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Absorption of Vitamin D₃-³H in Control Subjects and Patients with Intestinal Malabsorption *

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The preparation of radioactive vitamin D₃ and its absorption in the rat have been described by Norman and DeLuca (1) and by Schachter, Finkelstein, and Kowarski (2). Using tied intestinal loops and animals with artificial lymph fistulae, Schachter and his associates (2) have shown that maximal absorption of tritium-labeled vitamin D₃ takes place in the mid-jejunum and that its transfer into the blood is mainly via the lymph. Little information exists on the absorption of vitamin D in man, except for the observations of Kodicek (3), who found that between 13 and 23% of an oral dose of vitamin D₂-¹⁴C was recoverable from the feces of infants within 3 days.

The present paper deals with the preparation, purification, and radiochemical behavior of vitamin D₃ after random labeling with tritium and with its use in human subjects. The labeled vitamin D was purified by methods essentially similar to those previously described (2), with the main exception that the vitamin was recovered in crystalline form without preliminary esterification. Vitamin D absorption was assessed in control subjects and patients with various forms of intestinal malabsorption by measuring their plasma and fecal radioactivity after oral doses of vitamin D₃-³H. Malabsorption of the labeled vitamin was demonstrated in patients with adult celiac disease and in others with steatorrhea due to biliary or pancreatic dysfunction; several of these patients showed clinical evidence of vitamin D deficiency.

Methods

Preparation and purification of vitamin D₃-³H. Crystalline vitamin D₃¹ in 0.2- to 2-g quantities was labeled

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with tritium by random exchange by stirring for 24 hours in 70% acetic acid, containing 300 to 500 c of tritium in the presence of a catalyst prepared by prereduction of 0.2 to 0.5 g platinum oxide.² After removal of labile tritium the radioactive material, dissolved in hexane, was chromatographed on a silicic acid column and eluted with 100 ml 65% vol/vol benzene in hexane. Further purification was by thin-layer chromatography on Kieselgel H,³ containing rhodamine 6G, with 10% vol/vol acetone in hexane as solvent and unlabeled vitamin D₃ as a marker. The appropriate band was located under ultraviolet light and eluted with chloroform, dried *in vacuo*, and redissolved in a minimal volume of acetone. Crystallization was achieved after seeding and prolonged slow cooling to 4° C. The average yield was 20%.

Identification. The labeled crystalline material was identical to authentic vitamin D₃ in its mobility on thin-layer chromatography when using either chloroform or 10% vol/vol acetone in hexane as solvents, in its ultraviolet absorption spectrum, which showed a peak at 265 mμ, and on quantitative estimation with Nield, Russell, and Zimmerli's reagent (4). In addition, bioassay was carried out at two dilutions in paired rats from each of four rachitic litters, healing being assessed radiologically (5). The labeled vitamin was fully active when compared with a vitamin D₃ standard.

On thin-layer chromatography the main contaminant in the unpurified material moved with the same mobility as a precalciferol 3 marker, prepared by refluxing crystalline vitamin D₃ in benzene (6). This radioactive compound had an absorption peak at 262 mμ and reacted with Nield's reagent similarly to vitamin D₃. It was biologically inactive, but on storage at 4° C it was gradually converted into vitamin D₃-³H. These characteristics suggest that it was precalciferol 3, an isomer of vitamin D₃ (7).

Radiochemical behavior. The highest initial specific activity obtained with any batch of vitamin D₃-³H was 54.6 μc per mg. Further measurements of specific activity were performed at intervals after repeated repurification of the labeled vitamin by thin-layer chromatography. During the 5 days after crystallization the specific activity of a solution of this batch of vitamin D₃-³H in benzene decreased rapidly to 18.8 μc per mg, and this was followed by a more gradual decline in specific activity during the next 10 days (Figure 1). From the

² Radiochemical Centre, Amersham, England.

³ E. Merck, Darmstadt, Germany.

fifteenth day onward the specific activity remained relatively stable at between 13.6 and 16.4 μc per mg.

To assess the stability of the labeled vitamin on storage in benzene under nitrogen at -15°C , we determined its radiochemical purity at varying time intervals. Within 10 days of purification three decomposition products were observed to have accumulated, two being more polar than vitamin D_3 on thin-layer chromatography, the third and major radioactive product being less polar. Radiochemical decomposition into the latter component, which had the R_f of precalciferol 3, occurred exponentially. The time taken for 50% of the total radioactivity to be converted into this less polar compound was apparently related to the initial specific activity of each batch of vitamin $\text{D}_3\text{-}^3\text{H}$; a solution of specific activity 15 μc per mg had a half-life of 5 weeks, whereas the half-life was 13 weeks in another batch of specific activity 5.8 μc per mg. Because of the lability of the radioactive vitamin on storage, it was repurified by thin-layer chromatography not more than 5 days before use.

Procedure for absorption tests. The labeled vitamin, dissolved in 0.5 to 1 ml arachis oil, was given immediately after a light breakfast. The subjects received 0.1, 0.5, or 1.0 mg of vitamin $\text{D}_3\text{-}^3\text{H}$ of specific activity 5 to 15 μc per mg, delivered onto the tongue from a calibrated syringe and rinsed down with 250 ml of milk. Feces were collected in two successive 3-day pools and either stored at -15°C or analyzed immediately. Three consecutive 24-hour urine collections were made after the oral dose. Blood samples were withdrawn into heparinized containers at 2- to 4-hour intervals for 12 hours, then daily for 4 days; the plasma was separated and retained.

Plasma radioactivity. Three ml plasma was added by drops to 60 ml chloroform:methanol, 2:1, vol/vol. Twelve ml water was added; after separation of the phases the aqueous layer was removed (8). The chloroform layer was taken to dryness under a nitrogen stream and the residual lipid dissolved in 10 ml toluene containing 0.4% PPO (2,5-diphenyloxazole) and 0.01% POPOP [1,4-bis-2-(5-phenyloxazolyl) benzene]⁴ and transferred quantitatively into counting vials. Radioactivity was assayed in a Packard Tri-Carb liquid scintillation spectrometer, the counting efficiency being 25%. Quenching was insignificant except in the presence of jaundice or hemolysis. Results were expressed as disintegrations per minute per milliliter of plasma and were corrected for dose and body weight as follows: disintegrations per minute per milliliter plasma/(dose in microcuries/weight in kilograms).

Actual count rates in the plasma of normal subjects after a 15- μc dose varied between 300 cpm per ml at the peak of absorption and 50 cpm per ml at 72 hours, above a background of 40 to 50 cpm.

In some studies, 2.5 ml of plasma was layered under an equal volume of distilled water and centrifuged at 20,000 rpm for 6 hours in a Spinco model L preparative ultracentrifuge with 40.3 rotor. The upper fraction,

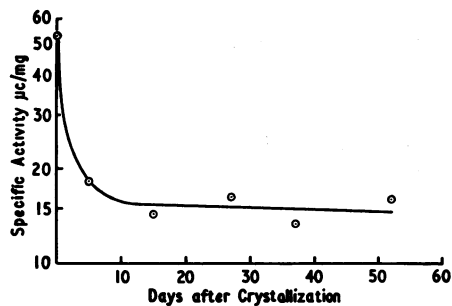


FIG. 1. SPECIFIC ACTIVITY OF VITAMIN $\text{D}_3\text{-}^3\text{H}$ REPURIFIED AT INTERVALS DURING STORAGE.

containing the chylomicrons, and the optically clear lower fraction were separated with a tube slicer and extracted in the same manner as the plasma samples. Lipid extracts from the upper layer were fractionated by thin-layer chromatography on Kieselgel H with the solvent system hexane: ether: acetic acid, 80:20:1, vol/vol/vol; triglyceride was determined (9) and constituted 84.6% by weight of the lipid. Free and ester cholesterol and phospholipid were present in small quantities. Recoveries of chylomicrons were satisfactory, in that few light-scattering particles were visible in the supernatant on dark-field microscopy.

Fecal radioactivity. Three-day pools were diluted with water and homogenized. Five- to 10-g portions were refluxed for 3 hours with 200 ml acetone and then for a further 3 hours with 200 ml of absolute ethyl alcohol. To remove fecal pigments, we combined the filtered extracts and passed them through a cation exchange resin column, as described by Lewis and Myant (10). The extract was then dried in a rotary evaporator and the residue redissolved in 5 ml ethanol. An aliquot of 0.1 ml was transferred to a counting vial and 10 ml PPO/POPOP/toluene added. Quenching corrections were made by addition of an internal tritium standard, the average correction factor being 20%.

Urinary radioactivity. Samples of 0.4 ml were taken from each 24-hour sample of urine and counted in 10 ml Bray's solution (11). Quenching was assessed by addition of internal standards.

Other investigations. The serum calcium, inorganic phosphate, and alkaline phosphatase were measured as described by Wootton (12). The serum albumin was measured by electrophoresis on cellulose acetate (13). Fecal fat excretion was measured either by the method of Van de Kamer, Ten Bokkel Huinink, and Weyers (14) or by that of Bowers, Lund, and Mathies (15); the dietary fat was between 50 and 70 g per day.

A radiological skeletal survey was carried out on all patients with steatorrhea. Iliac crest bone biopsies were obtained in three patients with the technique described by Stammers and Williams (16). The presence of osteoid seams wider than 15 μ in undecalcified sections was regarded as diagnostic of osteomalacia (17).

Subjects. Studies were carried out on 12 control subjects who were convalescent patients in the hospital,

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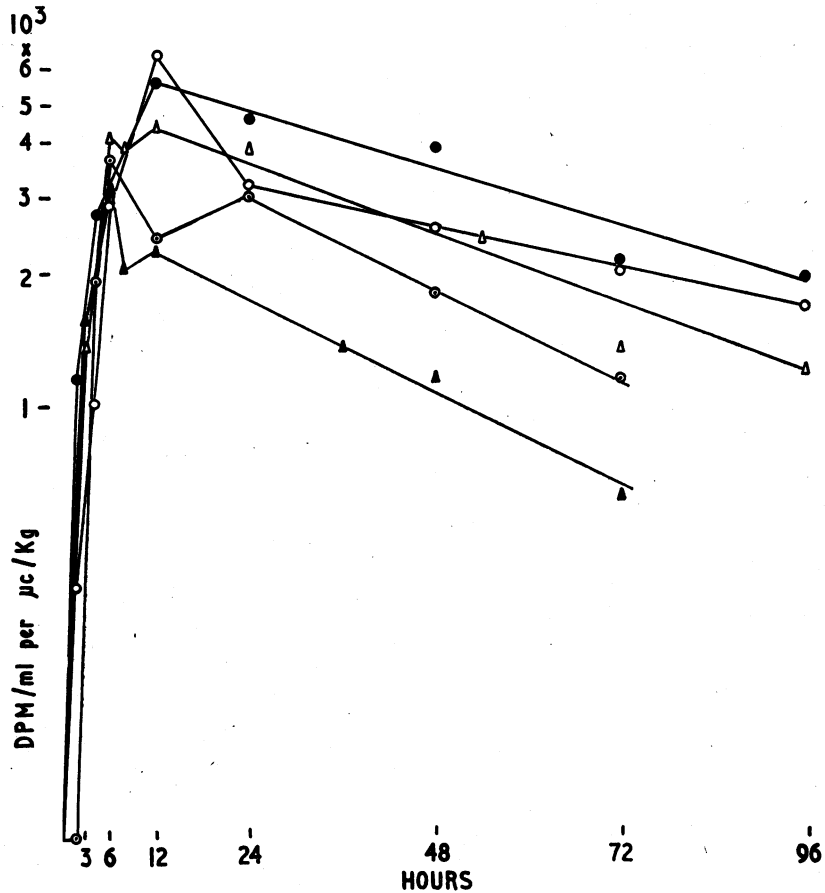


FIG. 2. PLASMA RADIOACTIVITY IN FIVE CONTROL SUBJECTS AFTER 1-MG ORAL DOSES OF VITAMIN D₃-³H (5 TO 15 µC), EXPRESSED AS DISINTEGRATIONS PER MINUTE PER MILLILITER OF PLASMA AND CORRECTED FOR DOSE AND BODY WEIGHT.

aged 45 years or more, and showed no evidence of bone disease or intestinal dysfunction. Studies were also carried out on ten patients with steatorrhea. Five of these patients had adult celiac disease, the diagnosis having been established by the finding of subtotal villous atrophy on jejunal biopsy (18) and by a satisfactory response to a gluten-free diet (19). Three patients had pancreatic

dysfunction, one who had undergone partial pancreatectomy and gastrojejunostomy, and two with chronic calcific pancreatitis. This diagnosis had been confirmed by the demonstration of a marked reduction in the volume, bicarbonate concentration, and enzyme activity of their duodenal aspirates during Lundh (20) and secretin tests (21) and by normal jejunal biopsies. The remaining two patients had biliary obstruction; one of these had primary biliary cirrhosis (serum bilirubin, 17 mg per 100 ml), and the other had a carcinoma of the head of the pancreas (serum bilirubin, 10 mg per 100 ml). Further clinical data relating to these patients are shown in Tables III and IV.

TABLE I

Proportion of radioactivity in chylomicrons in four control subjects after oral vitamin D₃-³H

Subject	Hours after dose	
	2-3	4-6
	% of total plasma radioactivity	
1	46	35
2	100	26
3	50	14
4	45	49
Mean	60	31

Results

Control subjects

Plasma radioactivity. The plasma radioactivity in five of the control subjects at varying time intervals after receiving 1 mg of vitamin D₃-³H containing 5 to 15 µc of ³H is shown in Figure 2. At 2 hours after the oral dose, radioactivity had ap-

peared in the plasma of four subjects, and by 6 hours it was increasing in all five; radioactivity then rose further to reach a peak at 6 to 12 hours after the oral dose, thereafter declining exponentially.

The mean plasma half-life after a 1-mg dose, calculated from observations in the five subjects whose results are illustrated in Figure 2, and from four other subjects in whom plasma curves were also measured, was 54 hours (range, 36 to 78 hours).

Distribution and nature of radioactivity in plasma. The distribution of radioactivity between chylomicrons and nonparticulate lipid was determined in plasma samples obtained during the early stages of absorption of the labeled vitamin in four control subjects. Data are shown in Table I: at 3 hours after the oral dose, 45 to 100% of plasma radioactivity was in the chylomicron fraction, and at 6 hours this had fallen to 14 to 49%.

Thin-layer chromatography was carried out on lipid extracts of pooled plasma samples taken from three other subjects at 12, 24, and between 48 and 72 hours after oral doses of vitamin D₃-³H containing 15 to 50 μ C of ³H; a marker of vitamin D₃ was run on each plate. The radioactivity in various regions of each chromatogram was assayed after elution and calculated as a percentage of total radioactivity eluted. An average of 81.2% (range, 73.4 to 88.8) was present in the region of the vitamin D₃ marker and 4.6% in the sterol ester region. The remaining counts were chiefly at the origin. The mean recovery of the radioactivity applied to the thin-layer plates was 70% (range, 65 to 73).

To investigate the stability of the tritium label during absorption into plasma, we gave a control subject 50 μ C of vitamin D₃-³H by mouth. Samples of 1 ml plasma were extracted with 20 ml chloroform:methanol, and 4 ml water was added. An 0.8-ml aliquot of the aqueous layer of each sample was added to 10 ml Bray's solution for radioassay. No water-soluble radioactivity was detectable in this subject's plasma at 3, 7, 12, and 24 hours after the dose, although absorption of lipid-soluble radioactivity was normal.

Net absorption. The absorption of vitamin D₃-³H was calculated by assuming that the radioactivity not recovered in the feces during the 6-day period had been absorbed.

Test doses of 0.1 mg of vitamin D₃-³H were

TABLE II
Net absorption of 0.1-, 0.5-, and 1-mg oral doses of vitamin D₃-³H in nine control subjects

Subject	Dose	Net absorption
	mg	%
1	0.1	55.3
2	0.1	98.7
3	0.5	83.1
4	0.5	84.3
5	1.0	62.4
6	1.0	67.7
7	1.0	80.1
8	1.0	81.2
9	1.0	91.3

given to two subjects, 0.5 mg to two others, and 1 mg to five subjects; these doses contained 1.5 to 15 μ C of ³H. The results of the absorption tests in these nine control subjects are shown in Table II.

The net absorption of either 0.5- or 1-mg doses of vitamin D₃-³H in seven control subjects ranged from 62.4 to 91.3% (mean 78.6). The absorption in the two control subjects who were given 0.1-mg doses was 55.3% and 98.7% (mean 77.0).

Nature of radioactivity in feces. Thin-layer chromatography was carried out on the fecal extracts from four subjects after oral doses of vitamin D₃-³H. The mean recovery of radioactivity from the region opposite a vitamin D₃ marker was 77.2% (range, 68.4 to 85.8), suggesting that most of the radioactivity in fecal extracts represented vitamin D₃.

The possibility that bacteria in the bowel might release water-soluble tritium was investigated *in vitro* by incubating vitamin D₃-³H with cultures of *Escherichia coli*, *Klebsiella aerogenes*, and *Streptococcus faecalis*. After 12 hours incubation at 37° C the cultures were extracted with chloroform:methanol and the phases separated with water. Only 1% of the total radioactivity was detectable in the aqueous phase, the rest remaining lipid soluble.

Urinary excretion. The mean urinary excretion of radioactivity in eight control subjects given 1 mg (15 μ C) of vitamin D₃-³H was 3.5% of the absorbed dose during the first 3 days (range, 0 to 4.8). Urinary radioactivity was mainly water soluble, less than 1% being extractable into chloroform. Excretion of urinary radioactivity was maximal during the first 24 hours after a dose.

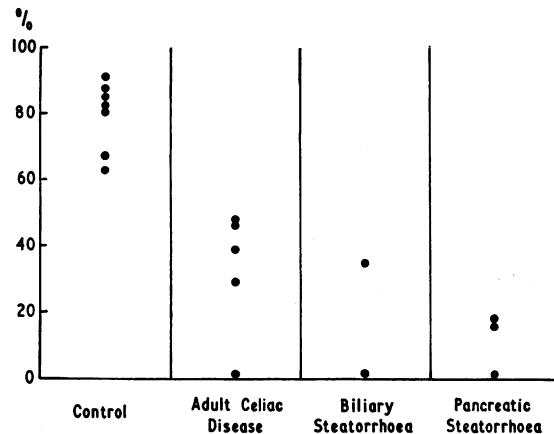


FIG. 3. NET ABSORPTION OF 0.5 TO 1 MG VITAMIN D₃-³H IN CONTROL SUBJECTS AND PATIENTS WITH INTESTINAL MALABSORPTION.

Patients with intestinal malabsorption

The net absorption of 0.5- to 1-mg doses of vitamin D₃-³H in the ten patients with different types of intestinal malabsorption is shown in Figure 3, together with the results in control subjects given similar doses. Clinical and biochemical data relating to these ten patients and individual results of vitamin D absorption tests are shown in Tables III and IV.

Adult celiac disease. The net absorption of 1 mg (15 μc) vitamin D₃-³H, calculated from the fecal radioactivity, was subnormal in all the five patients, ranging from nil to 47.6% (Figure 3).

The plasma radioactivity in these five patients is shown in Figure 4. In Patients 1 and 2, Table III, the level of plasma radioactivity was very low; these two patients also had the lowest net ab-

sorption of vitamin D₃-³H (nil and 29.1%). Both patients presented with hypocalcemia and severe steatorrhea but without symptoms of bone disease or elevation of their serum alkaline phosphatase (Table III).

In Patients 3, 4, and 5 the level of plasma radioactivity was less subnormal, rising to just below or within the normal range (Figure 4). Their net absorption of vitamin D₃-³H was also less abnormal (38.6, 46.5, and 47.6%). Two of these patients (No. 4 and 5) had a normal serum calcium, but all three had histological or biochemical evidence of osteomalacia. In two (Patients 4 and 5) symptoms of steatorrhea had been present since childhood; the third (Patient 3) had no diarrhea but gave a long history of vague ill health and bone pain.

Pancreatic steatorrhea. The net absorption of oral doses of 1 mg (15 μc) vitamin D₃-³H was grossly reduced in all three patients with pancreatic steatorrhea (Figure 3). Plasma radioactivity was measured in one patient (Patient 8, Table IV) and was markedly subnormal. In this patient, who had undergone partial pancreatectomy and gastrojejunostomy 11 years previously, the bone biopsy showed osteomalacia, but the serum calcium was normal. In the other two patients (Patients 6 and 7, Table IV) the serum calcium level and the radiological appearance of the bones were normal, in spite of marked steatorrhea and severely defective absorption of vitamin D₃-³H.

Biliary obstruction. The absorption of 0.5 to 1 mg (2.5 to 5 μc) vitamin D₃-³H in the two patients with obstructive jaundice was also markedly subnormal (0 and 28.4%). Neither of these patients

TABLE III
Details of patients with adult celiac disease

Patient no.	Age	Duration of symptoms	Serum				Bone X rays	Fecal fat	Vitamin D ₃ - ³ H
			Ca	P	Alkaline phosphatase	Albumin			
	years		mEq/L		King-Armstrong U	g/100 ml		g/24 hours	% absorption
1	56	9 months	4.0	1.3	10	3.3	Normal	40.0	0
2	58	12 months	3.5	2.0	11	4.2	Rarefaction	30.0	29.1
3	70	15 years	4.4	1.9	12	3.4	Rarefaction*	15.0	38.6
4	43	Since childhood	5.1	1.8	29	3.7	Rarefaction	6.4	46.5
5	58	Since childhood	4.9	1.9	26	4.3	Rarefaction*	14.0	47.6
Normal range			4.7-5.5	1.5-2.5	3-13	3.5-5.2		<6	62.4-91.3

* Bone biopsy showed osteomalacia.

had any radioactivity detectable in his plasma after the oral dose. The patient with primary biliary cirrhosis (Patient 9, Table IV) had been jaundiced for 11 years and had both hypocalcemia and marked skeletal rarefaction, suggesting osteomalacia, but this was not confirmed histologically, since a bone biopsy was not obtained.

Fecal fat excretion and vitamin D₃-³H absorption. The relationship between vitamin D₃-³H absorption and fecal fat excretion in the patients with intestinal malabsorption is shown in Figure 5. The five patients with adult celiac disease excreted between 6.4 and 40 g of fat daily, and there was a close correlation between the degree of steatorrhea and the severity of the defect in vitamin D₃-³H absorption in these patients. In the patients with steatorrhea secondary to biliary or pancreatic disease, on the other hand, this relationship was less obvious. One of the patients with obstructive jaundice, for example, showed total malabsorption of vitamin D₃-³H in the presence of mild steatorrhea (Patient 9, Table IV).

Discussion

The methods described in this paper for preparing and purifying tritiated vitamin D₃ are relatively simple, and the product conforms to the known physicochemical and biological properties of the vitamin. The specific activity of the labeled vitamin has varied from batch to batch. With oral doses of 0.1 to 1 mg, specific activities of 5 to 15 μC per mg were adequate for absorption tests. The smallest dose used was 1.5 μC , which was suffi-

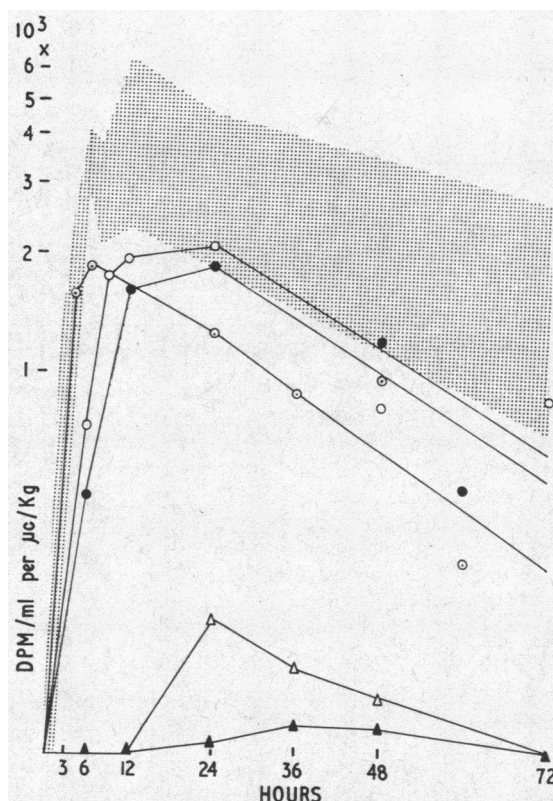


FIG. 4. PLASMA RADIOACTIVITY IN FIVE PATIENTS WITH ADULT CELIAC DISEASE AFTER 1 MG OF ORAL VITAMIN D₃-³H (15 μC). The shaded area represents the normal range. The symbols refer to patient numbers in Table III: Patient 1 ▲, 2 △, 3 ⊙, 4 ●, 5 ○.

cient to measure fecal excretion but not to estimate plasma radioactivity. Although a higher specific activity of up to 55 μC per mg was attainable, this

TABLE IV
Details of patients with steatorrhea due to biliary or pancreatic dysfunction

Patient no.	Age	Diagnosis	Duration	Serum				Bone X rays	Fecal fat	Vitamin D ₃ - ³ H
				Ca	P	Alkaline phosphatase	Albumin			
	years			mEq/L		King-Armstrong U	g/100 ml	g/24 hours	% absorption	
6	66	Chronic pancreatitis	5 months	4.7	2.2	8	4.2	Normal	23.6	0
7	68	Chronic pancreatitis	1 year	4.6	2.0	10	3.1	Normal	75.4	17.9
8	59	Partial pancreatectomy	11 years	5.0	2.2	12	4.6	Rarefaction*	11.5	16.3
9	40	Primary biliary cirrhosis	11 years	4.3	2.2	40	3.8	Rarefaction	13.0	0
10	60	Biliary obstruction	2 months			18	4.6		17.4	28.4
Normal range				4.7-5.5	1.5-2.5	3-13	3.5-5.2		<6	62.4-91.3

* Bone biopsy showed osteomalacia.

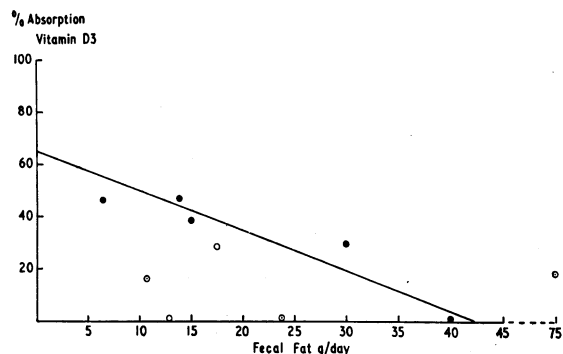


FIG. 5. RELATIONSHIP BETWEEN FECAL FAT EXCRETION AND NET ABSORPTION OF VITAMIN D_3 - 3H IN PATIENTS WITH VARIOUS TYPES OF STEATORRHEA. Adult celiac disease ●, pancreatic steatorrhea ○, biliary steatorrhea ○. The diagonal line ($y = 42.06 - 0.65x$) is the calculated regression for the five patients with adult celiac disease. For this group there is a high degree of correlation ($r = 0.94$, $p < 0.02$) between fecal fat excretion and vitamin D_3 - 3H absorption.

material was unsuitable owing to a rapid rate of decomposition and decline in specific activity. When the specific activity of vitamin D_3 - 3H was between 5 and 15 μ c per mg, it remained reasonably constant, although repurification by thin-layer chromatography was necessary before use.

In practice, vitamin D_3 - 3H was used within 5 days of repurification, and under these circumstances the tritium label appeared to be stable during absorption. The urinary excretion of water-soluble tritium did not exceed 5% of the absorbed dose in the first 3 days, and plasma water showed no detectable radioactivity. At least 80% of the radioactivity appearing in plasma after doses of pure vitamin D_3 - 3H behaved as vitamin D_3 on thin-layer chromatography. These results are comparable to those obtained by Schachter and co-workers (2), who found that 69.4% of the radioactivity in the lymph of rats given vitamin D_3 - 3H was in the free sterol zone, of which 81.7% behaved as vitamin D_3 .

After oral doses of 1 mg vitamin D_3 - 3H radioactivity in plasma did not reach a peak until 6 to 12 hours had elapsed. This contrasts with peak lipemia after a fatty meal, which is usually maximal at 3 to 4 hours. In the rat Schachter and co-workers (2) have demonstrated a delay in transfer of the absorbed vitamin across the intestinal mucosa, and it is possible that this also occurs in man. At 2 to 3 hours, from 45 to 100% of the

radioactivity in the plasma was present in the chylomicron fraction. By 6 hours, the plasma radioactivity had increased, but the proportion of labeled vitamin D in the chylomicrons had diminished, possibly due to liberation of vitamin D_3 - 3H from chylomicrons after entering the plasma. The association of vitamin D_3 - 3H with chylomicrons during early absorption is compatible with a predominantly lymphatic route of absorption in man, as has already been demonstrated in the rat (2).

At the time of peak plasma radioactivity about 40% of the absorbed dose was present in the plasma. The subsequent distribution of vitamin D after absorption in man is unknown. In the rat, vitamin D is initially deposited mainly in the liver (22), but in pigs it has been reported that blood is the main storage site (23). Vitamin D does not appear to have been excreted by the kidney to any significant degree, since only 1% of the urinary radioactivity was lipid soluble.

The plasma levels of absorbed vitamin D_3 - 3H varied considerably in different subjects, but there was less variation in the estimates of net absorption based on measurements of fecal radioactivity in a 6-day study in the same subjects. This may have been due to individual variation in the rate of clearance of an absorbed dose from plasma. When oral doses of 0.5 to 1 mg of vitamin D_3 - 3H were given to control subjects, they absorbed between 62.4 and 91.3% of the dose; these results are similar to those reported in infants by Kodicek (3), using vitamin D_2 - ^{14}C .

In contrast to the control subjects, all five patients with adult celiac disease showed malabsorption of vitamin D_3 - 3H calculated from their fecal excretion of radioactivity. The results in these patients show more obvious malabsorption of vitamin D_3 - 3H when measured by the net absorption of radioactivity than by studying plasma levels. There was a close correlation between the degree of malabsorption of vitamin D_3 - 3H and the fecal fat excretion in these patients, and both were presumably due to the mucosal abnormality. Similarly, there was also marked malabsorption of vitamin D_3 - 3H in the three patients with pancreatic steatorrhea. One possible mechanism for this could be that deficiency of pancreatic lipase caused vitamin D to be retained in solution within the intestinal lumen by unsplit dietary fat. Malabsorp-

tion of vitamin D_3 - 3H was also demonstrated in both of the patients with biliary obstruction, even in the presence of mild steatorrhea. The importance of bile acids in vitamin D absorption has been clearly established in the rat (2), and both our results and the clinical observations of Atkinson, Nordin, and Sherlock (24) suggest that they are equally essential in man.

There was marked hypocalcemia in two of the patients with adult celiac disease, both of whom had gross steatorrhea and severe malabsorption of vitamin D_3 - 3H . In contrast, two of the patients with pancreatic steatorrhea had a similar degree of malabsorption of fat and vitamin D_3 - 3H yet maintained normal serum calcium levels. A possible explanation for this difference may be the state of the intestinal mucosa in these patients, since the jejunal biopsy was abnormal in those with adult celiac disease, but was normal in both the patients with chronic pancreatitis. It is known that active transport of calcium across the jejunum is vitamin D dependent (25), and it has been shown that this action of vitamin D can be blocked by actinomycin D (26, 27). If vitamin D acts on the intestinal mucosa by stimulating the synthesis of a protein or enzyme responsible for calcium transport, as tentatively suggested by Zull, Czarnowska-Misztal, and DeLuca (26), then it seems possible that patients with a mucosal abnormality would show a more marked disturbance of calcium metabolism than those with a comparable degree of vitamin D deficiency but with a normal mucosa.

Summary

Tritium-labeled vitamin D_3 of specific activity 5 to 15 μ c per mg was prepared by random exchange and isolated in crystalline form. Its properties were those of the authentic vitamin.

Control subjects were given oral doses of 0.1 to 1 mg of vitamin D_3 - 3H , containing 1.5 to 15 μ c of tritium, in arachis oil, and their plasma and fecal radioactivity was assayed during the subsequent 6 days. Radioactivity was present in the plasma 3 hours after a dose and at this stage was largely located in the chylomicrons. It reached a peak at 6 to 12 hours and thereafter declined exponentially, with a mean half-life of 54 hours. The net absorption of 0.5- to 1-mg doses, calculated from the fecal excretion of radioactivity, ranged from 62.4 to 91.3%

The net absorption of 0.5- to 1-mg doses of vitamin D_3 - 3H was also measured in patients with various forms of steatorrhea. Malabsorption of vitamin D_3 - 3H was demonstrated in five patients with adult celiac disease, the degree of malabsorption being related to the fecal fat excretion. Malabsorption of vitamin D_3 - 3H was also demonstrated in three patients with pancreatic steatorrhea and in two patients with biliary obstruction.

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References

1. Norman, A. W., and H. F. DeLuca. The preparation of H^3 -vitamins D_2 and D_3 and their localization in the rat. *Biochemistry* 1963, **2**, 1160.
2. Schachter, D., J. D. Finkelstein, and S. Kowarski. Metabolism of vitamin D. I. Preparation of radioactive vitamin D and its intestinal absorption in the rat. *J. clin. Invest.* 1964, **43**, 787.
3. Kodicek, E. The fate of ^{14}C -labelled vitamin D_2 in rats and infants *in* *Drugs Affecting Lipid Metabolism*, Proceedings of the Symposium on Drugs Affecting Lipid Metabolism, S. Garattini and R. Paoletti, Eds. Amsterdam, Elsevier, 1961, p. 515.
4. Nield, C. H., W. C. Russell, and A. Zimmerli. The spectrophotometric determination of vitamins D_2 and D_3 . *J. biol. Chem.* 1940, **136**, 73.
5. Bourdillon, R. B., H. M. Bruce, C. Fischmann, and T. A. Webster. The quantitative estimation of vitamin D by radiography. *Spec. Rep. Ser. med. Res. Coun. (Lond.)* 1931, no. 158.
6. Shaw, W. H. C., J. P. Jefferies, and T. E. Holt. The determination of vitamin D and related compounds. Part I. Introduction and preparation of compounds in the irradiation series. *Analyst* 1957, **82**, 2.
7. Shaw, W. H. C., and J. P. Jefferies. The determination of vitamin D and related compounds. Part II. Analysis of irradiation products. *Analyst* 1957, **82**, 8.

8. Folch, J., M. Lees, and G. H. Sloane Stanley. A simple method for the isolation and purification of total lipides from animal tissues. *J. biol. Chem.* 1957, **226**, 497.
9. Carlson, L. A., and L. B. Wadström. Determination of glycerides in blood serum. *Clin. chim. Acta* 1959, **4**, 197.
10. Lewis, B., and N. B. Myant. Quantitative separation of radioactive sterols and bile acids in human faeces. *J. clin. Path.* 1965, **18**, 105.
11. Bray, G. A. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.* 1960, **1**, 279.
12. Wootton, I. D. P. *Micro-Analysis in Medical Biochemistry*, 4th ed. London, Churchill, 1964.
13. Kohn, J. Cellulose acetate electrophoresis and immunodiffusion techniques in *Chromatographic and Electrophoretic Techniques*, vol. 2, Zone Electrophoresis, I. Smith, Ed. London, Heinemann, 1960, p. 56.
14. Van de Kamer, J. H., H. ten Bokkel Huinink, and H. A. Weyers. Rapid method for the determination of fat in feces. *J. biol. Chem.* 1949, **177**, 347.
15. Bowers, M. A., P. K. Lund, and J. C. Mathies. A rapid, reliable procedure for the determination of total fecal lipids, with observations on the composition of the lipids excreted by human subjects in normal and pathological states. *Clin. chim. Acta* 1964, **9**, 344.
16. Stammers, F. A., and J. A. Williams. *Partial Gastrectomy*. London, Butterworths, 1963.
17. Ball, J. *Diseases of bone in Recent Advances in Pathology*, 7th ed., C. V. Harrison, Ed. London, Churchill, 1960, p. 293.
18. Crosby, W. H., and H. W. Kugler. Intraluminal biopsy of the small intestine. The intestinal biopsy capsule. *Amer. J. dig. Dis.* 1957, **2**, 236.
19. Bolt, R. J., J. A. Paris, A. B. French, and H. M. Pollard. Evaluation of histologic response to maintenance of long term, low gluten diet in patients with idiopathic nontropical sprue (abstract). *Gastroenterology* 1963, **44**, 818.
20. Lundh, G. Pancreatic exocrine function in neoplastic and inflammatory disease: a simple and reliable new test. *Gastroenterology* 1962, **42**, 275.
21. Dreiling, D. A. The pancreatic secretion in the malabsorption syndrome and related malnutrition states. *J. Mt Sinai Hosp.* 1957, **24**, 243.
22. Kodicek, E. The metabolism of vitamin D in Fourth International Congress of Biochemistry, Vienna, vol. 11, Vitamin Metabolism, W. Umbreit and H. Moliter, Eds. London, Pergamon, 1960, p. 198.
23. Quarterman, J., A. C. Dalgarno, A. Adam, B. F. Fell, and R. Boyne. The distribution of vitamin D between the blood and the liver in the pig, and observations on the pathology of vitamin D toxicity. *Brit. J. Nutr.* 1964, **18**, 65.
24. Atkinson, M., B. E. C. Nordin, and S. Sherlock. Malabsorption and bone disease in prolonged obstructive jaundice. *Quart. J. Med.* 1956, **25**, 299.
25. Schachter, D., and S. M. Rosen. Active transport of Ca^{45} by the small intestine and its dependence on vitamin D. *Amer. J. Physiol.* 1959, **196**, 357.
26. Zull, J. E., E. Czarnowska-Misztal, and H. F. DeLuca. Actinomycin D inhibition of vitamin D action. *Science* 1965, **149**, 182.
27. Norman, A. W. Actinomycin D and the response to vitamin D. *Science* 1965, **149**, 184.