Metabolism of phytol-U-^{14}C and phytanic acid-U-^{14}C in the rat

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ABSTRACT The metabolism of uniformly-labeled ^{14}C-phytol, ^{14}C-phytenic acid, and ^{14}C-phytanic acid was studied in the rat. Conversion of both phytol and phytenic acid to phytanic acid was demonstrated. Tracer doses of phytol-U-^{14}C given orally were well absorbed (30–66%), and approximately 30% of the absorbed dose was converted to $^{14}CO_2$ in 18 hr. After intravenous injection, 20% appeared in $^{14}CO_2$ in 4 hr. Phytanic acid-U-^{14}C given intravenously was oxidized at a comparable rate (22–37% in 4 hr) and was as rapidly oxidized as palmitic acid-1-^{14}C (21% in 4 hr).

Metabolism of these substrates was also studied in rats previously maintained on a diet containing 5% phytol by weight, which causes accumulation of phytanic acid, phytenic acid, and, to a lesser extent, phytol in blood and tissues. Despite the large body pools of preformed, unlabeled substrate in these animals, the fraction of an administered dose of phytol-U-^{14}C or phytanic acid-U-^{14}C converted to $^{14}CO_2$ was not significantly diminished. These studies indicate that the rat has an appreciable capacity to degrade the highly branched carbon skeleton of phytol and its derivatives.

Twenty-four hours after administration of phytol-U-^{14}C, the lipid radioactivity remaining in the body was widely distributed among the tissues, highest concentrations being found in liver and adipose tissue. Four hours after intravenous administration of phytanic acid-U-^{14}C, all of the major lipid classes in the liver contained radioactivity, most in triglycerides and phospholipids and least in cholesterol esters and lower glycerides. There was no demonstrable incorporation of mevalonate-2-^{14}C or acetate-1-^{14}C into liver phytanic acid when they were given intravenously to a rat previously fed phytol. Endogenous biosynthesis, if it occurs at all, must be extremely limited.

KEY WORDS phytol-U-^{14}C · phytanic acid-U-^{14}C · phytenic acid-U-^{14}C · absorption · metabolism · oxidation · incorporation · lipid classes · tissue distribution · rat · branched-chain fatty acids and alcohols · dihydrophytol-2,3-^{3}H · Refsum's disease

PHYTOL AND phytanic acid are widely distributed in food materials, although they represent minor constituents of the human diet. Phytol, as a component of the chlorophyll molecule, is found ubiquitously in green vegetables; it may be present in other dietary sources as well, but no analytic data are available. Phytanic acid has been found in butter fat (3), bovine plasma (4), bovine fat (5), sheep fat (6), and human serum (7, 8). It represents in most cases less than 1% of the total fatty acids.

Information about the metabolism of these C_{20} branched-chain compounds is very limited. In view of the recent demonstrations that heredopathia atactica polyneuritiformis (Refsum's disease) is associated with storage of large amounts of phytanic acid in blood and tissues (9–11), and that dietary phytol is a potential precursor of phytanic acid both in experimental animals (12–14) and in man (15, 16), it was of interest to establish in more detail the normal patterns of metabolism of these compounds. The present studies are concerned with the distribution and oxidation of phytol-U-^{14}C, phytenic acid-U-^{14}C, and phytanic acid-U-^{14}C in rats.[1] A prelimi-

A portion of this work was presented at the meetings of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April 1965 (1) and April 1966 (2).

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

[1] Compounds in the text referred to by their trivial names are: phytol (3,7,11,15-tetramethyl hexadec-2-en-1-ol); phytanic acid (3,7,11,15-tetramethyl hexadecan-1-oic acid); phytenal (3,7,11,15-tetramethyl hexadec-2-en-1-al); phytenic acid (3,7,11,15-tetramethyl hexadec-2-en-1-oic acid); dihydrophytol (3,7,11,15-tetramethyl hexadecan-1-ol).

nary report of portions of this work has appeared elsewhere (17).

METHODS AND MATERIALS

Male rats of the Sprague-Dawley strain, weighing less than 250 g, were fed, ad lib., either commercial chow in pellet form (Ralston Purina Co., St. Louis, Mo.) or the same material ground to a powder and mixed with 5% phytol by weight. The food intake of rats on the phytol-containing diet was always less than that of the control rats; 150-g animals on the phytol diet ingested approximately 8 g of diet daily (400 mg of phytol). These animals, subsequently referred to as phytol-fed animals, received this diet for 2 weeks or longer prior to study.

Phytol-U-^{14}C was purified from the nonsaponifiable lipid fraction of algae grown on $^{14}CO_2$ (New England Nuclear Corp., Boston, Mass., and Volk Radiochemical Co., Skokie, Ill.). After addition of about 1 mg of carrier phytol, the material was chromatographed on thin layers of silicic acid, with benzene–ethyl acetate 4:1 as developing solvent (12). The phytol zone contained approximately 40% of the original nonsaponifiable radioactivity. The isolated phytol-U-^{14}C was tested for radiopurity in several TLC systems. As a further criterion of purity, the labeled phytol was converted to the aldehyde or to the acetate derivative, and recoveries of radioactivity in these derivatives after TLC were determined. For the preparation of phytenal, MnO_2 was added directly to phytol (20 mg/g of phytol) and the mixture was stirred overnight at room temperature; the catalyst was removed by centrifugation. Phytyl acetate was prepared by reacting phytol with acetic anhydride in pyridine.

The phytol-U-^{14}C used in some experiments was purified on a preparative scale by oxidation to phytenal-U-^{14}C, followed by TLC and reduction of the isolated aldehyde to phytol-U-^{14}C. This reduction was carried out by reacting 5 ml of an ethanolic solution saturated with lithium borohydride with approximately 2 mg of phytenal at room temperature for 15 min; the regenerated phytol was extracted into hexane after addition of water to 30% ethanol concentration. Another portion of the phytol-U-^{14}C was purified by successive preparative TLC and GLC of the acetic acid ester, prepared as described above, followed by hydrolysis and isolation of the free alcohol. The radiopurity of the phytol used in all cases was at least 93%, judged by recovery of radioactivity with authentic carrier material on TLC or GLC.

Phytenic acid-U-^{14}C was prepared by oxidation first of phytol-U-^{14}C to phytenal-U-^{14}C with isolation of the aldehyde from the reaction medium by TLC in benzene–ethyl acetate 16:1. Freshly prepared silver oxide in solution was then added directly to the aldehyde in approximately 2:1 ratio and the mixture was stirred for 2 hr. After centrifugation, the supernatant solution was acidified with HCl and extracted with ether. The ether solution containing phytenic acid-U-^{14}C was washed with 20% NaCl and concentrated. For the preparation of phytanic acid-U-^{14}C, approximately equal amounts of palladized charcoal were added to phytenic acid-U-^{14}C in hexane, and the mixture was hydrogenated for 16 hr. Phytenic acid-U-^{14}C and phytanic acid-U-^{14}C were purified separately as methyl esters by TLC, with benzene–hexane 2:1 as developer. Mevalonic acid-2-^{14}C as the dibenzyl ethylenediamine salt (2.9 μc/μmole), sodium acetate-1-^{14}C (2.0 μc/μmole) and dihydrophytol labeled with tritium in the 2- and 3-positions (0.9 mc/μmole) were obtained from New England Nuclear Corp.

Phytol-U-^{14}C was administered orally via a stomach tube, mixed with 1.0 ml of vegetable oil, or intravenously into a penile vein as a suspension in Tween 20 (polyoxyethylene sorbitan monolaurate) (Atlas Chemical Co.; distributor, Gilman, Inc., Washington, D.C.). Tracer doses of phytanic acid were given by stomach tube as the sodium salt in slightly alkaline solution, or intravenously in the form of a serum albumin complex (5% albumin in 0.85% saline).

After receiving the ^{14}C-labeled compounds, the animals were placed in a sealed desiccator fitted with air inlet and outlet, through which CO_2-free air was drawn. The expired $^{14}CO_2$ was absorbed in wash bottles containing 20% NaOH, and aliquots of this solution were counted in a liquid scintillation counter (Packard Tri-Carb, Model 314 EX) on anthracene crystals (18). The other radioactive fractions were prepared in 0.5% diphenyl oxazole in toluene. The results with each method were corrected to the efficiency obtained in toluene. Total feces were collected, pooled with the total contents of the gastrointestinal tract at the time of sacrifice, and treated with a large volume of slightly acidified 1:1 ethanol–acetone. Radioactivity in this extract was determined and subtracted from the dose administered to calculate the amount of labeled phytol absorbed. The amount calculated in this way represents a minimum value since some radioactivity would be expected to be involved in the enterohepatic circulation of the animals (2). Individual tissues were treated with ethanol–acetone 1:1, and aliquots of these extracts were taken for radioassay.

The phytol content and the phytanic or phytenic acid content of liver were determined by GLC of the nonsaponifiable lipid fraction and of the methyl esters of the fatty acid fraction, respectively, on 15% ethylene glycol succinate polyester (EGS) on Gas-Chrom P or Chromosorb W (Applied Science Laboratories Inc., State College, Pa.). Radioactive methyl phytanate and methyl phytenate were separated on an EGS column and collected directly into narrow-gauge Teflon tubing cooled in a dry ice–ethanol bath. The individual fractions were washed

from the tubing directly into vials for subsequent radioassay. Recovery by this technique was 95–100% with known standards. The details of lipid fractionations are described in the preceding paper (12).

RESULTS

Tracer doses of phytol-U-^{14}C (0.2 mg; 2 μc) given orally to rats were well absorbed (30–66% of the administered dose). Two control rats which had received phytol-U-^{14}C in tracer doses were killed after 24 hr for studies of the distribution of absorbed radioactivity, shown in Table 1. In these two rats, a total of 3 and 9% of the administered dose of ^{14}C was recovered in the body tissues at this time. The concentration of radioactivity in liver and adipose tissue was several times higher than the average concentration of radioactivity in the whole body. A higher concentration of radioactivity was found in the intestine in experiment 2; this may have been due to contamination with radioactive intestinal contents since the intestine still contained a large percentage of the administered dose of radioactivity. Kidneys, brain, heart, lungs, spleen, and eyes contained lower concentrations and lower total amounts of radioactivity.

In experiment 2 of Table 1, aliquots of muscle and skin were also analyzed and were found to contain relatively low concentrations of radioactivity. However, the contribution to total body radioactivity of these tissues, as well as that of adipose tissue, is quite large, because of the mass of tissue involved. Assuming that total adipose tissue, skin, and muscle represent 7, 18, and 46% of body weight, respectively (19), and that concentration of lipid radioactivity was the same in the total masses as that in the aliquots, liver radioactivity accounted for the largest percentage of the recovered label (19%), with the adipose tissue and muscle compartments containing a similar proportion (16% in each), and skin somewhat less (8%). The percentage of the total radioactivity found, by direct analysis, in the total carcass was 40.4%, which agrees quite well with that calculated to be present in the total adipose tissue, muscle, and skin (40%). In these experiments, less than 1% of the radioactivity was found in each of the other organs analyzed.

TABLE 1 TISSUE DISTRIBUTION OF RADIOACTIVITY 24 HR AFTER AN ORAL DOSE OF PHYTOL-U-^{14}C TO NORMAL RATS*

Organ	Concentration of Radioactivity	
	Experiment 1	Experiment 2
	cpm/g	
Liver	8440	2980
Intestine	1510	3330
Kidney	1800	440
Brain	1100	500
Lungs	1930	450
Heart	1150	230
Spleen	1900	370
Eyes	530	190
Adipose tissue†	5650	1150
Muscle†	—	170
Skin†	—	210
Carcass‡	1010	170

* Approximately 2 μc was administered to each rat. The radioactivities recovered in the tissues in experiments 1 and 2 were 218,600 and 72,300 cpm, respectively. The average concentration of radioactivity in the whole rat was 1460 and 530 cpm/g for experiments 1 and 2, respectively. The plasma of the rat of experiment 1 was analyzed separately and contained 1.3% of the total recovered tissue radioactivity.

† Aliquots of these tissues were taken from the subcutaneous fat, hind leg muscles, and ventral skin tissue.

‡ Carcass represents the total tissues remaining after removal of the whole organs listed above.

TABLE 2 DISTRIBUTION OF RADIOACTIVITY IN RAT LIVER LIPID CLASSES 4 HR AFTER INTRAVENOUS ADMINISTRATION OF PHYTANIC ACID-U-^{14}C

	Percentage of Total Radioactivity*	
	Phytol-Fed (2)†	Control (2)†
Free fatty acids	15 (12, 18)	8 (6, 11)
Triglycerides	36 (22, 49)	40 (30, 49)
Mono- and diglycerides	5 (3, 7)	2 (1, 3)
Cholesterol esters	9 (8, 10)	6 (3, 9)
Phospholipids	16 (11, 21)	37 (32, 41)
Unidentified	18 (14, 22)	8 (7, 8)

* Mean value with range of values in parentheses.

† Number of animals (150–250 g) represented in parentheses.

The distribution of radioactivity among the various lipid classes in phytol-fed and control rats was studied 4 hr after intravenous administration of phytanic acid-U-^{14}C. In the liver, radioactivity was present in all lipid classes in both groups (Table 2). The largest percentage of radioactivity was found in triglycerides and phospholipids. Similar distribution was evident as early as 30 min after injection in the phytol-fed rat, lipid esters accounting for 70% of total radioactivity in lipids. Relative incorporation into phospholipids at 4 hr appeared to be less in the phytol-fed rats, but the small number of animals studied does not permit quantitative conclusions. Analysis of plasma lipids showed essentially the same distribution of radioactivity as in the liver lipids.

Free phytanic acid and methyl phytanate show slightly higher R_f values in the TLC systems employed here than do the corresponding straight-chain fatty acids and their esters, and can be resolved from them. Triglycerides containing phytanic acid in ester linkage might also be expected to be somewhat less polar. A slight increase in R_f and broadening of the triglyceride zone were found in the lipids from the phytol-fed rat, in which phytanic acid accounts for 25–30% of total liver fatty acids and 16% of triglyceride fatty acids. In the control animals, 6–18% of total radioactivity was found in the area slightly ahead of the main TLC triglyceride zone. This radioactivity was

included in Table 2 with the major triglyceride zone radioactivity of the normal rats on the assumption that it represented phytanic acid-containing triglycerides. If triglycerides containing branched-chain fatty acids appeared exclusively in this zone, they would represent about 30% of the radioactivity in triglycerides. About 70% of the radioactivity in the free fatty acid fraction was contained in phytanic acid in the control rats after 4 hr. At an earlier time (30 min) in a phytol-fed rat, lipid ester radioactivity was found largely as phytanic acid, as expected; it represented 62% of radioactivity in cholesterol esters, 80% in glycerides, and 74% in phospholipids.

The nature of the metabolic products derived from phytol-U-^{14}C and retained in the liver has been investigated. Twenty-four hours after oral administration, less than 10% of the absorbed radioactivity remained in the liver. Most of the nonsaponifiable radioactivity was isolated with phytol by TLC (Table 3). About 50% of the radioactivity in the saponifiable fraction was recovered with the phytanic acid band (which would include also phytenic acid). Most of the remaining acidic radioactivity was found near the origin of the plate and probably represented polar metabolites of phytol. The nature of these components has not yet been determined.

The possible roles of phytenic acid and dihydrophytol as precursors of phytanic acid were investigated. In one experiment, phytenic acid-U-^{14}C (0.1 μc, S.A. 14 μc/μmole) complexed with albumin was injected intravenously into a normal rat and the animal was sacrificed 30 min later. The total fatty acids were extracted from liver and subjected to TLC as methyl esters, with a small amount of carrier methyl phytanate added prior to chromatography. Since methyl phytenate is not resolved from methyl phytanate by this method, the methyl phytanate zone from TLC was collected and again subjected to GLC, which separated the two compounds, and the radioactivity associated with each was determined. Thirty-one per cent of the radioactivity was found with phytanate and 11% with phytenate.

In another experiment, dihydrophytol-2,3-^{3}H (16.2 μc, 0.018 μmole) complexed in a Tween 20 suspension was given intravenously to a phytol-fed rat. After 30 min the liver was removed and the branched-chain fatty acids were analyzed for radioactivity by GLC, as in the preceding experiment. Ninety per cent of the radioactivity was found with phytanate; <1% was associated with the phytenate zone.

As a test of the possibility that animals are capable of synthesizing phytanic acid de novo, a rat was injected intravenously with mevalonate-2-^{14}C (8.8 μc, 3 μmoles). A trap was provided for any phytanic acid formed by feeding the animal phytol, in order that sizeable amounts of phytanic acid might accumulate in the tissues. Another rat fed phytol in the same way was injected intravenously with sodium acetate-1-^{14}C (180 μc, 90 μmoles). The rats were killed 15 and 30 min after injection, respectively, and the methyl esters of the total fatty acids of the liver were fractionated by TLC. Radioactivity in the branched-chain fatty acids was determined by GLC as in the preceding experiment. No radioactivity was found in the phytanic acid fraction in either experiment.

TABLE 3 RADIOACTIVITY IN LIVER LIPID FRACTIONS FOLLOWING ORAL ADMINISTRATION OF PHYTOL-U-^{14}C TO CONTROL AND PHYTOL-FED RATS*

Liver Lipid Fraction	Percentage of Liver Lipid Radioactivity	
	Control	Phytol-Fed
Nonsaponifiable	71	27
Phytol	61	—
Saponifiable	29	73
Phytanic acid	14	35

* Total lipid radioactivity represented 3 and 10% of the absorbed dose, respectively, in the control and phytol-fed animal. The liver of the phytol-fed rat contained 0.12 mg of phytanic acid per g and 4.5 mg of phytol per g.

Oxidation of phytol-U-^{14}C to $^{14}CO_2$ in vivo was studied in rats after both oral and intravenous administration. After an initial lag period, the cumulative time curves of $^{14}CO_2$ production (Fig. 1) showed an essentially constant rate of $^{14}CO_2$ formation. As seen in Table 4, two control rats each had converted approximately 31% of the radioactive substrate to $^{14}CO_2$ after 18 hr. The animal previously maintained on a 5% phytol diet oxidized nearly the same fraction of phytol-U-^{14}C as did the two controls. This occurred in spite of the fact that the organs of the phytol-fed animal contained large amounts of nonradioactive phytol and phytanic acid (12). Intravenously administered phytol-U-^{14}C was much more rapidly oxidized, almost 20% of the dose appearing in $^{14}CO_2$ in only 4 hr.

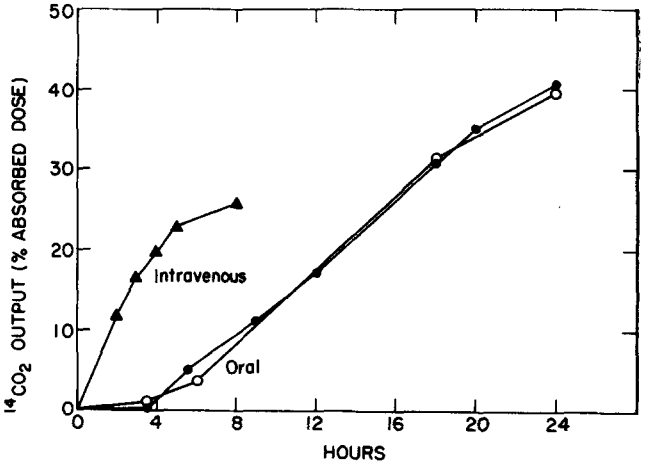


FIG. 1. Cumulative $^{14}CO_2$ production in the rat from phytol-U-^{14}C.

TABLE 4 OXIDATION OF PHYTOL-U-^{14}C AND PHYTANIC ACID-U-^{14}C TO $^{14}CO_2$ IN CONTROL AND PHYTOL-FED RATS

Radioactive Substrate*	Diet†	Per Cent of Absorbed Dose Converted to $^{14}CO_2$‡
Phytol-U-^{14}C		
Intravenous (4 hr)	Control (1)	19
Oral (18 hr)	Control (2)	31 (30, 31)
Oral (18 hr)	5% Phytol (1)	31
Phytanic acid-U-^{14}C		
Intravenous (4 hr)	Control (3)	32 (22–37)
Intravenous (4 hr)	5% Phytol (2)	21 (19, 23)
Oral (4 hr)	Control (1)	19
Palmitic acid-1-^{14}C		
Intravenous (4 hr)	Control (2)	21

* Preparation of substrates is described in the text. Specific activity of each compound was approximately 14 μc/μmole; doses administered varied from 0.1–1.0 μc. Duration of CO_2 collection, in hours, in parentheses.

† Number of animals (150–250 g) represented in parentheses.

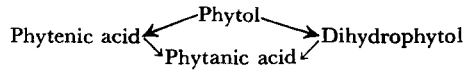
‡ Mean value, where applicable, with range of values in parentheses.

The oxidation of intravenously administered phytanic acid-U-^{14}C was investigated under similar conditions in two control rats and two phytol-fed rats (Table 4). Approximately one-fourth to one-third of the dose was oxidized to $^{14}CO_2$ in 4 hr in both groups. It therefore appears that the metabolism to $^{14}CO_2$ of phytanic acid-^{14}C, like that of phytol-^{14}C, was not greatly affected by the large body pool of nonradioactive phytanic acid. The rates of oxidation of parenterally administered phytanic acid-U-^{14}C and of phytol-U-^{14}C were very nearly the same and comparable to that of palmitic acid-1-^{14}C. Another rat given phytanic acid-^{14}C orally produced in 4 hr approximately the same amount of $^{14}CO_2$ as did the animals receiving this substrate intravenously, which indicates that the phytanic acid-^{14}C was absorbed extremely rapidly.

Information on the capacity of the organism to metabolize phytanic acid was obtained as follows. A control rat was intravenously injected with phytanic acid-U-^{14}C of high specific radioactivity, like that used in the other experiments presented in Table 4, while another control rat of similar size was injected with the same radioactive preparation containing 10 μmoles of added nonradioactive phytanic acid. The first animal converted 37% of the substrate to $^{14}CO_2$ in 4 hr; the second rat converted 32% to $^{14}CO_2$.

DISCUSSION

These radioisotopic studies demonstrate that phytol, administered either orally or intravenously to rats, is an effective biological precursor of phytanic acid. The conversion, requiring reduction of the 2,3-double bond and oxidation of the alcohol function to a carboxylic acid, could occur by two possible pathways:



As shown in the present study, labeled phytenic acid is converted to phytanic acid. In rats chronically fed phytol at high dose levels, significant amounts of phytenic acid, as well as phytanic acid, were demonstrated in plasma and tissues, whereas, except for traces in kidney, there were no detectable amounts of dihydrophytol (12). Phytenic acid and phytanic acid were also found in lymph after feeding phytol, but no dihydrophytol was detected.[2] These observations may suggest that prior oxidation to the carboxylic acid is the major pathway, although the lack of accumulation of dihydrophytol does not exclude this alternative route. Dihydrophytol-2,3-^{3}H is also converted in vivo to phytanic acid, and it has been shown that chronic feeding of high doses of dihydrophytol leads to the accumulation of phytanic acid (13). Thus, it may be that both pathways are potentially available.

The pathway for further degradation of phytanic acid to CO_2 remains to be elucidated. The presence of the β-methyl group of phytanic acid prevents the breakdown by classical β-oxidation. The rate of oxidation is nonetheless extremely rapid and comparable to that of palmitic acid under the same conditions. Eldjarn, Try, and Stokke (20) have suggested that a pathway other than β-oxidation or ω-oxidation may be involved. The only closely related structure whose degradative pathway has been determined in detail is that of farnesoic acid, studied by Seubert and Fass in a microbial system (21).

The branched-chain carbon skeleton of phytanic acid is that of a saturated diterpene. Consequently, it might be synthesized endogenously by the addition of a fourth isopentenyl pyrophosphate unit to farnesyl pyrophosphate to form geranyl geranyl pyrophosphate, a known intermediate in carotene biosynthesis (22). Geranyl geranyl pyrophosphate can be formed by this reaction in mammalian liver (23). Removal of the pyrophosphate, oxidation to the carboxylic acid, and reduction of the four double bonds could give rise to phytanic acid. This possibility was tested by injection of labeled mevalonic acid and labeled acetic acid, but no incorporation was observed. Attempts to demonstrate endogenous biosynthesis of phytanic acid in man have also been negative (16, 24). Although a very slow rate of incorporation of mevalonate cannot be excluded, it would appear that the major, or even only, source of phytanic acid in mammals is exogenous. The only species so far reported to have readily measurable levels of phytanic acid in blood (>1% of total fatty acids) are ruminants, which ingest large amounts of phytol in the form of chlorophyll (4, 7).

[2] Unpublished experiments.

Patients with Refsum's disease have been shown to convert orally administered phytol to phytanic acid (15, 16), and recent studies show that after 12 months on a butter-free, vegetable-free diet the phytanic acid level in these patients fell significantly (25). These observations point to the diet as the only significant source of either phytanic acid, phytol, or other phytanic acid precursors with the appropriate branched-chain structure.

It has been shown here that phytol is in part converted to phytanic acid, and that phytanic acid is oxidized rapidly. Therefore, it is clear that one pathway of phytol oxidation involves phytanic acid as an intermediate step. Furthermore, the fact that intravenously administered phytanic acid was oxidized to CO_2 as rapidly as intravenously administered phytol is compatible on kinetic grounds with the suggestion that phytanic acid is an intermediate in phytol oxidation, and that the rate-limiting step lies between phytanic acid and CO_2. However, the present data are not conclusive. Phytol may be converted to CO_2 directly by another pathway, especially if the branch methyl groups can undergo degradation independently of the chemical function on carbon-1 of the molecule (21), or if ω-oxidation represents a potential site for initial attack (26, 27).

The over-all capacity of the organism to metabolize phytol and phytanic acid is relevant to the physiological effects of dietary phytol in animals, as well as to the interpretation of the phytanic acid accumulation in Refsum's disease in humans. Previous studies have shown the accumulation of significant amounts of phytol, phytanic acid, and phytenic acid in phytol-fed rats, and the disappearance of these compounds from liver and plasma when the exogenous source of phytol is removed (10). The present experiments qualitatively demonstrate a rapid, extensive oxidation in vivo of phytanic acid-U-^{14}C, as well as of phytol-U-^{14}C, even in the presence of large body pools of these compounds. The amount of labeled substrate oxidized to $^{14}CO_2$ was not significantly affected by previous feeding of relatively large quantities of unlabeled phytol. This may in part reflect incomplete mixing of the tracer with the body pools. Most of the stored phytanic acid in the phytol-fed animals is present in ester forms, whereas the administered tracer material is present in free form. Initially there would be dilution only in a small fraction of the total body pool. However, observations as early as 30 min after administration of labeled phytanic acid show that most of it has already been incorporated into esters, still as phytanic acid. Without further information on the distribution and metabolic pathways, the extent of effective dilution cannot be explicitly determined, but some must occur. The results indicate that the fractional turnover of the pools with which the administered tracer mixes is not altered in animals with expanded body pools of phytanic acid.

All of the available data suggest that the normal mammalian organism has a large inherent capacity to oxidize or otherwise eliminate phytanic acid. If phytanic acid is to accumulate in the normal animal, dietary intake must be very great in order to exceed this normal metabolic capacity; in patients with Refsum's disease, accumulation probably occurs because the capacity is limited by virtue of an inborn error in metabolism (16, 24).

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