Synthesis of C₁₈- and C₂₀-Dihydrosphingosines, Ketodihydrosphingosines, and Ceramides by Microsomal Preparations from Mouse Brain*

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SUMMARY
Microsomal preparations from mouse brain were found to synthesize 3-ketodihydrosphingosine (2-amino-1-hydroxyoctadecan-3-one) from L-serine and palmitoyl coenzyme A. The reaction appeared to require pyridoxal phosphate but no metal ion. In the presence of TPNH the major sphingolipid product was dihydrosphingosine; smaller amounts of N-acetyldihydrosphingosine and N-acylphosphatidylcholine were also found. The kinetics of utilization of [¹⁴C]palmitoyl-CoA indicated that accumulation of dihydrosphingosine was followed by acylation to form the ceramide. Sphingosine, which was not accumulated in detectable amounts, was apparently acylated much more rapidly. Stearoyl-CoA also reacted, producing the corresponding C₂₀-sphingolipids, but oleoyl-CoA and lignoceroyl-CoA were inactive. At the optimal acyl CoA concentration, stearoyl-CoA was less than half as effective as palmitoyl-CoA in the formation of ketodihydrosphingosine.

EXPERIMENTAL PROCEDURE
Materials
Nonradioactive palmitoyl CoA and stearoyl CoA were synthesized by the procedure of Seubert (9), with acyl chlorides from the Hormel Institute, Austin, Minnesota. A mixture of C₁₈ and C₂₀ bases was prepared by hydrolysis of a ganglioside preparation (Grade III, Sigma Chemical Company). Labeled and unlabeled 3-ketodihydrosphingosine were gifts from Drs. 

The biosynthesis of dihydrosphingosine in vitro by a particulate enzyme preparation from yeast was recently demonstrated by Braun and Snell (1, 2) and Brady, Di Mari and Snell (3) to proceed in two steps:

1. Serine + palmitoyl-CoA → 3-ketodihydrosphingosine + CO₂
2. 3-Ketodihydrosphingosine + TPNH → dihydrosphingosine + TPN⁺

The initial condensation, forming 2-amino-1-hydroxyoctadecan-3-one, was dependent on pyridoxal phosphate; the second reaction, requiring TPNH, yielded the erythro, or natural form of the long chain base. Stoffel, LeKim, and Sticht noted the conversion of labeled ketodihydrosphingosine by rats into dihydrosphingosine (4) and independently observed the above sequential reactions in yeast as well as in liver (5). The yeast system also formed sphingosine, the allylic base, and recent evidence implicates 3-ketosphingosine as an intermediate (3).

In view of the abundant quantities and importance of sphingolipids in the nervous system, and because earlier reports (6, 7) on the biosynthesis of long chain bases in brain are at variance with the above observations, we have examined the biosynthesis of these amines in microsomal preparations of mouse brain. Brain contains an appreciable amount of the C₁₈ base, particularly in the gangliosides, as well as the more usual C₁₈ base (8), and so it was of particular interest to see whether the homologous bases are made by similar reactions and to investigate the chain length specificity of the synthetic system.

We report here our findings that in brain microsomes (a) 3-ketodihydrosphingosine is an intermediate in the biosynthesis of dihydrosphingosine; (b) dihydrosphingosine is accumulated and in part acylated to form ceramide; (c) sphingosine is found in ceramide form but does not accumulate as free base; and (d) the C₂₀ homologues are readily formed.

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Robert Brady and Esmond Snell. The procedure of Gaver and Sweeley (10) was used to synthesize N-acetyl-3-ketosphingosine and \(\text{N-acetyl-3-ketohydrosphingosine}\). Synthetic \(\text{dL-threo-sphingosine}\) and \(\text{dL-threo-dihydrosphingosine}\) were gifts from Dr. E. F. Jenny, Ciba, Basel. L-Serine-\(^{14}\)C, uniformly labeled, was obtained from Schwarz BioResearch and diluted to a specific activity of 2500 cpm per nmole. Other materials were obtained as described in the accompanying paper (11). Radioactive acyl-CoAs were usually used at a specific activity of 2500 cpm per nmole. Microsomes were prepared from the brains of 16-day-old mice (11), suspended in 0.25 M sucrose (final volume 0.6 ml per g of brain tissue), and dialyzed overnight against the same solution.

**Isolation of Biosynthetic Products and Their Derivatives**

Enzyme activity was measured by determining the incorporation of \(^{14}\)C-labeled palmitoyl-CoA or stearoyl-CoA into sphingolipids. The composition of the reaction mixtures is given in the legends to tables and figures; unless otherwise stated, each tube contained 3.5 to 4 mg of microsomal protein. A TPNH-generating system employed in some experiments consisted of TPN (1 mM), glucose 6-phosphate (2 mM), glucose 6-phosphate dehydrogenase (2 units, from yeast), and MgCl\(_2\) (0.2 mM). Enzyme reactions (0.5-ml final volume) were conducted in capped tubes at 34\(^\circ\)C with vigorous shaking and were terminated by addition of chloroform-methanol. Suspended protein was removed by filtration and unreacted acyl-CoA was removed by partitioning against aqueous KC1 as described in the accompanying paper (11). Except when analysis for ketohydrosphingosine was to be performed, the lipid residues were subjected to alkaline methanalysis (12). The methanalysis degrades ester-type lipids, which interfere with the identification of the bases. Carrier sphingolipids were added, and product separation was effected by thin layer chromatography. Recovery of labeled ketohydrosphingosine and ceramide through the filtration and partition steps was quantitative.

**Analytical Methods**

Thin layer chromatography was carried out with 0.5-mm layers of Silica Gel G (Merck) which were activated for 90 min at 110\(^\circ\)C and washed with chloroform. Stored plates were reactivated for 15 min prior to use, and sample spots or bands were applied with a Hamilton syringe. Development with the following solvent systems was carried out in paper-lined glass tanks.

**System A**—Sphingosine, dihydrosphingosine, and ketohydrosphingosine were separated with chloroform-methanol-water-ammonia (280:70:6:1) (13).

**System B**—Separation of the bases could be effected, while moving fatty acids, esters, and ceramides to the top of the plate by first using System A, then chloroform-methanol-acetic acid (90:2:8) (14). The plate was dried for 10 min at 60\(^\circ\)C before being placed in the second system.

**System C**—The amine was separated from cerebrosides and other sphingolipids with chloroform-methanol-ammonia (135:45:8), but the individual bases were not separated as widely from one another as in System A.

**System D**—Ceramides were separated from other components of incubation mixtures with chloroform-acetic acid (90:10). Long chain bases and more complex sphingolipids remain at the origin.

**System E**—Ceramides isolated by System D were extracted from the powder and characterized further with chloroform-methanol (90:10) on borate plates used without reactivation. The adsorbent layer consists of Silica Gel G-Na\(_2\)B\(_4\)O\(_7\)-10H\(_2\)O (20:1). This system provides excellent separation of fatty acylhydrosphingosine from fatty acylphosphinosine, a property observed independently by Morrison (15). It also provides slight but distinct separation of N-acetyldihydrosphingosine from N acetylsphingosine, as well as of their 3 keto analogues. Dinitrophenylated bases were also separated by this system. Overactivation of borate plates was found to decrease resolution.

**System F**—Separation of the dinitrophenylated amines on the basis of chain length was accomplished by reversed phase development on a tetrail-coated borate plate in methanol-water (90:10) saturated with tetrail (16).

**Hydrolysis of Biosynthetic Ceramides**

\(^{14}\)C-Labeled compounds were detected by radioautography on Kodak No-Screen x-ray film. Lipid spots were visualized by exposure to iodine vapor or by spraying with alkaline bromthymol blue. Compounds for rechromatography or analysis were aspirated (17) and eluted from the gel with chloroform-methanol (2:1) containing 1% water or dilute ammonia. For quantitative analysis, radioactive zones were scraped from the plate and transferred directly to counting vials. Radioactivity was determined by adding 0.5 ml of water and 11 ml of a standard 2,5-diphenyloxazole-1,4-bis[4-(methyl-5-phenyloxazolyl)]-benzene-toluene solution containing 9% BioSolV BBS-3 (Beckman solubilizer) and counting in a scintillation counter. The adsorbent did not interfere with counting efficiency.

**Identification of 3-Ketohydrosphingosine as Reaction Product**—Fig. 1 illustrates the \(^{14}\)C-labeled lipids extracted from reaction mixtures in which the substrates were \(^{14}\)C-labeled palmitoyl- or stearoyl-CoA and unlabeled L-serine, or \(^{14}\)C-L-serine plus unlabeled acyl-CoAs. In each instance a radioactive zone corresponding to 3-ketohydrosphingosine was evident. (The heavily labeled zones in the sphingosine and dihydrosphingosine region derived from labeled fatty acids are due to free fatty acid and phospholipid.) The radioactive zone derived from stearoyl-CoA is slightly but distinctly faster moving than that of the C\(_{18}\) standard and palmitoyl-derived material, indicating that it is the expected C\(_{18}\) homologue. Faster migration on thin layer chromatography plates of lipids containing longer fatty acids has been observed on a number of occasions in this and other laboratories; to this list must now be added a similar effect of longer amines as well as their fatty acyl amides (see later).

Additional evidence for the identification of the ketohydrosphingosines comes from the following experiments. The radioactivity in the keto base zone was greatly reduced by including a TPNH-generating system in the incubation mixtures; this was accompanied by the appearance of \(^{14}\)C-dihydrosphingosine (cf. Fig. 6). Exposure of the enzymatic reaction products obtained...
FIG. 1 (left). Radioautogram of two biosynthetic \( ^{14}C \)-labeled 3-ketodihydrosphingosines. Reaction mixtures, incubated for 30 min, contained L-serine (6 mM), acyl-CoA (0.4 mM), phosphate buffer (0.05 M, pH 7.4), and 3.5 mg of microsomal protein. Following addition of carrier 3-ketodihydrosphingosine (20 \( \mu \)g per tube) the extracted lipids were chromatographed in System A. The following substrates were used: 1, \( ^{14}C \)-stearoyl-CoA; 2, \( ^{14}C \)-palmitoyl-CoA; 3, \( ^{14}C \)-L-serine and unlabeled stearoyl-CoA; 4, \( ^{14}C \)-L-serine and unlabeled palmitoyl-CoA. Chromatographic standards: 3, dihydrosphingosine (D) and sphingosine (S); 4, \( ^{14}C \)-ketodihydrosphingosine. Spots visualized by iodine are indicated by the stippled areas.

in the absence of TPN to 1 N KOH in methanol-water at 50° prior to chromatography resulted in nearly complete disappearance of the labeled ketodihydrosphingosine, a characteristic previously noted for this compound (2). The correspondence in shape of the radioactive and carrier bands is good evidence of identity.

Treatment of the biosynthetic \( ^{14}C \)- and \( ^{15}C \)-ketosphingolipids with NaBH\(_4\), as described previously (2), resulted in nearly quantitative conversion to the respective reduced bases (dihydrosphingosines) as determined by thin layer chromatography in System A. Dinitrophenylation (20) of the NaBH\(_4\)-reduced keto bases and chromatography of the yellow derivatives confirmed that dihydrosphingosine is the reduction product. The identification of the primary \( ^{14}C \)-labeled condensation product as 3-ketodihydrosphingosine was further verified by acetylation of the keto base (10) and chromatography of the product. Multiple development of the chromatogram (Fig. 2) by a procedure similar to that of Brater, Di Mari, and Shell (3) demonstrated the cochromatography of the acetylated keto base with N-acetyl-3-ketodihydrosphingosine; no labeled N-acetyl-3-ketosphingosine was detectable by radioautography or direct counting of the silica gel.

Subcellular Localization of 3-Ketodihydrosphingosine Synthetase—An homogenate of mouse brains was fractionated centrifugally by the procedure of De Robertis et al. (21) to yield the following nominal fractions: nuclei and cell membrane fragments, mitochondria, myelin, nerve endings, lysosomes, microsomes, and particle-free supernatant fluid. Analysis of each fraction for its ability to catalyze the enzymatic incorporation of labeled stearoyl-CoA or palmitoyl-CoA into 3-ketodihydrosphingosine or the \( C_{19} \)-homologue revealed that nearly all of the biosynthetic activity for both homologues resided in the microsomal fraction. Less than 10% of the total activity was found in the myelin fraction, presumably because of contamination by microsomes.

Properties of 3-Ketodihydrosphingosine Synthetase—Biosynthetic activity in a 10-min incubation was proportional to microsomal concentration up to about 4.5 mg of protein. The time course for ketodihydrosphingosine synthesis from labeled pal-
mitoyl-CoA (Fig. 3) shows that maximal synthesis is reached by 30 min. The palmitoyl-CoA supply did not appear to be the limiting factor, since further addition of 0.1 μmole after 30 min did not enhance enzymatic activity. The synthetase was found to be maximally active over a pH range of 6.6 to 7.5.

The plot of enzyme activity as a function of the palmitoyl-CoA and stearoyl-CoA concentration (Fig. 4) demonstrates saturation of the enzyme at a low concentration (0.1 mM) of both substrates, with inhibition occurring at acyl-CoA levels above 0.5 mM. In the region of optimal acyl-CoA concentrations palmitoyl-CoA incorporation into the keto base was more than twice that of stearoyl-CoA. In contrast to the low concentration of acyl-CoA which is required for maximal enzyme activity, Fig. 5 shows that a higher concentration (3 mM) of L-serine is required for maximal activity.

Various reagents were tested for ability to stimulate or inhibit ketodihydrosphingosine synthetase activity of the dialyzed microsomal preparation. Table I shows that none of the salts or cofactors tested had an appreciable stimulatory effect at the concentrations which were employed; only KCl and cysteine significantly depressed enzymatic activity. Cysteine has previously been observed to inhibit the acyl-CoA-serine condensation reaction by virtue of its ability to form a complex with the pyridoxal phosphate coenzyme (2, 6). Inhibition by KCl may be due to the high ionic strength which was employed.

TPNH-dependent Biosynthetic Products—The inclusion of a TPNH-generating system in the enzymatic incubation mixture resulted in the appearance of dihydrosphingosine and two ceramides, with a concomitant reduction in the amount of observable keto base. A dihydro base was the only free base formed when serine and palmitoyl- or stearoyl-CoA were incubated, regardless of whether the amino acid or the acyl-CoA was the

![Fig. 3. Time course for the incorporation of 14C-palmitoyl-CoA (specific activity 2500 cpm per nmole) into 3-ketodihydrosphingosine. Reaction conditions and chromatographic separations are described in legend to Fig. 1.](image)

![Fig. 4. Synthesis of C17- and C20-ketodihydrosphingosines as a function of the 14C-acyl-CoA concentration (2500 cpm per nmole). The reaction mixture and isolation procedure are described in Fig. 1. A 10-min incubation was used.](image)

![Fig. 5. Incorporation of 14C-palmitoyl-CoA into 3-ketodihydrosphingosine as a function of serine concentration. Conditions as in Fig. 4.](image)

### Table I

| Additions to reaction mixture | Ketodihydrosphingosine synthesized (μmole) |
|-----------------------------|------------------------------------------|
| None                        | 1000                                     |
| MnCl₂, 0.5 mM               | 1100                                     |
| MgCl₂, 0.5 mM               | 890                                      |
| CaCl₂, 0.5 mM               | 950                                      |
| EDTA, 1 mM                  | 820                                      |
| EDTA, 1 mM; MnCl₂, 5 mM     | 980                                      |
| Pyridoxal phosphate, 1 mM; MnCl₂, 0.5 mM | 1030   |
| Cysteine, 4 mM              | 200                                      |
| KCl, 400 mM                 | 470                                      |
| Dithiothreitol, 1 mM        | 990                                      |
| TPN⁺, 1 mM                  | 930                                      |
| DPN⁺, 1 mM                  | 970                                      |
| FAD, 0.4 mM                 | 800                                      |
| ATP, 1 mM                   | 970                                      |

* 0.5 ml of a mixture containing L-serine (6 mM), 14C-palmitoyl-CoA (0.4 μmole), phosphate buffer (0.05 M, pH 7.4), 4.5 mg of microsomal protein, and the added components, incubated for 15 min at 34°C.
FIG. 6 (upper). Radioautogram of $^3$H-labeled Cl*- and Go-dihydrosphingosine. In addition to the components listed in Fig. 1, the reaction mixture contained a TPNH-generating system. The incubation time was 45 min. Carrier dihydrosphingosine (20 µg) was added to each tube, and lipids were chromatographed in labeled form (Fig. 6). In the case of Lane 2, derived from stearoyl-CoA, the radioactive product migrated slightly faster than the carrier C18 base. This chain length effect is not seen with Lane 4, but it is usually quite distinct.

Dihydrosphingosine was further identified by recovery of the radioactive zone from a chromatogram similar to Fig. 6, treatment of the base with 1-fluoro-2,4-dinitrobenzene and chromatographic comparison of the labeled derivative with authentic standards (Fig. 7).

We usually observed small amounts of radioactivity in the sphingosine region of chromatograms developed with System A followed by System B, but rechromatography of the eluted material with System C showed that the radioactivity was in other sphingolipid regions.

Confirmation that the dihydro base formed from stearoyl-CoA was actually the C18 homologue was obtained by reversed phase thin layer chromatography, which is somewhat more sensitive to chain length than ordinary thin layer chromatography. Lane 5 of Fig. 8 shows that the nitrophenylated base formed from radioactive stearoyl-CoA incubation corresponds to the carrier made from naturally occurring C18-dihydrosphingosine. Lane 1 furnishes additional evidence for the enzymatic formation of the erythro form of dihydrosphingosine.

Since brain microsomes can form ceramides from dihydrosphingosine and fatty acyl-CoA (11), one should expect to find N-acyldihydrosphingosine in the incubation mixtures containing the TPNH-generating system. Examination of the lipids formed from labeled palmitoyl-CoA and stearoyl-CoA, with the use of Solvent System D, showed the presence of both n- and a-dihydrosphingosine. This solvent system moved the dihydro compound distinctly faster than the allylic compound. Moreover, the bands from stearoyl-CoA were slightly faster moving than the bands from palmitoyl-CoA, owing to a difference of 4 carbon atoms in the ceramides.

Recovery of the ceramide zone from this plate and rechromatography with System E resulted in improved separation of the two ceramide types (Fig. 9). Radioactivity was seen in both saturated and unsaturated ceramides when either palmitoyl- or stearoyl-CoA was the precursor.

Confirmation of these identifications was made by extracting each radioactive zone, cleaving the amide link with acid, and examining the resultant bases and fatty acid methyl esters. Both the ester and amine spots were radioactive, as expected, and the unsaturated ceramides yielded some $^3$H in the O-methyl-sphingosine and three-sphingosine spots that arise artifactually from acid treatment of sphingosine-containing lipids.

A time course for the incorporation of labeled palmitoyl-CoA into the three TPNH-dependent sphingolipids is shown in Fig. 10. Dihydrosphingosine accumulated almost linearly over the 60 min.
Fig. 8 (upper). Radioautogram of the dinitrophenyl derivatives of C18 and C20 dihydrosphingosine. Dinitrophenyl derivatives of dihydrosphingosine, biosynthesized from 14C-palmitoyl-CoA and 14C-stearoyl-CoA, were separated with System E and then with System F. 1, DNP-erythro-C18-dihydrosphingosine-4C and DNP-three-C18-dihydrosphingosine; 2, DNP-erythro-C18 and C16-dihydrosphingosine; 3, DNP-three-C18-dihydrosphingosine; 4, DNP-three-C16-dihydrosphingosine; 5, DNP-erythro-C18-dihydrosphingosine.

Fig. 9 (lower). Radioautogram of 14C-labeled N-acyl-dihydrosphingosine and N-acylsphingosine. Ceramides were isolated by chromatography with System D then rechromatographed with System E. One fraction was chromatographed as in Fig. 6 for the isolation of dihydrosphingosine. Ceramides were isolated by chromotography of the other fraction in Systems B and E. The maximal dihydrosphingosine activity (at 60 min) corresponds to the formation of 1.4 nmoles per incubation tube, or 0.7% of the added palmitoyl-CoA.

FIG. 10. Time course for the enzymatic incorporation of 14C-palmitoyl-CoA into dihydrosphingosine, N-acyldihydrosphingosine, and N-acylsphingosine. Reaction conditions are described in the legend to Fig. 6. Extracted lipids were treated with alkali (12) and divided into two equal fractions. One fraction was chromatographed as in Fig. 6 for the isolation of dihydrosphingosine. Ceramides were isolated by chromatography of the other fractions in Systems B and E. The maximal dihydrosphingosine activity (at 60 min) corresponds to the formation of 1.4 nmoles per incubation tube, or 0.7% of the added palmitoyl-CoA.

Our data demonstrate that brain microsomes synthesize dihydrosphingosine by a mechanism similar or identical to that found in liver (5) and in the yeast Hansenula ciferri (1, 2, 5). As in the yeast enzyme system (1) the condensation of palmitoyl-CoA with serine was inhibited by cysteine (Table I), suggesting its pyridoxal phosphate dependence. Unlike the rat brain microsomal sphingolipid-synthesizing system described by Brady and Koval (5), added Mn++ and pyridoxal phosphate did not stimulate the synthesis of 3-ketodihydrosphingosine by our dialyzed preparations of mouse brain microsomes. Since the addition of Mn++ increases the synthesis of 3-ketodihydrosphingosine by the hepatic enzyme system, it is possible that the brain enzyme system is specific for palmitoyl-CoA.

DISCUSSION

Our data demonstrate that brain microsomes synthesize dihydrosphingosine by a mechanism similar or identical to that found in liver (5) and in the yeast Hansenula ciferri (1, 2, 5). As in the yeast enzyme system (1) the condensation of palmitoyl-CoA with serine was inhibited by cysteine (Table I), suggesting its pyridoxal phosphate dependence. Unlike the rat brain microsomal sphingolipid-synthesizing system described by Brady and Koval (5), added Mn++ and pyridoxal phosphate did not stimulate the synthesis of 3-ketodihydrosphingosine by our dialyzed preparations of mouse brain microsomes. Since the addition of Mn++ increases the synthesis of 3-ketodihydrosphingosine by the hepatic enzyme system, it is possible that the brain enzyme system is specific for palmitoyl-CoA.
of EDTA to the reaction mixture did not affect a significant inhibitory response, we conclude that Mn²⁺ is probably not involved in this reaction. Failure of pyridoxal phosphate to stimulate enzymatic activity suggests that this coenzyme remains bound to the enzyme during ordinary conditions of storage and manipulation of the microsomal preparation. Further similarity between brain and yeast particulate enzyme systems (1, 2) in the synthesis of 3-ketodihydrosphingosine is seen in the inhibitory effect of high levels of acetyl-CoA (Fig. 4) and the need for a somewhat higher concentration of serine.

Because of the markedly greater abundance of C₂₀ base in gray matter of brain, as contrasted with white matter (22), the possibility arises that the site of synthesis of these homologues is different in the two areas. The highest concentration of ganglioside, which contains most of the C₁₈ base (8), is apparently the synaptic junction between axon and neuron (23), so it is possible that the longer base is formed in that region. However, our comparison of subcellular fractions shows that both the C₁₈ and C₂₀ bases are formed primarily in microsomes. This observation is consistent with the microsomal localization of the other biogenic enzymes in sphingolipid metabolism.

Although dihydrosphingosine was isolated from the reaction mixture as a free base, as well as a ceramide, no free sphingosine could be unequivocally demonstrated. These findings are at variance with earlier reports of serine-¹⁴C incorporation into dihydrosphingosine and sphingosine (6, 7, 24), and the interconversion of dihydrosphingosine and sphingosine (8, 25). However, as well as Fujino (25), found that the paper chromatographic procedure used by Brady and Koval (6) to demonstrate this interconversion of bases does not separate the two amines. Our reaction mixtures, with or without one or several detergents (Tween 20, taurodeoxycholate, Triton X-100), did not accumulate detectable amounts of labeled sphingosine when 1 pmole of unlabeled sphingosine was added prior to incubation. In earlier, analogous experiments with brain homogenates Fujino and Zabin (24) reported the formation of some sphingosine, as well as dihydrosphingosine. The labeled "sphingosine," however, was characterized only by two isolation steps, using thin layer chromatography with one solvent system, but not radioautography. Except for hydrogenation and benzoylation, which would not affect the synthesis of 3-ketodihydrosphingosine to sphingosine under a variety of experimental conditions. Attempts to demonstrate the synthesis of 3-ketosphingosine (Fig. 2) were equally unsuccessful.

All our attempts to confirm the presence of sphingosine by chromatography of acetyl or dinitrophenyl derivatives of biosynthetic products were unsuccessful. Nor were we able to manipulate the microsomal preparation. Further similarity with earlier reports of serine-¹⁴C incorporation into sphingosine (25) revealed a very rapid synthesis of N-acylsphingosine at early time points, whereas N-acyldihydrosphingosine is synthesized at a lower rate during the same period. We interpret these data to indicate that sphingosine is synthesized by the microsomal preparation but that it is very rapidly acylated. The transient dip in the N-acylsphingosine radioactivity suggests that this ceramide may be utilized for the synthesis of more complex sphingolipids; presumably its short duration reflects a limited supply of endogenous substrates.

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