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Research Article

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Metabolism of Vitamin D_3-^3H in Vitamin D–Resistant Rickets and Familial Hypophosphatemia *

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Abstract. The fate of an intravenous dose of tritiated vitamin D_3 was studied in seven normal subjects, four children with vitamin D–resistant rickets, and four adults with a familial history of vitamin D–resistant rickets and persistent hypophosphatemia. An abnormal metabolism of vitamin D in vitamin D–resistant rickets was defined and characterized by a decrease in the plasma fractional turnover rate, a marked increase in plasma water-soluble metabolites, and a relative decrease in the conversion of vitamin D to a polar, biologically active metabolite. Alterations in vitamin D metabolism in the adults with persistent hypophosphatemia were similar but less severe than those of affected children with vitamin D–resistant rickets. It is tentatively concluded that the abnormalities in vitamin D metabolism documented in patients with vitamin D–resistant rickets and familial hypophosphatemia may account for the observed osseous and biochemical changes.

Introduction

Familial (hereditary) vitamin D–resistant rickets in its typical form presents a number of characteristics that tend to set it apart as a single, presumably genetically determined entity (1–3): (*a*) inheritance as a single-dose effect in the x-linked gene (sex-linked dominance); (*b*) hypophosphatemia as the most consistent, genetically associated abnormality and often the only abnormality in many affected family members (Fam-Hypo); (*c*) active rickets or osteomalacia in some family members, which fails to respond to treatment with nutritional doses of vitamins D_2 or D_3 (VDRR); (*d*) diminished net tubular reabsorption of inorganic phosphate; (*e*) decreased gastrointestinal absorption of calcium; and (*f*) at times, unusual histologic changes in bone and other changes including increased bone density (4).

Although attempts have been made to base a pathogenetic concept primarily on each one of the three major affected sites, i.e. renal, gastrointestinal, and osseous, no theory dependent on an abnormality intrinsic to one organ system adequately explains the pathological or functional abnormalities in the other two organ systems. Therefore, a unifying concept to explain the underling pathogenesis is still lacking.

Recent studies in two patients with VDRR by DeLuca, Lund, Rosenbloom, and Lobeck reveal a distinct abnormality in the metabolism of radioactive vitamin D, characterized by abnormal amounts of water-soluble vitamin D plasma metabolites 16 hr after vitamin D_3-^3H injection (5). The present study, undertaken to evaluate this metabolic abnormality in VDRR, provides further evidence that the metabolism of vitamin D in VDRR patients differs significantly from that observed in normal subjects.

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Methods

Four children with vitamin D–resistant rickets, four adults with persistent hypophosphatemia, and seven normal healthy volunteers were used for these studies. Three of the four children with vitamin D–resistant rickets had never received vitamin D therapeutically. The fourth (P. W., Table I) had been treated since age 7 with large doses of vitamin D and for the 8 month period immediately preceding the present study had been receiving 150,000 U of vitamin D per day. Three of the hypophosphatemic adults were of the same pedigree as the affected children (D. B. and L. B., J. E. and L. E., G. D. and L. D., Table I). The fourth adult hypophosphatemic patient (T. C., Table I) gave a history of severely bowed legs since childhood but had never received vitamin D therapeutically. She also had a strong family history of VDRR. The vitamin D–resistant subjects were selected on the basis of abnormal elevations in serum alkaline phosphatase, hypophosphatemia (Table I), and roentgenograms consistent with active rickets. The three adult relatives of the affected children (L. D., L. E., and L. B., Table I) were selected because of persistent hypophosphatemia and a family history of VDRR. All subjects were studied in ambulatory states during periods of hospitalization on a Clinical Research Center Ward.

1 hr before breakfast after a 12–15 hr overnight fast, 5–8 μc of randomly labeled vitamin D_3-^3H (D_3-^3H) with a specific activity of 72 μc/mg, prepared and purified by the method of DeLuca et al. (6), was dissolved in 0.5–1.0 ml of absolute ethanol and administered intravenously over a 30–45 sec period. 10–18-ml samples of heparinized blood were collected at 5, 15, 30, and 45 min and at 1, 2, 4, 8, 12, and 16 hr. 30–50 ml of blood was removed from each subject 24 hr after D_3-^3H injection. 10 ml of blood was then obtained at 12-hr intervals for the subsequent 24 hr period.

TABLE I

Clinical data

Subjects	Age	Sex	Serum calcium	Serum phosphorus	Serum alkaline phosphatase
	yr		*mg/100 ml*	*mg/100 ml*	*King-Armstrong units*
Vitamin D–resistant rickets					
D. B.	8	F	9.5	2.7	36.0
P. W.*	11	F	10.2	2.5	46.0
J. E.	8	F	10.2	3.2	29.2
G. D.	9	F	10.5	2.6	30.0
Familial hypophosphatemia					
L. D.	30	M	10.9	1.9	11.2
L. E.	36	F	9.6	2.4	3.5
L. B.	24	F	9.1	2.4	9.0
T. C.	20	F	9.0	2.2	6.5
Normals					
D. R.	32	M	10.2	3.5	5.7
K. M.	17	M	9.7	3.9	9.1
E. F.	10	F	10.0	4.3	16.5
J. L.	12	M	9.6	4.0	12.7
W. R.	27	M	9.4	3.3	4.9
H. D.	12	F	10.2	4.1	13.8
J. P.	38	F	9.9	2.9	3.0

* Treated since age 7 yr with large doses of vitamin D. For 8 month period immediately preceding D_3-^3H study she had been ingesting 150,000 U of vitamin D_2 per day.

During the first day, cumulative 6-hr urine collections were made. Thereafter, urine was collected during 8-hr periods for the remainder of the study. All urine collections were refrigerated and subsequently lyophilized. Cumulative fecal collections were made during the 48 hr study period; these were homogenized with water in a Waring Blendor and aliquots were lyophilized. All fecal collections were initiated and terminated with enemata to ensure their complete recovery.

Determination of total ^3H in biological samples. 1–2-ml samples of whole plasma, lyophilized feces, and urine were combusted according to the procedure of Kelly et al. (7), utilizing a Thomas Ogg Safety Igniter (A. H. Thomas Co., Philadelphia, Pa.).

The resulting ^3H water vapor was frozen on the bottom of a 2 liter Erlenmeyer flask and placed in a dry ice–acetone bath for 45 min. 20 ml of liquid scintillation counting solution A was added and the flask was allowed to stand in the crushed ice bath for 45 min. Then 18 ml of the solution was withdrawn and assayed for radioactivity. The efficiency of the tritium counting, which was strongly dependent on the amount of water vapor present in the sample, varied between 7 and 15%. Recoveries of ^3H-vitamin D_3 added to nonradioactive samples of plasma, urine, and feces ranged between 96 and 102%.

Extraction of radioactivity. The lipid-soluble radioactivity present in plasma, urine, and feces was extracted by the procedure of Bligh and Dyer (8) as previously described (9). After filtration to remove the denatured protein, the phases were allowed to separate overnight at 12°C. The denatured protein was found to contain little or no tritium after this extraction procedure. The aqueous-methanol layer was routinely reextracted three times with additional volumes of chloroform and these extracts were combined and concentrated by evaporation with nitrogen gas. 2 ml of the aqueous layer was added to counting solution B and assayed for radioactivity. 1–2-ml aliquots of chloroform-soluble radioactivity were added to counting solution C and also assayed for radioactivity.

Chromatography. Thin-layer chromatography apparatus and Cab-O-Sil silica gel G (Research Specialties Co., Richmond, Calif.) were used to prepare the thin-layer silicic plates. 10 μg of crystalline, nonradioactive vitamin D_3 were placed at the origin on each side of the plate as a marker. Aliquots of the chloroform-soluble extract were then applied across the origin of the plate between the marker vitamin D spots. The plates were then chromatographed in a solvent of 10% acetone in *n*-hexane (v/v) as previously described (6). After drying, the plates were sprayed with 0.20% $KMnO_4$ in 1% Na_2CO_3 to locate the marker vitamin D. The radioactivity was then located by scraping off successive 0.5

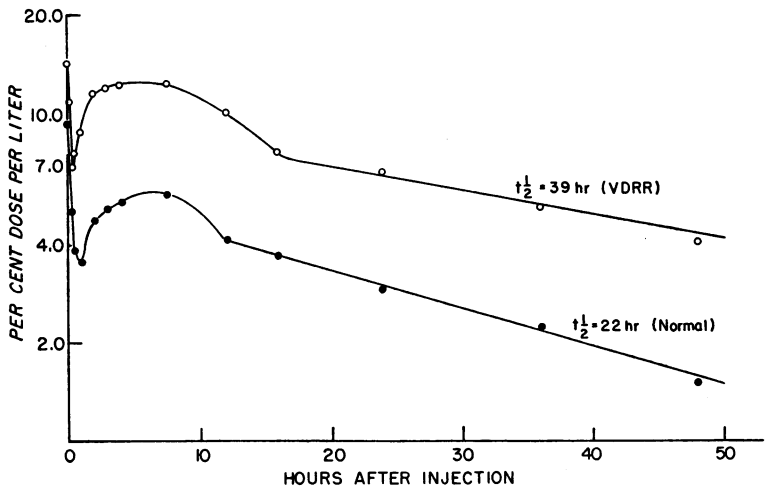

FIG. 1. PLASMA DISAPPEARANCE OF VITAMIN D_3-^3H FOLLOWING INTRAVENOUS ADMINISTRATION TO NORMAL SUBJECTS AND PATIENTS WITH UNTREATED VDRR. Each point on the curves represents the mean value obtained in seven normal subjects and four patients with VDRR.

cm segments of the silicic acid with a microscopic slide and the scrapings were placed in a counting vial containing liquid scintillation counting solution C. The radioactivity in the "vitamin D_3 band" was calculated either as a percentage of the injected dose or as a percentage of the total radioactivity in the combusted samples. The mean recovery of the radioactivity applied to the thin-layer plates was 91%. As noted previously (6), the marker vitamin D always migrated the same distance in the system whether chromatographed alone or with a lipid extract of the biological samples. The radioactivity migrating with the same R_f as the stable vitamin D markers was labeled "radioactive vitamin D_3."

Silicic acid column chromatography of chloroform extracts of 24-hr plasma and cumulative fecal and urine specimens was carried out essentially as described previously (10). After extraction by a modification of the method of Bligh and Dyer (8), the chloroform phase of plasma, urine, and fecal specimens was evaporated to dryness under vacuum with a flash evaporator and the residue redissolved in hexane. The hexane phase was then applied to silicic acid columns, 58 × 1.5 cm, and chromatographed with a hyperbolic gradient of diethyl ether in petroleum ether, whereupon serial collections of 10 ml were made. The collected fractions were evaporated; 20 ml of counting solution C was added and radioactivity measured in a liquid scintillation counter.

Measurement of radioactivity. Counting solution A was used to measure the radioactivity of combusted samples of plasma, urine, and feces; this solution consisted of 40 mg of dimethyl-POPOP (1,4-bis [5-phenyl-2-oxazoly]-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 tritium in aqueous extracts was prepared according to the method of Bray (11). Counting solution C 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. In each instance, observed counts per minute were converted to disintegrations per minute (dpm) by reference to ^3H-toluene standards (U. S. National Bureau of Standard). All samples were counted in an automatic Packard Tri-Carb Liquid Scintillation Counter, Model 314 XE (Packard Instrument Company, Inc., Downers Grove, Ill.).

Results

Metabolic fate of plasma D_3-^3H radioactivity

The plasma disappearance of D_3-^3H in normal, VDRR, and FamHypo subjects (Fig. 1) was biphasic with a rapid fall in the immediate postinjection period followed by a gradual rise for 5 hr and an exponential fall for the remainder of the 48 hr study. The mean ± SE plasma D_3-^3H half-times for normal, VDRR, and FamHypo subjects were 22 ± 1, 39 ± 4, and 25 ± 2 hr, respectively. Individual D_3-^3H half-times for normal, VDRR, and FamHypo subjects ranged from 19 to 25, 30 to 46, and 21 to 29 hr, respectively. Whereas a significant elevation ($P < 0.01$) in the mean plasma D_3-^3H half-time was obtained for the VDRR group, no significant difference existed between normal and FamHypo plasma D_3-^3H half-times at the 5% probability level.

Normally, over 95% of the plasma radioactivity for the first 48 hr after D_3-^3H injection is extracted by the nonpolar chloroform phase of the

TABLE II

Distribution of plasma radioactivity between the aqueous phase and chloroform phase 12 and 24 hr after intravenous vitamin D_3-^3H

Subjects	Aqueous 12 hr	Aqueous 24 hr	Chloroform 12 hr	Chloroform 24 hr
	Per cent total plasma radioactivity			
Vitamin D–resistant rickets				
D. B.	20.1	38.3	79.9	61.7
P. W.	13.4	16.5	86.6	83.5
J. E.	26.1	30.0	73.9	70.0
G. D.	15.0	29.2	85.0	70.8
Mean ± SE	18.6 ± 3.1	28.5 ± 5.4	81.4 ± 3.1	71.5 ± 5.4
Familial hypophosphatemia				
L. D.	9.2	13.4	90.8	86.6
L. E.	6.3	10.0	93.7	90.0
L. B.	0.0	4.0	100.0	96.0
T. C.	12.0	25.7	88.0	74.3
Mean ± SE	6.9 ± 3.0	13.3 ± 5.4	93.1 ± 3.0	86.7 ± 5.4
Normal				
D. R.	0.0	0.0	100.0	100.0
K. M.	1.0	3.6	99.0	96.4
E. F.	0.0	0.0	100.0	100.0
J. L.	1.1	4.8	98.9	95.2
W. R.	0.0	0.0	100.0	100.0
H. D.	1.2	4.2	98.8	95.8
J. P.	0.0	1.2	100.0	98.8
Mean ± SE	0.5 ± 0.1	1.9 ± 0.7	99.5 ± 0.1	98.1 ± 0.7

Bligh and Dyer extraction procedure; the remainder represents polar water-soluble metabolites (5, 12). In the present study, a consistent finding in VDRR and FamHypo subjects was an increase in the aqueous-soluble plasma radioactivity as compared to that in normal subjects. This is illustrated in Table II for the 12- and 24-hr plasma samples obtained in normal, VDRR, and FamHypo subjects. Whereas only 1.9 ± 0.7% radioactivity in the 24-hr plasma samples was normally rendered water-soluble by the Bligh and Dyer extraction procedure, an average of 28.5 ± 5.4 and 13.3 ± 5.4% total plasma radioactivity was found in the aqueous plasma extracts of VDRR and FamHypo subjects, respectively.

The results of silicic acid chromatography of chloroform extracts obtained from 24-hr plasma samples are noted in Table III. In the three groups, three fractions were clearly separated, two of which (peaks 2 and 4) are still unidentified. The material in peak 3 has been previously identified as unaltered vitamin D_3 (9, 12). The polar substance in peak 4 previously isolated from both rat (9, 10) and human (10, 12) plasma has been shown to possess potent biological activity (12, 13). Peak 1, as noted in Table III, previously identified in humans by Avioli, Lund, DeLuca, and McDonald as a vitamin D ester (9, 12), was characteristically absent from the plasma of normal and FamHypo subjects. However, in two of the four patients with VDRR, peak 1 represented 3–4% fractionated radioactivity. Subsequently, 24-hr plasma extracts from these same subjects (D. B. and J. E., Table III) were saponified by refluxing under nitrogen for 30 min in 10% KOH in methanol (v/v), and the saponified material was extracted with small volumes of diethyl ether. The extracts were combined and dried over anhydrous $CaSO_4$. The ether evaporated and the residue was dissolved in hexane.

TABLE III

*Distribution of plasma chloroform extracts during silicic acid column chromatography**

Subject	Peak 1	Peak 2	Peak 3	Peak 4
			Per cent total radioactivity	
Vitamin D–resistant rickets				
D. B.	3	4	90	3
P. W.	0	3	79	18
J. E.	4	2	86	8
G. D.	0	2	95	3
Mean ± SE			88 ± 4	8 ± 4
Familial hypophosphatemia				
L. D.	0	2	84	14
L. E.	0	3	85	12
L. B.	0	4	65	31
T. C.	0	1	82	17
Mean + SE			79 ± 5	19 ± 5
Normal				
D. R.	0	2	58	40
K. M.	0	3	68	29
E. F.	0	4	59	37
J. L.	0	7	67	26
W. R.	0	6	58	36
H. D.	0	4	71	25
J. P.	0	2	49	49
Mean ± SE			61 ± 3	35 ± 3

* The chloroform extracts of plasma obtained 24 hr after an intravenous dose of vitamin D_3-^3H were chromatographed on a 24 g silicic acid column with a gradient of diethyl ether in petroleum ether (9, 10). 10-ml fractions were collected. Peak 1 represents fractions 10–14; peak 2, fractions 17–23; peak 3, fractions 24–30; peak 4, fractions 59–65.

The hexane extract was then rechromatographed on silicic acid columns in the identical hyperbolic gradient system described earlier (10). As illustrated for J. E. in Fig. 2, saponification resulted in a disappearance of peak 1 in the VDRR subjects and an increase in the percentage of the recoverable radioactivity in peak 3.

In both normal and VDRR subjects saponification also induced a decrease in the amount of chloroform-soluble radioactivity that chromatographed as peak 4 (Figs. 2 and 3). Similar observations have also been made after chromatography of saponified plasma samples from animals injected with D_3-^3H (9). Whereas 35% of the radioactivity recovered during the chromatographic procedure was normally present in peak 4, an average of 9 and 19%, respectively, of chloroform-soluble, 24 hr plasma radioactivity was identified as peak 4 in VDRR and FamHypo (Table III). The observed decrease in peak 4 at 24 hr after intravenous D_3-^3H was also reflected in the thin-layer chromatograms, wherein an abnormal decrease in plasma metabolites more polar than vitamin D was observed in VDRR and FamHypo subjects throughout the entire 48 hr sampling period. In the only patient with VDRR who had been taking therapeutic doses of vitamin D (P. W., Table III) immediately before the D_3-^3H study, 18% of the chloroform-soluble phase was identified as peak 4. This represented a two- to sixfold increment over values obtained in the untreated VDRR subjects (D. B., J. E., and G. D., Table III). One patient with FamHypo demonstrated a relatively normal distribution of plasma radioactivity between peaks 3 and 4 (L. B., Table III). It is of interest that in this case the D_3-^3H content of plasma was consistently normal, as well as the distribution of 24 hr plasma radioactivity between the aqueous and chloroform phase (Table II). This subject had persistent hypophosphatemia but no evidence of bone disease either as a child or as a adult.

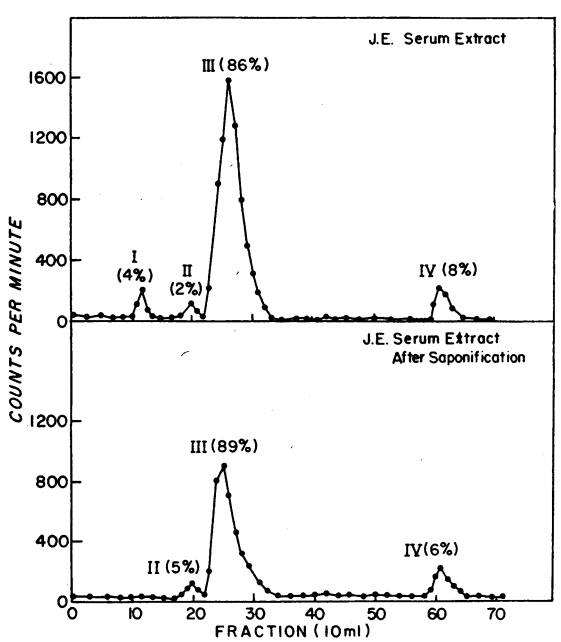

FIG. 2. SILICIC ACID COLUMN CHROMATOGRAPHY OF THE 24 HR PLASMA CHLOROFORM EXTRACT FROM A PATIENT WITH VDRR, BEFORE AND AFTER SAPONIFICATION.

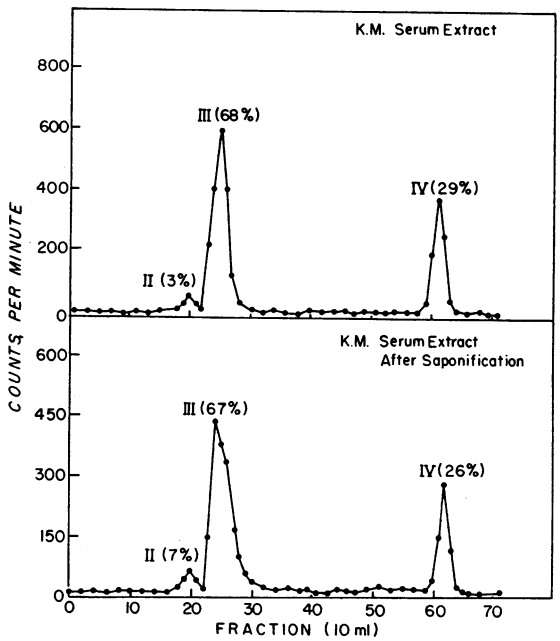

FIG. 3. SILICIC ACID COLUMN CHROMATOGRAPHY OF THE 24 HR PLASMA CHLOROFORM EXTRACT FROM A NORMAL SUBJFCT, BEFORE AND AFTER SAPONIFICATION.

Urinary and fecal radioactivity

For the 48 hr period after D_3-^3H injection the urinary excretion of tritiated material in the normal subjects ranged from 1.4 to 4.6% per administered dose, with a mean for the group of 2.1% (Table IV). Direct extraction of the freshly lyophilized urine by the Bligh and Dyer technique (8) yielded water-soluble material ranging from 84 to 89% total excreted radioactivity (mean

TABLE IV

*Cumulative 48 hr urine and fecal radioactivity after intravenous vitamin D_3-^3H**

Subjects	Total urine radioactivity	Water-soluble urine radioactivity	Total fecal radioactivity	Lipid-soluble fecal radioactivity	Fecal "vitamin D_3-^3H"‡
	% injected dose	*% total urine radioactivity*	*% injected dose*	*% total fecal radioactivity*	*% total fecal radioactivity*
Vitamin D–resistant rickets (4)	2.0 ± 0.3	92.4 ± 1.7	4.3 ± 0.7	76.7 ± 2.6	18.9 ± 4.3
Familial hypophosphatemia (4)	1.7 ± 0.4	86.9 ± 1.3	4.5 ± 0.5	72.7 ± 5.6	14.4 ± 6.5
Normal (7)	2.1 ± 0.3	85.5 ± 0.8	5.2 ± 0.5	58.7 ± 4.8	5.6 ± 3.8

* Values represent mean ±SE . The number of subjects in each group is noted in parentheses.
‡ Represents lipid-soluble material identified by silicic acid chromatography as vitamin D_3.

85.5 ± 0.8, Table IV). Thin-layer chromatography of the chloroform-soluble phase of extracted urine normally revealed little, if any, substance that migrated with the same R_f as the crystalline vitamin D_3 standard. Whereas the values for cumulative 48 hr urine radioactivity for VDRR and FamHypo (2.0 ± 0.3 and 1.7 ± 0.4, Table IV) were not unlike those found in normal subjects, from 89 to 96% (mean 92.4 ± 1.7, Table IV) of the urinary radioactivity was rendered water-soluble in patients with VDRR as compared with 85.5 ± 0.8% in normal subjects. No free D_3-^3H could be demonstrated in the organic phase of urine extracts of either VDRR or FamHypo patients.

Cumulative 48 hr fecal radioactivity was similar in normal, VDRR, and FamHypo subjects, averaging 5.2, 4.3, and 4.5% per injected dose, respectively. Whereas normally, 58.7 ± 4.8% total fecal radioactivity was chloroform-soluble, 76.7 and 72.7% fecal radioactivity was recovered in the organic chloroform phase in VDRR and FamHypo, respectively (Table IV). A threefold increment in the fecal radioactivity which migrated as vitamin D_3-^3H during chromatographic analysis was also observed in VDRR and FamHypo.

Discussion

The present study indicates that an abnormal metabolism of vitamin D exists in children with vitamin D–resistant rickets, as well as in their adult relatives with persistent hypophosphatemia. This derangement, more pronounced in the affected children than in their hypophosphatemic relatives, is manifested by a decrease in the fractional turnover rate of vitamin D, a marked increase in water-soluble metabolites in plasma, and a relative decrease in the conversion of vitamin D to a polar biologically active metabolite. Since previous experiments in man (12) and animals (9) indicated that significant metabolism of D_3-^3H normally obtains at 24 hr after its administration, only 24-hr plasma samples from normal, VDRR, and FamHypo individuals were subjected to more definitive column chromatographic separation procedures. The observed decrease in 24 hr plasma peak 4 content in VDRR and FamHypo subjects and the increase in D_3-^3H plasma half-time in these same individuals (Fig. 1) are consistent with an abnormal conversion of D_3-^3H to its more polar peak 4 metabolite. The abnormally low concentration of D_3-^3H polar metabolites noted on thin-layer chromatograms during the *entire* 48 hr study lends support to this hypothesis. Heretofore, relatively little information was available concerning the metabolism of vitamin D in man. Recent studies in human subjects from this laboratory have demonstrated the role of the hepato-biliary tract in the metabolism of vitamin D (12). Biologically active metabolites have also been isolated from plasma, bone, intestine, and kidney in rats (9, 10) and from plasma in man (5, 9, 12). Several investigators have often cited the paradox of markedly elevated blood levels of vitamin D during therapy of VDRR with "inherent resistance" to its action and have postulated a defect in the metabolism of vitamin D to account for the discrepancy (14, 15). In the past, experimental evidence to verify this hypothesis was absent. Recently DeLuca, Lund, Rosenbloom, and Lobeck administered D_3-^3H to two control subjects, an 18 yr old male with VDRR and his 42 yr old mother with a childhood history of VDRR (5). 16 hr after the D_3-^3H injection, these investigators found abnormally elevated concentrations of total plasma

radioactivity and an increase in plasma aqueous D_3-^3H metabolites up to values 20 times normal in both affected individuals.

In 1965 Scott et al. administered radioactive D_3 orally to 10 patients with vitamin D-resistant rickets and noted "significantly lower removal rates of plasma D_3-^3H" (16). These investigators also commented on a decrease in the intestinal absorption of D_3-^3H in the VDRR subjects, as reflected by a decrease in lipid-soluble radioactivity after the oral D_3-^3H dose. As noted in the present report, the plasma lipid-soluble radioactivity in subjects with VDRR is lower than normal after intravenous D_3-^3H. These latter observations of Scott et al. most likely represent the abnormal metabolism of vitamin D resulting in abnormally low lipid-soluble plasma radioactivity initially cited by DeLuca et al. (5), and confirmed in the present report, rather than a malabsorption of vitamin D. Since blood levels of vitamin D (measured by bioassay) have been shown to be normal in VDRR before treatment and markedly elevated after therapeutic oral doses (14, 17, 18), and since parenteral vitamin D therapy has reportedly failed to cure VDRR (19–21), any abnormality in the intestinal absorption of vitamin D in VDRR seems remote.

As noted in Table IV, the increase in plasma water-soluble metabolites noted in VDRR was also associated with an increase in water-soluble urine radioactivity. Normally, 10% of the urine radioactivity can be accounted for by water-soluble glucuronide conjugates and an additional 16% by water-soluble, acid-labile conjugates (12). When the water-soluble urine extracts of VDRR subjects were subjected to β-glucuronidase and acid hydrolysis according to previously described techniques (12), the concentrations of glucuronide and acid-labile substances were within normal limits. Inasmuch as the liver can apparently conjugate vitamin D and its metabolites normally in VDRR, the observed defect in D_3-^3H metabolism is probably extrahepatic.

Of the three major functional and/or morphological abnormalities associated with VDRR, in kidneys, gastro-intestinal tract, and bone, the findings of this study and of other recent work may be directly related to two abnormalities. Firstly, with regard to the well-documented subnormal gastro-intestinal absorption of calcium in VDRR (3, 14, 22–25), the material isolated as peak 4 in the present study, and found to be significantly decreased in amount in three of the four patients with VDRR 24 hr after D_3-^3H infusion (Table III), has recently been shown to be twice as effective as vitamin D in stimulating the intestinal transport of calcium (9, 13). Thus, a relative deficiency of the active peak 4 metabolite in patients with VDRR could possibly result in defective calcium absorption.

Secondly, it is possible that the decrease in vitamin D turnover in VDRR and, in particular, the decreased amounts of peak 4 metabolite are related to the bony abnormalities found in VDRR. Radioautographs of bone obtained after radioactive vitamin D injection demonstrate localized radioactivity in the cytoplasm of chondrocytes in the proliferating cartilage zone of growing bone (26); experiments by Lund and DeLuca (9, 13) reveal that the peak 4 metabolite represents the majority of radioactivity in rat bone while vitamin D, itself, is present in only small amounts. Furthermore, the peak 4 metabolite has been shown to be as biologically active as the parent vitamin in curing rickets in rats (9, 12).

That vitamin D (or its metabolites) is directly involved in bone metabolism, in addition to its effects on the availability of calcium, has long been suggested. Vitamin D deficiency in dogs (27) and rats (28) results in trabecular changes and increased noncalcium content of the shafts of long bones that are not directly related to the amounts of absorbed calcium and phosphate. Moreover, recent experiments by Au and Bartter (29) and Cruess and Clark (30) indicate that the vitamin D has a direct effect on bone metabolism independent of its known effects on the intestinal absorption of calcium. In bone from patients with VDRR, in addition to the typical rachitic changes, other abnormalities not found in vitamin D–deficiency rickets have been described. These include an irregular mosaic formation of the haversian system and trabecular (31–33) "halos" of low-density bone, together with a "dynamic" abnormality in osteoblastic development (4, 34), and abnormal metabolism of chondroitin sulfate and alkaline phosphatase (35). It may be suggested that these abnormalities are related in some way to the defects in vitamin D metabolism found in this study, through the mech-

anism of the direct effects of vitamin D or its metabolites on bone.

Any attempt to relate the decreased net renal tubular resorption of phosphate of both VDRR and FamHypo to the abnormalities in vitamin D metabolism becomes much more conjectural. One possibility is that the decreased intestinal absorption of calcium leads to secondary hyperparathyroidism which in turn results in phosphaturia and hypophosphatemia. This hypothesis has received much attention in the literature dealing with VDRR; it remains a proposal upon which there is considerable disagreement (see reference 3 for a comprehensive review of the evidence through 1964 and references 36 and 37 for recent negative evidence). The phosphaturia and hypophosphatemia observed in members of affected families who lack bony abnormalities and have normal calcium absorption, i.e. in whom there is no reason to suspect secondary hyperparathyroidism, are not readily explainded by this hypothesis. Another possible explanation would be to relate the renal abnormality that results in phosphaturia to the increase in plasma and urinary water-soluble D_3-^3H metabolites observed in patients with VDRR and FamHypo (Tables II and IV). The identity of these water-soluble metabolites has yet to be determined. To date, essentially no antirachitic vitamin D–like biological activity has been detected in any of the water-soluble D_3-^3H metabolites, which suggests that the metabolites most probably represent degradation products of vitamin D (9). It is possible that increased amounts of these steroids in the glomerular filtrate, and thus within the renal tubule, could act directly to decrease net phosphate reabsorption since three other steroids, cortisone, hydrocortisone, and diethylstilbesterol, have been shown to have such an effect (38–40).

Other aspects of the present observations still remain to be clarified. The observed decrease in D_3-^3H turnover in VDRR may relate to abnormal protein-binding of vitamin D in plasma or certain tissue receptor sites. The increase in biologically inactive water-soluble metabolites may also represent an accentuation of plasma vitamin D–degrading mechanisms that normally obtain but are abnormally perpetuated by consistent abnormal elevations in circulating vitamin D.

The reason for the increments in fecal lipid-soluble radioactivity in VDRR and FamHypo noted in Table IV is also presently obscure. Associated increments in fecal material which migrates with the same R_f as vitamin D_3 is also of note since this material lacks biological activity (9). It may represent endogenously secreted degradation products of vitamin D with relatively minor structural alterations that are not readily separated from the parent vitamin by the chromatographic procedures used in the present study; or the increments may be the result of bacterial decomposition of vitamin D metabolites (26).

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