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METABOLISM OF [1-^{14}C]LIGNOCERIC ACID IN THE RAT

S. GATT

Department of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem (Israel)

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SUMMARY

[1-^{14}C]Lignoceric (tetracosanoic) acid was synthesized and administered to rats. Following intravenous injection, the fatty acid accumulated in the liver, was slowly oxidized to $^{14}CO_2$ and incorporated into sphingolipids, cholesterol esters and glycerides; a large portion of the administered acid was found as free fatty acid for periods of 24 h or more. After intracerebral administration to young rats, lignoceric acid was incorporated into the brain sphingolipids and glycerides. Incorporation of tritiated water, [1-^{14}C]acetic and [1-^{14}C]palmitic acids under similar conditions was also studied. The relative specific activities of the sphingomyelins, cerebrosides and ceramides are discussed in the light of currently available theories concerning synthesis of the sphingolipids.

INTRODUCTION

The C_{24} fatty acids are major components of the fatty acid moiety of most sphingolipids[1]. Four such acids have been identified, namely, lignoceric (tetracosanoic), α-hydroxy lignoceric, nervonic and α-hydroxy nervonic. While all four are found in the cerebrosides[2,3], the hydroxy acids are absent from the spingomyelins[4]. The C_{24} fatty acids are present in negligible amounts in brain gangliosides[5,6], but constitute most of the fatty acid portion of the complex glycolipids of spleen and erythrocyte stroma[7]. In all sphingolipids the C_{24} fatty acids are bound to sphingosine in amide linkage. Lipids having these fatty acids in ester bond have not been identified as natural components of animal tissue.

The present communication describes the metabolic utilization of lignoceric acid when administered to rats by intravenous or intracerebral route. Preliminary communications have appeared[8,9].

MATERIALS

The following materials were used. Alumina: Savory and Moore, standardized according to Brockman; silicic acid: Mallincrodt, 100 mesh, suitable for chromatography; florisil: Floridin Co., Tellahassee, Fla. (U.S.A.), 100 mesh; bovine serum albumin: Pentex Inc., Kankakee, Ill. (U.S.A.). All solvents were U.S.P. or Reagent grade and were not distilled prior to use.

METHODS

Synthesis of [1-^{14}C]lignoceric acid

Lignoceric acid was isolated from peanut oil by the procedure of TAYLOR[10]. Following hydrolysis with 5 N NaOH at 85° for 3 h, the fatty acids were acidified, washed 3 times with hot water and left overnight at room temperature. The solid acids were collected by filtration and were recrystallized twice from 90% ethanol and 3 times from acetone. The melting point of the crystals was 75.5–76°. Five additional crystallizations from acetone raised the melting point to 78.5–79° and two further crystallizations from benzene to 80°. This material was still contaminated with small amounts of C_{22} and C_{26} fatty acids; it was used for the introduction of a radioactive carboxyl by the following modification of ANKER's method for palmitic acid[11]. Lignoceric acid was converted to the silver salt which was then decarboxylated with Br_2. 800 mg of the tricosyl bromide were dissolved in 25 ml of absolute ethanol and refluxed with 65 mg K^{14}CN for 30 h. Due to its low solubility, the tricosyl cyanide thus obtained could not be hydrolyzed with aqueous alkali and was therefore refluxed with 20% KOH in 90% ethanol for 12 h. The reaction mixture was diluted with water, extracted with ether and the free lignoceric acid was obtained by heating the potassium salt with H_2SO_4 on a water bath. It was then extracted with light petroleum (b.p. 60–80°) and recrystallized several times from acetone. The melting point was 79.5–80° and the neutralization equivalent 368.5. Gas–liquid chromatography on a polyethyleneglycol adipate column using a Pye Argon chromatograph showed that traces of docosanoic and hexacosanoic acids were still present.

Administration to animals and isolation of lipids

a. Intravenous administration: Neutralized suspensions of [1-^{14}C]lignoceric acid were injected into the tail vein of adult rats under light ether anaesthesia and the animals were killed by cervical dislocation. For determination of total radioactivity, the organs were extracted with 20 volumes of a mixture of chloroform – methanol (2:1, v/v). For fractionation of the liver lipids, the organ was minced and heated with acetone. The acetone was removed on a water bath and the tissue dried overnight in a vacuum desiccator over P_2O_5. It was then ground in a mortar, extracted 3 times with acetone and 4 times with light petroleum (b.p. 40–60°). The two extracts were combined, evaporated *in vacuo* and taken up in a small volume of light petroleum. The phospholipids were precipitated with acetone in presence of ethanolic $MgCl_2$ (ref. 12), and were subsequently reprecipitated 3 times with acetone. The supernatant was taken twice through a column of MgO–celite[12] to remove free fatty acids and then chromatographed on a silicic acid–celite column in order to seperate cholesterol esters and tri-, di- and monoglycerides[13]. The tissue remaining after the acetone and light petroleum extractions, was extracted 3 times with boiling ethanol, the extracts being filtered and evaporated *in vacuo*. The crude sphingolipid preparation thus obtained was purified by dissolving in 1–2 ml of boiling ethanol, adding 5–10 ml boiling acetone and chilling at 4°. This procedure was repeated several times to constant radioactivity.

b. Intracerebral administration: The radioactive precursors were administered to 16 day old rats by intracerebral injection as described by NICHOLAS AND THOMAS[14]; no anaesthesia was used and the fatty acids were given as a suspension of their

sodium salt at a pH of about 7.5. Palmitic acid was sometimes injected as a complex with bovine serum albumin. The animals were killed by cervical dislocation and the brain was extracted with 19 volumes of a mixture of chloroform – methanol (2:1, v/v) or chloroform – methanol – water (64:32:4, v/v). The extract was evaporated *in vacuo* and chromatographed on alumina using the procedure of LONG AND STAPLES[15] with the following modification. An extract obtained from 1–5 g brain was dissolved in a mixture of chloroform – methanol (98:2, v/v) and put on a column containing 13 g alumina. Neutral lipids were eluted with 150–250 ml of the same solvent mixture, and the lecithins and sphingomyelins with 150–250 ml of a mixture of chloroform – methanol (1:1, v/v). The cerebrosides were then eluted with a stepwise gradient of increasing concentrations of water in a mixture of chloroform – methanol (1:1, v/v). 15–25-ml fractions of 1–6 % and 150–250-ml fractions of 6.5 % water in chloroform – methanol (1:1, v/v) were collected. The cerebrosides which were eluted with solvents containing 4–6.5 % water were heavily contaminated with free lignoceric acid which was eluted on the downward slope of the cerebroside peak. They were then rechromatographed on florisil with a mixture of chloroform–methanol (4:1, v/v)[3] and subsequently, on silicic acid using a stepwise gradient of chloroform with increasing concentrations of methanol; the cerebrosides were eluted with 10 % methanol in chloroform.

The lecithin–sphingomyelin mixture was subjected to mild alkaline hydrolysis in 0.4 N methanolic KOH containing 10 % water for 2 h at 37°. After neutralization, chloroform and water were added to give a mixture of chloroform – methanol – water (8:4:3, v/v). The phases were separated, the lower phase washed once with "pure solvents upper phase"[16], evaporated to dryness and the sphingomyelins obtained by rechromatography on alumina. The free ceramide fraction was isolated as already described[17]. Isolation of the sphingosine and fatty acid moieties of the sphingolipids, and the analytical procedures used were reported previously[18].

Radioactive assay

Samples were either plated on lens paper on planchets and counted at infinite thinness in an end window counter, or alternatively, counted in a Packard Tri-Carb liquid scintillation counter. $Ba^{14}CO_3$, resulting from respiratory CO_2 was plated at infinite thickness and the results corrected to infinite thinness according to standard curves.

RESULTS

Dispersion of [1-^{14}C]lignoceric acid

[1-^{14}C]Lignoceric acid was neutralized, suspended in water and incubated with bovine or human serum albumin. In spite of the low free fatty acid content of the albumin, and the high protein to acid ratio (up to one mole albumin per mole fatty acid), the mixture was not homogeneous and particles separated on cooling. Such an incubation mixture was then subjected to paper electrophoresis in 0.05 M sodium barbital buffer (pH 8.5). Of two parallel paper strips, one was stained with amido black to detect the albumin and the other was taken through a strip counter for radioactivity termination. Tracings of such an experiment may be seen in Fig. 1. It is evident that most of the radioactivity did not move with the albumin but remained at the origin. A third paper strip was cut into several consecutive pieces,

eluted with a mixture of chloroform – methanol (2:1, v/v) and the radioactivity determined. Only about 3 % of the total radioactivity was found with the albumin. Under the same conditions, incubation of [1-^{14}C]palmitic acid with albumin resulted in the migration of most of the radioactivity with the protein. It is thus evident that

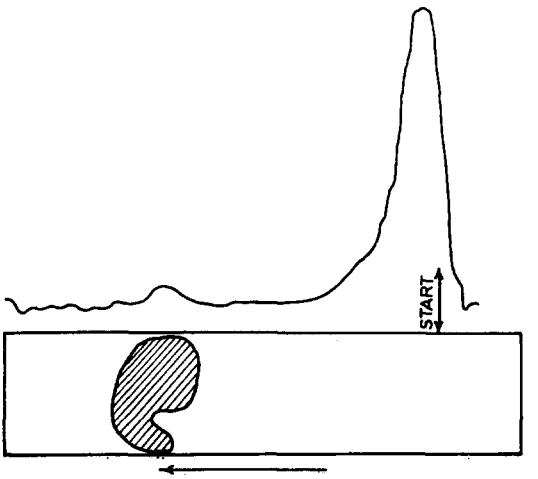

Fig. 1. Incubation of lignoceric acid and serum albumin. Paper-electrophoresis patterns of an incubation mixture of [1-^{14}C]lignoceric acid with bovine serum albumin. Lower part: tracing of the albumin stained with amido black. Upper part: tracing of radioactivity as recorded in a strip counter.

lignoceric acid did not form a lipoprotein complex with albumin. Therefore, a suspension of [1-^{14}C]lignoceric acid was prepared as follows. The fatty acid was dissolved in hot ethanol and introduced slowly into boiling water to which 0.7 equivalent of KOH or NaOH had been added. The ethanol was then removed by repeated evaporation and addition of water. Such a suspension was stable for several weeks in the refrigerator.

Administration by intravenous injection

Distribution of [1-^{14}C]lignoceric acid in rat organs: [1-^{14}C]lignoceric acid was injected into the tail vein of adult rats. At the specified times, the animals were killed and the organs extracted with a mixture of chloroform – methanol (2:1, v/v). The results are summarized in Table I. It is evident that the injected acid was removed from the blood within 15 min and concentrated mostly in the liver. Small amounts were found also in the lung and spleen, but practically none in brain or testicular adipose tissue. After 24 h, the liver still contained most of the remaining body radioactivity, the value was, however, lower than that observed 15 min after injection; brain and adipose tissue still had almost no radioactivity.

Oxidation of [1-^{14}C]lignoceric acid to respiratory CO_2: Fig. 2 represents a time curve of the expired $^{14}CO_2$ following intravenous injection of [1-^{14}C]lignoceric acid to fasted or fed rats and to rats forced fed with glucose. About 20 % of the injected radioactivity was recovered in the expired CO_2 within 7 h, and as is evident oxidation

TABLE I

DISTRIBUTION OF [1-^{14}C]LIGNOCERIC ACID IN RAT ORGANS

[1-^{14}C]Lignoceric acid was injected into the tail vein of rats and the organs removed and extracted with 19 volumes of a mixture of chloroform – methanol (2:1, v/v). Expt. 1: 3 μmoles [1-^{14}C]lignoceric acid were given and the animal sacrificed after 15 min. Expt. 2: 2 μmoles [1-^{14}C]lignoceric acid were given and the animal sacrificed after 26 h. Expt. 3: 15 μmoles [1-^{14}C]lignoceric acid were given in 4 injections at 2-h intervals and the animal sacrificed 24 h after first injection.

Organ	*% of injected radioactivity*		
	Expt. 1	*Expt. 2*	*Expt. 3*
Liver	50	18	18
Lung	8		0.5
Spleen	3	1	0.4
Brain	—	—	—
Kidney	—	0.4	0.1
Adipose tissue	—		0.1
Blood	—		0.3
Muscle			3

of the fatty acid was not effected by the nutritional state of the animal. In all cases there was a lag of about 30 min after injection, followed by an increased oxidation during the next 2 h and then by a gradual decrease in the evolution of $^{14}CO_2$. Fig. 3 shows the change in the specific activities of the expired CO_2 with time. The shape of the curves was similar with rats in the three nutritional states, the differences in the specific activities being due, apparently, to different degrees of dilution with endogenous, non radioactive CO_2.

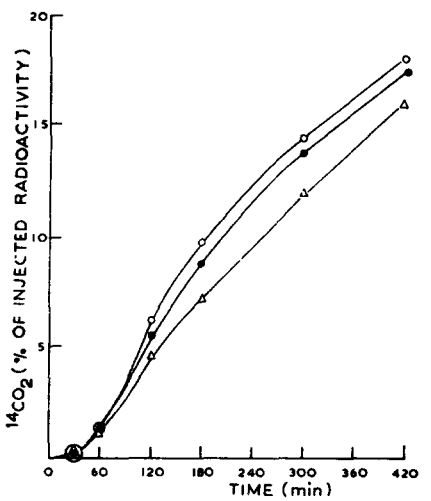

Fig. 2. Oxidation of [1-^{14}C]lignoceric acid to $^{14}CO_2$. 6 μmoles [1-^{14}C]lignoceric acid (0.2 μC per μmole) were given by intravenous injection to adult rats and respiratory $^{14}CO_2$ collected. O—O, fasted rat; ●—●, fed rat; △—△, rat forced fed with glucose prior to administration of the fatty acid.

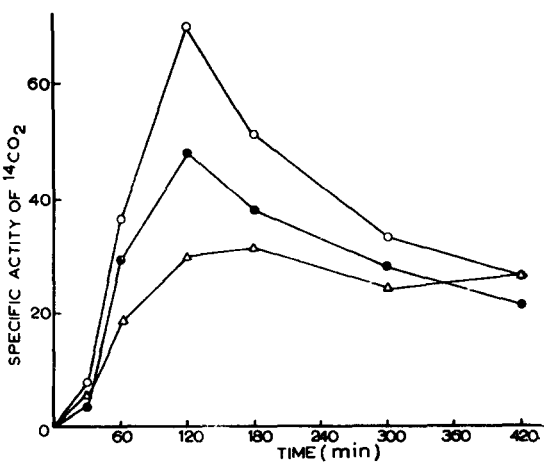

Fig. 3. Specific activity of expired $^{14}CO_2$. Conditions same as in Fig. 2. Results expressed as counts/min per dish of $Ba^{14}CO_3$ counted at infinite thickness. O—O, fasted rat; ●—●, fed rat; △—△, rat forced fed with glucose prior to administration of the fatty acid.

Incorporation of [1-^{14}C]lignoceric acid into rat-liver lipids: Fig. 4 represents a time course of the incorporation of [1-^{14}C]lignoceric acid into three lipid fractions of rat liver. The amount of free lignoceric acid is also represented but on a scale exceeding that of the lipids by a factor of 10. The three lipid fractions (neutral lipids, glycerophosphatides and sphingolipids) exhibit a peak incorporation about 7 h after injection, followed by a slow decrease in the radioactivity. Most striking was the high content of free fatty acid present (about 50 and 8% of the injected radioactivity) 1 and 24 h respectively after administration.

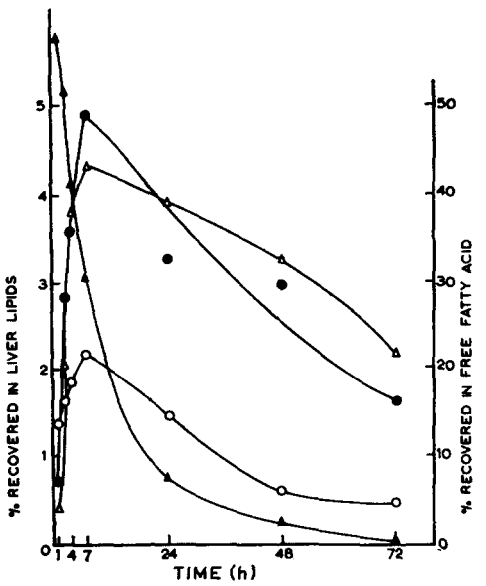

Fig. 4. Incorporation of [1-^{14}C]lignoceric acid into rat lipids. 6 μmoles of [1-^{14}C]lignoceric acid (0.2 μC/μmole) were given by intravenous injection to rats. At the specified times, the animals were sacrificed and the liver lipids fractionated as described in section METHODS. ▲—▲, free fatty acid; O—O, neutral fat; ●—●, glycerophosphatides; Δ—Δ, sphingolipids.

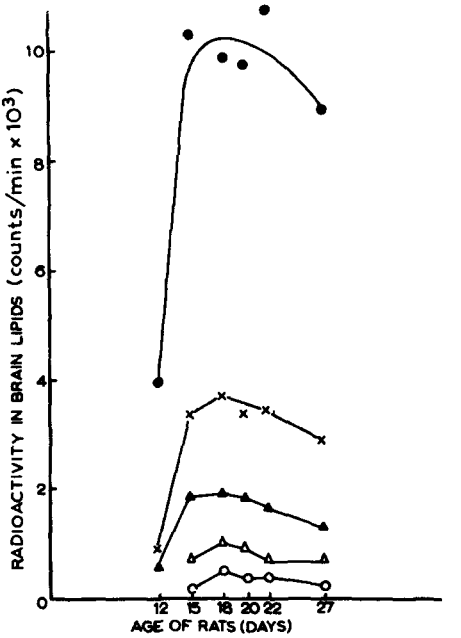

Fig. 5. Effect of age of animals on incorporation of tritiated water into brain lipids. 0.05 ml ^3H_2O (200 mC/ml) were given by intraperitoneal injection to rats. The animals were sacrificed after 24 h and the brain lipids isolated as described in section METHODS. O—O, sphingomyelin; Δ—Δ, cerebrosides; ▲—▲, neutral fraction; ×—×, lecithin + sphingomyelin; ●—●, total lipid extract.

Of special interest was the presence of [1-^{14}C]lignoceric acid in neutral lipids and glycerophosphatides. Since lignoceric acid has hitherto been found only in amide bond with sphingosine, the identity of the radioactive portion of the liver lipids was further investigated. The samples of each lipid fraction of the experiment described in Fig. 4 were pooled; the sphingolipids were taken through a mixed resin bed[19] or alternatively, through alumina[15]. In both cases, the radioactivity resided mostly in the sphingomyelins and only negligible activity was found in the cerebroside fraction. The glycerophosphatides were precipitated with $CdCl_2$ (ref. 20) and following removal of the salt, chromatographed on alumina[21]. The lecithin thus obtained was radioactive but still contained a small contamination of sphingomyelin, as shown by paper chromatography[22].

The neutral fraction was separated into cholesterol esters, tri-, di- and monoglycerides[13]. The radioactivity of these fractions was as follows (given as percent of the neutral lipids): cholesterol esters 11 %, triglycerides 42 %, diglycerides 20 %, monoglycerides and non identified lipids 27 %. Due to the much smaller content of the diglycerides, their specific activity exceeded that of the triglycerides by about 25-fold.

The incorporation of tritiated water into brain lipids of rats of varying age is shown in Fig. 5. The highest incorporation was obtained with 16–18 day old rats. This agrees with the observation of BURTON AND BRADY[23] on the incorporation of [^{14}C]galactose into brain cerebrosides.

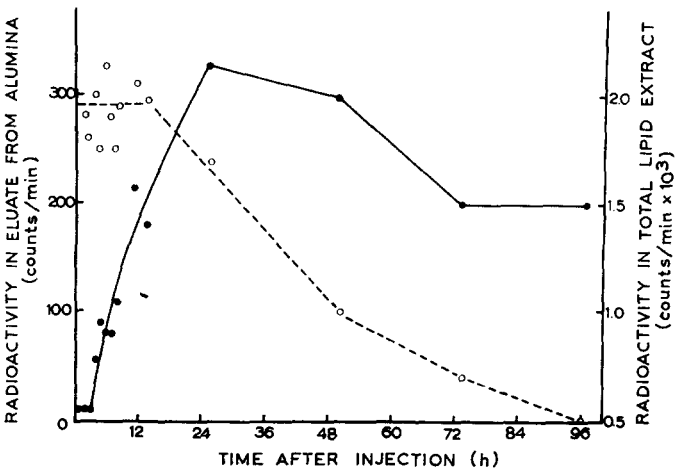

Fig. 6. Time course of incorporation of tritiated water into brain lipids. 0.05 ml ^3H_2O (200 mC/ml) was given by intraperitoneal injection to 16 day old rats. The animals were sacrificed at the specified times and the brain lipids isolated as described in section Methods. O---O, total lipid extract; ●—●, eluate from alumina.

A time curve of the incorporation of [1-^{14}C]lignoceric acid into brain lipids is shown in Fig. 6. The lipids described were a mixture of neutral lipids, lecithin and sphingomyelins obtained from an alumina column by elution with a mixture of chloroform – methanol (1:1, v/v)[15]. In contrast to the results observed in the liver following intravenous administration, the incorporation into brain lipids in the first 5 h was low, and the expired CO_2 during this period had practically no radioactivity. The maximal incorporation occurred 24 h after injection, and a portion of the administered compound remained as free fatty acid for as long as 96 h. In view of these two experiments, the radioactive precursors were administered to 16–18 day old rats and the animals sacrificed after 24 h.

Administration by intracerebral injection

Table II summarizes the incorporation of [1-^{14}C]lignoceric acid into four brain lipid fractions. For comparison, results obtained with tritiated water, [1-^{14}C]acetic and [1-^{14}C]palmitic acids under similar experimental conditions are also presented. With all precursors the radioactivity of the sphingolipids did not exceed 5–10 % of that of the neutral and phosphoglycerides (90 % or more of the radioactivity of

TABLE II

INCORPORATION OF RADIOACTIVE PRECURSORS INTO BRAIN LIPIDS

0.05 ml of neutralized suspensions of [1-^{14}C]lignocerate (0.5 μmole, 0.2 μC/μmole), tritiated water (200 mC/ml), [1-^{14}C]acetate (2.2 μmoles, 4.5 μC/μmole) and [1-^{14}C]palmitate (0.5 μmole, 2 μC/μmole) were given by intracerebral injection to 16 day old female rats and the animals sacrificed after 24 h. The brain lipids were isolated as described in section METHODS.

Lipid fraction	*% of injected radioactivity*			
	[1-^{14}C]-Lignocerate	*Tritiated water*	*[1-^{14}C]-Acetate*	*[1-^{14}C]-Palmitate*
Lipid extract	35	$5.5 \cdot 10^{-3}$	2.7	13
Neutral fraction	2.5	$1 \cdot 10^{-3}$	0.45	1
Lecithins and sphingomyelins	3.9	$2.25 \cdot 10^{-3}$	0.98	6.2
Cerebrosides	0.17	$1.7 \cdot 10^{-4}$	0.07	0.22
Free ceramides	0.15	$3.4 \cdot 10^{-5}$	0.01	0.05

TABLE III

RELATIVE SPECIFIC RADIOACTIVITIES OF BRAIN SPHINGOLIPIDS

All values are expressed as mμmoles precursor incorporated per μmole lipid or its sphingosine and fatty acid moieties. Experimental conditions are same as in Table II.

Lipid fraction	*[1-^{14}C]-Lignocerate*	*Tritiated water*	*[1-^{14}C]-Acetate*	*[1-^{14}C]-Palmitate*
Sphingomyelins	2.1	1.5	0.75	2.0
Sphingomyelins, sphingosine moiety	0.05	0.66	0.3	1.0
Sphingomyelins, fatty acid moiety	2.0	0.84	0.45	0.82
Cerebrosides	0.78	6.7	2.6	1.4
Cerebrosides, sphingosine moiety	0.16	2.8	0.75	0.7
Cerebrosides, fatty acid moiety	0.60	2.6	0.85	0.6
Free ceramides	4.0	3.9	0.84	1.0
Free ceramides, sphingosine moiety	0.15	1.6	0.46	0.7
Free ceramides, fatty acid moiety	3.2	1.4	0.36	0.23

the lecithin–sphingomyelin mixture resided in the lecithin moiety). With [1-^{14}C]-lignoceric acid, over 70 % of the radioactivity of the brain extract was present as free fatty acid.

Table III shows the relative specific radioactivities of three sphingolipid fractions and their sphingosine and fatty acid moieties. Since the specific activities of the four substrates used differed from each other, the data are presented, for ease of comparison, as mμmoles precursor incorporated per 1 μmole purified sphingolipid fraction.

It may be seen that the specific activities of the sphingomyelins exceeded those of the cerebrosides when the long-chain fatty acids were used as precursors; with the low-molecular-weight precursors the reverse was true. The specific activities of the free ceramides exceeded, in most cases, those of the sphingomyelins and were lower than those of the cerebrosides with the low-molecular-weight precursors. The presence of low radioactivity in the sphingosine moiety of the lipids following administration of [1-^{14}C]lignoceric acid is probably due to an imcomplete separation of the sphingosine and fatty acid methyl esters in the method used[18]. In the case of palmitic

acid, the radioactivity in the sphingosine moiety is due to the fact that this acid is a precursor in the synthesis of sphingosine[24-26].

DISCUSSION

In animal tissue, lignoceric acid occurs in the sphingolipids bound to sphingosine in amide linkage. It was therefore surprising to observe that following administration to rats, [1-^{14}C]lignoceric acid was found not only in this group of compounds but also in the cholesterol esters, neutral and phosphoglycerides. The pattern of utilization of this fatty acid differed considerably, however, from that of lower fatty acids, such as palmitate or stearate. Following intravenous administration to rats, both palmitic and lignoceric acid are taken up by the liver. However, while palmitic acid is fully incorporated into the neutral fat and phospholipids, most of the lignoceric acid was found as free fatty acid. Furthermore, with palmitic acid the peak incorporation into the neutral and phosphoglycerides occurs after about 15 and 60 min respectively[27]; with lignoceric acid the corresponding value for all lipid fractions was about 7 h after adminstration. It has already been shown by KORNBERG AND PRICER[28] that the rate of "activation" of fatty acids with more than 18 C atoms is very low. This might therefore suggest that the free lignoceric acid which is transported to the liver undergoes a very slow "activation" and the lignoceryl coenzyme A formed transfers its lignocerate moiety not only to sphingosine, but also to glycerol, resulting in its presence in lipids with both amide and ester bonds. These linkages are split slowly and the lignoceric acid released is either reincorporated or degraded. Contrary to lower fatty acids, it is, however, not "mobilized" for use as metabolic fuel at low carbohydrate levels. This is concluded from the finding that oxidation of [1-^{14}C]-lignoceric acid to respiratory CO_2 is independent of the nutritional state of the animal. The lower fatty acids are oxidized much faster in the fasted than in the fed or glucose-fed rats[29,30].

The enzymic mechanisms synthesizing the amide bond of the sphingolipids have not been elucidated to date. It is therefore impossible to propose the pathways through which lignoceric acid might be utilized for either ester or amide-bond synthesis. As has already been mentioned, lignoceric acid has not been found in glycerides from animal source. The possibility exists, however, that it might be present in these compounds, but in amounts so small that they were not detected by the methods employed. Alternate explanations might be that the C_{24} and the lower fatty acids are not synthesized by the same enzymic systems or that they are formed at different locations within the cell. During its synthesis *de novo*, lignoceric acid might be formed by an enzyme complex which utilizes it for the synthesis of the sphingolipids only, and does not transfer it to the substrates which accept the lower fatty acid. When administered, however, from outside, the C_{24} fatty acid which is transported to the liver in unphysiological quantities is subjected to a metabolic utilization similar to that of the lower fatty acids, *i.e.* incorporation into cholesterol esters, neutral and phosphoglycerides.

In other experiments[31], [1-^{14}C]lignoceric acid was fed to rats and its absorption in the intestine followed by cannulation of the thoracic duct. The radioactive fatty acid was present in the lymph mostly as neutral glycerides. This, again, shows that when given from an external source, lignoceric acid undergoes a metabolic utilization similar to that of the lower fatty acids.

Following intravenous administration of [1-^{14}C]lignoceric acid practically no radioactivity was found in the brain. To investigate its metabolic utilization in this organ, the radioactive fatty acid was given by direct intracerebral injection to young rats. Similar to the result in the liver, a portion of the compound was present as free fatty acid for as long as 96 h. The rate of incorporation into brain lipids was, however, slower than into the liver lipids, the peak incorporation occuring only 24 h after administration. An incorporation into neutral lipids, phosphoglycerides and all sphingolipids was observed. Of special interest were the relative specific activities of the cerebrosides and sphingomyelins (Table III). As measured by the incorporation of tritiated water or [1-^{14}C]acetate, the total synthesis of the cerebrosides was about four times greater than that of the sphingomyelins; with [1-^{14}C]lignocerate, however, the incorporation into the sphingomyelins was about three times higher than into the cerebrosides. This indicates that lignoceric acid is about twelve times more efficient a precursor for the sphingomyelins than for the cerebrosides. This difference becomes even more striking when the fatty acid composition of these two brain sphingolipids is compared. While brain sphingomyelins have stearic acid as their major component[4,32,33], the C_{24} fatty acids constitute most of the fatty acids of the cerebrosides[2,3]. In view of this composition, a higher incorporation of lignoceric acid into the cerebrosides would have been anticipated. The opposite results observed might be explained as follows. It has been proposed that the cerebrosides and sphingomyelins are synthesized by two different metabolic pathways[34-36]; the sphingomyelins from ceramides and CDP choline and the cerebrosides from psychosine (sphingosine galactose) and the coenzyme A derivative of the fatty acid. The possibility exists that the enzymes condensing sphingosine and fatty acids to give ceramides can utilize preformed, long-chain fatty acids. Those forming cerebrosides from psychosine might, however, be more strict in their requirements and use mostly fatty acids formed by a synthesis *de novo* at the site of the reaction (*i.e.* by an enzyme complex synthesizing both fatty acid and cerebroside). Thus, the administered [1-^{14}C]-lignoceric acid will be introduced mostly into the sphingomyelins in spite of a greater synthesis of the cerebrosides as evident when using tritiated water and acetate as precursors. The possibility should, however, not be overlooked that the data in Table III might be a consequence of the experimental procedure used. Following intracerebral injection, a large portion of the lignoceric acid administered remains in the brain as free fatty acid and is slowly utilized for sphingolipid synthesis. The different incorporation into the sphingomyelins and cerebrosides might be due to an easier access of the fatty acid to the sphingomyelin synthesizing enzymes which might be present in a different cell location than those synthesizing the cerebrosides.

The specific activities of the free ceramide fractions exceeded that of the sphingomyelins with most precursors used, and were lower than those of the cerebrosides with tritiated water and acetate. As has already been shown[17], the fatty acid composition of the brain ceramides is similar to that of the sphingomyelins but differs from that of the cerebrosides. This, together with the higher specific activities of the ceramides might indicate that these compounds are precursors for sphingomyelins but not for cerebrosides. Similar conclusions were also reached from experiments with intact human brain[17], and are also in agreement with the pathways proposed by KENNEDY, BRADY and co-workers[34-36].

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