CONVERSION OF U-C^{14}-PHYTOL TO PHYTANIC ACID AND ITS OXIDATION IN HEREDOPATHIA ATACTICA POLYNEURITIFORMIS

D. Steinberg, J. Avigan and C. Mize
Laboratory of Metabolism, National Heart Institute
Bethesda, Maryland

L. Eldjarn and K. Try
Institute of Clinical Biochemistry, University of Oslo
Oslo, Norway

S. Refsum
Department of Neurology, University of Oslo
Oslo, Norway

Received May 14, 1965

Heredopathia atactica polyneuritiformis (Refsum's disease) is a well-defined clinical entity characterized by retinitis pigmentosa, hypertrophic peripheral neuropathy, cerebellar ataxia, and other features (Refsum, 1946). The familial pattern, with recessive mode of inheritance, was recognized early and was presumed to indicate a specific underlying metabolic error (Refsum, 1960). In 1963, Klenk and Kahlke first reported the occurrence of very high levels of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) in the tissues and serum of patients with Refsum's disease, and this has been confirmed in a number of additional cases (Kahlke, 1964; Richterich, et al, 1963; Hansen, 1965a). The origin of the phytanic acid remains to be established.

One major possibility is that phytanic acid arises by biosynthesis from endogenous precursors. Because of its polyterpenoid structure, Kahlke (1964) suggested that it might be formed by the addition of a fourth isopentenyl pyrophosphate residue to farnesyl pyrophosphate, a reaction shown to occur in plants (Grob, Kirschner and Lynen, 1961) and in mammalian liver (Wells, Schelble and Porter, 1964). Subsequent reduction of the double bonds and oxidation to the carboxylic acid would yield phytanic acid.

A second possibility is that the phytanic acid has an exogenous origin, either dietary phytanic acid itself or precursors of it. Phytanic

acid occurs in butter fat (Hansen and Shorland, 1953; Sonneveld et al, 1962), ox plasma (Lough, 1964), and ox perinephric fat (Hansen, 1965b). The amounts available from these sources, however, are very low. Phytol, which accounts for approximately one third of the mass of the chlorophyll molecule, might represent a significant dietary source. It has been shown that the normal rat absorbs labeled phytol, oxidizes it readily and converts it in part to phytanic acid (Steinberg, Avigan, Mize and Baxter, 1965). Furthermore, when a 5% phytol diet is fed to rats, phytanic acid accumulates, accounting for over 20% of the total fatty acids in liver and plasma after three weeks. Since there is nothing to suggest that patients with Refsum's disease have an unusual intake of phytol or other potential precursors, accumulation of exogenously derived phytanic acid would imply a defect in oxidation or excretion. Based on a reduced output of sebacic acid after administration of loading doses of tricaprin to patients with Refsum's disease, Eldjarn (1965) has recently proposed that the enzymatic defect may be at the level of omega-oxidation of fatty acid structures.

Both of the hypotheses for the origin of phytanic acid discussed above were tested in the present studies. Evidence consistent with a defect in breakdown of phytol and/or phytanic acid in Refsum's disease has been obtained.

Methods

A tracer dose of mevalonic acid-2-C^{14} (25 μc) was injected intravenously into subjects fasted overnight. Venous blood samples were drawn for determination of radioactivity in nonsaponifiable lipids, saponifiable lipids and, in some samples, phytanic acid. The fatty acids were converted to methyl esters and methyl phytanate was isolated by TLC (after addition of carrier in the case of the normal controls). U-C^{14}-phytol was isolated from the nonsaponifiable lipid fraction of algae grown on $C^{14}O_2$, and its radiopurity, as verified by TLC and GLC, was greater than 90%. It was mixed with 0.34 mg of unlabeled phytol and 20-30 ml of vegetable oil, and given by

mouth after an overnight fast. Respiratory $C^{14}O_2$ was collected and plasma lipids were isolated and assayed for radioactivity as described above. Total lipid radioactivity in the feces also was measured.

Results

Phytanic acid accounted for 4.8% of the total plasma fatty acids in the first subject with Refsum's disease (T.E.); none was detectable in the plasma of the control subject (J.K.). The incorporation of mevalonic acid-2-C^{14} into plasma phytanic acid was in both subjects virtually at the lower limits of the sensitivity of the methods used and in most samples was less than 2% of that in cholesterol (Table I). Most of the small amount of radioactivity in the acid fraction did not co-chromatograph with phytanic acid. Furthermore, the irregular time course suggests that even the small apparent incorporation into phytanate may not be real. On the other hand, incorporation into plasma cholesterol in the two subjects (Table I) was comparable and within the range of values previously found in a series of normal and hypercholesterolemic subjects (Steinberg, Avigan and Feigelson, 1961).

Table I

Radioactivity of Plasma Lipids (cpm/ml) after Intravenous Injection of Mevalonic Acid-2-C^{14} in a Control Subject (J.K.) and in a Patient with Refsum's Disease (T.E.)*

Time (hrs)	Nonsaponifiable Lipids**		Saponifiable Lipids		Phytanic acid	
	J.K.	T.E.	J.K.	T.E.	J.K.	T.E.
4	418	434	3	18	--	4.8 (1.1)***
8	441	389	2	24	--	3.0 (0.8)
24	406	316	--	10	--	0 (0)
96	304	236	7	16	1	< 1 (< 0.4)
168	297	179	7	33	1	10.0 (5.6)

* Both subjects received 25 μc of DL-mevalonic acid-2-C^{14} intravenously after an overnight fast. Total plasma cholesterol levels: J.K., 319 mg%; T.E., 208 mg%. Plasma phytanic acid levels: J.K., not detected; T.E., 15 mg%.

** Over 85% in cholesterol according to TLC.

*** Radioactivity isolated with methyl phytanate acid band on TLC as a percentage of the radioactivity in nonsaponifiable lipids.

The conversion of orally administered U-C^{14}-phytol to $C^{14}O_2$ in a control subject (M.C.) and in a subject with Refsum's disease (K.M.) were

strikingly different (Figure 1). From these data, it can be calculated that the normal subject oxidized 26.2% of the administered dose to $C^{14}O_2$ in 12 hours; by contrast, the subject with Refsum's disease oxidized only 2.7% in 12 hours. This latter figure represents a maximum since the preparation of labeled phytol may have contained as much as 10% of radioimpurities. In both subjects more than 80% of the administered dose was absorbed.

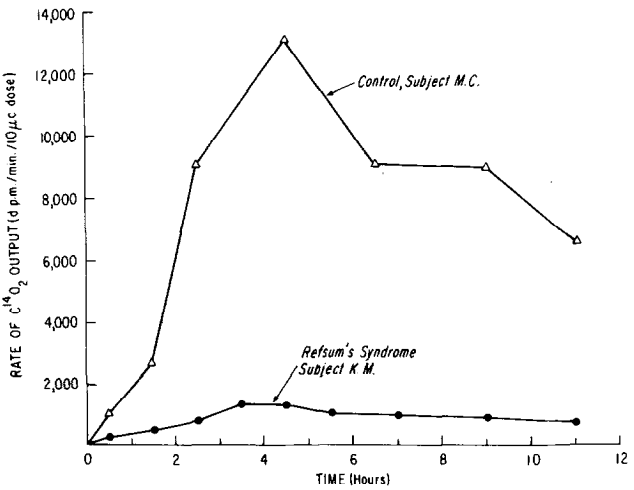

Figure 1. Rate of $C^{14}O_2$ production from orally administered U-C^{14}-phytol in control subject and in a patient with Refsum's disease.

Radioactive phytanic acid was found in the plasma lipids of both subjects (Figure 2). There was no detectable mass of phytanic acid in the control subject; it accounted for 15% of the total plasma fatty acids in the patient. In both subjects, a peak of radioactivity was reached at 6 hours. This probably represents radioactivity in chylomicrons, consistent with the finding in rats that absorption of labeled phytol occurs largely via the lymphatics and that some phytol is converted to phytanic acid in the course of absorption (Steinberg, et al, 1965). In the control subject, the radioactivity in phytanic acid then fell rapidly to low levels and had disappeared by the fifth day. In contrast, in the patient the radioactivity in the plasma phytanic acid showed a second large peak at 2 days and then

fell rather slowly. It can be calculated that in this patient 7.8% of the administered dose was present in the form of phytanic acid in the plasma compartment alone on the second day.

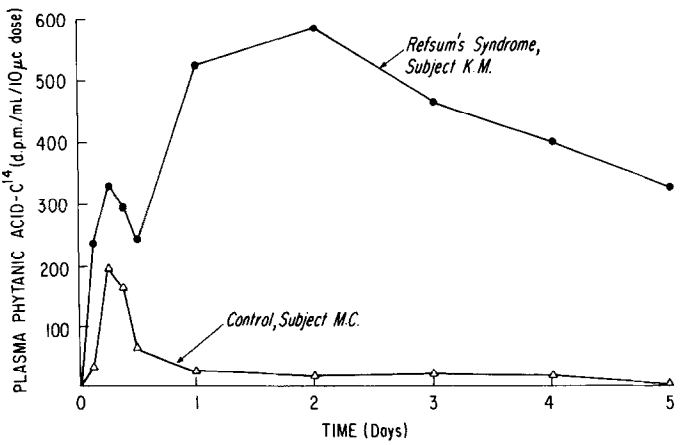

Figure 2. Radioactivity in plasma phytanic acid after oral administration of U-C^{14}-phytol in control subject and in a patient with Refsum's disease.

Discussion

The present results with mevalonic acid-2-C^{14} suggest that endogenous biosynthesis is unlikely to be a major source of plasma phytanic acid. Biosynthesis cannot be excluded conclusively, however. Trace amounts of radioactivity co-chromatographing with phytanic acid were found. Furthermore, the metabolic pathways or the sites of synthesis may differ from those for cholesterol biosynthesis.

On the other hand, phytol is shown to be an excellent precursor of phytanic acid. In the patient with Refsum's disease, 7.8% of the administered tracer dose was present in the plasma as phytanic acid at 2 days. The normal subject showed an initial rise in plasma phytanic acid radioactivity similar to that in the patient, suggesting that absorption and initial conversion of phytol to phytanic acid proceeded similarly in the two subjects. After 12 hours, however, the difference in the curves was striking. The conversion of labeled phytol to $C^{14}O_2$ during the first 12

hours in the patient with Refsum's disease was only about 10% of that in the control subject. The results are consistent with the hypothesis that in the subject with Refsum's disease there is a relative block in the further metabolism of phytanic acid. Because the blood and tissues of the patient contained large amounts of phytanic acid, the lower rate of $C^{14}O_2$ production might reflect dilution by this preformed unlabeled phytanic acid. However, it has been shown that conversion of orally administered phytol-U-C^{14} to $C^{14}O_2$ in rats fed high doses of phytol (which leads to accumulation of phytanic acid) is not significantly different from that in control rats without demonstrable tissue phytanic acid (Steinberg, et al, 1965). It should be noted that the possibility of conversion of phytol directly to $C^{14}O_2$ without prior conversion to phytanic acid has not been ruled out. An enzymatic defect in such a pathway would also explain the present results.

The quantitative importance of dietary phytol or other exogenous precursors as sources of the phytanic acid accumulating in patients with Refsum's disease remains to be determined. Whatever the sources of phytanic acid, the present results taken together suggest that an error in the pathway for oxidation of the branched-chain structure of phytol and/or of phytanic acid most likely represents the basic enzymatic defect leading to storage of phytanic acid in Refsum's disease. This is consistent with the results of Eldjarn (1965) as discussed above.

Addendum. After this work was completed, a paper by Stoffel and Kahlke (1965) appeared reporting incorporation of dietary phytol (H^3-labeled) into plasma phytanic acid in a patient with Refsum's disease.

References

Eldjarn, L. Scand. J. Clin. Lab. Invest. 17, 178 (1965).
Grob, E. C., Kirschner, K. and Lynen, F. Chimia 15, 308 (1961).
Hansen, R. P. Biochim. et Biophys. Acta, in press (1965a).
Hansen, R. P. Chem. and Ind. No. 7, 303 (1965b).
Hansen, R. P. and Shorland, F. B. Biochem. J. 55, 662 (1953).
Kahlke, W. Klin. Wchschr. 42, 1011 (1964).
Klenk, E. and Kahlke, W. Z.f. physiologische Chemie 333, 133 (1963).
Lough, A. K. Biochem. J. 91, 584 (1964).

Refsum, S. Acta psychiat. Scand., Suppl. 38 (1946).
Refsum, S. World Neurology 1, 334 (1960).
Richterich, R., Kahlke, W., Van Mechelen, P. and Rossi, E. Klin. Wchschr. 41, 800 (1963).
Sonneveld, W., Haverkamp-Begemann, P., van Beers, G. I., Keuning, R. and Schogt, J. C. M. J. Lipid Res. 3, 351 (1962).
Steinberg, D., Avigan, J. and Feigelson, E. B. J. Clin. Invest. 40, 884 (1961).
Steinberg, D., Avigan, J., Mize, C. and Baxter, J. Biochem. Biophys. Res. Comm. 19, 412 (1965).
Stoffel, W. and Kahlke, W. Biochem. Biophys. Res. Comm. 19, 33 (1965).
Wells, L. W., Schelble, W. J. and Porter, J. W. Fed. Proc. 23, 426 (1964).

ERRATUM

Vol. 19, No. 5 (1965) in the communication by I. B. Weinstein and D. Grünberger, (pages 647-653), reference 7 on page 653 should read as follows: Grünberger, D., and Mandel, H.G., unpublished studies (1965).