Chem. Phys. Lipids 5 (1970) 139–158 © North-Holland Publ. Co., Amsterdam.

STUDIES ON THE BIOSYNTHESIS AND DEGRADATION OF SPHINGOSINE BASES

WILHELM STOFFEL

Institut für Physiologische Chemie der Universität zu Köln, Köln, West Germany

Summary

Our studies in vivo and in vitro on the biosynthesis and the degradation of long chain sphingosine bases allow now to formulate the following reaction sequences. In the *biosynthesis* palmitoyl-CoA and L-serine are condensed with the pyridoxal phosphate dependent 3-dehydrosphinganine-L-serine synthetase with decarboxylation to yield 2S-3-dehydrosphinganine. The 3-keto-group is reduced by a NADPH dependent D-3-dehydrosphinganine reductase to D-erythro or 2S,3R-sphinganine. The B-side hydride ion is transferred to the 3-keto-group. Both enzymes are microsomal lipoprotein enzymes, of which so far the reductase has been purified 84 fold.

The *degradation* of sphinganine is initiated with a kinase reaction yielding the sphinganine-1-phosphate ester. This is cleaved by a pyridoxal phosphate dependent sphinganine-1-phosphate:phosphoryl ethanolamine lyase to palmitaldehyde and phosphoryl ethanolamine. These cleavage products are preferably reutilized for the synthesis of phosphatidyl ethanolamine and alkenyl phospholipids.

Finally the biosynthetic and degradation pathway taken together represent an additional route of serine transformation for the synthesis of the hydrophilic part of phospholipids.

The most abundant long chain bases, present as a basic component in all sphingolipid classes of the animal and plant kingdom are 4*t*-sphingenine, sphinganine and 4-hydroxy-sphinganine. Their chemical structures and particularly their stereochemistry have been conclusively established by Carter and his collaborators[1–4]), although a number of valuable contributions from other laboratories[5–11]) had been made over the past 100 years. The chemical structures of these three long chain bases are given in fig. 1. Another reason for presenting these structures is to indicate the specific positions of the radioactive labels ^{3}H or ^{14}C in these molecules, which we obtained by chemical synthesis or biosynthesis[12, 13]).

The trifunctional structure of sphinganine and the tetrafunctional one of 4*t*-sphingenine and 4-hydroxy-sphinganine are related to several unresolved problems with regard to the biosynthesis, degradation, mutual transformation and incorporation into the more complex structures named after the common basic component sphingosine, sphingolipids.

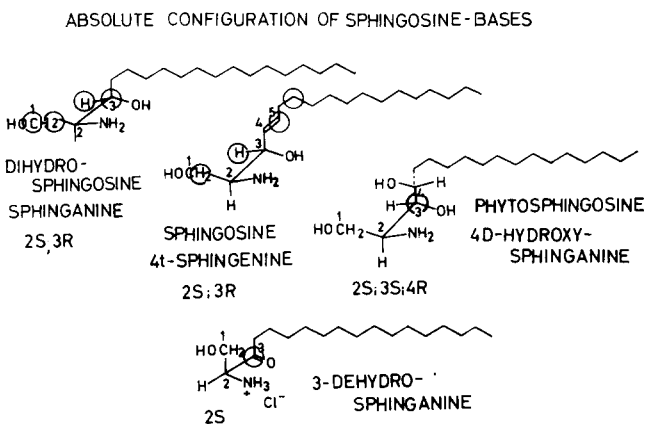


Fig. 1. Chemical structures of long chain bases. ^{3}H and ^{14}C labeled positions of synthetic compounds are encircled.

Aided by the ^{3}H and ^{14}C labeled compounds indicated in fig. 1 and the experiments described below, we undertook investigations *in vivo*[12–20]) and *in vitro*[21–24]) in order to get insight into the metabolism of the long chain bases, into their *biosynthesis and degradation.*

The origin of carbon atoms 1 and 2 from serine and that of the alkane chain of the long chain bases from acetate via palmitate is well established for sphinganine, 4*t*-sphingenine[25–27]) and 4-hydroxy-sphinganine[28]). However the mechanism of the synthesis suggested as a condensation of palmitaldehyde and serine by Brady *et al.*[29,30]) was incompatible with the mechanism derived from experiments, carried out independently in Snell's[31–33]) and the author's laboratory[21,22]). These studies elucidated the biosynthesis of sphinganine which is outlined in the second part of this paper.

In the first part the results of our studies on the catabolism of the long chain bases are described, a field, which was fully neglected until we began our studies.

Degradation of long chain bases[13–20])

Studies in vivo

Specifically labeled sphinganine, 4*t*-sphingenine and 4-hydroxy-sphinga-

nine were administered into the portal vein of rats. The surprising result of these experiments revealed, that [3-^{14}C] sphinganine in its free form is rapidly degraded as measured by the respiratory $^{14}CO_2$ collected in time intervals. More than 25% of the injected radioactivity appeared as $^{14}CO_2$ within the first 4 h.

The subsequent analysis of the complex liver lipids by silicic acid chromatography, fig. 2, indicated that the ester lipids, triglycerides (TG), phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) were heavily labeled, however among the sphingolipids only the ceramide and sphingomyelin fractions were radioactive.

After hydrolysis the total radioactivity of the different lipid classes resided in palmitic acid and 5–10% in stearic acid except the ceramides and sphingomyelins, which were also labeled in the base moiety. Dauben degradation proved that carbon atom 1 of palmitic acid, representing C-3 of [3-^{14}C] sphinganine was labeled and that stearic acid carries the label at C-3 indicating, that an elongation of palmitic acid had occurred to some extent. Like the *erythro* form, the *threo* isomer of L-sphinganine and also [7-$^{3}H_2$] sphingenine yielded labeled palmitate. With these experiments we had obtained convincing evidence, that carbon atoms 3 to 18 are liberated as 16 carbon atom fragment. The results of an experiment with [3-^{14}C] sphinganine and [7-$^{3}H_2$] 4*t*-sphingenine are summarized in table 1.

TABLE 1

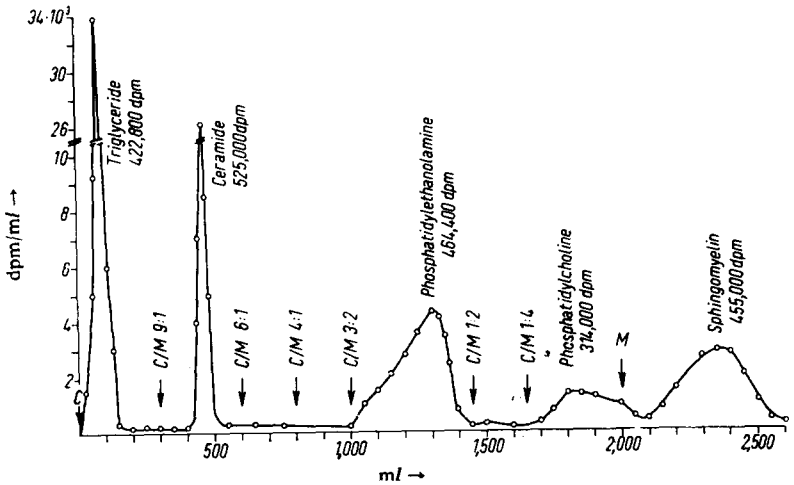
Distribution of radioactivity in lipid classes and their fatty acids after administration of [3-^{14}C] *erythro*-D,L-sphinganine and [7-$^{3}H_2$] *erythro*-D,L-4t-sphingenine.

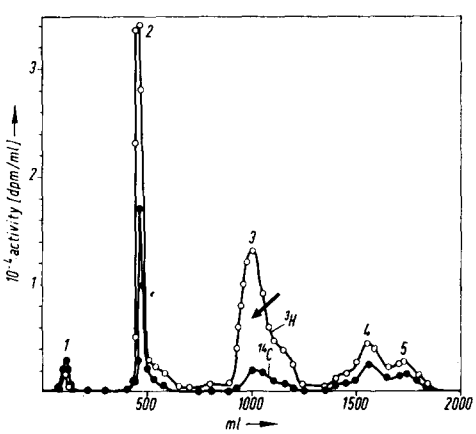
Radioactive lipids	[3-^{14}C] sphinganine			[7-$^{3}H_2$] sphingenine		
	Total radioactivity	% of total radioactivity in 16:0	18:0	Total radioactivity	% of total radioactivity in 16:0	18:0
Triglycerides	422800	80	20	1075000	92	8
Ceramides	525000	90	10	624000	73	27
Phosphatidyl ethanolamines	464000	62	38	340000	75	25
Phosphatidyl cholines	314000	70	30	670000	72	28
Sphingomyelins	450000	90	10	650000	85	15

In studies *in vivo* with [3-^{14}C] *erythro* eicosasphinganine we observed, that C-3 to C-20 appeared as [1-^{14}C] stearic acid and [3-^{14}C] 4-hydroxy-sphinganine yielded a C_{16}-fragment, which was identified as [1-^{14}C] α-hydroxy-palmitate.

On the other hand [1-$^{3}H_2$]- or [1-^{14}C] sphinganine and [1-$^{3}H_2$] 4*t*-sphin-

genine used in experiments *in vivo*, released C-1 and C-2 as an unit namely as labeled *ethanolamine*, which was isolated from the aqueous extract of liver as phosphoryl ethanolamine or liberated from phosphatidyl ethanolamine by phospholipase C treatment. A minor part is present in its quater-





fraction	^3H [dpm]	^{14}C [dpm]
neutral lipids	—	$1.6 \cdot 10^6$
ceramide	$0.683 \cdot 10^6$	$0.315 \cdot 10^6$
phosphatidyl ethanolamine	$8.00 \cdot 10^6$	$2.02 \cdot 10^6$
phosphatidyl choline + sphingomyelin	$2.62 \cdot 10^6$	$1.59 \cdot 10^6$

Fig. 2. Distribution of radioactivity in rat liver lipids, separated by silicic acid chromatography after administration of (a) [3-^{14}C] D,L-sphinganine, and (b) [1-^{3}H; 3-^{14}C] D,L-sphinganine. C = chloroform, M = methanol, TG = triglycerides, CER = ceramides, PE = phosphatidyl ethanolamine, PC = phosphatidyl choline, S = sphingomyelin.

nized form as choline in phosphatidyl choline and sphingomyelin. The labeled free phosphorylethanolamine and the phosphoryl bases released from PE, PC and sphingomyelin were characterized by paper electrophoresis or as DNP-derivative.

We had synthesized [3-^{14}C] 3-dehydrosphinganine (3-ketodihydrosphingosine) for studies of the biosynthesis of the long chain bases. When this 3-dehydro-derivative was administered intravenously to rats it behaved almost identical to sphinganine, except that most of it was recovered as sphinganine in ceramide and sphingomyelin (see fig. 7, p. 149) (table 2).

TABLE 2

Distribution of radioactivity in rat liver lipids separated by silicic acid chromatography after administration of [3-^{14}C] 3-dehydrosphinganine

Fractions*	Radioactivity dpm·10^5	% of total extracted radioactivity
1. triglycerides cholesterol esters	3.58	36
2. ceramides	1.20	12
3. phosphatidyl ethanolamine	0.91	9
4. intermediate fraction	0.24	3
5. phosphatidyl choline	1.59	16
6. sphingomyelin	2.33	24

* Column: 2.5 × 40 cm; prewashed silicic acid Mallinckrodt.

Fraction	% of total radioactivity of long chain bases		
	Sphinganine	Sphingenine	3-O-Methyl-sphingenine*
Ceramide (88000 dpm)	27	63	10
Sphingomyelin (192000 dpm)	12.5	75	12.5

* Arises as a by-product during acid hydrolysis in methanol.

The experiments *in vivo* with 3-dehydrosphinganine suggested to us, that this compound might be a common intermediate in the biosynthesis and degradation. However, when we proceeded to studies *in vitro* with rat liver subcellular fractions (homogenate, mitochondria, microsomes and cytosol) it became immediately evident, that 3-dehydrosphinganine is only an intermediate in the biosynthesis and not in the degradation of sphinganine.

Studies in vitro

Since the degradation *in vivo* proceeds very rapidly, studies *in vitro* were indicated.

[3-^{14}C] sphinganine was incubated with different subcellular fractions of rat liver. Degradation to palmitic acid and palmitaldehyde occurred when the microsomal and mitochondrial fractions were used as enzyme source. The degradation could be increased by the addition of ATP and Mg^{++}, suggesting that a kinase reaction leads to sphinganine-1-phosphate prior to its cleavage (fig. 3).

The rate of the degradation was measured by the appearance of labeled palmitaldehyde and palmitate, which we characterized as dimethylacetal and methylesters respectively.

The ATP-requirement together with our early observation that C-1 and

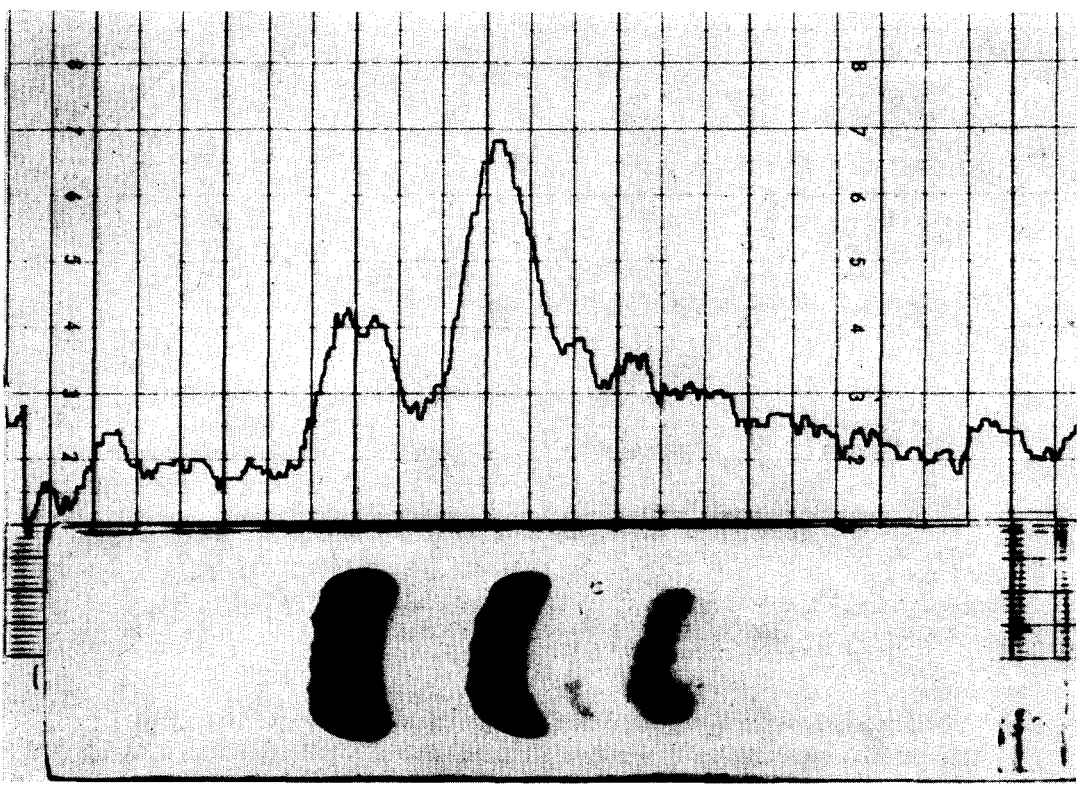


Fig. 3. Radio thin-layer scan of reaction products after incubation of [3-^{14}C] sphinganine with rat liver microsomal fraction. The incubation mixture contained in a volume of 2.0 ml:0.2 μmoles [3-^{14}C] D,L-sphinganine, 5 μmoles ATP, 5 μmoles $MgCl_2$, 5 mg protein, 100 μmoles phosphate buffer *p*H 7.4.

C-2 are released as phosphoryl ethanolamine suggested that sphinganine-1-phosphate is the primary product in the degradation and the substrate of the cleavage reaction. We therefore synthesized [1-^{14}C]- and [3-^{14}C] sphinganine-1- phosphate. This substrate was rapidly degraded by an enzyme localized in the microsomal and, to a less extent, in the mitochondrial fraction. The enzymatic activity is dependent on pyridoxal phosphate, which can be washed out by prolonged dialysis or inhibited by desoxypyridoxal phosphate.

Palmitaldehyde and not palmitic acid is the primary cleavage product: when [3-^{3}H; 3-^{14}C] sphinganine-1-phosphate was incubated with the enzyme fraction the palmitaldehyde isolated as product had retained the ^{3}H/^{14}C-ratio of the substrate, palmitate being derived from palmitaldehyde by the oxidation.

Complementary [1-^{14}C; 3-^{3}H] sphinganine-1-phosphate yielded stoichiometric amounts of ^{14}C-phosphoryl ethanolamine and ^{3}H-palmitaldehyde (figs. 4a and 4b).

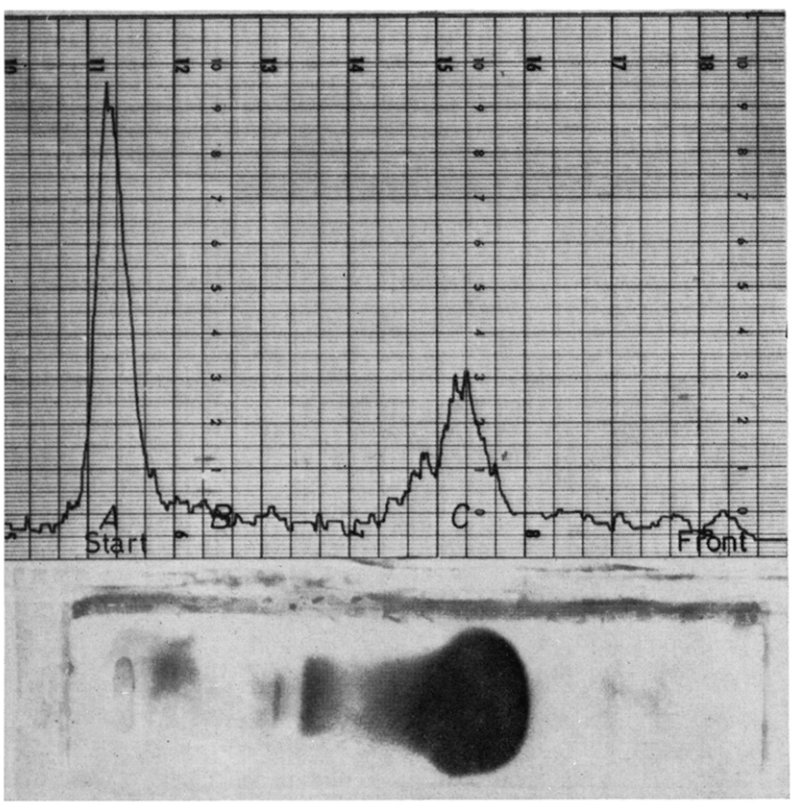


fig. 4a

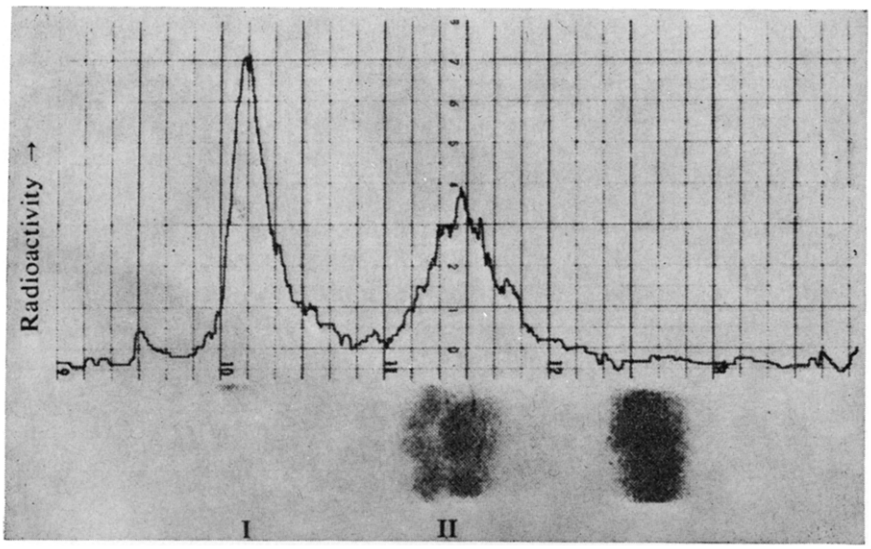


fig. 4b

Fig. 4. TLC-analysis of the aldolase reaction with (a) [3-^{14}C] sphinganine-1-phosphate and (b) [1-^{14}C; 3-^{3}H] sphinganine-1-phosphate; (4a) A: sphinganine-1-phosphate; B: position of palmitic acid; C: palmitaldehyde; solvent system: petroleum ether/ether/acetic acid 60:10:1; plasmal reagent; (4b) I: sphinganine-1-phosphate; II: phosphoryl ethanolamine.

The microsomal and mitochondrial fractions contain a very active sphinganine-1-phosphate hydrolase, the action of which leads to a decrease in substrate concentration. However NaF in concentration between 2 and 5×10^{-2} M inhibits not only the phosphatase but also the palmitaldehyde oxidase; the cleavage enzyme, which we name sphinganine-1-phosphate: phosphoryl ethanolamine lyase remained unimpaired.

The lyase has a wide specificity with regard to the chain length and stereochemistry of the substrate: short chain bases e.g. 2-amino-1,3-dihydroxyheptane and 2-amino-1,3-dihydroxydodecane are cleaved in the same way as the long chain bases with either the *erythro-* or *threo* isomeric form of sphinganine-1-phosphate.

The lyase is a pyridoxal phosphate dependent enzyme, which is strongly inhibited by deoxypyridoxal phosphate. Sulfhydryl reagents such as pCMB, N-ethylmaleinimide and iodoacetamide lead to a loss of the enzymic activity. On the basis of these observations we propose the following mechanism of the aldolase reaction (fig. 5).

The enzyme is present in the microsomal fraction of liver, brain, spleen, muscle, mucosa and lungs with highest activity of liver and brain.

Recently we reported on the *reutilization of the two fragments*, which are formed on enzymatic cleavage, phosphoryl ethanolamine and palmitaldehyde[34]). In comparative studies in vivo using equimolar amounts and a 25 fold excess of [3-^{14}C] serine over [1-^{3}H] 4*t*-sphingenine we observed, that the base of phosphatidyl ethanolamine was predominantly supplied from

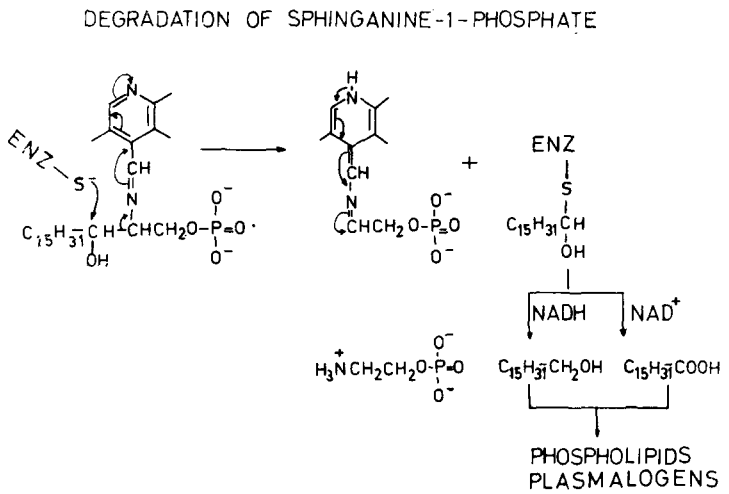


Fig. 5. Suggested mechanism of the sphinganine-1-phosphate-phosphoryl ethanolamine lyase (aldolase) reaction.

C-1 and C-2 of the long chain bases 4*t*-sphingenine and sphinganine likewise in these experiments. Taking the results of the biosynthesis of the long chain bases into account this means another pathway for serine as precursor of the base moiety of phospholipids. Palmitaldehyde derived from C-3 to C-18 is either oxidized to palmitic acid and used for acylation reactions in the biosynthesis of ester lipids or degraded by β-oxidation.

Furthermore when [3-^{3}H] sphinganine is injected in the brains of young rats (10–25 days) during the myelinisation period, 94% of the recovered radioactivity were found in *plasmalogens*. Also liver contains a considerable amount of labeled plasmalogens. The palmitaldehyde was characterized as its dimethylacetale (table 3).

When doubly labeled [3-^{3}H; 3-^{14}C] sphinganine with a ^{3}H/^{14}C ratio of 10.6 was administered this ^{3}H/^{14}C ratio was reduced to 5.2 in palmitaldehyde dimethylacetale isolated from the alkenylethers (plasmalogens). Since [3-^{3}H; 3-^{14}C] sphinganine phosphate is cleaved *in vitro* by the aldolase to [1-^{3}H; 1-^{14}C] palmitaldehyde without the loss of tritium the further transformation of palmitaldehyde to the alkenylether must pass a reaction,

TABLE 3

Utilization of [1-^{3}H] palmitaldehyde liberated from [3-^{3}H] sphinganine for plasmalogen synthesis in rat brain and liver.

	Liver		Brain	
	(dpm)	% of total radioactivity	(dpm)	% of total radioactivity
Total lipid extract	620000	–	220000	–
Total lipid extract (after rubber dialysis)	600000	100	220000	100
Sphinganine	31000	51.5	13500	6
Palmitaldehyde dimethylacetale	290000	48.5	206500	95
Fatty acid methyl esters	–	–	–	–

which leads to the loss of half of the ^{3}H-activity. These observations stimulated experiments with regard to the pathway of palmitaldehyde into plasmalogens, the results of which will be reported elsewhere[35]).

The following scheme (fig. 6) summarizes the reactions leading to the degradation of sphinganine and the reutilization of degradation products.

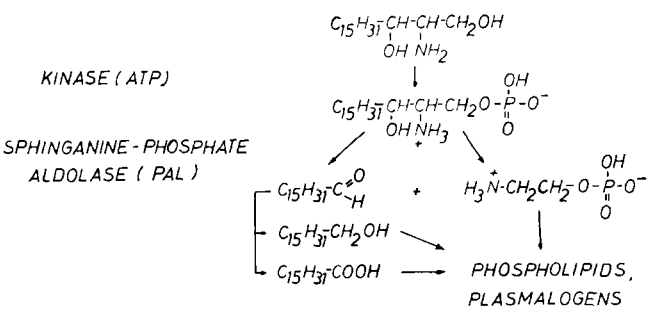


Fig. 6. Summary of reactions leading to the degradation of sphinganine and the reutilization of degradation products.

Biosynthesis of sphingosine bases[21–24])

In the course of our investigations on the degradation of sphinganine we studied the metabolic pathway of [3-^{14}C] 3-dehydrosphinganine *in vivo* (see p. 149). This 3-keto-derivative was rapidly transformed into sphinganine and 4*t*-sphingenine as well degraded to palmitate and CO_2. Fig. 7 summarizes the analysis of the long chain bases of ceramides and sphingomyelins after the administration of [3-^{14}C] 3-dehydrosphinganine. These experiments

proved that the 3-keto group is reduced to form sphinganine and suggested that sphinganine biosynthesis proceeds *via* this 3-keto derivative under physiological conditions.

These observations demanded studies of the biosynthesis *in vitro*, which we carried out in two steps:

1. enzymatic formation of 3-dehydrosphinganine,
2. enzymatic reduction of the 3-keto functional group to yield sphinganine.

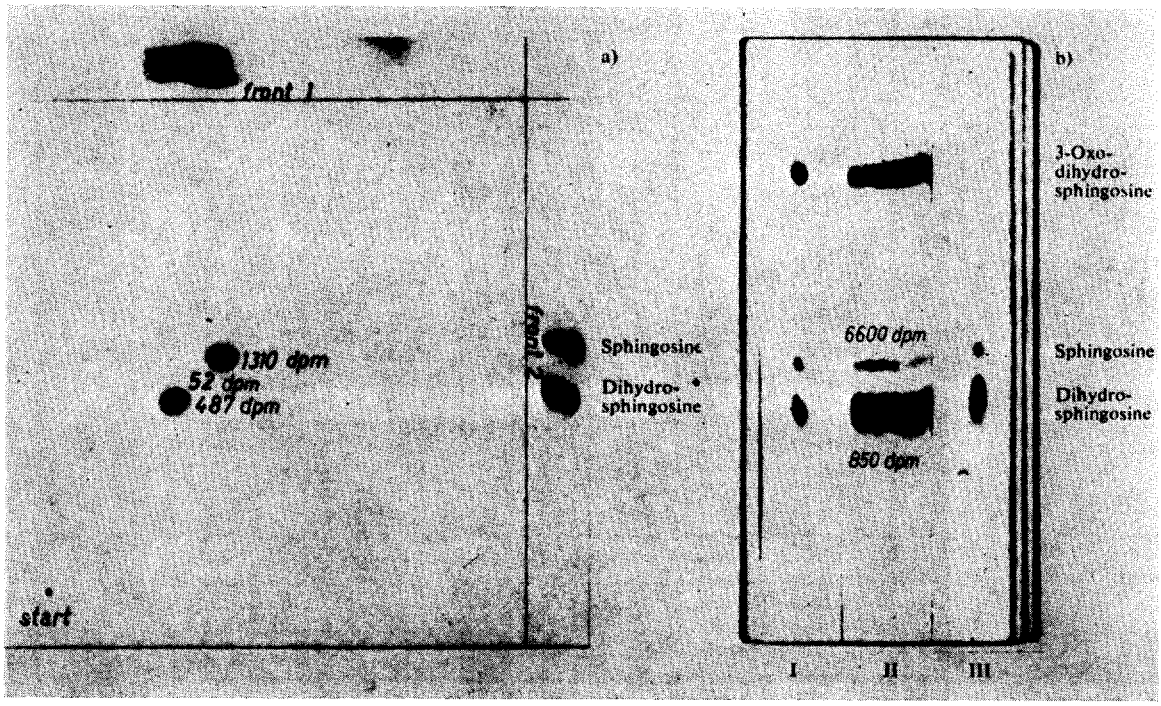


Fig. 7. Analysis of long chain bases of (a) ceramides, (b) sphingomyelin after administration of [3-^{14}C] 3-dehydrosphinganine. (a) Two-dimensional thin-layer chromatography of long chain bases of ceramide from rat liver after intravenous administration of [3-^{14}C] 3-dehydrosphinganine. Solvent system: chloroform/methanol/2 N NH_4OH 40:10:1. (b) One-dimensional thin-layer chromatography of the bases of sphingosine. Solvent system: chloroform/methanol/water 65:25:4, charred with 5% $Na_2Cr_2O_7$ in conc. H_2SO_4/water 1:1. I = III: Test; II. React. product + carrier.

1. *Condensation reaction*

The oxidation level of 3-dehydrosphinganine implied a condensation of palmitoyl-CoA and serine. We studied this reaction with different enzyme sources, the most favorable being the microsomal fraction of liver and the

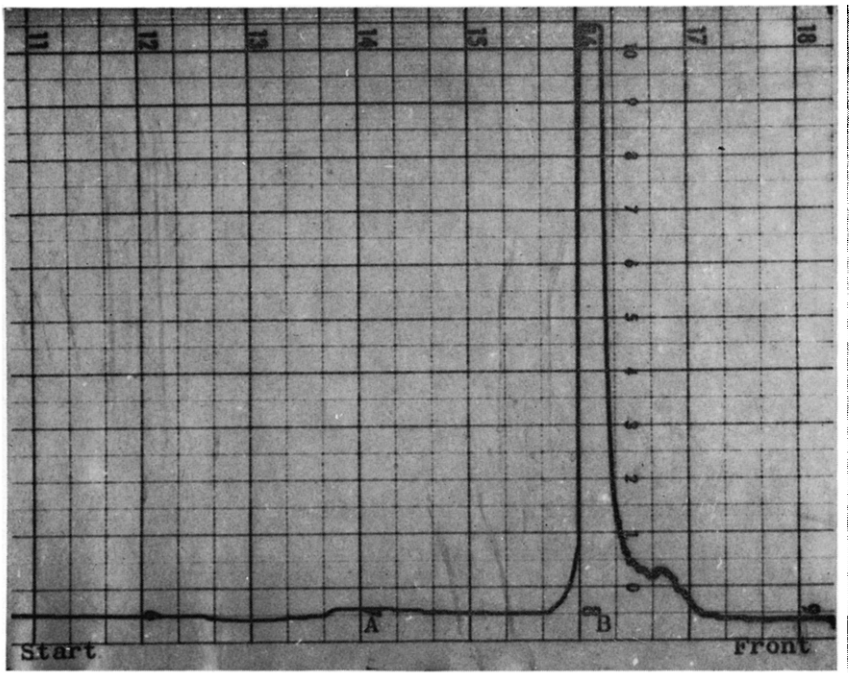


Fig. 8a

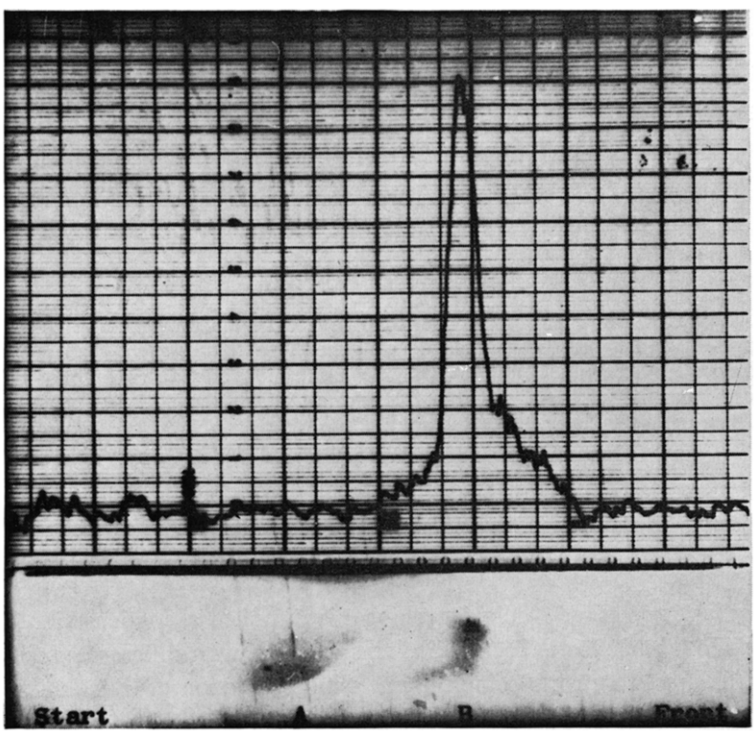


Fig. 8b

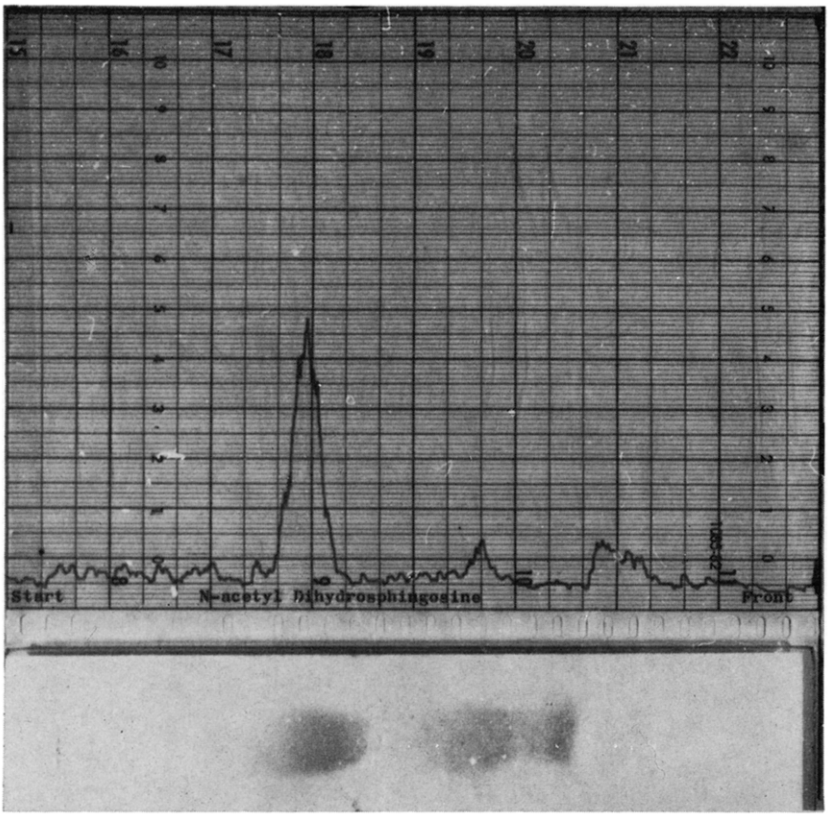


Fig. 8c

Fig. 8. Radio thin-layer scan of (a) the purified reaction product of the condensation reaction of palmitoyl-CoA and [3-^{14}C] D,L-serine with rat liver or *Hansenula cif.* microsomal fraction; (b) after N-acetylation of the reaction product; (c) after $NaBH_4$-reduction of N-acetylated reaction product. The incubation mixture contained in a volume of 2.0 ml: palmitoyl-CoA (0,5 μmoles); [3-^{14}C] D,L-serine (1 μmole,spec. act. 1×10^6 dpm/μmole); pyridoxal phosphate (0.5 μmoles); microsomal protein (5 mg); incubation for 2 hr at 37°C.

yeast *Hansenula ciferrii*. The latter produces large amounts of the fully or partially acetylated long chain bases 4-hydroxy-sphinganine and sphinganine.

With palmitoyl-CoA or palmitate, ATP, CoASH and [3-^{14}C] serine as substrates and these microsomal fractions as enzyme source we were able to isolate 3-dehydrosphinganine. The reaction product was identified by comparison with authentic synthetic 3-dehydrosphinganine. After acetylation the stable N-acetyl derivative cochromatographed with authentic N-acetyl-3-dehydrosphinganine. $NaBH_4$-reduction transformed this product into N-acetyl sphinganine, figs. 8a, 8b and 8c.

The *in vitro* synthesis of 3-dehydrosphinganine together with the studies *in vivo* prove that the biosynthesis of sphinganine begins with the condensation of palmitoyl-CoA and serine with decarboxylation. The 3-dehydrosphinganine synthetase (acyl-CoA-serine C-2-acyl transferase) is a microsomal enzyme which contains firmly bound pyridoxal phosphate as coenzyme. Addition of pyridoxal phosphate leads to the stimulation of the condensation reaction. The stereospecificity of the reaction will be discussed at a later point.

2. *Reduction of 3-dehydrosphinganine* in vitro

In the experiments *in vivo* described above, we had already observed that 3-dehydrosphinganine is reduced to sphinganine and furthermore transformed into 4*t*-sphingenine. We screened the cell fractions of rat liver, heart and *Hansenula ciferrii* for reductase activity, table 4, and cofactor requirement, table 5. It is evident from table 5 that the enzyme is absolutely dependent and specific for NADPH.

TABLE 4

Subcellular distribution of 3-dehydrosphinganine reductase in heart muscle cell

Cell fraction	Conversion (%)
Mitochondria	4
Microsomes	21
Supernatant	3

The reaction product is sphinganine according to the following equation:

$$[3\text{—}^{14}C]\ \text{3-dehydrosphinganine} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{3-dehydrosphinganine reductase}} [3\text{—}^{14}C]\ \text{sphinganine} + \text{NADP}^+.$$

It was characterized by radio-thin-layer chromatography. The free base cochromatographed with authentic sphinganine, its N-acetyl derivative with authentic N-acetyl-sphinganine. In GLC the trimethylsilyl-N-acetyl derivative had a retention time identical with erythro-1,3-di-O-trimethylsilyl-N-acetyl-sphinganine, fig. 9.

TABLE 5

Cofactor requirement of reductase.

	sphinganine formed					
	Rat liver enzyme			*Hansenula cif.* enzyme		
	(dpm)	(nmoles)	(%)	(dpm)	(nmoles)	(%)
Complete	36600	79	16	74200	158	32
− NADPH	9200	20	4.0	11100	24	4.7
− NADPH + NADH	9450	21	4.1	10750	23	46

Each incubation contained in a total volume of 2 ml: 5 mg of enzyme, 0.5 mmole [3-^{14}C] 2-amino-1-hydroxy-octadeca-3-one (2.3×10^5 dpm), NADPH generating system (2.0 μmoles $NADP^+$, 20 μmoles G-6-P, 12 μg G-6-PDH), 200 μmoles Na^+, K^+-phosphate buffer pH 6.8, 10 μmoles $MgCl_2$, 10 μmoles thioglycol, 2h at 37°. The NADH generating system contained: 2.0 μmoles NAD^+, 50 μl ethanol, 12 μg ADH.

No *threo*-isomer is formed. The reductase has a high chain length specificity for the substrate around C_{18} with a slower rate for the C_{16} and C_{20} homologues. Substrates with chain lengths below C_{16}, e.g. 2-amino-1-hydroxy-tetradecane-3-one or 1-amino-1-hydroxy-heptane-3-one are not reduced at all. The K_m for 3-dehydrosphinganine is 2.5×10^{-5} and for the C_{20} homologue 3.5×10^{-5} M.

The *p*H optimum of this enzyme is around *p*H 7.0. The enzymic activity remains unimpaired even by higher concentration of Triton X-100. The solubilization of the enzyme by this non-ionic detergent permits to follow the enzymatic reduction spectrophotometrically at 340 mμ and furthermore to purify the enzyme from the beef liver microsomal fraction. The procedures which led to a 84 fold purification are summarized in table 6.

TABLE 6

Purification steps of 3-dehydrosphinganine reductase.

Purification steps	Protein (mg)	Spec. act. (unit/mg)	Total activity	Yield	Purification factor
Microsomal fraction	12500	0.3	3700	100	1
Triton X-100	7450	0.5	3780	100	1.7
$(NH_4)_2SO_4$	3230	1.0	3230	89	3.3
Ca-apatit adsorption	320	7.5	2400	66	25
Density gradient	37	25	930	25	84

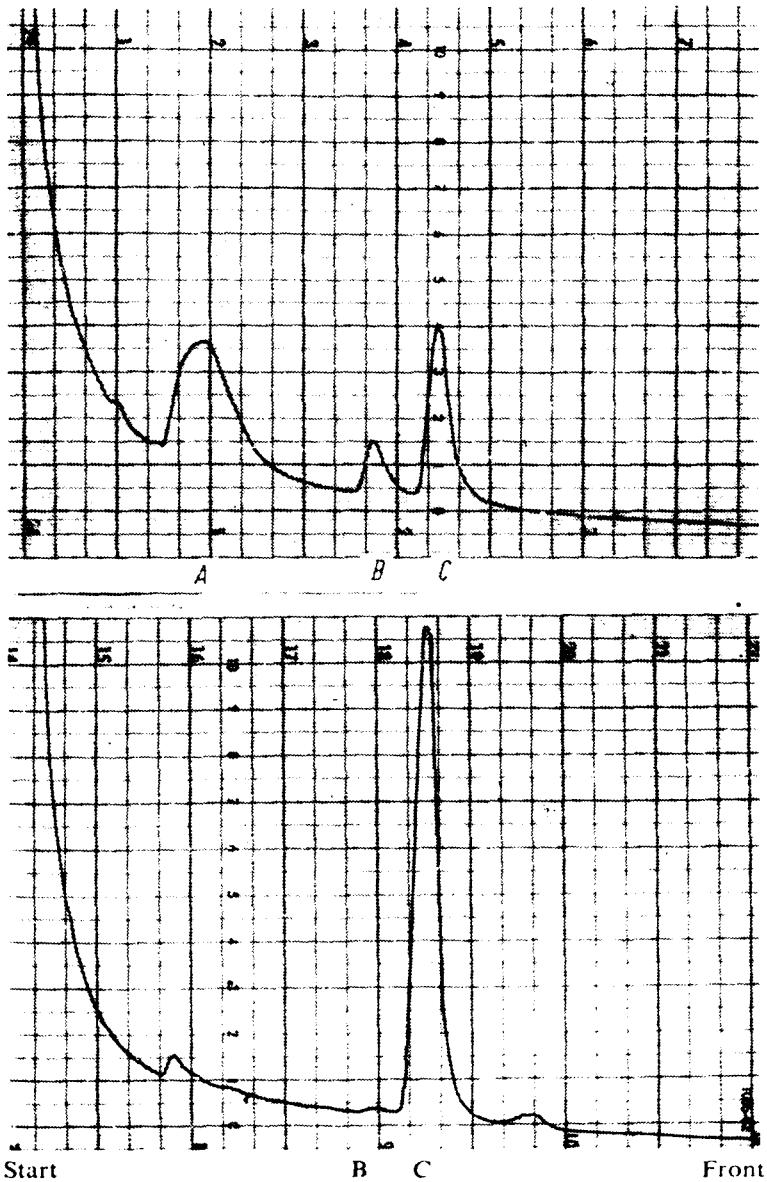


Fig. 9. GLC-analysis of enzymatically reduced 3-dehydrosphinganine. Upper chromatogram: test mixture of TMS-derivatives of (A) N-acetyl-3-dehydrosphinganine (2-acetamido-1-hydroxy octadecane-3-one); (B) *threo*-N-acetyl-sphinganine; (C) *erythro*-N-acetyl-sphinganine. Lower chromatogram: reaction product of reduction of 3-dehydrosphinganine with NADPH after N-acetylation and silylation.

The enzyme is present in all organs investigated so far with highest activity in liver, brain, heart and muscle, table 7.

The degree of the reduction of our synthetic 3-dehydrosphinganine never exceeds 50% because our substrate is racemic at C-2. When the condensation of palmitoyl-CoA and serine and the reduction with NADPH are combined, the 3-dehydrosphinganine initially formed, is completely reduced to sphinganine. We studied the stereospecificity of the reductase using as hydride

TABLE 7

Distribution of D-3-dehydrosphinganine reductase in organs of the rat.

Organ	Spec. act. (mU/mg)+
liver	0.73
spleen	0.23
muscle	0.50
heart muscle	0.48
lung	0.43
brain	0.46
kidney	0.125

+1 mU = 1 n mole sphinganine formed by 1 mg of protein per minute.

donor specifically labeled A-and B-NADPT. 3-NADPT was prepared by a combined hexokinase-glucose-6-phosphate dehydrogenase reaction with [1-^{3}H] D-glucose as substrate and A-NADPT after enzymic oxidation of B-NADPT with lactic dehydrogenase and pyruvate and subsequent reduction with radioactive glucose-6-phosphate. Table 8 summarizes the results which prove that only ^{3}H – from the B-side is transferred to the 3-keto group. 95% of the theoretical amount of ^{3}H were incorporated from B-NADPT.

The reduction of the 3-keto group introduces another optically active center. In order to prove the configuration of biosynthesized sphinganine the reaction product from an incubation of 3-dehydrosphinganine with

TABLE 8

Stereospecificity of reductase reaction; transfer of 4B-^{3}H from NADPH.

Specifically labeled NADPH	Total radioactivity (dpm) in sphinganine		% of ^{3}H radioactivity transferred
	^{3}H	^{14}C	
A – NADP^{3}H	1.6×10^5	1.675×10^4	2.8
B – NADP^{3}H	9.9×10^6	1.445×10^4	95

B-NADP^{3}H and purified reductase has been isolated and diluted with authentic *erythro*-D, L-sphinganine. Addition of D-glutamic acid induced the crystallization of D-*erythro*-sphinganine-D-glutamate ($[\alpha]_D^{30} = +19.2°$; theor. $+18°$). The D-form contained 93% of the radioactivity. The L-form was isolated from the mother liquid as L-glutamate and proved to be almost free of radioactivity. The experiments clearly demonstrated that only the D-isomer of D,L-3-dehydrosphinganine is enzymatically reduced to D-*erythro*-sphinganine. On this basis we name the enzyme more specifically D-3-dehydrosphinganine: NADPH oxido-reductase.

The asymmetric carbon atom 2 of naturally occurring sphinganine which has the 2S;3R structure is derived from L-serine (=R-serine). Therefore an inversion of the asymmetric C-2 must occur during the decarboxylating

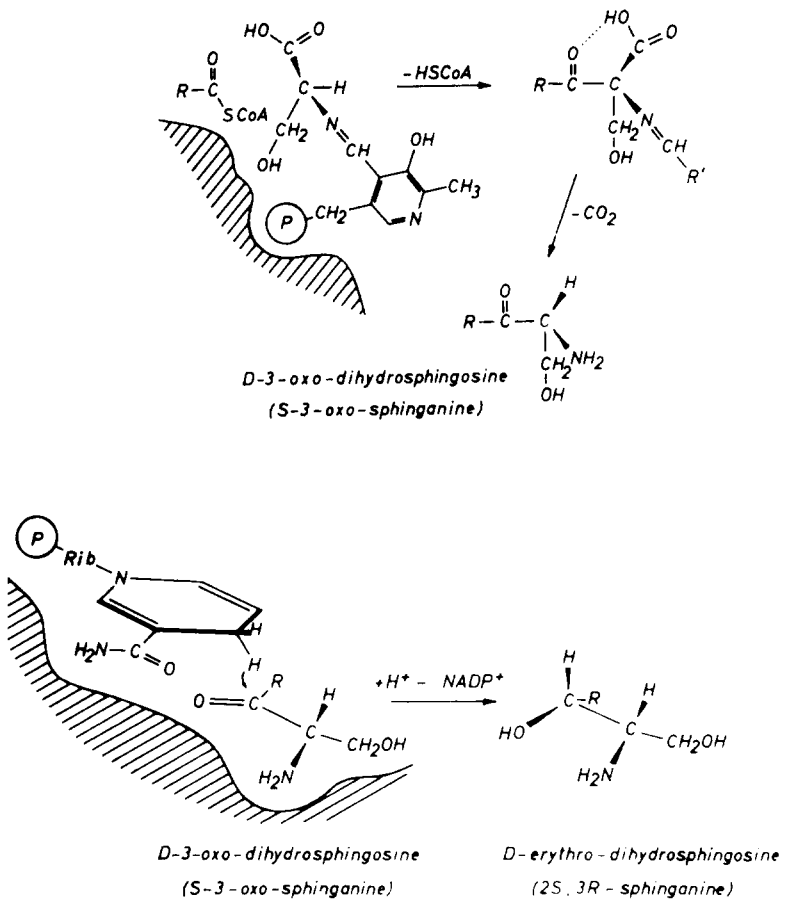


Fig. 10. Stereospecificity of the condensation and reduction reaction in the biosynthesis of sphinganine.

condensation. In the subsequent reduction with NADPH the second asymmetric C-atom of R-configuration at C-3 is introduced. The stereospecificity of the condensation and reduction reaction in the biosynthesis of 4*t*-sphingenine is summarized in fig. 10.

Fig. 11 represents a summary of the reactions in the biosynthesis and the degradation of long chain bases.

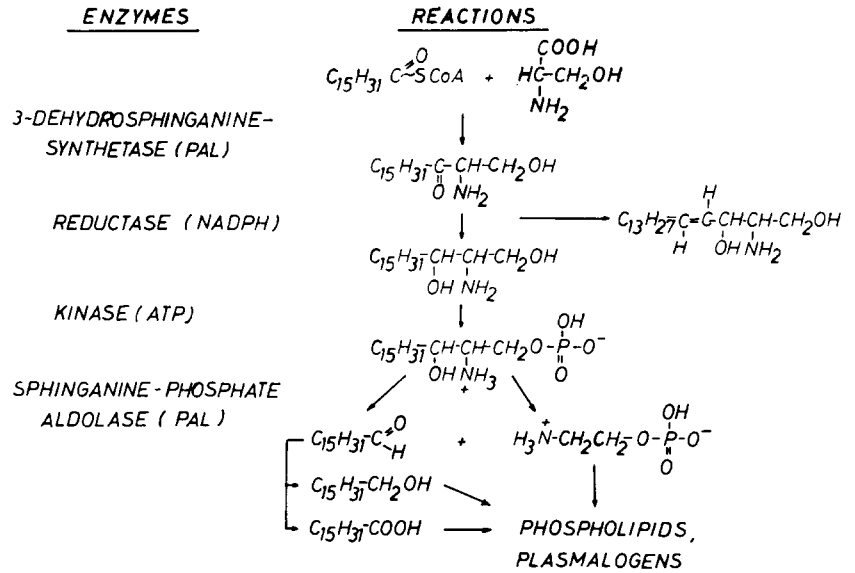


Fig. 11. Reaction sequences and enzymes involved in biosynthesis and degradation of long chain bases.

Acknowledgment

These studies were carried out together with Drs. G. Sticht and D. LeKim. Their continuous and admirable efforts merit particular acknowledgment. The support of this work by the Deutsche Forschungsgemeinschaft and the Bundesministerium für Bildung und Wissenschaft is gratefully acknowledged.

References

1) H. E. Carter, F. J. Glick, W. P. Norris and G. E. Philips, J. Biol. Chem. **142** (1942) 449
2) H. E. Carter and C. G. Humiston, J. Biol. Chem. **191** (1951) 727
3) H. E. Carter, D. Shapiro and B. Harrison, J. Am. Chem. Soc. **75** (1953) 1007
4) H. E. Carter and D. Shapiro, J. Am. Chem. Soc. **75** (1953) 5131
5) J. L. W. Thudichum, J. Prakt. Chem. **25** (1882) 19
6) P. A. Levene and C. J. West, J. Biol. Chem. **16** (1913/14) 549
7) P. A. Levene and C. J. West, J. Biol. Chem. **18** (1914) 481

8) E. Klenk and W. Diebold, Z. Physiol. Chem. **198** (1931) 25
9) E. Klenk and H. Faillard, Z. Physiol. Chem. **299** (1955) 48
10) J. Kiss, G. Fodor and D. Banfi, Helv. Chim. Acta **37** (1954) 1471
11) K. Mislow, J. Am. Chem. Soc. **74** (1953) 5155
12) W. Stoffel and G. Sticht, Z. Physiol. Chem. **348** (1967) 1561
13) W. Stoffel, G. Sticht and D. LeKim, Z. Physiol. Chem. **349** (1968) 1149
14) W. Stoffel and G. Sticht, Z. Physiol. Chem. **348** (1967) 941
15) W. Stoffel and G. Sticht, Z. Physiol. Chem. **348** (1967) 1345
16) W. Stoffel, D. LeKim and G. Sticht, Z. Physiol. Chem. **348** (1967) 1570
17) W. Stoffel and R. Henning, Z. Physiol. Chem. **349** (1968) 1400
18) W. Stoffel, G. Sticht and D. LeKim, Z. Physiol. Chem. **350** (1969) 63
19) R. Henning and W. Stoffel, Z. Physiol. Chem. **350** (1969) 827
20) W. Stoffel and A. Scheid, Z. Physiol. Chem. **350** (1969) 1593
21) W. Stoffel, D. LeKim and G. Sticht, Z. Physiol. Chem. **349** (1968) 664
22) W. Stoffel, D. LeKim and G. Sticht, Z. Physiol. Chem. **349** (1968) 1637
23) W. Stoffel, G. Sticht and D. LeKim, Z. Physiol. Chem. **349** (1968) 1745
24) W. Stoffel, D. LeKim and G. Sticht, Z. Physiol. Chem. **350** (1969) 1233
25) I. Zabin and J. F. Mead, J. Biol. Chem. **205** (1953) 271
26) I. Zabin and J. F. Mead, J. Biol. Chem. **211** (1954) 87
27) D. B. Sprinson and A. Coulon, J. Biol. Chem. **207** (1954) 585
28) M. L. Greene, T. Kaneshiro and J. H. Law, Biochim. Biophys. Acta **98** (1965) 582
29) R. Brady and G. J. Koval, J. Biol. Chem. **233** (1958) 26
30) R. Brady, J. V. Formica and G. J. Koval, J. Biol. Chem. **233** (1958) 1072
31) P. E. Braun and E. E. Snell, Proc. Nat. Acad. Sci., USA **58** (1967) 298
32) P. E. Braun and E. E. Snell, J. Biol. Chem. **243** (1968) 3775
33) R. N. Brady, S. J. DiMari and E. E. Snell, J. Biol. Chem. **244** (1969) 491
34) W. Stoffel and D. LeKim, Z. Physiol. Chem., in press.
35) W. Stoffel and D. LeKim, Z. Physiol. Chem., in press