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**Metabolism of Sphingosine Bases, XIX** [1]

# On the Origin of Phytosphingosine (4*D*-Hydroxysphinganine) in Mammalian Tissues

*Gerd Assmann and Wilhelm Stoffel**

(Received 21 April 1972)

**Summary:** 4*D*-Hydroxysphinganine (phytosphingosine) occurs as a component of sphingolipids of different mammalian organs particularly of intestine and kidneys. Evidence has been obtained from experiments in which radioactive 4*D*-hydroxysphinganine was administered orally that a considerable amount of the labelled base passes through the intestinal mucosa and is used in the biosynthesis of sphingolipids of parenchymatous organs. The base is also degraded to a large extent in the mucosa. 4*D*-Hydroxysphinganine was found to be incorporated more particularly into sphingomyelin and cerebrosides of the intestinal wall, liver and kidney. 4*D*-Hydroxysphinganine, present in the sphingolipids of the mammalian cell, originates from dietary sources. Neither 4*t*-sphingenine (sphingosine) nor sphinganine (dihydrosphingosine) are precursors of 4*D*-hydroxysphinganine in the mammalian cell.
The metabolism of 4*D*-hydroxysphinganine after both oral and intravenous administration has been studied.

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*Stoffwechsel von Sphingosinbasen, XIX: Über den Ursprung von Phytosphingosin (4D-Hydroxysphinganin) im Gewebe von Säugetieren*

**Zusammenfassung:** 4*D*-Hydroxysphinganin (Phytosphingosin) findet man als Baustein von Sphingolipiden verschiedener Säugetierorgane, vor allem der Darmschleimhaut und Nieren. Mit Hilfe oral applizierten, radioaktiv markierten 4*D*-Hydroxysphinganins konnte der Beweis erbracht werden, daß die Base zwar zu einem erheblichen Teil in der Darmschleimhaut abgebaut wird, jedoch ein beträchtlicher Anteil unverändert die Darmwand passiert und in die Sphingolipide der parenchymatösen Organe eingebaut wird. Die stärkste Inkorporation des markierten 4*D*-Hydroxysphinganins fand sich jeweils im Sphingomyelin und Cerebrosid des Darms, der Leber und der Niere. Das in der tierischen Zelle befindliche 4*D*-Hydroxysphinganin hat seinen Ursprung in der Nahrung. 4*D*-Hydroxysphinganin wird weder aus 4*t*-Sphingenin (Sphingosin) noch Sphinganin (Dihydrosphingosin) in der tierischen Zelle gebildet.
Der Stoffwechsel des 4*D*-Hydroxysphinganins nach oraler wird mit dem nach intravenöser Verabreichung verglichen.

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*Enzymes:*

Sphinganine kinase, ATP:sphinganine 1-phosphotransferase (EC 2.7.1.?; not yet listed)

Sphinganine-1-phosphate aldolase, sphinganine-1-phosphate alkanal-lyase (EC 4.1.2.?; not yet listed).

[1] XVIII. Commun.: Stoffel, W. & Assmann, G. (1972) *this J.* **353**, 965–970, preceding.

4*D*-Hydroxysphinganine (D-*ribo*-2-aminooctadecan-1,3,4-triol) represents the major long chain base component of glycosphingolipids of macroplants such as soybeans and corn[2]. It has also been identified in protozoa[3], in various fungi[4] and yeasts[5]. Among the latter the most remarkable is the yeast *Hansenula ciferrii*[6,7]. This yeast excretes large amounts of tetraacetyl-4*D*-hydroxysphinganine into the medium.

Substantial amounts of the $C_{18}$ and $C_{20}$ homologous bases have been reported recently as being present in the ceramide hexoside fraction of human kidney *in addition to 4t-sphingenine and sphinganine*[8–12]. These three long chain bases also occur together in cerebrosides of rat and bovine kidney[13]. Sphingomyelin and three main gangliosides of bovine kidney are characterized by their 4*D*-hydroxysphinganine content[14]. 4*D*-Hydroxy-$C_{18}$-sphinganine has also been isolated in variable amounts from the acid hydrolysate of the total sphingolipid fraction of rat intestines[15]. In a more thorough study the relative contribution and the distribution of the three long chain bases of mucosal sphingolipids have been determined[16]. 4*D*-Hydroxy-$C_{18}$- and -$C_{20}$-sphinganines were present in the sphingomyelin and cerebroside fractions.

The ceramide part of a fucose containing glycosphingolipid isolated from human adenocarcinoma consisted predominantly of acylated 4*D*-hydroxysphinganine with a high content of 2-hydroxyfatty acids[17].

Recently we described a 4*D*-hydroxysphinganine containing ceramide fraction isolated from the glycosphingolipids of pig platelets[18].

Regarding the results of this communication our present knowledge about the biosynthesis and degradation should be briefly outlined.

The biosynthesis of 4*D*-hydroxysphinganine has been studied in this and other laboratories[19–22]. Palmitate and serine, too, were recognized as the precursors of 4*D*-hydroxysphinganine, when the growth medium of *Hansenula ciferrii* was supplemented with radioactive sources of these compounds. No conclusion regarding the stage at which the 4*D*-hydroxy group is introduced was possible. Experiments in this laboratory proved that sphinganine is the immediate precursor of 4*D*-hydroxysphinganine in *Hansenula ciferrii*[23]. The mechanism of the hydroxylation on the other hand is not clear, whereas the stereochemistry of the hydrogen elimination from carbon atom 4 of sphinganine has been elucidated[23,24]. 4*t*-Sphingenine is not transformed to 4*D*-hydroxysphinganine, as one could visualize, *via* a hydration step. The degradation of 4*D*-hydroxysphinganine is well understood. The primary hydroxy group is first phosphorylated in a kinase reaction[25]. The 1-phosphoryl derivative is then cleaved in an aldolase-type reaction, similar to that demonstrated for 4*t*-sphingenine and sphinganine 1-phosphate, to

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phosphorylethanolamine and 2-hydroxypalmitaldehyde. The accompanying paper[1] summarizes these studies *in vitro*. Other fragments such as pentadecanoic acid[26] and α-hydroxypalmitate[27] have been described as degradation products of 4*D*-hydroxysphinganine. They have to be regarded as secondary products. We have clearly demonstrated that the pyridoxal phosphate dependent microsomal aldolase catalyzes the same reaction, regardless of the substitution at the C-4 position, yielding palmitaldehyde, 2*trans*-hexadecenal or 2-hydroxypalmitaldehyde when sphinganine, 4*t*-sphingenine and 4*D*-hydroxysphinganine are the substrates respectively[1].

Quite considerable amounts of long chain bases are resorbed under balanced nutritional conditions *via* sphingomyelins, glycosphingolipids and gangliosides from a wide variety of dietary sources. It has been demonstrated that 4*t*-sphingenine originating from sphingomyelin is readily degraded[28]. Orally administered 4*t*-sphingenine and sphinganine, in addition to being degraded, can be resorbed unchanged and incorporated into complex sphingolipids[29].

The appearance of 4*D*-hydroxysphinganine as a long chain base in a variety of complex structures of intestinal mucosa and kidney and other organs raised the question whether this 4*D*-hydroxysphinganine originates from dietary sources. We therefore traced the metabolic pathway of orally administered labelled 4*D*-hydroxysphinganine into the sphingolipids and glycosphingolipids of different organs and compared these results with the pathway of intravenously administered 4*D*-hydroxysphinganine. Since 4*D*-hydroxysphinganine has been found predominantly in sphingolipids of the intestinal wall and kidney, these organs and the liver of the rat were the „target organs" of our studies.

## Results

### *I. Preparation of substrates*

Uniformly labelled [$^{14}$C]4*D*-hydroxysphinganine was prepared biosynthetically by supplementing the medium of *Hansenula ciferrii* with sodium [1-$^{14}$C]acetate. The free base (specif. radioactiv. 0.11 μCi/μmol] was obtained from the tetraacetyl derivative as described under Experimental. [5,6-$^{3}H_4$]4*D*-Hydroxysphinganine was also biosynthesized by *Hansenula ciferrii* (specif. radioactiv. 3.3 μCi/μmol). In this case [3,4-$^{3}H_4$]palmitic acid specif. radioactiv. 40 μCi/μmol) was added to the growth medium. 17% of the initial radioactivity was recovered as 4*D*-hydroxysphinganine.

The long chain base was characterized by three features: The tris-*O*-(trimethylsilyl) derivative of *N*-acetyl-4*D*-hydroxysphinganine yielded one single band in gas liquid chromatography. Periodate oxidation with subsequent radio gas chromatography proved that only pentadecanal was radioactive. The mass spectrum of tetraacetyl-4*D*-hydroxysphinganine revealed the typical fragments.

### *II. Metabolism of orally administered [5,6-$^{3}H_4$]-4D-hydroxysphinganine and [U-$^{14}$C]4D-hydroxysphinganine in the rat*

1) 85 μmol of 4*D*-hydroxysphinganine was administered orally to an adult rat which had been starved for 24 h. After 24 h the organs listed in Table 1 were isolated, their total lipids extracted and their radioactivities determined. More than one quarter of the radioactivity administered was still present in the total organism; predominantly in the intestinal wall, in the intestinal content, in the liver and the carcass.

Table 1. Distribution of radioactivity in organs of the rat after oral administration of [5,6-$^{3}H_4$]4*D*-hydroxysphinganine (85 μmol; specif. radioactiv. 3.3 μCi/μmol).

| Organs | $10^{-6} \times {}^{3}H$ radioactiv. [dpm] | % of radioactiv. administered |
|---|---|---|
| Liver | 13.22 | 2.2 |
| Intestinal wall | 33.60 | 5.6 |
| Intestinal content | 32.35 | 5.4 |
| Lungs | 1.82 | 0.3 |
| Kidney | 1.46 | 0.25 |
| Brain | 0.23 | 0.04 |
| Heart | 0.70 | 0.10 |
| Pancreas | 1.12 | 0.20 |
| Spleen | 0.76 | 0.13 |
| Carcass | 72.8 | 12.1 |

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[29] Anderson, R. (1972) Doctoral thesis, Cologne, unpubl.

Table 2. Distribution of $^3H$ radioactivity in lipid fractions of liver and intestinal wall after oral administration of [5,6-$^3H_4$]4*D*-hydroxysphinganine to the rat.

| Lipid fractions | Liver $10^{-6} \times {}^3H$ activ. [dpm] | [%] | Intestinal wall $10^{-6} \times {}^3H$ activ [dpm] | [%] |
|---|---|---|---|---|
| Neutral lipids | 2.3 | 40 | 1.40 | 10.8 |
| Ceramides | 0.28 | 4.8 | 1.02 | 7.9 |
| Cerebrosides | 0.28 | 4.8 | 3.60 | 27.8 |
| Phosphatidylethanolamine<br>4*D*-Hydroxysphinganine | 0.07 | 1.2 | 2.20 | 17 |
| 4*D*-Hydroxysphinganine 1-phosphate | – | – | 0.92 | 7.1 |
| Phosphatidylcholine | 1.20 | 20.3 | 1.20 | 9.3 |
| Sphingomyelin | 0.60 | 10.2 | 0.80 | 6.2 |
| Intermediate fractions | 1.10 | 18.9 | 1.80 | 13.9 |

The lipid extracts of the liver and intestinal wall were separated on silicic acid columns in the fractions summarized in Table 2.

This table indicates that the sphingolipid fractions which are free of any alkali labile compounds contain appreciable amounts of radioactivity, particularly in ceramides, cerebrosides and sphingomyelins. The rather high radioactivity in cerebrosides and the presence of 4*D*-hydroxysphinganine 1-phosphate in the lipids of the intestinal wall should be mentioned. The elution patterns are given in Fig. 1a and b.

The purified cerebrosides and sphingomyelins of rat liver were then analyzed for their long chain base and fatty acid compositions and for their respective radioactivity distributions.

Sweeley[30] hydrolysis was applied to the two sphingolipid fractions. The fatty acids were released quantitatively from the ceramide part however the long chain bases resided to a large extent in psychosine and sphingosylphosphorylcholine and the rest were present as free bases, both hydrolysis products, however, were prone to periodate oxidation and allowed therefore the structural analysis of the long chain bases *via* their aldehydes, including the distribution of the radioactivity[31]. Fig. 2a and b represent the radio gas liquid chromatograms of the aldehydes released from the long chain bases of the cerebrosides and sphingomyelin of rat liver after periodate oxidation. The radioactivity was concentrated in both cases in pentadecanal. The fatty acids of cerebrosides were only very weakly labelled and therefore rather more than 90% of the radioactivity in cere-

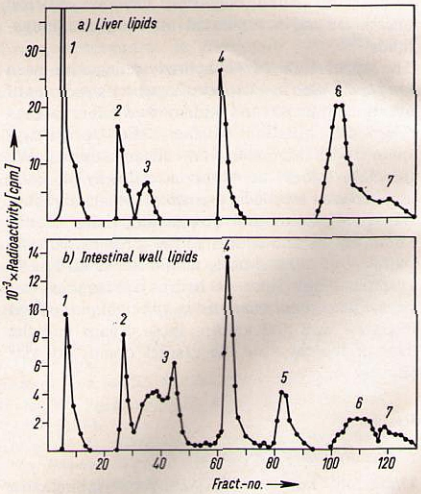


Fig. 1. Elution pattern of lipids in silicic acid chromatography after oral administration of [$^3H$]4*D*-hydroxysphinganine to a rat.

1: Neutral lipids, 2: ceramide, 3: cerebrosides, 4: phosphatidylethanolamine, phosphatidylserine, 4*D*-hydroxysphinganine, 5: 4*D*-hydroxysphinganine 1-phosphate, 6: phosphatidylcholine, 7: sphingomyelin. Volume of fractions: 10 m*l*.

[30] Gaver, R. C. & Sweeley, C. C. (1965) *J. Amer. Oil Chemists' Soc.* **42**, 294–298.

[31] Stoffel, W. & Assmann, G. (1972) *this J.* **353**, 65–74.

brosides of the rat liver corresponded to radioactiv 4*D*-hydroxysphinganine. About 75% of the radioactivity of sphingomyelin resided in hydroxysphinganine, the rest in the fatty acids. It should be mentioned that the mass peaks in radio gas liquid chromatography of pentadecanal reflect the actual amounts of 4*D*-hydroxysphinganine incorporated into cerebrosides and sphingomyelins.

Similarly the cerebroside and sphingomyelin fractions of the intestinal wall were hydrolyzed prior to base and fatty acid analyses. Again the cerebrosides were almost exclusively labelled in the long chain base 4*D*-hydroxysphinganine but not in the fatty acids. 96% of the radioactivity was recovered as pentadecanal. The mass peak of this aldehyde in radio gas liquid chromatography is also the most prominent one, Fig. 3. Sphingomyelin on the other hand contained about equal radioactivities in 4*D*-hydroxysphinganine and fatty acids. Radio gas liquid chromatographic analysis of their methyl esters localized 70% of the radioactivity in pentadecanoic acid, 27% in heptadecanoic acid and 3% in nonadecanoic acid. No 2-hydroxypalmitic acid could be found. The ceramide fraction of the intestinal wall also yielded exclusively labelled 4*D*-hydroxysphinganine on acid hydrolysis, the other bases being unlabelled.

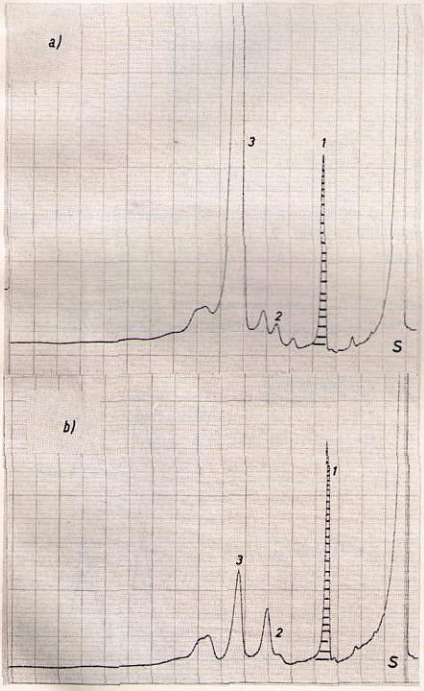


Fig. 2. Radio gas chromatogram of aldehydes released from the long chain bases of a) liver sphingomyelin and b) liver cerebrosides.

1: Pentadecanal, 2: hexadecanal, 3: 2*t*-hexadecenal, S: start. 15% EGS on kieselguhr, 130°C; 60 ml/ argon flow; radioactivity is located under shaded areas.

2) In another 24 h experiment [U-$^{14}$C]4*D*-hydroxysphinganine (specif. radioactiv. 1.5 μCi/μmol) had been administered twice, the second charge 15 h after the initiation of the experiment. A total of 15 μCi labelled base was used. 11% of the administered radioactivity was recovered in the following organs: liver 15%, intestinal wall 80%, lungs 1.8%, kidney 1.8% and brain 1.4%.

In this and another experiment with [4,5-$^{3}$H$_4$]4*D*-hydroxyphinganine only the lipids of liver and intestinal wall were further analyzed. After mild alkaline hydrolysis 50% of the radioactivity of the liver lipids and 90% of the intestinal wall lipids were found to be non-saponifiable. Cerebrosides and sphingomyelin were purified by preparative thin-layer chromatography or by silicic acid chromatography according to Sweeley[32], Fig. 4. The cerebroside fraction contained 67% and the sphingomyelin fraction 33% of the non-saponifiable radioactive sphingolipids of the intestinal wall. Acid hydrolysis of the cerebroside fraction released 25% of the radioactivity as fatty acid and 75% as free long chain bases or as psychosine, Fig. 5. The band containing the long chain base (2 in Fig. 5b) was chromatographed as its trimethylsilyl derivative and identified as the major base by its mass peak. It was partly degraded with periodate and the radioactivity determined in the aldehydes by discontinuous radio gas liquid chromatography. Here again the total radioactivity resided in pentadecanal. Pentadecanoic (65%) and heptadecanoicacid (35%) were the labelled fatty acids of cere-

[32] Vance, D. E. & Sweeley, C. C. (1967) *J. Lipid Res.* **8**, 621–630.

brosides. Sphingomyelin bases and fatty acids were analyzed using the same techniques. No 2-hydroxypalmitic acid was present. The total radioactivity resided in the pentadecanal band upon radio gas chromatography of the periodate oxidation product of the long chain bases of sphingomyelin.

The fatty acid methyl esters separated during thin-layer chromatography (solvent system: dichloromethane) into two radioactive bands, a major band with penta- and heptadecanoic acids as main radioactive components and a minor band which turned out to be 2-hydroxypalmitic acid.

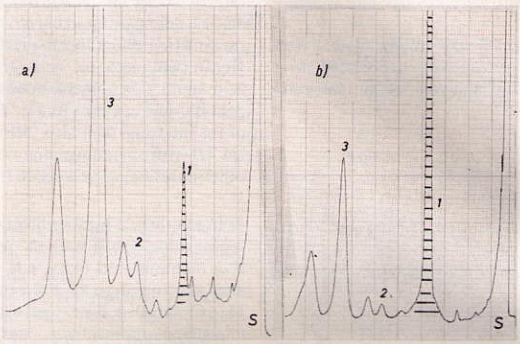


Fig. 3. Radio gas chromatogram of aldehydes released from a) sphingomyelin and b) cerebrosides of intestinal wall after oral administration of [4,5-$^3H_4$]4*D*-hydroxysphinganine to a rat.
1: Pentadecanal, 2: hexadecanal, 3: 2*t*-hexadecenal, S: start.

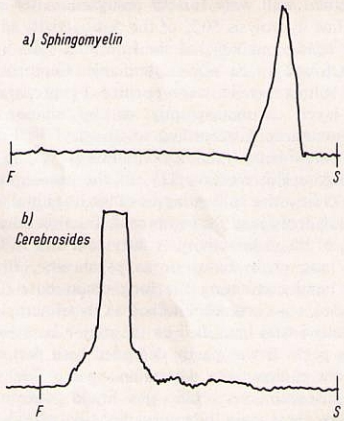


Fig. 4. Radio thin-layer chromatograms of purified a) sphingomyelin and b) cerebrosides of rat intestinal wall after oral administration of [4,5-$^3H_4$]4*D*-hydroxysphinganine to a rat.
Solvent system: chloroform/methanol/water 65:25:4.

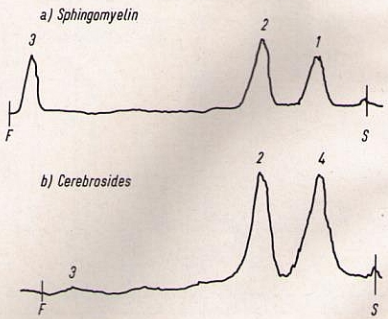


Fig. 5. Radio thin-layer chromatograms of products after acid hydrolysis of a) sphingomyelin and b) cerebrosides of intestinal wall.
1: sphingenyl phosphorylcholine, 2: long chain bases, 3: fatty acids, 4: psychosine. Solvent system: chloroform/methanol/2N $NH_4OH$ 40:10:1.

*III. Metabolism of intravenously administered [5,6-$^3H_4$]4D-hydroxysphinganine*

42.25 μmol [5,6-$^3H_4$]4*D*-hydroxysphinganine (specif. radioactiv. 3.2 μCi/μmol) was administered intravenously to two rats; the base was dissolved in 2 ml 17% Triton WR 1339. The total lipids of the liver, intestine, kidneys, lungs, spleen, heart and brain were isolated 24 h after the injection. About 12% of the injected radioactivity was recovered from these organs and the percentage distribution is given in the following Table 3.

Table 3. Distribution of radioactivity in organs after intravenous injection of 42.25 μmol [5,6-$^3H_4$]4*D*-hydroxysphinganine into two rats.

| Organ | $10^{-6} \times {}^3H$ activ. [dpm] | % of inj. radioactivity |
|---|---|---|
| Liver | 15 | 5.0 |
| Intestine | 10 | 3.0 |
| Kidney | 3 | 1.0 |
| Lung | 2.9 | 1.0 |
| Spleen | 2.3 | 0.75 |
| Heart | 1.8 | 0.60 |
| Brain | 0.5 | 0.16 |

The lipid classes, particularly the sphingolipids ceramides, cerebrosides and sphingomyelins, were further separated and analyzed for their long chain bases and fatty acids, Table 4.

It is remarkable that a rather high concentration of 4*D*-hydroxysphinganine 1-phosphate is present in kidneys. This phosphate ester has been characterized by its chromatographic behaviour in thin-layer chromatography, hydrolysis to 4*D*-hydroxysphinganine and phosphate by the microsomal phosphatase and periodate oxidation of the base with subsequent gas liquid chromatography of labelled pentadecanal. Cerebrosides and sphingomyelins were most strongly labelled and these fractions were submitted to further analyses of the long chain bases and fatty acids of the sphingolipids in the liver and kidneys. The techniques for analysis of the long chain bases and of the fatty acid composition were the same as described in the previous section. Cerebrosides and sphingomyelins of liver and kidneys were hydrolyzed and the long chain bases determined by radio gas chromatography of their aldehydes obtained after periodate oxidation. The total $^3H$-radioactivity resided exclusively in pentadecanal which is derived from 4*D*-hydroxysphinganine both of the cerebroside and sphingomyelin fractions. The fatty acids of kidney and liver cerebrosides and sphingomyelins contained only about 5–10% of the total radioactivity of the fractions and these were concentrated in penta- (80%) and heptadecanoic acids (20%) as revealed by radio gas chromatography.

Table 4. Distribution of $^3H$ radioactivity in lipid fractions of liver and kidney after intravenous injection of [4,5-$^3H_4$]4*D*-hydroxysphinganine into two rats.
Separation by silicic acid column chromatography.

| Lipid class | Liver $10^{-6} \times {}^3H$ activ. [dpm] | [%] | Kidney $10^{-6} \times {}^3H$ activ. [dpm] | [%] |
|---|---|---|---|---|
| Neutral lipids | 4.80 | 31.6 | 7.50 | 27.4 |
| Ceramides | 0.33 | 2.18 | 0.74 | 2.7 |
| Cerebrosides | 1.50 | 9.90 | 3.00 | 10.75 |
| Phosphatidylethanolamine<br>Lysophosphatidylethanolamine<br>4*D*-Hydroxysphinganine | 2.4 | 15.80 | 1.85 | 6.75 |
| 4*D*-Hydroxysphinganine 1-phosphate | – | – | 3.75 | 13.70 |
| Phosphatidylcholine | 2.8 | 18.4 | 3.20 | 11.70 |
| Sphingomyelin* | 1.8 | 11.9 | 6.40 | 23.40 |
| Intermediate fractions | 1.60 | 10.5 | 1.0 | 3.60 |

* Sphingomyelin was purified by mild alkaline hydrolysis and silicic acid chromatography.

## Discussion

The occurrence of 4*D*-hydroxysphinganine as a long chain base in a number of complex sphingolipids of different mammalian organs, but especially of intestine and kidneys, raised the question whether this long chain base originates from dietary sources. This appeared to be the most reasonable assumption since we were able to demonstrate its synthesis from sphinganine in the yeast *Hansenula ciferrii* but never found any transformation of labelled sphinganine or 4*t*-sphingenine into 4*D*-hydroxysphinganine in mammalian tissues. The results reported in this paper prove that orally administered labelled 4*D*-hydroxysphinganine passes the intestinal mucosa and is transported to different organs unchanged. The base is also degraded to a large extent. The base is mainly incorporated into cerebrosides and sphingomyelin of the intestinal wall and of the liver. Analysis of the long chain bases and fatty acids revealed that among the bases only 4*D*-hydroxysphinganine was radioactive. The radioactivity of the fatty acids was concentrated in pentadecanoic and its elongation product heptadecanoic acid. Pentadecanoic acid is formed from the primary degradation product 2-hydroxyhexadecanal[1] by oxidation and decarboxylation. These two odd-numbered fatty acids always occur as trace components in the total fatty acid mixture of the different ester lipids and dietary 4*D*-hydroxysphinganine might well contribute as a source of these and even longer odd-number chain acids.

The results of experiments in which 4*D*-hydroxysphinganine was administered orally and intravenously are very similar. They are in full agreement with other experiments in which the contribution of dietary sphinganine and 4*t*-sphingenine[29] in the synthesis of more complex sphingolipids in different organs in the rat has been studied. All three bases are utilized after passage of the intestinal mucosa. No comparative quantitative data about the contribution of the endogenous synthesis of the long chain bases and the uptake of these bases from the diet can be derived as yet.

We would like to acknowledge gratefully the support of these studies by the *Deutsche Forschungsgemeinschaft*, Bad Godesberg.

## Experimental

3-Hexadecynoic acid was synthesized in this laboratory[33]. Catalytic reduction in a $^3H_2$-atmosphere yielded [3,4-$^3H_4$]palmitic acid, specif. radioactiv. 130 μCi/μmol.

*Hansenula ciferrii* was grown in two 1 *l cultures*[6,7]. The medium was supplemented with 100 μmol ammonium [3,4-$^3H_4$] palmitate, specif. radioactiv. 40 μCi/μmol. The medium and cells were extracted as described before and the total lipid extract saponified with 0.5N KOH in methanol at room temperature overnight. *N*-Acetyl-4*D*-hydroxysphinganine was isolated by preparative thin-layer chromatography (solvent system: chloroform/methanol 8:1). The acetyl derivative was hydrolyzed according to Gaver and Sweeley[30], yield: 74 mg (211 μmol), specif. radioactiv. 3.4 μCi/μmol. Periodate oxidation yielded pentadecanal which was found to contain the total radioactivity on radio gas chromatographic analysis.

For oral administration 84.5 μmol (285 μCi) [5,6-$^3H_4$]-4*D*-hydroxysphinganine was dissolved in 0.5 ml of an ethanol/water (1:1) mixture and introduced into the stomach. 24 h after the administration the different organs were isolated, extracted with chloroform/methanol (2:1 and 1:2) and the total radioactivity counted.

[U-$^{14}$C]4*D*-Hydroxysphinganine was prepared by growing *Hansenula ciferrii* in a medium supplemented with sodium [1-$^{14}$C]acetate as described for [5,6-$^3H_4$]4*D*-hydroxysphinganine, specif. radioactiv. 0.15 μCi/μmol.

42.25 μmol (136 μCi) [5,6-$^3H_4$]4*D*-hydroxysphinganine was dissolved in 2 ml of 17% Triton WR 1339 and injected into the tail vein of the rat. The animals were killed after a 24 h period and the lipids of liver, intestine, kidneys, lungs, spleen, heart and brain extracted. The liver lipid extract of the [$^{14}$C]4*D*-hydroxysphinganine experiment and the liver and kidney lipids of the experiment with intravenously injected 4*D*-hydroxysphinganine were analyzed. In the former experiment the ester lipids were hydrolyzed according to Dawson[34] and the sphingolipids separated by silicic acid column chromatography. Sphingomyelin and cerebrosides were finally purified by preparative thin-layer chromatography. Intestinal wall and liver lipids were fractionated on a silicic acid column by increasing methanol concentrations in chloroform as described before[35] and the distribution of the radioactivity determined. The main sphingolipids, namely, ceramides, cerebroside, 4*D*-hydroxysphinganine 1-phosphate and sphingomyelin were characterized by their chromatographic properties and by degradation analysis[25]. The distribution in the different long chain bases was determined by radio gas liquid chromatography of the correspond-

[33] Ecker, W. (1967) Diplomarbeit, Cologne.

[34] Dawson, R. M. C. (1954) *Biochim. Biophys. Acta* **14**, 374–379.

[35] Stoffel, W. & Sticht, G. (1967) *this J.* **348**, 941–943.

ing aldehydes released by periodate oxidation[36] of the free bases. Psychosine and sphingosylphosphorylcholine were determined after treatment of the cerebroside and sphingomyelin. The fatty acids were extracted with petroleum ether/ether 1:1 and esterified with diazomethane. The esters were separated into normal unsubstituted and 2-hydroxyfatty acid esters by thin-layer chromatography using dichloromethane. Radioactive bands were localized by scanning the thin-layer plate in a Berthold scanner, model LB 2722 or a Packard scanner, model 7201. Radio gas liquid chromatographic analyses were carried out discontinously on a Packard gas chromatograph, model 7600, with an argon ionisation detector. Long chain bases were chromatographed as trimethylsilyl ethers on 1% SE 30, 2 m column, 60 ml argon flow at 225°C. The bases were reacted with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide[37]. Thin-layer chromatographic systems used for analytical and preparative separations were as follows:

complex lipids: chloroform/methanol/water 65:25:4;
ceramides: chloroform/methanol 8:1;
n- and hydroxyfatty acid methyl esters: dichloromethane;
long chain bases: chloroform/methanol/2N $NH_4OH$ 40:10:1.

[36] Sweeley, C. C. & Moscatelli, E. A. (1959) *J. Lipid Res.* **1**, 40–47.

[37] Donicke, M. (1969) *J. Chromatogr.* **42**, 103–104.