A comparative study of straight chain and branched chain fatty acid oxidation in skin fibroblasts from patients with peroxisomal disorders

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Abstract The β-oxidation of stearic acid and of α- and γ-methyl isoprenoid-derived fatty acids (pristanic and tetramethylheptadecanoic acids, respectively) was investigated in normal skin fibroblasts and in fibroblasts from patients with inherited defects in peroxisomal biogenesis. Stearic acid β-oxidation by normal fibroblast homogenates was several-fold greater compared to the oxidation of the two branched chain fatty acids. The effect of phosphatidylcholine, α-cyclodextrin, and bovine serum albumin on the three activities suggests that different enzymes are involved in the β-oxidation of straight chain and branched chain fatty acids. Homogenates of fibroblasts from patients with a deficiency in peroxisomes (Zellweger syndrome and infantile Refsum's disease) showed a normal ability to β-oxidize stearic acid, but the oxidation of pristanic and tetramethylheptadecanoic acid was decreased. Concomitantly, $^{14}CO_2$ production from the branched chain fatty acids by Zellweger fibroblasts in culture (but not from stearic acid) was greatly diminished. The Zellweger fibroblasts also showed a marked reduction in the amount of water-soluble metabolites from the radiolabeled branched chain fatty acids that are released into the culture medium. The data presented indicate that the oxidation of α- and γ-methyl isoprenoid-derived fatty acids takes place largely in peroxisomes in human skin fibroblasts. —**Singh, H., S. Usher, D. Johnson, and A. Poulos.** A comparative study of straight chain and branched chain fatty acid oxidation in skin fibroblasts from patients with peroxisomal disorders. *J. Lipid Res.* 1990. **31:** 217–225.

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Long-chain fatty acids (LCFA), i.e., fatty acids with 10–22 carbons in length, are thought to be converted to coenzyme A derivatives by long-chain fatty acyl CoA synthetases and incorporated into cellular lipids. Long-chain acyl CoA synthetases are known to be located in microsomes, peroxisomes, and mitochondria (1–6). The acyl CoA synthetases located at the microsomal membranes are thought to be involved in cellular lipid biosynthesis, whereas those located at the mitochondrial and peroxisomal membranes direct fatty acid catabolism in mitochondria and peroxisomes, respectively. While straight chain fatty acids such as palmitic and stearic acids are degraded by β-oxidation, the branched chain fatty acids with a β-methyl group such as phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) theoretically cannot be degraded by β-oxidation. Thus, phytanic acid must be catabolized initially by α-oxidation, i.e., one carbon cleavage. The α-oxidation product, pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) is thought to be degraded by β-oxidation.

In vivo and in vitro studies on very long-chain fatty acids (VLCFA), i.e., fatty acids with >22 carbons, indicate that VLCFA are degraded mainly in peroxisomes (6–13). Recently, we observed that not only VLCFA but also branched chain fatty acids such as phytanic and pristanic acid accumulate in the plasma of patients with inherited defects in peroxisomal biogenesis (Zellweger syndrome, infantile Refsum's disease) indicating the probable involvement of peroxisomes in the α- and β-oxidation of branched chain fatty acids (14). However, while there have been some reports on the α-oxidation of phytanic acid by human skin fibroblasts (8, 15), the degradation of branched chain fatty acids which are theoretically capable of being β-oxidized has not been investigated. In the present study, we report on the comparative oxidation of straight chain and branched chain fatty acids in normal skin fibroblasts, in fibroblasts from patients with defects in peroxisomal biogenesis, and in fibroblasts from patients with peroxisomal defects affecting specifically either the oxidation of VLCFA (X-linked adrenoleukodystrophy) or phytanic acid (Refsum's disease). Our data indicate that

Abbreviations: LCFA, long-chain fatty acids: VLCFA, very long-chain fatty acids; C16:0 acid, palmitic acid; C18:0 acid, stearic acid; C19:0 br. acid, pristanic acid or 2,6,10,14-tetramethylpentadecanoic acid; C20:0 br. acid, phytanic acid or 3,7,11,15-tetramethylhexadecanoic acid; C21:0 br. acid, 4,8,12,16-tetramethylheptadecanoic acid; TLC, thin-layer chromatography; ALD, adrenoleukodystrophy; RCP, rhizomelic chondrodysplasia punctata; BME, basal modified Eagle's medium; DTT, dithiothreitol.

peroxisomes are necessary for normal β-oxidation of isoprenoid-derived fatty acids by human skin fibroblasts.

MATERIALS

[1-^{14}C]Stearic acid (59 mCi/mmol) was purchased from New England Nuclear, Boston, MA; L-[U^{14}C]glycerol-3-phosphate (171 mCi/mmol), [1-^{14}C]glutamic acid (58 mCi/mmol), and sodium [^{14}C]cyanide (55.7 mCi/mmol) were purchased from Amersham International, Buckinghamshire, England. Methane sulfonyl chloride was obtained from Aldrich Chemical Co., Milwaukee, WI. Preswollen DEAE-cellulose (DE-52) and DEAE-cellulose filter papers (DE-81) were supplied by Whatman Chemical Separation Ltd., England. Reversed phase KC-18 thin-layer chromatographic (TLC) plates were supplied by Whatman Inc., Clifton, NJ, and TLC silica gel 60 plates by E. Merck, Darmstadt, West Germany. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO. Analytical grade solvents were purchased from May and Baker Australia Pty. Ltd., Melbourne, Victoria, or Ajax Chemicals, Sydney, New South Wales. Basal modified Eagle's medium was purchased from Flow Laboratories, McLean, VA, and fetal calf serum was from GIBCO, New Zealand Ltd., New Zealand. Dulbecco's phosphate-buffered saline (Ca^{2+}- and Mg^{2+}-free) and trypsin-versene solutions were obtained from Commonwealth Serum Laboratories, Melbourne, Victoria.

Skin fibroblast cultures were established in the Department of Chemical Pathology, The Adelaide Children's Hospital, from skin biopsies of patients with peroxisomal disorders, and from individuals with no prior evidence of abnormality. The approval for these studies was granted by the Human Ethics Committee of the Adelaide Children's Hospital. The diagnoses of X-linked adrenoleukodystrophy (ALD), Refsum's disease, infantile Refsum's disease, Zellweger syndrome, and rhizomelic chondrodysplasia punctata (RCP) were based on case histories and on clinical and biochemical investigations. Biochemical investigations were carried out in our laboratory which included plasma C26:C22 ratios and phytanic acid content, and skin fibroblast phytanic acid oxidation, dihydroxyacetone phosphate acyltransferase (DHAP-AT) activities (14, 16). Case histories and biochemical findings of some of the patients have been reported (16–20).

METHODS

Synthesis of 2,6,10,14-tetramethylpentadecan 1-[^{14}C]oic acid (C19:0 br. acid)

5,9,13-Trimethyltetradecanoic acid prepared by 2-carbon elongation of hexahydrofarnesol (21), was converted to 6,10,14-trimethylpentadeca-[1-^{14}C]oic acid by a standard one carbon elongation procedure involving sodium [^{14}C]cyanide. The [1-^{14}C]acid was converted to its acid chloride with oxalyl chloride, to the diazomethylketone with diazomethane, and thence to the [2-^{14}C]chloromethylketone with concentrated HCl in diethyl ether (22). A solution of the [2-^{14}C]chloromethylketone in ethanol (2 ml) and 10% (w/v) aqueous sodium hydroxide (1 ml) was stirred at 80°C for 90 min. The mixture was cooled and extracted three times with 1 ml hexane each time. The ethanolic solution was acidified to pH 2.0 with concentrated HCl and extracted three times with 2 ml hexane. The hexane extract was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated to give a residue that was chromatographed on a silica gel 60 plate (20 × 20 cm) with hexane-diethyl ether-acetic acid 40:10:1 (by volume). The band with R_f 0.4, which stained with iodine vapor, was removed and extracted with ethyl acetate. Evaporation of the ethyl acetate afforded the [1-^{14}C]pristanic acid as a colorless oil. Gas–liquid chromatography–mass spectrometry of the product from treatment with diazomethane showed one homogenous peak, EI-MS *m/z* 314 ([^{14}C]-M^+, 10.8%), 312 ([^{12}C]-M^+, 1.5%), 40 (100%), specific radioactivity 55.7 mCi/mmol.

Synthesis of 4,8,12,16-tetramethylheptadecan-[1-^{14}C]oic acid (C21:0 br. acid)

Dihydrophytol prepared from phytol by reduction with Raney nickel and hydrogen (23) was converted to the methane sulfonate with methane sulfonyl chloride and pyridine (24). The methane sulfonate was reacted with sodium [^{14}C]cyanide in dimethylsulfoxide and the resultant [^{14}C]nitrile was hydrolyzed with sodium hydroxide in ethanol–water, as described for preparation of a related compound (25), to afforded the [1-^{14}C]tetramethylheptadecanoic acid. Gas–liquid chromatography–mass spectrometry of the methyl ester showed one homogenous peak, EI-MS *m/z* 342 ([^{14}C]-M^+, 5.7%), 340 ([^{12}C]-M^+, 0.7%), 89 (100%), specific radioactivity 55.7 mCi/mmol.

Preparation of fibroblast homogenates

Human skin fibroblasts were routinely grown under sterile conditions for 2–4 weeks in tissue culture flasks (75 cm^2) in basal modified Eagle's (BME) medium containing 10% (v/v) fetal calf serum. Approximately 5 × 10^5 cells were plated per 75-cm^2 flask and the medium was changed twice a week. The passage number in each cell line was kept to a minimum. The cells usually reached confluency stage within 2 weeks and remained viable for several weeks. The cells were harvested from the tissue culture flask with trypsin. Briefly, the culture medium was removed, the cells were rinsed with Dulbecco's phosphate-buffered saline (Ca^{2+}- and Mg^{2+}-free), and incubated at 37°C for 2–5 min with trypsin-versene solution. The cells

were collected by low speed centrifugation (400 g × 5 min) and the cell pellet was washed three times with 3–4 ml of Dulbecco's phosphate-buffered saline. The washed cell pellet was dispersed in 0.3–0.4 ml of 0.25 M sucrose and the homogenate was prepared by controlled sonication (5 sec) using a microtip (Ystrom). The homogenate was centrifuged at 400 g × 10 min to remove unbroken cells and the supernatant was used for enzyme assays.

β-Oxidation of [1-^{14}C]-labeled fatty acids by fibroblast homogenates

For [1-^{14}C]stearic acid or [1-^{14}C]pristanic acid oxidation, 30–40 nmol of fatty acid and 50 μg of phosphatidylcholine (dipalmitoyl) were pipetted into a glass tube; the solvent was removed under nitrogen, and 0.6–0.8 ml of water was added. The mixture was sonicated for 10–15 min in an ultrasonic water bath (Branson Ultrasonic Cleaner, Shelton, CT) and 25 μl of the substrate was used for each assay. For [1-^{14}C]C21:0 br. acid oxidation, the substrate (30–40 nmol) was pipetted into a glass tube, the solvent was removed under nitrogen, and 0.6–0.8 ml of α-cyclodextrin (20 mg/ml) was added. The mixture was sonicated as above and 25 μl of the substrate was used for each assay.

For comparative oxidation of stearic acid and pristanic acid under identical conditions, the reaction mixtures consisted of Tris-HCl buffer, pH 8.0 (50 mM), NaCl (40 mM), KCl (2 mM), $MgCl_2$ (2 mM), dithiothreitol (DTT) (1 mM), ATP (5 mM), L-carnitine (0.2 mM), NAD^+ (0.2 mM), FAD (60 μM), coenzyme A (120 μM) and [1-^{14}C]-labeled fatty acid (4–8 μM) dispersed in phosphatidylcholine (final concentration 10 μg/ml) in a total volume of 0.2 ml. The assay conditions for C21:0 br. acid oxidation were the same as above, except that the fatty acid was dispersed in α-cyclodextrin (final concentration 2.5 mg/ml) solution by sonication (see above). The reaction was started by the addition of 25–50 μg fibroblast protein, incubated at 37°C for 60 min, and the reaction was stopped with 4 ml of chloroform–methanol 2:1 (v/v). Twenty μl of 10 M HCl was added, and the two phases were separated by the addition of 0.8 ml water. The lower chloroform phase was discarded and the aqueous phase was washed twice with 2 ml hexane. The washed aqueous phase was made alkaline (pH > 10) with 50 μl of 10 M KOH and heated at 60°C for 16 h. The mixture was cooled, acidified (pH 1.0–1.5) with 40 μl of 10 M HCl, and washed twice with 2 ml hexane. The radioactivity retained in the aqueous phase (radiolabeled acetate or propionate) was determined.

Incubation of [1-^{14}C]-labeled fatty acids with fibroblasts in culture

Radiolabeled fatty acids were treated with 10–20 μl of 0.6 M NaOH and then incubated at 37°C for 30–60 min with fetal calf serum to bind fatty acids to serum proteins. The mixture was diluted with BME to give a fetal calf serum concentration of 0.5–1.0% (v/v), and the medium was filtered (8, 26). Human skin fibroblasts were grown in 25-cm^2 flasks to confluency, the culture medium was removed, and the cells were equilibrated with 3 ml BME containing 0.5% (v/v) fetal calf serum. After 24 h, the medium was replaced with 3 ml BME containing 0.5–1.0% (v/v) fetal calf serum and radiolabeled fatty acids (2–5 nmol). The culture flasks were sealed, incubated at 37°C (5 h to 3 days) and released $^{14}CO_2$ was measured (26). The cells attached to tissue culture flasks were rinsed with Dulbecco's phosphate-buffered saline (Ca^{2+}- and Mg^{2+}-free) and the radioactivity in the medium was determined. The cells were harvested, lysed, and the radioactivity was determined. The lipids from the medium and the cells were extracted according to the method of Bligh and Dyer (27), and the radioactivity in the chloroform phase (lipids) and in the aqueous phase (water-soluble) was determined. The protein content in the cell lysate was determined according to the method of Bradford (28). Lipids extracted from skin fibroblasts were transesterified, and the released fatty acid methyl esters were purified by preparative TLC and subjected to reverse phase TLC and radioautography (10, 29).

RESULTS

All three fatty acids were converted to water-soluble products by fibroblast homogenates. The major water-soluble product released from stearic acid was earlier shown to be acetyl CoA (10); the major radiolabeled products formed from C19:0 br. and C21:0 br. fatty acids were propionyl CoA and acetyl CoA, respectively. Under the same conditions [1-^{14}C]phytanic acid was not converted to a water-soluble product. The method of presentation of substrate influenced the rate of β-oxidation of fatty acids by fibroblast homogenates. Stearic acid β-oxidation was 2-fold higher when the substrate was suspended in phosphatidylcholine (dipalmitoyl) than in α-cyclodextrin, whereas pristanic acid β-oxidation was 1.5-fold higher in α-cyclodextrin than in phosphatidylcholine. Addition of 100 μg/incubation of bovine serum albumin (BSA) (fraction V, fatty acid-free) had no effect when stearic acid was dispersed in phosphatidylcholine, whereas BSA stimulated stearic acid β-oxidation 2-fold when the substrate was dispersed in α-cyclodextrin. BSA inhibited (40–50%) pristanic acid β-oxidation irrespective of whether the substrate was dispersed in phosphatidylcholine or α-cyclodextrin. In contrast to stearic acid and pristanic acid, C21:0 br. acid, β-oxidation by fibroblast homogenates could be demonstrated only when the substrate was dispersed in α-cyclodextrin. Inclusion of BSA (100 μg/incubation) in

the incubation inhibited (>80%) C21:0 br. acid β-oxidation by fibroblast homogenates.

Cofactor requirements for branched chain fatty acid β-oxidation were similar to those described earlier for stearic acid β-oxidation by fibroblast homogenates (10). β-Oxidation of fatty acids was negligible in the absence of ATP, Mg^{2+}, and coenzyme A. Addition of NAD^+, L-carnitine, DTT, and FAD further stimulated oxidation of straight chain and branched chain fatty acids. Under optimal conditions the oxidation of the substrates by fibroblast homogenates was linear up to 2 h and at least up to 50 μg of fibroblast protein per assay.

In normal skin fibroblast homogenates, β-oxidation of stearic acid was approximately fourfold and eightfold greater than for pristanic acid and C21:0 br. acid oxidation, respectively (**Table 1**). Stearic acid β-oxidation was normal whereas pristanic acid and C21:0 br. acid oxidation in homogenates of Zellweger fibroblasts were <20% of controls (Table 1). Stearic acid β-oxidation was also nearly normal in fibroblast homogenates of infantile Refsum's disease whereas pristanic acid and C21:0 br. acid oxidation was <30% of controls. Both straight chain and branched chain fatty acid β-oxidation was nearly normal or above normal in fibroblast homogenates of patients with other peroxisomal disorders, namely, Refsum's disease, X-linked adrenoleukodystrophy, and rhizomelic chondrodysplasia punctata (Table 1).

Normal fibroblasts in culture converted radiolabeled stearic, pristanic, and C21:0 br. acids to radiolabeled carbon dioxide and water-soluble products. Radiolabeled carbon dioxide production from the three fatty acids increased with incubation period up to 72 h (**Fig. 1**). Radiolabeled water-soluble products in the cells plus medium increased with incubation period up to 24 h from stearic and pristanic acids, whereas that from C21:0 br. acid increased up to 72 h (Fig. 1). The initial rates of oxidation (carbon dioxide plus water-soluble products as an index of oxidation) of stearic and C21:0 br. acids were similar and were several-fold lower compared to the initial rate of oxidation of pristanic acid.

Radiolabeled fatty acids were rapidly taken up from the culture medium by fibroblasts. During a 24-h incubation period, >85% of stearic and pristanic acids and >65% of C21:0 br. acid added to the culture medium were taken up by fibroblasts. In the same period, 16–19% of stearic, 53–56% of pristanic, and 12–14% of C21:0 br. acids added to the culture medium were converted to carbon dioxide and water-soluble products. Higher oxidation of the three fatty acids was observed at longer incubation periods. For example, in a 48-h incubation period, 17-26%, 65-67%, and 16-23% of the added stearic, pristanic, and C21:0 br. acids, respectively, were oxidized to carbon dioxide and water-soluble products. During the same period (48 h) 50–70%, 5–13%, and 36–45% of the added stearic, pristanic, and C21:0 br. acids, respectively, were detected in cellular lipids. A similar proportion of the radioactivity from the three fatty acids was found in cellular lipids at a longer incubation period of 72 h.

Radiolabeled carbon dioxide production from the two branched chain fatty acids (pristanic and C21:0 br. acids) was greatly reduced in Zellweger fibroblasts compared to controls. However, carbon dioxide production from radiolabeled stearic acid was normal in Zellweger fibroblasts in culture (**Table 2**). Water-soluble products formed during 1–3 days of incubation of the three fatty acids with fibroblasts in culture were mainly released into the culture medium. Compared to controls, formation of radiolabled water-soluble products from the two branched chain fatty acids was greatly reduced both at 22-h and 48-h or 72-h incubation periods in Zellweger fibroblasts (**Table 3**). Under similar conditions, formation of water-soluble products from radiolabeled stearic acid was normal in Zellweger fibroblasts in culture (Table 3).

Reverse phase TLC analysis of methyl esters derived

TABLE 1. Fatty acid β-oxidation by skin fibroblast homogenates

Cell Source	Radiolabeled Acetate or Propionate Produced from [1-^{14}C]-Labeled Fatty Acids		
	Stearic Acid	Pristanic Acid	21:0 br. Acid
	pmol · h^{-1} · mg protein^{-1}		
Control (n = 12)	1625 ± 283	434 ± 163	204 ± 58
Zellweger syndrome (n = 6)	1706 ± 529	32 ± 14	37 ± 22
Infantile Refsum's disease (n = 5)	1305 ± 356	86 ± 46	57 ± 27
Refsum's disease (n = 4)	1541 ± 314	438 ± 194	211 ± 57
X-Linked adrenoleukodystrophy (n = 5)	1612 ± 380	314 ± 79	178 ± 44
Rhizomelic chondrodysplasia punctata (n = 3)	1322 ± 169	638 ± 239	326 ± 83

Fatty acid β-oxidation assays were performed in duplicate on freshly prepared fibroblast homogenates as described under Methods. The values represent means ± SD and n is the number of different cell lines tested.

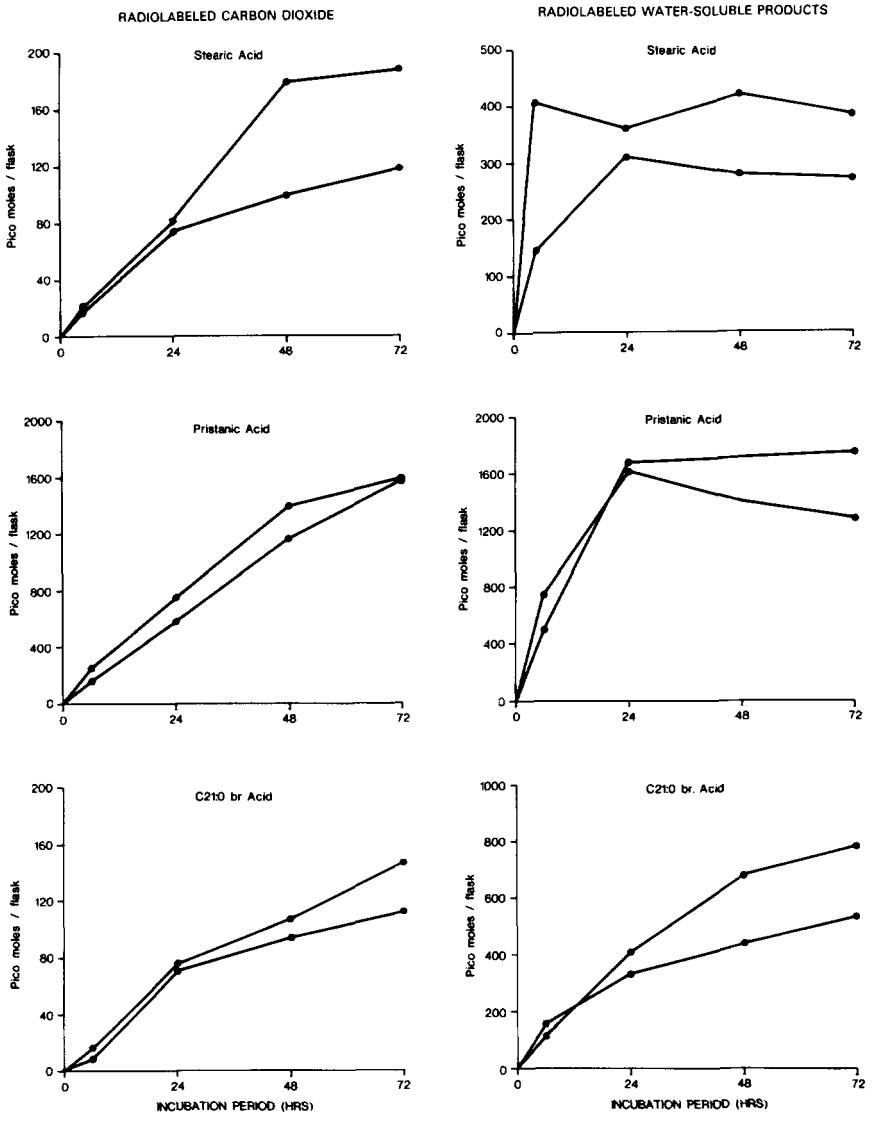

Fig. 1. [1-^{14}C]-labeled fatty acids bound to fetal calf serum and incubated with normal fibroblasts in culture, as described in the text. After the indicated times, radiolabeled carbon dioxide and water-soluble products (in medium plus cells) were measured as described in the text. Each point represents a mean of two observations.

from the lipid extracts of cells incubated with [1-^{14}C]stearic and [1-^{14}C]C21:0 br. fatty acids contained a number of other radiolabeled fatty acids tentatively identified as saturated 14- to 24-carbon chain fatty acids. The observation that Zellweger fibroblasts incubated with [1-^{14}C]C21:0 br. acid did not convert the substrate to elongation products migrating in the region of C22:0 and C24:0 fatty acid suggests that C22:0 or C24:0 fatty acids formed in control fibroblasts were straight chain fatty acids produced by elongation of the unlabeled precursor with radiolabeled acetate (formed by β-oxidation of the radiolabeled substrate). Elongation products of pristanic and C21:0 br. acids could not be detected in any of the cell extracts examined (**Fig. 2**). Traces of other fatty acids were detected in [1-^{14}C]pristanic acid incubations but these were not identified. Zellweger fibroblasts showed a relatively normal uptake of all three fatty acids. The formation of saturated 14- to 24-carbon fatty acids from C21:0 br. acid was greatly reduced in Zellweger fibroblasts, but the formation of the corresponding products from stearic acid was normal (Fig. 2).

DISCUSSION

Straight chain saturated, and α-methyl and γ-methyl fatty acids can be degraded by β-oxidation by skin fibroblast homogenates. In contrast, β-methyl fatty acids, such as phytanic acid, cannot undergo β-oxidation and thus must be degraded initially by α-oxidation. The α-oxi-

TABLE 2. Oxidation of radiolabeled fatty acids by human skin fibroblasts in culture

	Radiolabeled CO_2 Produced from Fatty Acids					
	Stearic Acid		Pristanic Acid		C21:0 br. Acid	
Cell Source	22 h	48 h	22 h	48 h	22 h	72 h
	pmol · h⁻¹ · mg protein⁻¹					
Normal subjects						
1		3.1		35.3		3.4
2		4.1		25.0		7.0
3	2.0		163.3		9.5	
4	3.4		194.7		16.1	
Zellweger syndrome patients						
1		1.8		0.1		0.2
2		3.1		0.1		0.1
3	3.8		0.4		1.5	
4	2.3		16.3		1.3	

[1-^{14}C]-Labeled fatty acids were added to cell cultures, incubated at 37°C as indicated, and the released $^{14}CO_2$ was measured as described under Methods. The data presented at 22 h are the means of two observations, whereas a single observation was made at 48-h or 72-h time points.

TABLE 3. Production of water-soluble product(s) from [1-^{14}C]-labeled fatty acids by human skin fibroblasts in culture

	Radiolabeled Fatty Acids Converted to Water-Soluble Product					
	Stearic Acid		Pristanic Acid		C21:0 br. Acid	
Source	22 h	48 h	22 h	48 h	22 h	72 h
	pmol · h⁻¹ · mg protein⁻¹					
Cells						
Normal subjects						
1		6.4		5.1		1.7
2		8.3		3.7		2.2
3	9.4		20.6		16.6	
4	12.9		16.0		10.8	
Zellweger syndrome patients						
1		6.0		0.4		0.3
2		4.8		0.5		0.5
3	10.5		5.1		3.4	
4	8.0		12.2		2.6	
Culture medium						
Normal subjects						
1		44.1		71.1		29.9
2		53.5		64.7		20.6
3	32.6		524.3		65.8	
4	46.5		478.2		144.5	
Zellweger syndrome patients						
1		39.9		0.5		0.6
2		33.0		0.8		1.0
3	54.0		3.2		10.8	
4	33.6		29.2		10.1	

Fibroblasts in culture were incubated with [1-^{14}C]-labeled fatty acids as described in Methods and the legend to Table 2. After collection of $^{14}CO_2$ at the end of the incubation period, the cells and the medium were collected and water-soluble radioactivity was determined as described in the text.

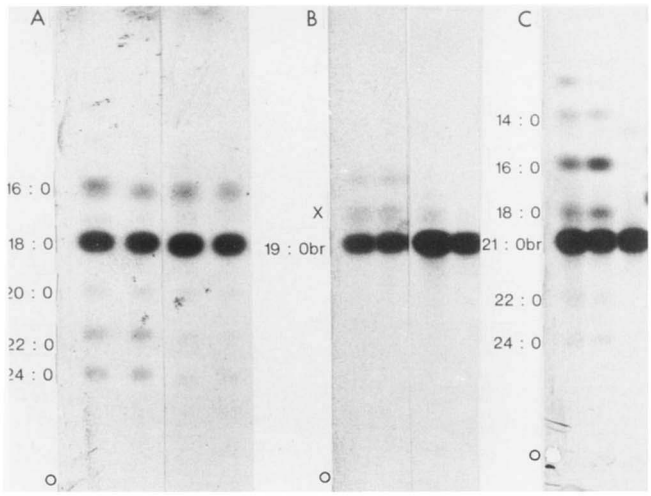

Fig. 2. Reverse phase TLC of [1-^{14}C]-labeled fatty acids (as their methyl esters) present after incubation with [1-^{14}C]stearic acid (A), [1-^{14}C]pristanic acid (B), and [1-^{14}C]21:0 br. acid (C) with normal and Zellweger fibroblasts in culture as described in the text. The first two lanes (from left to right) are control cell lines and the third and fourth are Zellweger cell lines. The tentative identification of fatty acids is shown (number of carbon atoms:number of double bonds) and is based on a comparison of their chromatographic mobility with labeled fatty acid standards; O, origin; X, unidentified contaminant. The figures shown are radioautograms prepared after a 2-week exposure. The faint radioactive band in Fig. 2C (Zellweger fibroblasts) between 14:0 and 16:0 appears to be an autoradiographic artefact.

dation of [1-^{14}C]phytanic acid has been shown to be deficient in skin fibroblasts of Refsum's disease patients (8, 15, 26). Our earlier observations that phytanic acid accumulates in plasma from peroxisome-deficient patients and that phytanic acid oxidation is deficient in their fibroblasts in culture (8, 14) suggest that α-oxidation of phytanic acid occurs in peroxisomes in humans. A direct demonstration of phytanic acid α-oxidase activity in purified peroxisomes from human tissues would provide further confirmation to the above hypothesis.

The finding that branched chain fatty acid β-oxidation activity was deficient only in fibroblasts from patients who have been reported to have gross reductions in peroxisomal numbers (30, 31) and not in other disorders such as RCP, X-linked ALD, and Refsum's disease where peroxisomal biogenesis is not affected (8, 32–34) is strong evidence for the role of peroxisomes in isoprenoid-derived fatty acid oxidation. Our recent observations (14) that plasma levels pristanic acid in RCP, X-linked ALD, and Refsum's disease are normal are further evidence that the β-oxidation of this fatty acid is not impaired in these disease states although α-oxidation is clearly affected in RCP (16). X-linked ALD fibroblasts showed a reduction in peroxisomal β-oxidation of VLCFA (11), but it is clear from Table 1 that the oxidation of branched chain fatty acids is relatively normal. Current evidence suggests that the defect in X-linked ALD is in a peroxisomal VLCFA CoA synthetase (11, 35), an enzyme distinct from the corresponding enzyme that catalyzes the activation of long chain fatty acids (6). As the β-oxidation of branched chain fatty acids is not impaired in these patients it would seem likely that VLCFA and branched chain fatty acyl CoA synthetases are different. However, whether a specific branched chain fatty acyl CoA synthetase is present in peroxisomes and is involved in the regulation of the β-oxidation of these fatty acids is not known but is under current investigation.

A significantly large proportion of the added fatty acid was degraded to carbon dioxide and water-soluble compounds during the 1- to 3-day incubation period. Higher apparent oxidation of pristanic acid compared to stearic and C21:0 br. acids at all time points (Fig. 1) is the net result of the differences in uptake, esterification to lipids and turnover of lipids labeled with fatty acids of different structures. It should be pointed out that these observations do not take into account the dilution of radiolabeled stearic acid with endogenous fatty acid and the fatty acid present in fetal calf serum used in cell culture.

The data in Tables 2 and 3 showing a marked reduction in the formation of water-soluble metabolites by Zellweger fibroblasts from pristanic acid and C21:0 br. fatty acid provide supportive evidence of the in vitro data shown in Table 1. Although the characterization of these metabolites was not attempted, under similar conditions polyunsaturated fatty acids produce significant amounts of amino acids that are secreted almost entirely into the cell culture medium (36). It is possible that these compounds are formed from the radiolabeled acetate or propionate released by β-oxidation and transported to mitochondria where they enter the citric acid cycle and the citric acid intermediates are subsequently transaminated. Recent observations from our laboratory indicate that the conversion of pristanic acid to CO_2 requires propionyl CoA carboxylase, a mitochondrial enzyme catalyzing the conversion of propionate to methylmalonate (37), because CO_2 production from pristanic acid is greatly reduced in fibroblasts from patients with propionyl CoA carboxylase deficiency (38). The nature of the water-soluble metabolites produced from propionate released from pristanic acid is not known. As these metabolites are produced even in propionyl CoA carboxylase-deficient cells, they may not be formed in mitochondria. The possibility that peroxisomes are involved in their synthesis is under investigation. Our data also show that, in contrast to C16:0, C18:0, C20:4 and C24:4 fatty acids (36), neither of the branched chain fatty acids investigated is chain-elongated, indicating that the same enzymes that elongate the straight chain saturated and polyunsaturated fatty acids are unable to catalyze the elongation of branched chain fatty acids.

The mitochondrial β-oxidation system appears to be specific for straight chain fatty acids. Introduction of methyl group in α- or γ-position of saturated fatty acids

prevents their oxidation in mitochondria, but it is not known whether the rest of the molecule influences enzyme specificity. The specificity of mitochondrial β-oxidation may result from the specificity of *a*) the mitochondrial acyl CoA synthetase that converts fatty acids to their coenzyme A derivatives, *b*) the acyl CoA-carnitine transferases that translocate fatty acyl CoAs across the mitochondrial membrane, and *c*) the fatty acyl CoA β-oxidation complex present in mitochondrial matrix. It appears that the peroxisomal β-oxidation system has much broader substrate specificity or the peroxisomes have separate β-oxidation enzymes that degrade saturated straight chain and branched chain fatty acids. We reported recently that specific LCFA and VLCFA CoA synthetases are present in peroxisomal membranes (6) and possibly coupled to the peroxisomal β-oxidation complex present in the peroxisomal matrix. We hypothesize that, in addition to LCFA and VLCFA CoA synthetases, a branched chain acyl CoA synthetase is present in peroxisomal membranes which directs branched chain fatty acid oxidation in skin fibroblasts.

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