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Metabolism of Sphingosine Bases, XIV[1]

Sphinganine (Dihydrosphingosine), an Effective Donor of the Alk-1′-enyl Chain of Plasmalogens[2]

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Summary: Studies on the utilization of palmitaldehyde released in the sphinganine breakdown have been carried out in rats, during the myelination period, with specifically labelled precursors. An effective incorporation of [1-^{3}H]palmitaldehyde released from [3-^{3}H]sphinganine into plasmalogens (alk-1′-enylether of the phospholipids) takes place, when this long chain base is injected intraperitoneally or intracerebrally. [3-^{3}H;3-^{14}C]-sphinganine leads to the incorporation of doubly labelled palmitaldehyde, which has been recovered from the plasmalogens by acid hydrolysis and possesses a ^{3}H/^{14}C-ratio half that of the substrate sphinganine. The loss of the ^{3}H-activity can be best interpreted on the basis of the reduction of palmitaldehyde to hexadecanol (cetyl alcohol), subsequent formation of a hexadecyl glycerol ether derivative and dehydrogenation of the alkyl ether to the vinyl ether moiety. An alcohol dehydrogenase responsible for the reduction of palmitaldehyde to hexadecanol has been shown to be present in the 100000 × *g* supernatant of rat liver homogenate.

Palmitaldehyde released from sphinganine is a far better precursor than palmitic acid in these experiments *in vivo*. A significant contribution by reduction of one of the ester groups of diacylglycerolphospholipids to the corresponding plasmalogen can be excluded on the basis of our experiments with 2-[^{14}C]linoloyl-1-[^{3}H]stearoyl-*sn*-glycerol-3-phosphorylethanolamine. The experiments reported in this communication further extend our knowledge about the reutilization of the degradation products of the long chain bases for the biosynthesis of phospholipids and plasmalogens in particular.

Zusammenfassung: *Stoffwechsel von Sphingosinbasen, XIV: Sphinganin (Dihydrosphingosin), ein wirksamer Donator für die Alk-1′-enyl-Kette der Plasmalogene.* Die Verwertung des Palmitinaldehyds, der im Sphinganin-Abbau freigesetzt wird, wurde in Ratten während der Myelinisierungs-Periode (10—22 Tage) untersucht; hierzu wurden spezifisch markierte Vorstufen verwendet. Es findet ein erheblicher Einbau von [1-^{3}H]Palmitinaldehyd, der aus [3-^{3}H]Sphinganin entsteht, in die Plasmalogene (Alkenyläther der Phosphatide) nach intraperitonealer oder intracerebraler Injektion der langkettigen Basen statt.

[3-^{3}H;3-^{14}C]Sphinganin führt zum Einbau von doppelt markiertem Palmitinaldehyd, der durch saure Hydrolyse der Plasmalogene erhalten wird.

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Enzymes:

Alcohol dehydrogenase, alcohol:NAD oxidoreductase (EC 1.1.1.1)
Sphinganine-1-phosphate aldolase, sphinganine-1-phosphate alkanal-lyase (EC 4.1.2.?; not yet listed)
Sphinganine-1-phosphatase, sphinganine-1-phosphate phosphohydrolase (EC 3.1.3.?; not yet listed)

[1] XIII. Commun.: W. STOFFEL, G. ASSMANN and E. BINCZEK, this journal **351**, 635 [1970].

[2] A preliminary report has been given by W. STOFFEL at the Internat. Symposium on Sphingolipids, May 1969, Michigan State University, East Lansing, Michigan, USA.

Das $^{3}H/^{14}C$-Verhältnis des Palmitinaldehyds ist auf die Hälfte des Substrats Sphinganin abgefallen. Dieser Tritium-Verlust läßt sich am besten so erklären, daß der Palmitinaldehyd zu Hexadecanol (Cetylalkohol) reduziert wird, anschließend zur Synthese eines Hexadecyl-glycerinäther-Derivates verwendet wird und dann eine Dehydrogenierung der Alkyläther- zur Vinyläthergruppe mit Verlust der einen Hälfte des Tritiums erfolgt. Eine für die Reduktion des Palmitinaldehyds verantwortliche Alkohol-Dehydrogenase ist im $100000 \times g$-Überstand des *Rattenleber-Homogenats vorhanden.*

In diesen Experimenten erwies sich der aus dem Sphinganin freigesetzte Palmitinaldehyd als eine weitaus günstigere Vorstufe als die Palmitinsäure. Eine Plasmalogen-Biosynthese durch Reduktion der 1-ständigen Acylgruppe der Esterphospholipide zum entsprechenden Plasmalogen konnte durch Versuche mit 2-[^{14}C]Linoloyl-1-[^{3}H]stearoyl-*sn*-glycerin-3-phosphoryläthanolamin ausgeschlossen werden.

Die Ergebnisse der beschriebenen Experimente stellen einen weiteren Beitrag zu unserer Kenntnis über die Wiederverwertung der Abbauprodukte der langkettigen Basen für die Biosynthese von Phospholipiden und besonders der Plasmalogene dar.

Studies in this laboratory have demonstrated, that the degradation of the long chain bases sphinganine, 4*t*-sphingenine and 4-hydroxysphinganine is initiated by the enzymatic phosphorylation of the primary hydroxy group. The product is then cleaved in an aldolase type reaction[1,3–7]. The products are palmitaldehyde, hexadec-2-en-1-al and 2-hydroxypalmitaldehyde respectively, which correspond to carbon atoms 3 to 18, and phosphorylethanolamine representing carbon atoms 1 and 2. The enzyme sphinganine-1-phosphate alkanal-lyase is dependent on pyridoxal phosphate as coenzyme. These degradation products are largely reutilized for the biosynthesis of phospholipids and triglycerides. The palmitaldehyde is oxidized to palmitic acid, which is then incorporated as an acyl moiety of ester lipids. Phosphorylethanolamine is used as such or after methylation to phosphorylcholine to give 3-*sn*-phosphatidylethanolamine and 3-*sn*-phosphatidylcholine. The pathway of serine *via* the biosynthesis of the long chain bases and their degradation into the hydrophilic part of the phospholipids represents another route of serine utilization.

We now pursued the question, whether palmitaldehyde, released as the primary cleavage product from sphinganine 1-phosphate, might also be the donor of the alkenyl ether chain of plasmalogens. A suitable object for these studies is the brain of *young rats. During the 10th and 22nd* day of the postnatal period the weight of the rat brain increases most rapidly due to the deposition of myelin. Parallel to this process plasmalogen biosynthesis is very active[8–11]. The most abundant class of plasmalogens in brain are the 1-alk-1′-enyl-2-acyl-*sn*-glycerol-3-phosphorylethanolamines.

There are many but very contradictory reports and hypotheses on the origin of the vinyl ether group and the formation of this linkage in the 1-position of the glycerophosphate skeleton.

Burton[12] and Craig *et al.*[13] speculate, that palmitaldehyde might be utilized for the formation of the vinyl ether moiety of plasmalogens and Ellingboe and Karnovsky[14] and Friedberg and Greene[15] obtained experimental evidence for this pathway in the digestive gland of the starfish, Bell and White[16] in rat brain and Baumann *et al.*

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[17] in *Clostridium butyricum*. VIGNAIS and ZABIN[18] have described the enzymic reduction of palmitate to palmitaldehyde.

On the other hand CARR *et al.*[19] and BICKERSTAFFE and MEAD[20] derived from their experiments the conclusion that palmitaldehyde must be first oxidized to palmitate, which in turn is incorporated into the phospholipid molecule as an ester group in the 1-glycerol position. The reduction of the ester to the vinyl ether group was suggested as occurring on the completed phospholipid molecule. THOMPSON[21] deduced from the slow transfer of radioactivity, that the alkenyl ether of neutral and phospholipids is formed *via* the alkyl glyceryl ether, the pathway of their synthesis being unknown. Admittedly the incorporation of radioactivity in these and the other experiments was low. Recently SNYDER *et al.*[22,23] reported on the biosynthesis of the alkyl ether bond of glyceryl ether in a cellfree system of preputial tumors. They observed the transfer of the long chain alcohol in the presence of ATP and CoA to glyceraldehyde phosphate and dihydroxyacetone phosphate. The mechanism and the basis of the cofactor requirement is unknown. WOOD *et al.*[24] proposed, that alkyl ethers give rise to the corresponding alk-1′-enyl ethers. So far the following pathways in the biosynthesis of plasmalogens have been discussed:

1. reduction of palmitate to palmitaldehyde and its direct utilization for plasmalogen biosynthesis,

2. reduction of the ester group in position 1 of the complete phospholipids to the corresponding alk-1′-enyl ether,

3. initial formation of an alkyl glyceryl ether with subsequent dehydrogenation to the vinyl ether.

In this communication we present the results of experiments which demonstrate, that 1. carbon atoms 3 to 18 of sphinganine during the myelination period of young rats are effectively incorporated into the 1-position of phospholipids forming plasmalogens, 2. palmitaldehyde, which is liberated by the enzyme sphinganine-1-phosphate alkanal-lyase from sphinganine 1-phosphate, is neither oxidized to palmitate before the incorporation into plasmalogens nor utilized as such but must be first reduced to hexadecanol (cetyl alcohol) before the incorporation and desaturation to plasmalogens can occur. An alcohol dehydrogenase with high affinity for long chain aldehydes has been shown to be present in rat liver. 3. Evidence will be presented, that the simultaneous supply of palmitate and sphinganine leads mainly to the utilization of palmitaldehyde derived from sphinganine and not to the reduction of palmitate for the biosynthesis of plasmalogens. 4. The reduction and transformation of 3-*sn*-phosphatidylethanolamine to the corresponding plasmalogen could not be observed in our experiments, which were carried out with 2-[^{14}C]linoloyl-1-[^{3}H]stearoyl-*sn*-glycerol-3-phosphorylethanolamine in young rats during the myelination period.

Results

Experiments *in vivo* were carried out with [3-^{3}H]-sphinganine, [3-^{3}H;3-^{14}C]sphinganine, [3-^{3}H]-sphinganine simultaneous with [1-^{14}C]palmitic acid and 2-[^{14}C]linoloyl-1-[^{3}H]stearoyl-*sn*-glycerol-3-phosphorylethanolamine. These substrates were administered intraperitoneally or intracerebrally to young rats 10 to 22 days of age once or in several intervals as indicated in the experiments.

The phospholipids of the brains and livers of 5 to 10 animals were pooled and were separated from neutral and other dialysable lipids by rubber dialysis[25]. The vinyl ether linkage of the alkenylglycerolphospholipids of the phospholipid fraction was cleaved by acid methanolysis yielding the dimethyl acetals together with the fatty acid methyl esters. The two were either separated by thin-layer chromatography (solvent system: 1,2-dichloroethane) or the dimethyl acetals were purified by thin-layer chromatography after saponification of the methyl esters.

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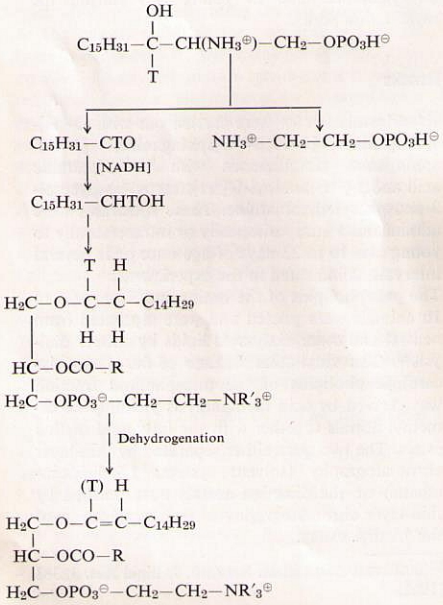
In previous experiments[3,4] we have shown that [3-^{3}H;3-^{14}C]sphinganine 1-phosphate yielded [1-^{3}H;1-^{14}C]palmitaldehyde and phosphoryl-ethanolamine in the aldolase reaction. The ^{3}H/^{14}C-ratio in the palmitaldehyde remained unchanged and identical to that of the substrate. The following arguments formed the basis of the experiments reported here: if this [^{3}H;^{14}C]palmitaldehyde is utilized for the biosynthesis of the alk-1′-enyl moiety of plasmalogens, the ^{3}H/^{14}C-ratio should give information whether the aldehyde is first oxidized to palmitic acid, which would lead to the loss of the ^{3}H-activity, or incorporated at the oxidation state of the aldehyde, in which case the ^{3}H/^{14}C-ratio remains unchanged or finally it is reduced to the alcohol incorporated to form the alkyl ether and subsequently dehydrogenated to the corresponding vinyl ether bond, which would reduce the ^{3}H/^{14}C-ratio to one half of its original value. The hydrogen atoms at the carbon atom 1 of the alkyl ether chain should be indistinguishable for the dehydrogenating enzyme, if a strong isotope effect does not interfere in this enzyme reaction. Fig. 1 visualizes these arguments.



1. Experiments with [3-^{3}H]DL-sphinganine

a) Intraperitoneal administration: Five injections of [3-^{3}H]sphinganine at intervals of 48 h were carried out between the 10th and 22nd postnatal days. Dimethyl acetals were isolated from the phospholipids after treatment with methanolic HCl and separation from fatty acid methyl esters by preparative thin-layer chromatography. The radioactivity of portions was determined. Analysis of the dimethyl acetal fraction by gas-liquid chromatography proved that the radioactivity was present only in the band eluting with palmitaldehyde dimethyl acetal.

The analytical data of one of the repeated experiments, which on the average yielded a recovery of 2% of the injected radioactivity, are summarized in Table 1.

The ^{3}H-label in position 3 of sphinganine led to clear cut results. Only two compounds were labelled, the substrate [3-^{3}H]sphinganine and the cleavage product [1-^{3}H]palmitaldehyde recovered from the plasmalogens. Any further oxidation to palmitic acid caused the loss of the ^{3}H-radioactivity. Whereas the liver had retained half of the recovered radioactivity as sphinganine and the other half was incorporated into plasmalogens, only 4% of the radioactive lipids of brain consisted of sphinganine while plasmalogens, which were recovered as palmitaldehyde dimethyl acetal, represented 96% of the lipid radioactivity.

b) Intracerebral administration: In order to bypass the blood liquor barrier [3-^{3}H]sphinganine was injected into the front brain of young rats of the same age and under the conditions described under „Experimental". After 2 h the lipids of the brains were extracted and separated by silicic-acid chromatography. 22% (1.08×10^6 dpm) of the injected radioactivity was recovered. Its distribution is indicated in Table 2.

Two observations should be mentioned: a) the phosphatidylcholine and phosphatidylethanolamine fractions were heavily labelled, b) the cerebrosides, which were obtained in pure form, were also labelled in the long chain bases.

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Fig. 1. Suggested pathway of [1-^{3}H;1-^{14}C]palmitaldehyde derived from [3-^{3}H;3-^{14}C]sphinganine into plasmalogens.

Table 1. Incorporation of [1-^{3}H]palmitaldehyde derived from [3-^{3}H]DL-sphinganine into plasmalogens of rat liver and brain.

	Liver Radioactivity		Brain Radioactivity	
		Rate of total lipid extract		Rate of total lipid extract
	[dpm]	[%]	[dpm]	[%]
Total lipid extract	620 000	—	220 000	—
Lipid extract after rubber-dialysis (phospho- and sphingolipids)	600 000	100	220 000	100
Palmitaldehyde dimethyl acetal	290 000	48.5	206 000	94
Sphinganine	310 000	51.5	13 500	4

Table 2. Distribution of radioactivity (in dpm and % of total radioactivity) in lipid fractions after intracerebral injections of [3-^{3}H]DL-sphinganine into young rats (10 to 22 days of age).

Lipid fraction	Radioactivity [dpm]	[%]
Neutral lipids, free fatty acids	31100	2.8
Ceramide	133000	11.7
Cerebrosides	39500	3.5
Phosphatidylethanolamine, sphinganine	151000	13.3
Phosphatidylcholine, lysophosphatidylethanolamine	480000	42.1
Sphingomyelin	115000	10.1
Sum of intermediate fractions	135000	12

The phospholipid fractions were further analyzed. The phosphatidylethanolamine fraction contained only 3.3% of [^{3}H]sphinganine which elutes together with phosphatidylethanolamine and represents non-metabolized substrate. The phosphatidylcholine fraction, on thin-layer chromatography, accompanied a strongly ninhydrin and plasmalogen positive substance which proved to be lysophosphatidylethanolamine. Most of the radioactivity (about 80%) of this fraction resided in this compound. The two phospholipid fractions were treated with HCl/methanol and the dimethyl acetals separated and purified by preparative thin-layer chromatography. Radio-gas chromatography identified the only radioactive compound as palmitaldehyde dimethyl acetal.

2. Experiments with [3-^{3}H; 3-^{14}C]DL-sphinganine

a) Incorporation of [1-^{3}H; 1-^{14}C]palmitaldehyde into plasmalogens: Similar experiments to those described before were carried out with [3-^{3}H; 3-^{14}C]sphinganine. This substrate should allow an extension of the observations of the previous experiment with regard to the extent to which plasmalogens are formed from the precursor palmitaldehyde derived from sphinganine. It should also give an insight into the fate of the hydrogen atom of the aldehyde group at carbon atom 1 and therefore into the origin of the alkenyl group in general.

When the plasmalogens were hydrolyzed, the dimethyl acetals accounted for 8% of the ^{14}C-activity recovered in the total lipid extract of brain. 80% of the total ^{14}C-activity was present in the fatty acid methyl ester fraction and 12% in sphinganine, Table 3.

Table 3. Incorporation of [1-^{3}H; 1-^{14}C]palmitaldehyde into plasmalogens after intracerebral injection of [3-^{3}H; 3-^{14}C]sphinganine. 1 μmol of [3-^{3}H; 3-^{14}C]DL-sphinganine had been injected intracerebrally per rat (5.75×10^5 dpm ^{14}C, 9.826×10^7 dpm ^{3}H).
^{3}H/^{14}C-ratio = 152.

Fraction	^{14}C-Radioactivity [%]	Ratio ^{3}H/^{14}C
Palmitaldehyde dimethyl acetal	7.7	70
Fatty acid methyl esters	80.0	4.5
Long chain bases	12.3	152

It is evident from the $^3H/^{14}C$-ratio in the palmitaldehyde dimethyl acetal, that the aldehyde has lost approximately half of the ^3H-activity during the reactions involved in the incorporation of palmitaldehyde into plasmalogens. The reduction of the isotope ratio by one half favours the mechanism discussed together with the basic arguments of these experiments. This would require an alcohol dehydrogenase for long chain aldehydes which would reduce palmitaldehyde to hexadecanol before a chimyl alcohol derivative is formed and the vinyl ether group established by dehydrogenation.

b) Reduction of palmitaldehyde to hexadecanol: The results of the previous experiment and its interpretation led us to demonstrate the enzymic reduction of palmitaldehyde to hexadecanol. We tested the different rat liver fractions for a long chain alcohol dehydrogenase and found that the 100000 $\times g$ supernatant fraction of rat liver contains a very active alcohol dehydrogenase, which could be concentrated by a heat step (2 min at 50°C) and ammonium sulfate precipitation between 55 and 85% saturation. It cannot be excluded that different alcohol dehydrogenases for groups of aldehydes with chain lengths C_1 and C_2, C_3 to C_6 and C_8 to C_{16} are present in this enzyme fraction, a question, which we did not further pursue. The enzyme(s) require NADH or NADPH as coenzyme. Commercially available alcohol dehydrogenase from horse liver exhibits a different chain length specificity toward the groups of aldehydes with 2 to 6 carbon atoms.

The kinetics of both enzyme preparations are given in Fig. 2.

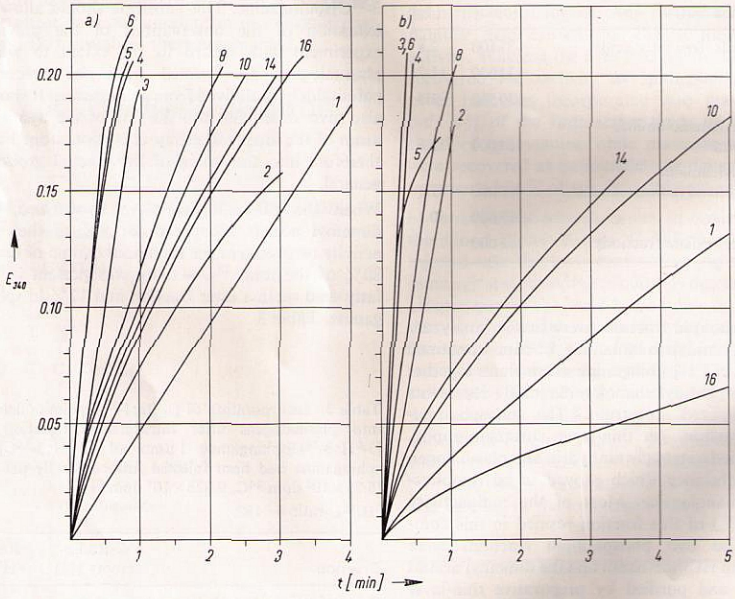

Fig. 2. Kinetics of the reduction of aldehydes with different chain lengths by alcohol dehydrogenases a) from rat liver (preparation from 100000 $\times g$ supernatant of rat liver homogenate) and b) from horse liver. The figures of the curves give the chain lengths of the aldehydes.

3. Comparative study regarding the donor function of [3-^{3}H]sphinganine and [1-^{14}C]palmitic acid for the alk-1'-enyl ether group

[3-^{3}H]Sphinganine (1 μmol, 2.6×10^6 dpm) and [1-^{14}C]palmitic acid (1 μmol, 1.6×10^6 dpm) dissolved in 20 μl of a 17% Triton WR 1339 were injected intracerebrally. The ^{3}H/^{14}C-ratio was 1.37. 32% of the ^{14}C-radioactivity of palmitic acid (1.217×10^6 dpm) was trapped as $^{14}CO_2$ in the respiratory air within the first 6 h of the experiment. The rats were killed after 70 h, the brain lipids extracted and separated by silicic acid chromatography. 4.41% of the ^{14}C- and 21.5% of the ^{3}H-activity were recovered in the lipid extract. The distribution of the radioactivity in the lipid fractions is given in Table 4.

The phosphatidylethanolamine and phosphatidylcholine fractions were again analyzed for their plasmalogen content by acid hydrolysis and the dimethyl acetal and fatty acid methyl ester fractions separated by preparative thin-layer chromatography and the ^{3}H- and ^{14}C-distribution determined in these fractions. The results are summarized in Table 5.

The analytical data obtained from this comparative study indicate that the degradation product palmitaldehyde derived from sphinganine is a far better precursor than palmitic acid when administered in equimolar concentration. These data correspond well with those of the experiment discussed under part 2 and point out that the incorporation of [1-^{14}C]palmitic acid into plasmalogens is surprisingly low.

Table 4. Distribution of ^{3}H- and ^{14}C-radioactivity in brain lipids after intracerebral injection of equimolar amounts of [3-^{3}H]DL-sphinganine and [1-^{14}C]palmitic acid.

Fraction	Radioactivity [dpm]		Rate of total radioactivity [%]		Ratio
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H/^{14}C
Neutral lipids (fatty acids, ceramides)	31 000	14 850	2.7	8.8	2.1
Ceramides, cerebrosides	133 000	7 600	11.7	4.5	17.5
Cerebrosides	39 500	2 450	3.5	1.5	6.1
Phosphatidylethanolamine, sphinganine	151 000	23 900	13.3	14.3	6.3
Phosphatidylcholine, lysophosphatidylethanolamine	480 000	63 000	42.1	37.5	7.6
Sphingomyelin	94 000	4 400	8.3	2.6	21.4
Intermediate fractions	135 000	29 500	11.9	17.6	—

Table 5. Comparative donor function of [3-^{3}H]DL-sphinganine and [1-^{14}C]palmitic acid for the synthesis of plasmalogens.

Fraction	^{3}H [dpm]	^{14}C [dpm]	^{3}H-Activity [%]	^{14}C-Activity [%]
Phosphatidylcholine	455 000	66 000	100	100
Dimethyl acetals	306 000	3 350	67.5	5.1
Fatty acid methyl esters	34 800	60 500	7.7	92
Phosphatidylethanolamine	21 000	3 350	100	100
Dimethyl acetals	8 340	113	39.8	3.4
Fatty acid methyl esters	2 385	2 513	11.4	75.0

4. Experiments with 2-[^{14}C]linoloyl-1-[^{3}H]stearoyl-sn-glycerol-3-phosphorylethanolamine

Using the conditions of the previous experiment *1.5 μmol of 2-[^{14}C]linoloyl-1-[^{3}H]stearoyl-sn-glycerol-3-phosphorylethanolamine*, specif. radioactiv. 1.875×10^6 dpm ^{3}H/μmol and 1.25×10^5 dpm ^{14}C/μmol (^{3}H/^{14}C = 15) per rat were injected intracerebrally. After 24 h the brains of 6 animals were pooled and their lipids extracted. 37% (4.9×10^6 dpm) of the ^{3}H- and 22.8% (2.68×10^5 dpm) of the ^{14}C-activity were recovered. The neutral lipids (6.35×10^5 dpm) ^{3}H and 3.3×10^4 dpm ^{14}C) were separated from the phospho- and sphingolipid fraction (4.40×10^6 dpm ^{3}H and 2.30×10^5 dpm ^{14}C) by rubber dialysis. The latter, nondialysable lipid was hydrolyzed by acid methanolysis and the dimethyl acetal and methyl ester fractions separated by preparative thin-layer chromatography. 0.71% (3.14×10^4 dpm) of the ^{3}H-activity and 0.47% (1.075×10^3 dpm) of the *^{14}C-activity were associated with the dimethyl acetal* fraction. In another experiment this proportion even dropped below 0.20% of the ^{3}H- and ^{14}C-activity.

Discussion

The elucidation of the mechanism of the breakdown of the long chain bases and of the properties and distribution of the enzyme sphinganine-1-phosphate alkanal-lyase (aldolase) responsible for this pyridoxal phosphate dependent reaction raised questions with regard to the pathways of the breakdown products palmitaldehyde and phosphorylethanolamine. In a previous paper[3,7] we have already reported, that the palmitaldehyde is rapidly oxidized to palmitic acid, which is further degraded by β-oxidation or incorporated as an acyl group in ester- or sphingolipids. Phosphorylethanolamine is efficiently reutilized as a building block of phosphatidylethanolamine and, fully methylated, of phosphatidylcholine. The high specific activity of the lyase in brain tissue and the predominant occurrence of plasmalogens in the phosphatidylethanolamine fraction of brain lipids prompted these studies, which should either prove or disprove the utilization of palmitaldehyde released from sphinganine phosphate ester for the synthesis of the vinyl ether moiety of plasmalogens. Suitable studies should be possible with rat brain during the myelination period with its concomitant active plasmalogen synthesis. In addition a specific labelling pattern of the palmitaldehyde released from sphinganine should give insight into the still largely obscure plasmalogen biosynthesis and allow a decision on whether the alkenyl ether originates from palmitic acid, palmitaldehyde, hexadecanol or the preformed corresponding diacyl-*sn*-glycerol-3-phosphorylethanolamine, each of which has been proposed during past years as precursor of plasmalogens.

Our experiments described in this paper demonstrate that [3-^{3}H]sphinganine injected either intraperitoneally or intracerebrally has a rapid turnover and effectively donates [1-^{3}H]palmitaldehyde for the biosynthesis of the alkenyl ether group. When doubly labelled [3-^{3}H;3-^{14}C]sphinganine was administered, the ^{3}H/^{14}C-ratio of [1-^{3}H;1-^{14}C]-palmitaldehyde released from the plasmalogens by acid hydrolysis dropped to approximately one half of the ^{3}H/^{14}C-ratio of the substrate sphinganine. This loss of ^{3}H can best be interpreted by *the following sequence: [^{3}H;^{14}C]palmitaldehyde* is first reduced to cetyl alcohol (hexadecanol), the ^{3}H/^{14}C-ratio being unchanged. The [^{3}H;^{14}C]-cetyl alcohol is incorporated into an alkyl ether derivative of glycerol 3-phosphate, which is subsequently dehydrogenated to the corresponding alk-1′-enyl ether derivative. Both reaction mechanisms are so far unknown. Studies regarding the stereospecificity of the reduction of palmitaldehyde to cetyl alcohol, its incorporation into plasmalogens and the introduction of the *cis*-olefinic bond of the alkenyl ether will be reported in a subsequent paper. The alkyl ether formation of glycerol phosphate has been demonstrated in preputial tumor cells and the cell free system by SNYDER *et al.*[22]. The subsequent dehydrogenation between C-1 and C-2 of the alkyl ether chain would yield the corresponding alk-1′-enylether. Here is no discrimination between tritium and hydrogen atoms at carbon atom 1 in the hydrogen elimination, which explains the decrease of the ^{3}H/^{14}C-ratio to one half. These results are at variance with those of BELL and WHITE[16] but agree with those of WOOD *et al.*[24].

Our experiments also exclude a direct incorporation of palmitaldehyde. Furthermore we could demonstrate the reduction of palmitaldehyde to hexadecanol with an alcohol dehydrogenase present in the $100000 \times g$ supernatant fraction of rat liver. Taking all these facts into consideration the result obtained in the experiments with doubly labelled phosphatidylethanolamine, which excludes a trans-

formation of the diacylphospholipid into the corresponding plasmalogen, is no longer surprising. It is also worthwhile to mention the comparative study with palmitic acid and sphinganine injected in equimolar quantities into the brains of young rats. Surprisingly little of the palmitic acid, compared to the palmitaldehyde released from sphinganine, was incorporated into the plasmalogens.

The studies reported in this paper extend the understanding of the reutilization of the degradation products of sphinganine. Carbon atoms 3 to 18 are incorporated as palmitic acid in ester linkages or as hexadecanol in the vinyl ether linkage of plasmalogens *via* the common intermediate palmitaldehyde. Carbon atoms 1 and 2, released as phosphorylethanolamine, are used for the formation of the hydrophilic group of the phospholipids.

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Experimental

[3-^{3}H]DL-Sphinganine, specif. radioactiv. 5.1×10^6 dpm/μmol, [3-^{3}H;3-^{14}C]DL-sphinganine, specif. radioactivity 7.3×10^7 dpm ^{3}H/μmol; 4.8×10^5 dpm ^{14}C/μmol; ^{3}H/^{14}C = 152, [1-^{14}C]palmitic acid, specif. radioactiv. 1.1×10^6 dpm/μmol, and 2-[^{14}C]linoloyl-1-[^{3}H]stearoyl-*sn*-glycerol-3-phosphorylethanolamine, specif. radioactiv. 1.875×10^6 dpm ^{3}H/μmol, 1.25×10^5 dpm ^{14}C/μmol; ^{3}H/^{14}C = 15, were synthesized in this laboratory[26,27]. The long chain bases were dissolved in 17 % Triton WR 1339. For intracerebral injections 10 μl portions containing 1 μmol and for intraperitoneal injection 25 μl portions containing 2.5 μmol of sphinganine were administered. For the intracerebral injection the needle was pushed through the sagittal suture approximately 3—5 mm deep. Most of the experimental animals survived without marked symptoms.

The animals were killed in an atmosphere of CO_2 by suffocation and their brains and livers carefully removed. Brain and liver lipids were extracted with chloroform/methanol according to FOLCH *et al.*[28]. Neutral lipids were removed by rubber dialysis according to EBERHAGEN and BETZING[25] and their radioactivity determined. Silicic acid chromatography of the residue was carried out as described before[29]. Phosphatidylethanolamine and phosphatidylcholine fractions were refluxed for 2 h with 5 % HCl/methanol, the dimethylacetals and methylesters extracted with petroleum ether, the extracts washed with 5 % $NaHCO_3$ and dried over Na_2SO_4. Occasionally the esters were saponified with 1.5 % methanolic KOH overnight at room temperature and the dimethyl acetals recovered from the unsaponifiable fraction by extraction with petroleum ether.

Dimethyl acetals were separated and purified by thin-layer chromatography in 1,2-dichloroethane. The radioactive bands were eluted with ether and the radioactivity of portions determined in a Tricarb liquid scintillation spectrometer, Packard, Model 3380. Thin-layer chromatograms were scanned with the Berthold radio-chromatogram scanner. Fatty acid methyl esters and dimethyl acetals were further analyzed by gas-liquid chromatography, stationary phase: 15 % ethylenglycol succinate polyester on Kieselguhr, column temperature 160°C, column length 200 cm. The radioactivity was monitored discontinuously.

Horse liver alcohol dehydrogenase was purchased from Boehringer Mannheim GmbH. The kinetics of the alcohol dehydrogenase reaction were measured spectrophotometrically at 340 nm and recorded automatically. The incubation mixtures contained in a volume of 2.0 ml: 0.1 μmol of substrate (aldehyde), 1 μmol of NADH or NADPH, 200 μmol of potassium/sodium-phosphate buffer, pH 6.8, containing 2 μmol of thioglycol and 2 μmol of $MgCl_2$ and protein as indicated. Aldehydes with more than 6 carbon atoms were dissolved with Triton X-100 (1 μmol of aldehyde and 1 mg of Triton X-100 in 20 μl of phosphate buffer).

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