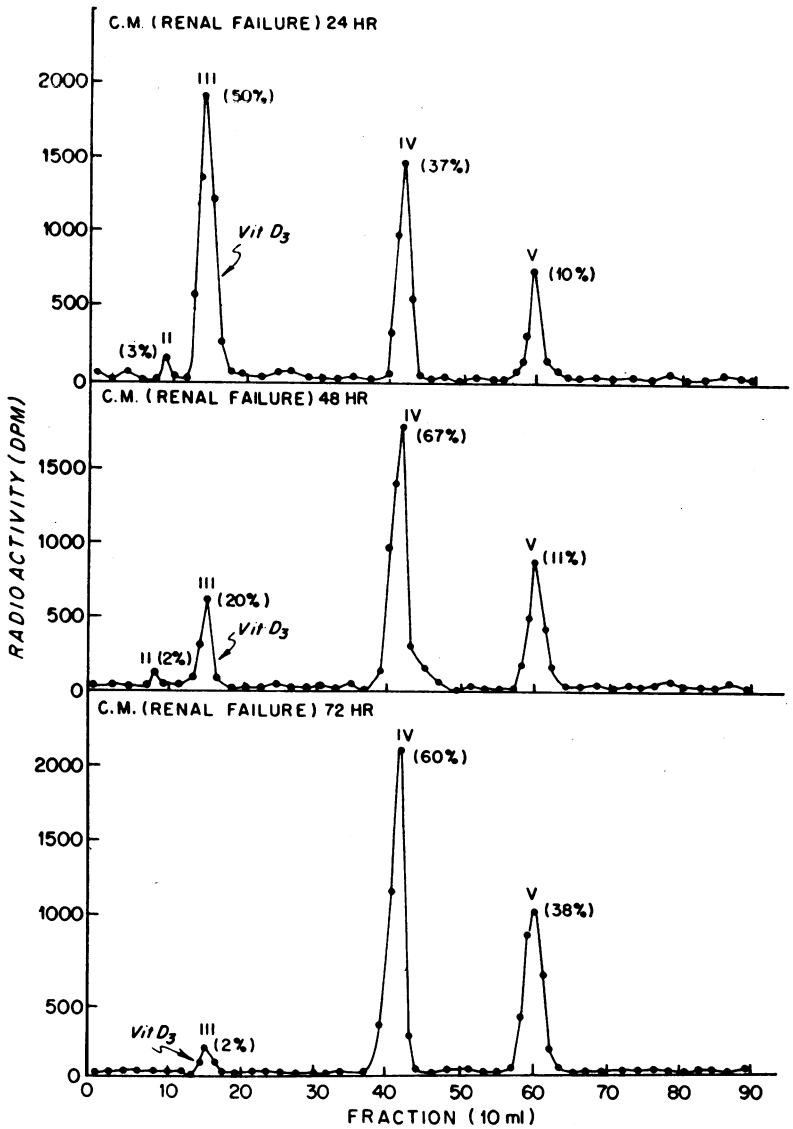

FIGURE 2 Column chromatographic profile of chloroform extracts of 10 ml of plasma obtained in C.M., a patient with chronic renal failure, 24, 48, and 72 hr after an oral dose of D_3-^3H. Chromatographic techniques used were identical with those described for Fig. 1.

subsequently confirmed these observations in animals demonstrating a defect in intestinal calcium transport in chronic experimental renal insufficiency which could not be reproduced by acidosis or uremia of short duration (22). Liu and Chiu initially considered a "resistance" to vitamin D action in the uremic individual as a cause for defective intestinal calcium absorption (1). Stanbury and Lumb (3, 4) and Dent and coworkers (2), commenting on the lack of bone healing in uremic patients given small doses of vitamin D, have also suggested that the "acquired vitamin D resistance" results primarily in the defective absorption of calcium from the gastrointestinal tract. The subsequent studies of Fletcher, Jones, and Morgan made during the therapeutic trials of alkali, aluminum hydroxide, vitamin D, and calcium supplements in uremic osteodystrophic patients appear confirmatory in this regard (5).

The present studies, describing the metabolic

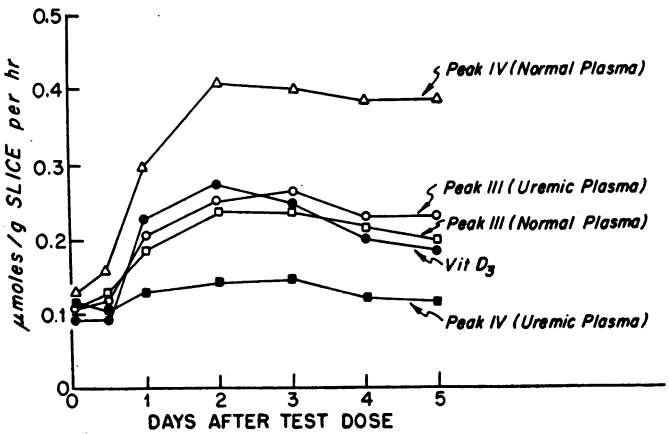

FIGURE 3 O_2-dependent accumulation of Ca by duodenal slices at various intervals after 0.25 IU of crystalline vitamin D_3, and amounts of peak 3 isolated from normal, □—□, and uremic, ○—○, plasma, and peak 4, isolated from normal, △—△, and uremic, ■—■, plasma corresponding to 0.25 IU of vitamin D_3. The amount of each compound tested was estimated on the basis of the specific activity of the parent D_3-^3H: 242,000 dpm = 1 µg. Duodenal slices from vitamin D–depleted rats were tested using the experimental methods described by Schachter and coworkers (16), except that the incubation medium was modified to contain 2×10^{-4} M $CaCl_2$ and 0.004 M KCl.

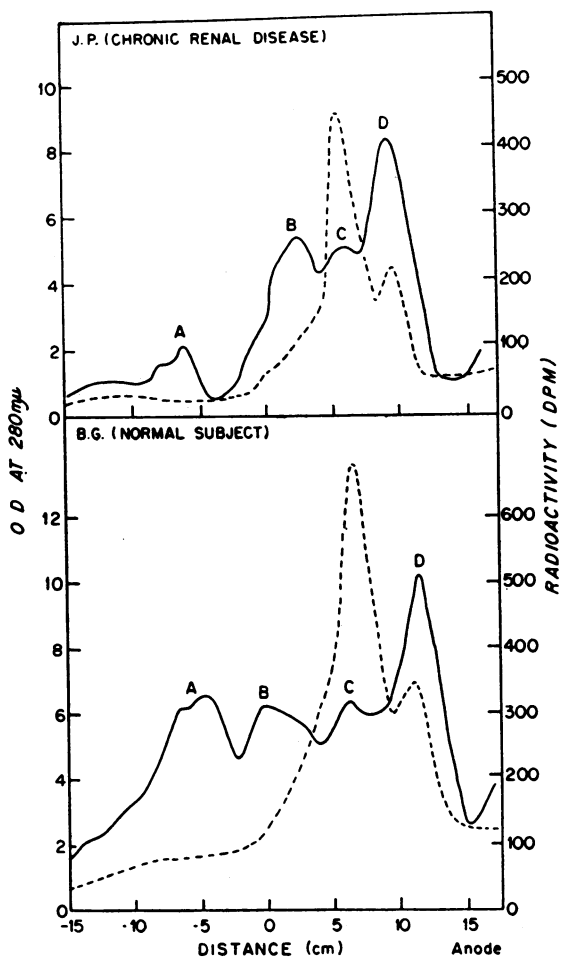

FIGURE 4 Polyvinyl-block electrophoretic patterns obtained from the plasma of a normal subject (B.G.) and a patient with chronic renal disease (J.P.). The solid line represents absorption at 280 mµ and the dashed line, radioactivity.

fate of a relatively large oral dose of vitamin D_3, demonstrate a distinct derangement in vitamin D metabolism in patients with chronic renal disease as manifested by an increase in the fractional turnover rate of vitamin D_3 in plasma, an increased formation of biologically inactive vitamin D_3 metabolites, and an abnormal urinary excretion of both vitamin D and biological active metabolites. The normal absorption of vitamin D_3 observed in CRD subjects (Table VI) is consistent with prevailing opinions (1, 3) and other unpublished experience from this laboratory documenting the failure of parenteral vitamin D therapy to promote bone healing in renal osteodystrophy when oral therapy has proved ineffectual. The isolated peak 4 metabolite rapidly appears in the plasma of human subjects as vitamin D_3 is metabolized (Fig. 1). Moreover, peak 4 is normally much more efficient than the parent vitamin D in inducing calcium transport by the intestine (Fig. 3), is biologically active in promoting healing of rachitic bone (Table IV), and is repeatedly found in the blood of normal human subjects (9, 10), patients with familial vitamin D–resistant rickets (10), and in the tissues of rachitic chicks given vitamin D_3-^3H (8). These data are consistent with the concept that a peak 4 metabolite is the metabolically active form of vitamin D_3. The paradoxical decreased biological activity of peak 4 isolated from the plasma of CRD subjects is consistent with a biochemical heterogeneity of peak 4 in chronic renal disease and the formation of abnormal amounts of a biological inactive vitamin D_3 metabolite (or metabolites) with structures so similar to the biologically potent metabolite in

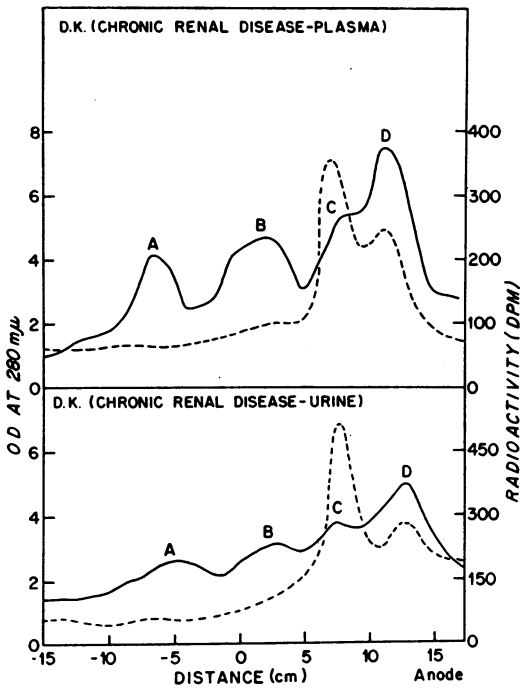

FIGURE 5 Polyvinyl-block electrophoretic patterns obtained from the plasma and urine of a subject (D.K.) with chronic renal disease. The solid line represents absorption at 280 mμ and the dashed line, radioactivity.

peak 4 that biochemical separation could not be achieved by the chromatographic techniques utilized. Recent work by Neville and DeLuca (8), indicating that the peak 4 fraction in normal rats is also not homogeneous, suggests that the biochemical changes observed in the uremic syndrome results in an abnormally high concentration of a biologically inactive metabolite(s) in peak 4 which, like peaks 2 and 5 (Table III), may be normally present in insignificant amounts.

The present study suggests that the observed decreased biological activity of peak 4, increased formation of impotent peak 5, and rapid turnover and abnormal excretion of vitamin D_3 in CRD subjects alter the physiological expression of the vitamin and its active metabolite and results in both the diminished intestinal absorption of calcium and the defective mineralization of bone. The cause for the observed derangement of vitamin D_3 metabolism in these subjects is presently still conjectural. The transport of vitamin D in blood has been studied in considerable detail in animals (23–25) and man (26–27). There is general agreement that the vitamin and its metabolite(s) are bound to albumin, α-globulins, and lipoproteins in stable and nonultrafilterable forms. These observations combined with reports of Schrade, Böhle, and Becker (28) and Klahr, Tripathy, and Bolanos (29), demonstrating lipiduria and lipoproteinuria in patients with chronic renal disease as well as other studies revealing abnormal protein synthesis in azotemic patients (30) and low molecular weight (α-globulin) proteinuria in chronic renal disease (31), led to a qualitative electrophoretic analysis of plasma and urinary proteins in subjects with CRD after D_3-^{3}H administration. As noted in Fig. 4, the plasma radioactivity (which proved to be comprised of peak 3 and peak 4) migrated in both normal and CRD subjects with proteins identified immunologically as albumin and α-globulins. Moreover, as illustrated for D.K. in Fig. 5, plasma and urinary protein-radioactivity relationships were similar in the same uremic individuals. These findings collectively suggest that the abnormal urinary excretion of vitamin D_3 and peak 4 observed in CRD subjects was due primarily to the associated proteinuria.

It is our present opinion that the proteinuria and associated renal "leak" of biologically active substances contained in peak 3 and peak 4, albeit characteristic of chronic renal failure, cannot account for the observed rapid disappearance of D_3-^{3}H from the plasma of these patients. Identical D_3-^{3}H studies performed in G.S. (Table I), a patient with the nephrotic syndrome without uremia, but excreting 8.0 g of protein per day, revealed a normal plasma D_3-^{3}H half-time of 29.0 hr and a normal plasma distribution of vitamin D_3-^{3}H and its metabolites at 24, 48, and 72 hr (Table III) despite an obvious urinary loss of vitamin D_3 (Table V). In this case, electrophoretic analysis of plasma and urine also revealed peak 3 and peak 4 migrating with both albumin and the lower molecular weight α-globulins. This isolated observation suggests that the biochemical derangements associated with uremia and prolonged renal insufficiency may be necessary prerequisites for the induction of vitamin D "resistance."

Many of the abnormalities present in patients with chronic renal disease such as anemia (32), hypertension (33), glucose intolerance (34), thrombocytopenia (35), encephalopathy (36), and polyneuropathy (37) are often improved by dialy-

TABLE V

Cumulative 72-Hr Urinary Radioactivity in Normal and CRD Subjects

Patient	Total ^3H	Lipid-soluble radioactivity	Peak 3*	Peak 4*	Peak 5*
	% administered dose	*% urinary radioactivity*		*% lipid-soluble radioactivity*	
Chronic renal disease					
J. T.	0.47	88.4	29.6	44.7	25.7
T. D.	0.24	65.7	53.0	36.0	11.0
J. P.	0.09	68.7	16.7	72.8	10.5
D. K.	0.31	71.1	9.4	76.2	14.4
S. S.	0.62	89.7	18.2	7.7	74.1
A. W.	0.38	77.1	65.2	24.2	10.6
C. M.	0.26	84.0	35.5	54.2	10.3
C. B.	0.32	25.4	7.8	69.5	22.7
Mean	0.34‡	71.3‡	29.4	48.2	22.4
±SE	±0.06	±7.3	±7.4	±8.7	±7.7
Nephrotic syndrome					
G. S.	1.28	24.0	54.8	9.2	36.0
After renal transplantation					
J. P.	1.96	10.6	0	0	100.0
C. M.	1.72	8.7	0	0	100.0
After chronic dialysis					
T. D.	0.52	69.1	55.0	39.0	6.0
Normal subjects					
B. G.	1.47	6.1	0	0	100.0
C. W.	1.97	8.7	0	0	100.0
R. M.	2.00	13.4	0	0	100.0
D. W.	1.25	5.7	0	0	100.0
G. J.	1.78	9.8	0	0	100.0
Mean	1.69	8.7			
±SE	±0.15	±1.4			

CRD, chronic renal disease.

* Resolution of chloroform extracts of lyophilized urine using chromatographic techniques and fraction collection as described in Table III.

‡ $P < 0.001$.

sis. In contrast, the decreased intestinal absorption of calcium is usually unaffected by dialysis, progressive renal osteodystrophy, and skeletal demineralization observed despite the reversal of hypocalcemia, hyperphosphatemia, and acidosis to normal (33, 38). These observations are consistent with our findings that hemodialysis (T.D., Tables I, II, III, V) in contrast to homotransplantation (J. P. and C.M., Tables I, II, III, V) was ineffectual in altering the deranged metabolism of vitamin D_3.

Animal evidence indicates that the liver is the site of the maximum concentration of D_3-^3H after its oral or intravenous administration (39). Other studies reveal that absorbed vitamin D_3-^3H is taken up by hepatic cells and excreted into bile (9). Thus, the liver appears to be an obvious site for the metabolic transformation of vitamin D_3. Schachter, Kowarski, and Finkelstein have also demonstrated that, in the rat, vitamin D acts directly on the small intestine without previous inactivation in another organ (40). Since, after an intravenous dose of vitamin D_3-^3H, over 80% of the intestinal radioactivity represents the biologically active peak 4 metabolite (41), the metabolism and subsequent transformation of vitamin D by the intestine may also be significant. The observed rapid turnover of vitamin D_3 in patients

TABLE VI

Fecal and Absorbed Vitamin D_3-^3H in Normal and CRD Subjects

Subject	Fecal D_3-^3H		"Net" absorbed D_3-^3H
	% total chloroform-soluble radioactivity	*% administered dose*	*% administered dose*
Chronic renal disease			
J. T.	73.9	9.1	90.9
T. D.	70.2	10.3	89.7
J. P.	85.9	12.9	87.1
D. K.	77.2	9.4	90.6
S. S.	84.2	13.2	86.7
A. W.	80.7	7.2	92.8
C. M.	76.9	11.3	88.7
C. B.	68.1	12.0	88.0
Mean	77.1*	10.7‡	89.3‡
±SE	±2.2	±0.7	±0.7
Nephrotic syndrome			
G. S.	80.3	13.2	86.8
After renal transplantation			
J. P.	77.3	11.7	88.3
C. M.	79.1	9.8	90.2
After chronic hemodialysis			
T. D.	85.3	11.7	88.3
Normal subjects			
B. G.	69.4	8.2	91.7
C. W.	71.8	9.3	90.7
L. M.	63.5	12.7	87.3
D. W.	78.4	9.7	90.3
G. J.	75.0	8.9	91.1
Mean	71.6	9.8	90.2
±SE	±2.5	±0.8	±0.8

* $P < 0.2 > 0.1$.
‡ $P < 0.5 > 0.4$.

with chronic renal disease with simultaneous increments in biologically impotent metabolites in peak 4 and peak 5 may represent alterations in specific hepatic or intestinal enzyme activity concerned with the metabolic transformation of vitamin D_3 induced by an abnormal nonultrafilterable substance(s). The hypothesis that alterations in enzyme systems regulating vitamin D metabolism occur in chronic uremia is not unique since enzymic systems concerned with protein synthesis, respiration, anerobic glycolysis, transamination, glutamic acid decarboxylation, oxidative phosphorylation, and brain nucleotidase activity have been reportely inhibited by uremic serum (30, 42, 43).

Finally, despite the reported lack of "resistance" to large vitamin D doses (10,000 U q.d. × 3 days) of uremic rat intestine (22), one must still consider the possibility that the chronic uremic state in man imposes an end organ (bone and intestine) resistance to the action of physiological doses of vitamin D and its biologically active metabolite(s). This resistance could result from biochemical alterations at the specific end organ receptor sites and (or) competitive inhibition between vitamin D and structurally similar biologically inactive metabolites in bone and intestine. To substantiate these hypotheses, studies are needed to evaluate the metabolism of vitamin D by bone, liver, and intestine of uremic animals, to determine by in vitro analysis the effect of uremic serum per se on vitamin D, and to define the relative biological

activities of small doses of vitamin D and its metabolites on the bone and intestine of uremic animals.

ACKNOWLEDGMENTS

This work was supported in part by grants AM06404 from the National Institute of Arthritis and Metabolic Diseases; Clinical Research Center grant FR-36 from the General Clinical Research Center Branch, Division of Research Facilities and Resources; training grant AM05027 from the National Institute of Arthritis and Metabolic Diseases; and grant AM09976 from the National Institute of Arthritis and Metabolic Diseases. Dr. Avioli is a Career Research Development Awardee (7-K3-GM 22-676-03).

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