PHYTANIC ACID FORMATION AND ACCUMULATION IN PHYTOL-FED RATS

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The presence of high concentrations of phytanic acid (3,7,11,15-tetramethylmyristic acid) in the lipids of blood and post-mortem tissues of a patient with heredopathia atactica polyneuritiformis (Refsum's disease) has been reported by Klenk and Kahlke (1963) and by Richterich et al (1963). This disease was first described as a clinical entity by Refsum (1946) who recognized it as genetically transmitted, recessive, and presumably attributable to a specific metabolic error (Refsum, 1960). The primary clinical features include peripheral hypertrophic neuropathy, ataxia and retinitis pigmentosa. Phytanic acid has now been demonstrated in the plasma lipids of at least nine cases of Refsum's disease (Kahlke, 1964), but the origin of this acid, which has not been reported in the plasma of normal individuals, remains to be established.

Phytanic acid has been found in butter fat (Hansen and Shorland, 1953; Sonneveld et al, 1962), ox plasma (Lough, 1964), and ox perinephric fat (Hansen, 1965), but the concentrations are very low. Phytol (3,7,11,-15-tetramethylhexadec-2-en-1-ol), which is a major constituent of the chlorophyll molecule (accounting for 1/3 of its mass), might represent a significant dietary source, if absorbed and converted to phytanic acid.

Phytol was obtained commercially and its purity (> 90%) was verified by gas-liquid chromatography (GLC) and by analysis of its nuclear magnetic resonance spectrum. Phytanic acid was synthesized from phytol in three steps: oxidation to the aldehyde with manganese dioxide; oxidation of the aldehyde to phytenic acid (3,7,11,15-tetramethylhexadec-2-enoic acid)

with silver oxide; hydrogenation with palladium-charcoal to phytanic acid. It was found to be chromatographically identical with a sample of this compound obtained from Prof. E. Klenk. U-C^{14}-phytol was purified by thin-layer chromatography (TLC) of the nonsaponifiable lipids extracted from algae grown on $C^{14}O_2$. Its radiopurity was confirmed by TLC of derivatives (phytyl acetate and phytenal) and also by radioassay of the fraction collected with carrier phytol from a preparative GLC column. Tissue samples were extracted with alcohol-acetone (1:1); serum and lymph samples with chloroform-methanol (2:1). Phytol was extracted after saponification and quantified (as the free alcohol) by GLC using 15% ethylene glycol-succinate as the liquid phase. Phytanic acid concentrations were determined by GLC of methyl esters prepared from the total saponifiable lipid fraction. Phytol and phytanic acid for radioassay were isolated by TLC, as the free alcohol and as the methyl ester, respectively.

As shown in Table I, phytanic acid was not detectable in the fatty acids of liver or serum in control rats, but accumulated in animals fed 1% or 5% phytol added to a Purina chow diet. After three weeks on the 5% phytol diet, phytanic acid accounted for over 20% of the total fatty acids in liver and in plasma. Identification of phytanic acid in the

Table I

Phytanic Acid Accumulation in Liver and Plasma of Phytol-Fed Rats

Phytol in Diet	Phytanic Acid as Percentage of Total Fatty Acids	
	Liver	Plasma
None	N.D.*	N.D.*
1% (for 3 weeks)	2.3	2.1
5% (for 3 weeks)	21.4	27.0

* Not detected under conditions used (less than 0.2% of total fatty acids).

liver was established by TLC, GLC and mass spectrometry. At this time the liver contained also 0.51 mg of phytol per gram of liver. In a sep-

arate study, two rats were fed 5% phytol for 7 days, at which time phytanic acid accounted for 4.9% of the liver fatty acids in one of them. Phytol was then removed from the diet of the other rat, and when it was sacrificed 9 days later the phytanic acid level in the liver had dropped by 70% (1.4% of the total fatty acids).

In Table II is shown the fate of a tracer dose (0.1 mg) of U-C^{14}-phytol fed to two control rats and to a rat previously maintained on a 5% phytol diet. The percentage absorption was variable (29 to 65% in a 24-hour period). Of the absorbed dose, 31 to 40% was recovered as $C^{14}O_2$.

Table II

U-C^{14}-Phytol Absorption, Oxidation and Deposition in Liver after Oral Dosage*

	Control Rat	Control Rat	Phytol-fed Rat**
	(Total radioactivity, c.p.m.)		
Dose administered	2,350,000	2,340,000	4,680,000
Feces + intestinal contents	795,000	1,672,000	1,638,000
Dose absorbed	1,555,000	668,000	3,042,000
Respiratory $C^{14}O_2$	619,000(40%)+	234,000(35%)+	930,000(31%)+
Total liver lipids	40,000(2.6%)	14,280(2.1%)	295,000(9.7%)
Nonsaponifiable liver lipids	28,400(1.8%)	--	106,000(3.5%)
Total saponifiable liver lipids	11,600(0.7%)	--	187,000(6.2%)
Liver phytanic acid	5,700(0.4%)	--	91,000(3.0%)

* Tracer dose (approx. 0.2 mg) given by gastric intubation in 1 ml cottonseed oil. Control rats sacrificed at 24 hours; phytol-fed rat at 18 hours.

** Fed a diet containing 5% phytol by weight for 14 days prior to dosing with C^{14}-phytol.

+ Numbers in parentheses give percentage of absorbed dose converted to $C^{14}O_2$ and to C^{14}-labeled liver lipids.

Of the nonsaponifiable lipid radioactivity recovered in the liver of a control rat, 85% migrated with phytol; 49% of the saponifiable lipid radioactivity was recovered with phytanic acid, the remainder being

associated with more polar components not yet identified. The phytol-fed rat oxidized somewhat less of the absorbed dose, and more accumulated in the liver. A larger fraction of the liver lipid radioactivity was found in the fatty acid fraction, and 48% of this was recovered with phytanic acid.

Four rats in which the thoracic lymph duct had been cannulated were given tracer doses of U-C^{14}-phytol in 0.5 ml vegetable oil by stomach tube. Total count recoveries (from lymph, tissues, CO_2, urine, intestinal contents and feces) ranged from 85 to 100%. The percentage of the administered dose absorbed in 24 hours (taken as the C^{14} radioactivity recovered in lymph, tissues, and CO_2, but excluding the relatively small amount in the urine, which was collected with the feces) varied from 40 to 59%. Of this absorbed radioactivity, 85 to 90% was recovered in the lymph in three experiments, and 48% in the other. In two of the first three experiments referred to, H^3-palmitic acid was administered with the phytol for comparison of absorption; over 90% of the absorbed H^3 radioactivity was recovered in the lymph. Seventy to 90% of the C^{14} radioactivity in the lymph lipid was in the nonsaponifiable fraction, and over 85% of this migrated with phytol. About 60% of the C^{14} radioactivity of the saponifiable fraction migrated with phytanic acid. Prior to saponification of the lipids, the phytol and the phytanic acid appeared to be present principally as esters or other combined forms. Larger doses of phytol were given to several rats. Using 100 mg of carrier phytol, 15% of the dose was recovered in the lymph. Of a 500 mg dose (fed without oil), a much smaller percentage was so recovered.

The present studies establish for the first time that phytol is converted in the mammalian organism to phytanic acid, i.e., the Δ^2-double bond can be reduced and the hydroxyl function oxidized to a carboxyl function. The accumulation of phytanic acid in normal rats fed phytol -- to levels comparable to those seen in plasma and liver of patients with Refsum's disease -- suggests that an exogenous source of phytol such as

chlorophyll in the diet may serve as a precursor of the phytanic acid accumulating in Refsum's disease. On the other hand, the studies reported also show that phytol (and/or its metabolic products) is readily oxidized to CO_2. Appreciable storage of phytanic acid in normal rats occurred only when the level of phytol in the diet was high. Furthermore, the stored phytanic acid in the liver was eliminated rather rapidly when phytol was withdrawn from the diet. These findings suggest further that in Refsum's disease there may be a metabolic error in the handling of phytol and/or phytanic acid.

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