Contribution of ω-oxidation to fatty acid oxidation by liver of rat and monkey[1]

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Abstract Contributions of ω-oxidation to overall fatty acid oxidation in slices from livers of ketotic alloxan diabetic rats and of fasted monkeys are estimated. Estimates are made from a comparison of the distribution of ^{14}C in glucose formed by the slices from ω-^{14}C-labeled compared to 2-^{14}C-labeled fatty acids of even numbers of carbon atoms and from [1-^{14}C]acetate compared to [2-^{14}C]acetate. These estimates are based on the fact that *1*) the dicarboxylic acid formed via ω-oxidation of a ω-^{14}C-labeled fatty acid will yield [1-^{14}C]acetate and [1-^{14}C]succinate on subsequent β-oxidation, if β-oxidation is assumed to proceed to completion; *2*) only [2-^{14}C]acetate will be formed if the fatty acid is metabolized solely via β-oxidation; and *3*) ^{14}C from [1-^{14}C]acetate and [1-^{14}C]succinate is incorporated into carbons 3 and 4 of glucose and ^{14}C from [2-^{14}C]acetate is incorporated into all six carbons of glucose. From the distributions found, the contribution of ω-oxidation to the initial oxidation of palmitate by liver slices is estimated to be between 8% and 11%, and the oxidation of laurate between 17% and 21%. Distributions of ^{14}C in glucose formed from ^{14}C-labeled palmitate infused into fasted and diabetic rats do not permit quantitative estimation of the contribution of ω-oxidation to fatty acid oxidation in vivo. However, the distributions found also indicate that, of the fatty acid metabolized by the whole animal in the environment of glucose formation, at most, only a minor portion is initially oxidized via ω-oxidation. As such, ω-oxidation cannot contribute more than a small extent to the formation of glucose.

Supplementary key words β-oxidation · fatty dicarboxylic acids · palmitic acid · lauric acid · starvation · diabetes mellitus · glucose

A microsomal fraction of liver catalyzes the hydroxylation of the terminal carbon of a number of fatty acids. These ω-hydroxyl fatty acids can be further oxidized by enzymes in the soluble as well as the microsomal fractions of liver to their corresponding dicarboxylic acids (1, 2). It has been suggested that the importance of ω-oxidation is perhaps that β-oxidation of the dicarboxylic acids to completion would yield succinic acid and thus could result in a net formation of carbohydrate from fatty acids (3). Ellin (4) has suggested that a possible function of ω-oxidation of fatty acids is to detoxify fatty acids that might poison cells by uncoupling the mitochondrial respiratory chain.

In 1949 Lorber, Cook, and Meyer (5) fed [1-^{13}C]-octanoate and [7-^{14}C]octanoate to rats fasted for 24 hr and found the distribution of the isotopes in liver glycogen consistent with the metabolism of octanoate via β-oxidation. Incorporation of carbon 1 of the octanoate into carbons 3 and 4 of the glucose unit of glycogen, however, exceeded that of carbon 7 by about 30%. In 1968, Antony and Landau (6) introduced a method for quantitating the contribution of ω-oxidation in vitro. Liver slices from rats fasted for 24 hr were incubated with [16-^{14}C]palmitate and [18-^{14}C]-stearate, and the distribution of ^{14}C among the carbon atoms of the glucose unit of glycogen from the slices was determined and compared with the distribution obtained with [2-^{14}C]acetate. Via β-oxidation of the labeled fatty acids, [2-^{14}C]acetate would be formed; via ω-oxidation of the fatty acids and then β-oxidation of the resulting dicarboxylic acids, [1-^{14}C]acetate would be formed. The ^{14}C from [1-^{14}C]acetate labels carbons 3 and 4 of glucose, while that from [2-^{14}C]-acetate labels all six carbons of glucose in their metabolism by liver (7). From the distributions, the contribution of ω-oxidation to the initial oxidation of palmitate and stearate was estimated to be less than 5%.

Wada and coworkers (8, 9) found a decrease in the concentration of blood ketone bodies in both 24-hr fasted and alloxan-induced diabetic rats upon the administration of dicarboxylic fatty acids, suggesting that ω-oxidation could be important for the production of succinyl CoA. They estimated that in starving or diabetic rats about 15% of palmitic acid is subjected to ω-oxidation and then to β-oxidation.

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Björkhem (10) reported that starvation or diabetes stimulates ω-hydroxylation of stearate by rat liver homogenates 3- to 7-fold, while Pettersen (11) found that normal rats excrete small quantities of hexanedioic acid and traces of octanedioic acid, and that ketotic rats excrete much more. Pettersen also reported (12) increased amounts of these dicarboxylic acids in the urine of juvenile diabetics when they were ketonuric. He stated that probably only a minor part of the dicarboxylic acids formed was excreted while most was broken down to succinyl CoA. While no data were provided to support these statements, this pathway was suggested as being of possible importance in the diabetic. Björkhem (13) concluded that ω-oxidation may be more important under conditions of starvation and diabetes than in the normal state. There is also evidence for the occurrence of ω-oxidation in Jamaican vomiting sickness (14).

We (15) recently administered 2-amino-4-phenylbutyric acid and specifically ^{14}C-labeled fatty acids to rats. The acetylated amino acid excreted in their urines was hydrolyzed and the resulting acetates were degraded. From the distribution of ^{14}C in the acetates, we concluded that about 40% of the initial oxidation of fatty acid was via ω-oxidation when the rats were diabetic and nonketotic, but was less when they were ketotic or nondiabetic. These estimates are for the extent of ω-oxidation in the environment of the acetylation. Using ^{14}C distribution in glucose as a reflection of the relative quantities of [1-^{14}C]acetate and [2-^{14}C]acetate formed from the fatty acids allows estimates of the contribution of ω-oxidation in the environment of glucose formation, presumably involving the parenchymal cells of the liver (16). We have made such estimates using slices of livers from alloxan diabetic rats and from starved monkeys and applied the method of estimation to the rat in vivo.

EXPERIMENTAL

Labeled substrates

Sodium [1-^{14}C]acetate, sodium [2-^{14}C]acetate, and [1-^{14}C]palmitic acid were purchased from New England Nuclear Corp., Boston, MA. [2-^{14}C]Palmitic acid and [3-^{14}C]palmitic acid were purchased from the Applied Science Laboratories, Inc., State College, PA. [10-^{14}C]Palmitic acid, [16-^{14}C]palmitic acid, and [12-^{14}C]lauric acid were purchased from the Commission d'Energie Atomique, Départment des Radioéléménts, Gif-sur-Yvette, France. Their purity was established as previously described (15).

In vitro studies

Monkey. Three rhesus monkeys, also being used in another study, were fasted for 72 hr. Their livers were removed under ketamine–pentobarbital anesthesia and transported to the laboratory in cold isotonic saline. A different liver was used in each of the three experiments. The livers were sliced within 45 min to 1 hr after removal. There were two flasks in the first experiment, six flasks in the second, and five flasks in the third. The incubation procedure was essentially as previously described (6, 17). Briefly, each flask contained about 2 g of slices. There were 10 ml of a high potassium bicarbonate medium containing 100 μmol of glucose, 50 μmol of sodium acetate, 20 μmol of fatty acid, and 6.7 μmol of essentially fatty acid-free bovine albumin (Sigma Chemical Co., St. Louis, MO). Either the fatty acid or the acetate was labeled and each flask in an experiment contained 10 μCi of a labeled acid different from that in the other flasks. The contents were gassed with 95% O_2–5% CO_2 to give a pH in the medium of 7.4. The flasks with contents were sealed and shaken. Incubations were at 37°C and were terminated with 6% perchloric acid after 2 hr.

Rats. Female Sprague-Dawley rats, weighing between 250 and 330 g, were fed a stock diet. Diabetes was induced after 24 hr of fasting by the intravenous injection of alloxan, 40 mg/kg, and the rats were guillotined 5 days after injection. Four experiments were performed. In the first experiment the livers from three rats were sliced and the same representation of slices was distributed into each incubation flask. Blood glucose concentrations at the time of killing, as determined by use of glucose oxidase (Glucostat, Worthington Biochem. Corp., Freehold, NJ), were 570, 610 and 642 mg/dl, respectively, and ketonurias were small (measured by Ketostix, Ames Co., Elkhart, IN). In each of the other experiments the livers from two rats were sliced and distributed. The rats in the second experiment had blood glucose concentrations of 572 and 588 mg/dl, respectively, and they were in moderate ketosis with blood β-hydroxybutyrate concentrations of 1.1 and 2.0 mmol/liter as determined according to the method of Persson (18). In the third experiment blood glucose concentrations were 586 and 340 mg/dl and β-hydroxybutyrate concentrations were 14.2 and 8.2 mmol/liter, respectively. In the fourth experiment glucose concentrations were 329 and 333 mg/dl and β-hydroxybutyrate concentrations were 4.0 and 4.4 mmol/liter, respectively. The incubation procedure was the same as for slices from monkey liver, except that the period of incubation was 90 min.

In vivo studies

Ten female Sprague-Dawley rats were catheterized through their right jugular veins by the method of Popovic and Popovic (19). Five days later each rat was fasted for 48 hr and then infused with 5 ml of 0.45% saline containing 20 μmol of a labeled fatty acid (10 μCi) or 50 μmol of labeled acetate (10 μCi), as their sodium salts, and 6.7 μmol of essentially fatty acid-free bovine albumin. At the end of 5 hr of infusion, the rat was guillotined. Three other rats were treated identically, except that infusion was for only 1 hr.

Five rats with diabetes, induced with streptozotocin (65 mg/kg i.v.) (20), were also treated identically, except that they were infused for 5 hr with no prior fasting. Their blood glucose concentrations at the time of death ranged between 340 and 580 mg/dl.

Isolation of glucose

On completion of incubation of the monkey liver slices, glycogen in the slices was isolated and hydrolyzed to glucose. To isolate the glycogen, the slices were plunged into hot 30% KOH containing carrier glycogen, and the dissolved glycogen was precipitated with ethanol. It was purified by repeated precipitations with ethanol from water and from trichloroacetic acid solution, and hydrolysis was done in sulfuric acid (21). The resulting glucose was purified first by passage through a mixed-bed ion exchange resin (Amberlite MB-3, Rohm and Haas Co., Philadelphia, PA), and then by preparative paper chromatography using *n*-butanol–acetic acid–water 4:1:5 (by volume) (22). The area with the mobility of glucose, identified by using guide spots, was eluted.

In incubation of liver slices of the diabetic rats, glucose was isolated from the medium, because ^{14}C incorporation into the glycogen was too low for reliable measurements.

To isolate glucose from the perchloric acid-containing medium, the medium was extracted with petroleum ether and then neutralized with potassium hydroxide and cooled; the potassium perchlorate that precipitated was removed by centrifugation. The supernatant was deionized, the effluent was concentrated and subjected to preparative paper chromatography, and the glucose was eluted.

The $^{14}CO_2$ that evolved on addition of perchloric acid at the termination of incubation in experiments with monkey slices was collected and assayed for activity to obtain a measure of the quantity of fatty acid oxidized.

Blood was collected from the rats that were infused at the time they were guillotined. The blood was deproteinized with barium hydroxide and zinc sulfate (23) and then centrifuged. The supernatant was deionized and chromatographed and the glucose was isolated as described above for glucose in the incubation medium.

In some cases, as is indicated in Table 2, glycogen was isolated from the livers of infused rats as an alternative to isolating glucose from the blood. The liver was excised and plunged into 30% KOH immediately after the rat was guillotined and the procedure for forming and isolating the glucose was then identical to that for liver slices.

Degradation of glucose

Glucose from glycogen, medium, and blood, after addition of carrier glucose, was partially degraded using *Leuconostoc mesenteroides* to yield CO_2, ethanol, and lactate. The ethanol and lactate were degraded chemically to yield successively each of their carbons as CO_2 and each CO_2 quantity was assayed for ^{14}C (24, 25). The specific activities of the individual carbons are recorded in the tables, with the specific activity of carbon 4 or 5 set equal to 100 and the activities of the other carbons presented relative to these. The specific activity of ^{14}C recovered, as obtained by summing the specific activities of the individual carbons, totaled 77–109% of the specific activity of the glucose degraded, as obtained by combusting a sample of the glucose to CO_2. This is recorded as percent recovery in the tables.

Repeat degradations of samples of uniformly labeled glucose were performed.[3] The 95% confidence limits for the specific activity of a given carbon of glucose were within ±8% of the determined activity.

Incorporation of ^{14}C from [1-^{14}C]acetate and [2-^{14}C]acetate into glucose

To make the estimations that follow, the relative amount of incorporation into glucose of acetate carbon 1 compared to carbon 2 was determined. This was done, as described above, by incubating slices from the livers of three 72-hr fasted monkeys and three alloxan-diabetic rats in the presence of unlabeled palmitate with [1-^{14}C]acetate and with [2-^{14}C]acetate. For the experiments with monkeys, ^{14}C activity was then measured in the glycogen in the liver slices; for the experiments with rats it was measured in the glucose of the medium in which the liver slices had been incubated. Carrier glycogen (30 mg) was added to the

[3] Antony, G., and K. Kumaran, unpublished observations.

TABLE 1. Distribution of ^{14}C in the glucose unit of glycogen from 72-hour fasted monkey liver slices incubated with ^{14}C-labeled acetate or ^{14}C-labeled fatty acids

Labeled Substrate	Expt. No.	% Added ^{14}C Converted to:		^{14}C in Carbons						% Recovery
		CO_2	Glucose	1	2	3	4	5	6	
[1-^{14}C]acetate	2	19.0	4.4	1	2	65	100	2	1	92
[2-^{14}C]acetate	1	11.4	2.8	68	65	17	14	100	98	98
	2	9.0	10.1	85	70	16	19	100	82	96
	3	10.3	4.1	68	70	13	15	100	97	95
[2-^{14}C]palmitate	2	1.1	0.1	46	60	12	15	100	99	103
	3	2.3	0.4	67	72	12	16	100	96	99
[10-^{14}C]palmitate	2	1.1	1.0	68	74	17	18	100	100	97
	3	2.7	0.2	69	76	14	21	100	102	96
[16-^{14}C]palmitate	2	0.9	0.6	71	74	19	22	100	99	97
	3	2.1	0.3	67	70	16	18	100	99	100
[12-^{14}C]laurate	1	3.1	0.4	70	69	28	28	100	100	84
	2	2.1	0.03	76	65	27	27	100	101	109
	3	6.0	0.5	69	63	27	37	100	106	93

slices and carrier glucose (30 mg) was added to the medium. The glycogen was hydrolyzed to glucose and glucosazones were prepared (21, 26) from the glucose in the medium and from that derived from the glycogen. The glucosazones were oxidized to CO_2 which was assayed for ^{14}C.

Statistics

Distributions of ^{14}C in glucose were analyzed using a modified Wilcoxon rank test (27) and a two-sided, paired Student's *t* test (28).

RESULTS

^{14}C incorporation into glycogen by monkey liver slices was on the average 3.1 times more from [2-^{14}C]-acetate than from [1-^{14}C]acetate (individual ratios were 3.6, 2.8 and 3.0, respectively). Incorporation of ^{14}C into glucose by liver slices from the three alloxan-induced diabetic rats averaged 3.0 times more from [2-^{14}C]acetate than from [1-^{14}C]acetate (individual ratios were 2.8, 3.0, and 3.2, respectively). Incubating rat liver slices under similar conditions, Strisower, Kohler, and Chaikoff (26) also obtained a ratio of 3.0.

Distributions of ^{14}C in the glucose units from the monkey liver glycogens are recorded in **Table 1,** along with the percentage of the added ^{14}C recovered in CO_2, and in the glucose that was degraded following purification. Loss during purification was not determined. The percent of the ^{14}C added as ^{14}C-labeled acetate that was recovered in $^{14}CO_2$ ranged from 9.0 to 19.0% and in glucose from 2.8 to 10.1%. Between 0.9 and 6.0% of the added ^{14}C from labeled fatty acids was collected in CO_2 and 0.03–1.0% was recovered in glucose.

Distributions from [2-^{14}C]acetate were similar to those from [2-^{14}C]palmitate. In comparison to these distributions, incorporation of ^{14}C from [16-^{14}C]-palmitate and, especially, from [12-^{14}C]laurate into carbons 3 and 4 of glucose was greater relative to carbons 1, 2, 5, and 6. Incorporation from [10-^{14}C]palmitate into carbons 3 and 4 was also somewhat more than from [2-^{14}C]palmitate. ^{14}C from [1-^{14}C]acetate was primarily in carbons 3 and 4, with trace incorporation into the other four carbons. The sum of the ^{14}C activities in carbons 3 and 4 of glucose formed from [10-^{14}C]palmitate, [16-^{14}C]palmitate, and [12-^{14}C]-laurate was significantly greater ($P < 0.05$) (27) than from [2-^{14}C]acetate and [2-^{14}C]palmitate.

Distribution of ^{14}C among the carbons of the glucose formed by the liver slices from the alloxan diabetic rats are recorded in **Table 2,** along with the recovery of added ^{14}C in glucose. The results are similar to those for monkeys. Again, using the rank test (27), incorporations into carbons 3 plus 4 from the terminally labeled fatty acids exceeded those from [2-^{14}C]acetate and [2-^{14}C]palmitate ($P < 0.05$). By the paired Student's *t* test (28), the sum of the relative specific activities in carbons 3 and 4 was significantly greater ($P < 0.01$) in the incubation with [16-^{14}C]-palmitate than in that with [2-^{14}C]acetate. ^{14}C from [1-^{14}C]acetate and [3-^{14}C]palmitate was incorporated primarily into carbons 3 and 4, although there was notable incorporation from [3-^{14}C]palmitate into the remaining four carbons.

Distributions of ^{14}C in blood glucose after 5 hr infusions into the fasted rats are recorded in **Table 3.** ^{14}C

TABLE 2. Distribution of ^{14}C in the glucose formed by alloxan diabetic rat slices incubated with ^{14}C-labeled acetate or ^{14}C-labeled fatty acids

Labeled Substrate	Expt. No.	% Added ^{14}C Converted to Glucose	^{14}C in Carbons 1	2	3	4	5	6	% Recovery
[1-^{14}C]acetate	1	0.5	2	3	64	100	1	0	91
[2-^{14}C]acetate	1	2.7	86	85	16	17	100	97	96
	2	1.1	71	73	18	18	100	92	97
	3	1.7	78	62	15	20	100	97	93
	4	8.7	78	68	14	20	100	96	102
[2-^{14}C]palmitate	1	0.2	81	85	16	18	100	97	94
	2	0.1	78	73	18	20	100	98	94
[3-^{14}C]palmitate	1	0.06	7	15	79	100	11	10	88
	3	0.05	7	6	52	100	3	5	86
	4	0.03	8	12	58	100	3	3	93
[16-^{14}C]palmitate	1	0.3	67	67	21	22	100	95	97
	2	0.06	76	78	23	24	100	98	99
	3	0.02	85	91	26	25	100	97	97
	4	0.04	77	145[a]	28	23	100	97	96
[12-^{14}C]laurate	1	0.2	68	61	22	23	100	94	98
	2	0.3	77	68	25	32	100	88	100
	3	0.3	73	61	33	58	100	98	86
	4	0.2	81	62	22	36	100	101	101

[a] This determination is undoubtedly in error, but the source of error has not been found.

from [1-^{14}C]palmitate and [3-^{14}C]palmitate was again incorporated primarily into carbons 3 and 4. Incorporation of ^{14}C into carbons 3 and 4 from [2-^{14}C]acetate, [2-^{14}C]palmitate, [16-^{14}C]palmitate, and [12-^{14}C]laurate relative to incorporation into carbons 1, 2, 5, and 6 was much greater than in the in vitro studies. Except for a lesser incorporation from [2-^{14}C]acetate into carbons 3 and 4 than from the other substrates, there were no discernible differences among the labeled substrates in their patterns of incorporation. Thus, the sums of incorporation into carbons 3 and 4 from [2-^{14}C]palmitate were 124 and 125, from [16-^{14}C]palmitate they were 138 and 120; and from [12-^{14}C]laurate they were 129 and 117; while those from [2-^{14}C]acetate were 94 and 88.

The data for 5-hr infusions into streptozotocin-induced diabetic rats are recorded in the upper portion of **Table 4.** Distributions were similar to those for fasted rats. The sum of ^{14}C in carbons 3 and 4 from [2-^{14}C]palmitate was 111, from [16-^{14}C]palmitate it was 97 and 120, and from [2-^{14}C]acetate it was 96. Distributions of ^{14}C in glucose after 1-hr infusions

TABLE 3. Distribution of ^{14}C in glucose from glycogen from fasted rats infused for 5 hours with ^{14}C-labeled acetate or ^{14}C-labeled palmitate

Labeled Substrate	^{14}C in Carbons 1	2	3	4	5	6	% Recovery
[2-^{14}C]acetate	94[b]	92	46	48	100	96	100
	91[a]	86	45	43	100	92	100
[1-^{14}C]palmitate	6[a]	4	83	100	4	4	87
[2-^{14}C]palmitate	94[b]	89	55	69	100	97	96
	96[b]	91	64	61	100	92	109
[3-^{14}C]palmitate	9[a]	8	90	100	8	7	104
[16-^{14}C]palmitate	102[a]	65	67	71	100	94	80
	94[a]	84	53	67	100	97	101
[12-^{14}C]laurate	83[a]	94	65	64	100	94	99
	96[b]	80	58	59	100	98	86

[a] Glucose from liver glycogen.
[b] From blood glucose.

TABLE 4. Distribution of ^{14}C in blood glucose from streptozotocin diabetic rats infused for 5 hours and fasted rats infused for 1 hour with ^{14}C-labeled acetate or ^{14}C-labeled palmitate

Labeled Substrate	^{14}C in Carbons 1	2	3	4	5	6	% Recovery
Diabetic Rats							
[1-^{14}C]acetate	1	1	65	100	2	1	94
[2-^{14}C]acetate	91	88	45	51	100	88	95
[2-^{14}C]palmitate	95	52	42	69	100	106	82
[16-^{14}C]palmitate	92	88	52	45	100	100	100
	91	54	44	76	100	98	87
Fasted Rats							
[2-^{14}C]acetate	87	78	38	44	100	96	77
	93	83	40	46	100	96	93
[16-^{14}C]palmitate	82	74	45	52	100	81	87

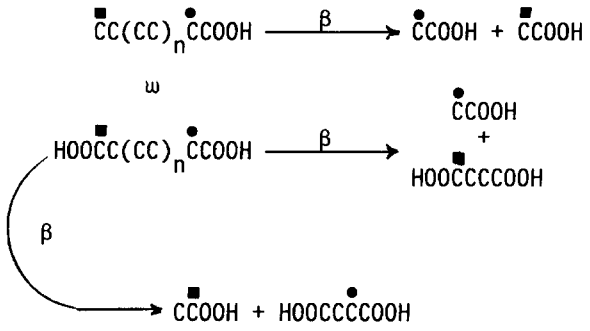

Fig. 1. Fate of carbon 2 (●) and the ω carbon (■) of an even carbon-containing fatty acid on β-oxidation or ω-oxidation and subsequent β-oxidation.

into fasted rats are also shown in Table 4. ^{14}C incorporation into carbons 3 and 4 may be somewhat lower than for 5-hr infusions. The sum in carbons 3 and 4 from [2-^{14}C]acetate was 82 and 86, and from [16-^{14}C]-palmitate it was 97.

All the distributions in the tables show greater activity in carbons 4, 5, and 6 than in carbons 1, 2, and 3. This is due to nonisotopic equilibration of triose phosphates and/or the transaldolase exchange reaction (21, 29).

DISCUSSION

The fates of carbon 2 and the ω carbon of an even carbon-containing long-chain fatty acid via β- and ω-oxidations are depicted in **Fig. 1.** If carbon 2 or the ω carbon is labeled with ^{14}C, [2-^{14}C]acetate is formed via β-oxidation. Omega-oxidation of the fatty acid will yield a dicarboxylic acid, and β-oxidation can be initiated from either end. If initiated from the α carbon, and if β-oxidation continues to the formation of succinate (i.e., β-oxidation "proceeds to completion"), the labeled product from the 2-^{14}C-labeled fatty acid will be [2-^{14}C]acetate, and from the ω-^{14}C-labeled fatty acid it will be [1-^{14}C]succinate. If initiated from the ω carbon, the products will be [1-^{14}C]acetate from the ω-^{14}C-labeled fatty acid and [2-^{14}C]succinate from the 2-^{14}C-labeled fatty acid.

The essentially identical distributions of ^{14}C in glucose formed by liver slices from [2-^{14}C]acetate and from [2-^{14}C]palmitate (Tables 1 and 2) are in accord with β-oxidation of the palmitate to form [2-^{14}C]acetate. It is also in accord with an initial ω-oxidation and then β-oxidations of the resulting hexadecanedioic acid, since the labeled products formed at the completion of all the β-oxidation would be [2-^{14}C]acetate and [2-^{14}C]succinate, and [2-^{14}C]succinate will label glucose as does [2-^{14}C]acetate (30). The greater incorporations of ^{14}C into carbons 3 and 4 of glucose from [16-^{14}C]palmitate and [12-^{14}C]laurate than from [2-^{14}C]palmitate are in accord with the occurrence of ω-oxidation followed by β-oxidation of the ω-labeled dicarboxylic acids to yield [1-^{14}C]acetate and [1-^{14}C]succinate, since [1-^{14}C]succinate will label glucose as does [1-^{14}C]acetate (30).

In **Table 5** the sum of the relative incorporations into carbons 3 and 4 and all six carbon atoms are recorded for the distributions in Tables 1 and 2. From these values, and from the greater incorporation of ^{14}C from [2-^{14}C]acetate than from [1-^{14}C]acetate into glucose, the relative contribution of ω-oxidation to the initial oxidation of the fatty acids can be estimated (6). These estimates are recorded in the last column of Table 5. The method of estimation will now be illustrated using data from the incubations of palmitate with monkey liver slices.

From **Table 5,** incorporation into carbons 3 and 4 from [16-^{14}C]palmitate is 38, and from [2-^{14}C]palmitate it is 28, so that incorporation attributed to [1-^{14}C]-acetate formation (or its equivalent, [1-^{14}C]succinate), is 38 − 28 = 10. Thus, incorporation of ^{14}C from 348 molecules of [2-^{14}C]acetate and 10 molecules of [1-^{14}C]acetate would approximate the distribution found with [16-^{14}C]palmitate. This does not include a correction for the fact that incorporation into the glucose unit of glycogen of ^{14}C from [2-^{14}C]acetate is 3.1 times that from [1-^{14}C]acetate. Thus 8.2% [(100)(3.1)(10)]/[348 + 3.1(10)] of the total number of molecules of the palmitate oxidized were oxidized initially via ω-oxidation. Using the data from [2-^{14}C]palmitate and [12-^{14}C]laurate, and calculating in an identical manner, 21.1% of the laurate oxidized was initially converted to dodecanedioic acid by the monkey slices.

TABLE 5. Sum of relative incorporations of ^{14}C into carbons 3 and 4 of glucose and into all six carbons of glucose (mean and range) from Tables 1 and 2 and estimates of the contribution of ω-oxidation from these data

Labeled Substrate	Sum of C-3 and C-4	Sum of C-1 through C-6	% via ω-Oxidation[a]
Fasted Monkey Liver			
[2-^{14}C]acetate	31 (28–35)	367 (362–372)	
[2-^{14}C]palmitate	28 (27–28)	348 (332–363)	
[10-^{14}C]palmitate	35 (35)	380 (377–382)	11.1
[16-^{14}C]palmitate	38 (34–41)	378 (370–385)	8.2
[12-^{14}C]laurate	58 (54–64)	398 (395–402)	21.1
Diabetic Rat Liver			
[2-^{14}C]acetate	35 (33–36)	380 (372–401)	
[2-^{14}C]palmitate	36 (34–36)	392 (387–397)	
[16-^{14}C]palmitate	48 (43–51)	416 (372–470)	8.4
[12-^{14}C]laurate	63 (45–91)	396 (368–423)	17.0

[a] For method of calculation, see discussion.

These estimates are in accord with the conclusion that the microsomal fraction of liver is more active in the oxidation of lauric than palmitic acid (31). The percentages are somewhat greater than the less than 5% estimated to be the contribution of ω-oxidation to palmitate and stearate metabolism in slices from rats fasted for 24 hr. This indicates that there is some increase in contribution with prolonged starvation and in diabetes mellitus.

These estimates depend upon several assumptions. It is assumed that the ^{14}C of [1-^{14}C]succinate is treated the same as that of [1-^{14}C]acetate in its conversion to glucose (26, 30, 32). It is further assumed that the two carboxyls of the dicarboxylic acid formed from the fatty acid by ω-oxidation are equivalent, so that β-oxidation would be as likely to be initiated and continue from one end of the molecule as the other (Fig. 1). There is good evidence that dicarboxylic acids are metabolized via β-oxidation and that their oxidation is via mono-CoA intermediates (33–35). Pettersen (11) administered [16-^{14}C]palmitate to one and [1-^{14}C]palmitate to another ketotic diabetic rat and isolated hexanedioic acid from their urines. The percentage of the administered ^{14}C recovered in the dicarboxylic acid was the same for both rats. This supports carbon 1 and the ω carbon of palmitate having the same fate during the β-oxidations that follow ω-oxidation of palmitate.

It is also assumed that α-oxidation makes a negligible contribution to the oxidation of the fatty acid. We have good evidence to support this, because the distribution from [2-^{14}C]acetate is similar to that from [2-^{14}C]palmitate. If α-oxidation and then β-oxidation of [2-^{14}C]palmitate occurred, [1-^{14}C]acetate would be formed and the distribution in carbons 3 and 4 from [2-^{14}C]palmitate would have exceeded that from [2-^{14}C]acetate.

The assumption of greatest importance is that the yield of [2-^{14}]acetyl CoA on β-oxidation of a fatty acid will be the same whether carbon 2 or the ω carbon of the fatty acid is labeled with ^{14}C. This seems unlikely to be fulfilled in the circumstance of ketosis, where many of the carbons of fatty acid are incorporated into ketone bodies. This is so because asymmetry in the ^{14}C-labeling of acetoacetate formed by liver slices from [3-^{14}C]butyrate (36, 37) and [7-^{14}C]octanoate (37) can be explained only by preferential incorporation of the terminal two carbons of these acids into acetoacetate, and hence hydroxybutyrate. The entrance of the ω carbon compared to carbon 2 of a fatty acid into the free acetyl CoA pool must then be less, and it is from this pool that incorporation into glucose occurs. To the extent that the ω carbon of the fatty acid oxidized via β-oxidation does not have the opportunity to find expression in glucose, our estimates of ω-oxidation relative to overall fatty acid oxidation are too high.

Greater incorporation into carbons 1, 2, 5, and 6 relative to carbons 3 and 4 of ^{14}C from [3-^{14}C]palmitate than from [1-^{14}C]acetate or [1-^{14}C]palmitate (Table 2 and the single value in Table 3) is also in accord with the occurrence of ω-oxidation with the resulting formation of [2-^{14}C]succinate from the [3-^{14}C]-palmitate.

After ω-oxidation, a fatty acid labeled in other than its first four or last four carbons will not yield labeled succinate if β-oxidation is initiated and continued from either end of its dicarboxylic acid. Thus, oxidation of [10-^{14}C]palmitate solely by β-oxidation should yield [2-^{14}C]acetate, while its oxidation to hexadecanedioic acid followed by β-oxidation should yield [1-^{14}C]-acetate from one-half of its molecules and [2-^{14}C]acetate from the other half. To estimate the extent of ω-oxidation, the previous calculation then has to be altered by doubling the excess incorporation into carbons 3 and 4, since only one-half of the molecules formed via ω-oxidation are converted to [1-^{14}C]acetate and none to [1-^{14}C]succinate. The percent of ω-oxidation estimated using [10-^{14}C]palmitate (Table 5) has been calculated in this manner. The estimate would not be altered by the failure of the ω carbon of palmitate to have the same access as carbon 2 of palmitate to the acetyl CoA pool.

If these estimates are applicable to man, they allow a measure of the quantity of glucose that could be formed via ω-oxidation. The quantity of fatty acid taken up by the liver of starved and of diabetic man is about 0.3 mol/24 hr (39, 40). Taken as palmitate, this equates to 77 g of palmitate. Assuming that 10% of the initial oxidation is via ω-oxidation, even if all of the palmitate were oxidized so that 9 g of dicarboxylic acid were formed, the quantity of succinate that could be formed from the 9 g of dicarboxylic acid could yield a maximum of 2.6 g of glucose. This net yield of glucose from fatty acids would be small relative to estimated yields of glucose from other sources (39, 40). Omega oxidation may, as suggested by Ellin (4), serve primarily to detoxify fatty acids under conditions where their concentrations reach toxic levels.

For the above estimations, the dicarboxylic acid is assumed to be oxidized completely via β-oxidation in the liver. If β-oxidation of the dicarboxylic acid formed by ω-oxidation of a terminally ^{14}C-labeled fatty acid does not go to completion, then oxidation at the labeled end of the dicarboxylic acid will yield [1-^{14}C]-acetate and an unlabeled dicarboxylic acid of more than four carbons. Oxidation at the unlabeled end of the dicarboxylic acid will yield unlabeled acetate and a labeled dicarboxylic acid of more than four carbons.

This dicarboxylic acid could be excreted or metabolized in another tissue in the whole animal where its label would not find expression in glucose. Then one-half of the molecules of the fatty acid oxidized would find expression through incorporation of ^{14}C into glucose and one-half would not. In contrast, every molecule of [2-^{14}C]fatty acid oxidized via β-oxidation will yield [2-^{14}C]acetate. As such, if one assumes that following ω-oxidation there is at least a single β-oxidation but that β-oxidation does not proceed to completion, the estimates of the percent of ω-oxidation have to be doubled. If β-oxidation did not occur following ω-oxidation, there would be no incorporation of label into glucose and the extent of ω-oxidation would be underestimated. In the case of [10-^{14}C]palmitate, as already noted, there must have been at least four β-oxidations after ω-oxidation for the formation of [1-^{14}C]acetate.

The experiments of Table 3 and 4 were an attempt to use our approach to estimate the contribution of ω-oxidation in vivo. The different pattern obtained in vivo with [2-^{14}C]acetate than with [2-^{14}C]palmitate (Table 3) may indicate that, compared to the [2-^{14}C]-acetate, the [2-^{14}C]acetate formed from the [2-^{14}C]-palmitate is oxidized in a different pool (tissue) to at least some degree (41, 42). The distribution from [2-^{14}C]palmitate, rather than [2-^{14}C]acetate, must then be used for comparison with the distribution from [16-^{14}C]palmitate. While incorporations into carbons 3 and 4 vary, no differences between the patterns can be discerned.

Incorporation of ^{14}C from [2-^{14}C]palmitate into carbons 3 and 4 relative to the other carbons of glucose exceeds that theoretically possible from incorporation solely from [2-^{14}C]acetate (or [2,3-^{14}C]succinate) into glucose, i.e., the theoretical relative specific activities for the six carbons are 100, 100, 50, 50, 100, and 100 (26). The distribution of ^{14}C in glucose excreted by a diabetic dog given [6-^{14}C]palmitate (32) was similar to that we have observed with [2-^{14}C]palmitate. Since ^{14}C from $^{14}CO_2$ is incorporated by liver exclusively into carbons 3 and 4 of glucose (7), this could explain the distributions observed (32). Thus, metabolism of the ^{14}C-labeled fatty acids to $^{14}CO_2$, the ^{14}C of which was then incorporated into glucose by liver, could have masked a contribution from products formed from the metabolism of the fatty acids in the liver via ω-oxidation. That this did not occur in our in vitro studies can be attributed to dilution of the $^{14}CO_2$ by unlabeled CO_2 in the medium and gas phase. However, that a contribution by ω-oxidations was not discernible in our in vivo studies indicates again that, at most, only a small portion of initial fatty acid oxidation in the environment of glucose formation was via ω-oxidation, and this occurred in the whole rat.

As noted in the introduction, we (15) recently reported results of experiments in which [2-^{14}C]palmitate, [10-^{14}C]palmitate, [16-^{14}C]palmitate, and [12-^{14}C]laurate were administered to diabetic rats fed 2-amino-4-phenylbutyric acid. The acetylated amino acid was isolated from the urine of the rats; it was then hydrolyzed and the resulting acetate was degraded. Relative to the labeling of the acetate from [2-^{14}C]palmitate, carbon 1 was labeled more than carbon 2 of acetate by [10-^{14}C]palmitate and the ω-^{14}C-labeled fatty acids. From these distributions, 20–40% of the initial oxidation of the fatty acids would be by ω-oxidation, larger percentages than we have estimated from the distributions in glucose.

The present estimates are for the contribution of ω-oxidation in the environment of glucose formation. This, then, is presumably a measure of the initial oxidation of fatty acids primarily in the parenchymal cells of the liver. The estimates made from the acetylation of phenylaminobutyric acid are for fatty acid oxidation in the environment of that acetylation, the site of which is unknown. However, acetylation of aromatic compounds has been reported to occur in the reticuloendothelial cells and not in the parenchymal cells of the liver (43).

Wada and Usami (9) estimated that about 15% of palmitic acid underwent ω-oxidation and then β-oxidation in starved or diabetic rats, based upon their observation of differences between the percentage incorporation of ^{14}C from [1-^{14}C]palmitate and from [U-^{14}C]palmitate into glucose. They provided no data to support their statement that "if palmitate is not subjected to ω-oxidation, the percentage incorporation from [1-^{14}C]palmitate and [U-^{14}C]palmitate should be equal." [1-^{14}C]Acetate is formed from [1-^{14}C]palmitate solely via β-oxidation and [1,2-^{14}C]acetate is formed from [U-^{14}C]palmitate. We show here, and Strisower et al. (26) and we (6) previously showed, that ^{14}C from [1-^{14}C]acetate is not incorporated into glucose to as great an extent as from [2-^{14}C]acetate in vitro. Abraham, Chaikoff, and Hassid (32) recovered in glucose about 6% of the ^{14}C of [1-^{14}C]palmitate and 14% of the ^{14}C of [6-^{14}C]palmitate they administered to diabetic dogs. This would also support a greater yield in glucose from [2-^{14}C]acetate than from [1-^{14}C]acetate and in vivo. Further, that the administration of even carbon-containing fatty dicarboxylic acids decreased the concentrations of ketone bodies in the blood of rats was taken by Wada and Usami (9) to suggest that ω-oxidation may be important for the production of succinyl CoA. However,

odd carbon-containing fatty dicarboxylic acids also decreased the concentrations and, as they noted, these acids should not yield succinyl CoA.

Thus, incorporation of ^{14}C from specifically ^{14}C-labeled fatty acids into glucose formed by liver slices of fasted monkeys and alloxan diabetic rats indicate a relatively small contribution by ω-oxidation to fatty acid oxidation and, thus, the formation of only small quantities of long chain fatty dicarboxylic acids in the environment of glucose formation. Even if β-oxidation of these dicarboxylic acids proceeds to the formation of succinate in liver, the glucose formed as a maximum from the succinate could then make only a small contribution to overall glucose formation.

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