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THE BIOSYNTHESIS OF LIPIDS IN THE NERVOUS SYSTEM: a Colloquium organized on behalf of the Lipid Group and the Neurochemical Group by M. I. Gurr (Sharnbrook) and H. F. Bradford (London)

Relationship between Sphingolipid and Phospholipid Metabolism

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The two complex classes of phospholipids and sphingolipids have been clearly distinguished from each other on the basis of their chemical structures. They differ in the complexity of their hydrophilic group (except for sphingomyelin and phosphatidylcholine, with an identical group) and in their hydrophobic moiety with a diglyceride or plasmalogenic diglyceride structure with ester and ether bonds on the one hand and the ceramide structure with an amide bond between fatty acids and long-chain bases of the sphingosine type on the other.

The different chemical structures of these lipid classes may be responsible for their very divergent functions in membranes, especially those of mammalian origin. They are also reflected in differences in physicochemical properties.

Recent studies have elucidated the metabolic interrelationship between these two lipid classes (for review see Stoffel, 1971). It is well known that complex sphingolipids are hydrolysed in the cell step by step, beginning at the hydrophilic group and finally

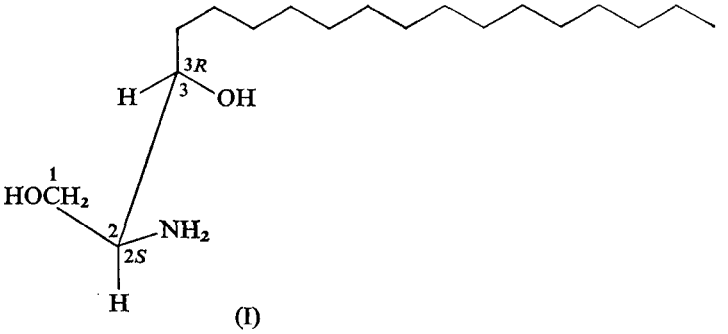
ending up with ceramide, the hydrophobic part. The glycosidases and sphingomyelinase responsible for these reactions are localized in the lysosomes and are characterized by their acidic pH optima. The degradation of ceramide by a ceramidase yields fatty acids and the long-chain bases sphingosine and dihydrosphingosine, and to a smaller extent phytosphingosine. These are the predominant long-chain bases present in mammalian sphingolipids. Intermediates of their metabolism form links between the sphingolipid and phospholipid classes.

Metabolism of sphingosine bases

The chemistry of the long-chain bases sphingosine, dihydrosphingosine and phytosphingosine, the determinant components of the large and complex group of sphingolipids and glycosphingolipids, has been reviewed by Karlsson (1970).

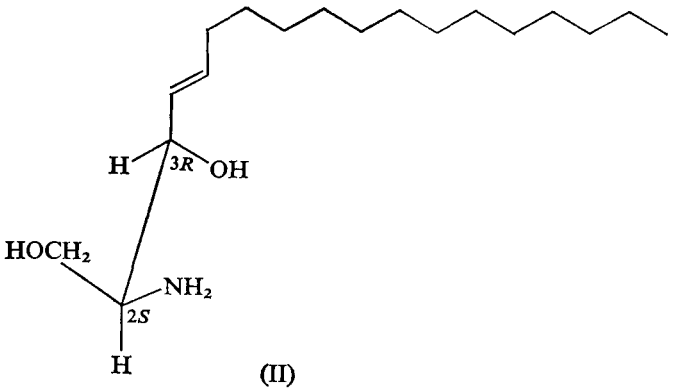
In the present communication the biochemistry and the interrelationship of the metabolism of sphingosine bases and of phospholipids and plasmalogens in particular is considered.

The first question is concerned with reactions leading to the synthesis of the unique 2-amino-1,3-dihydroxy-alkane or -alk-4-*trans*-ene system with the two optically active centres at C-2 and C-3, which have a 2*S*,3*R* D-*erythro* configuration. The main long-chain base is sphingosine (II). Dihydrosphingosine (I) and also phytosphingosine (III), the latter previously thought to be present only in fungi and yeasts, are also present in respectable amounts with a rather specific distribution. We have demonstrated that phytosphingosine comes from exogenous sources and is incorporated into complex glycosphingolipids (Assmann & Stoffel, 1972).



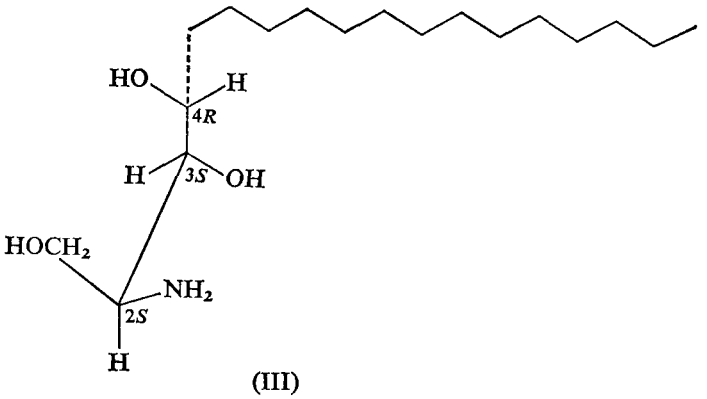
(I)

Dihydrosphingosine [(2*S*,3*R*)-sphinganine]



(II)

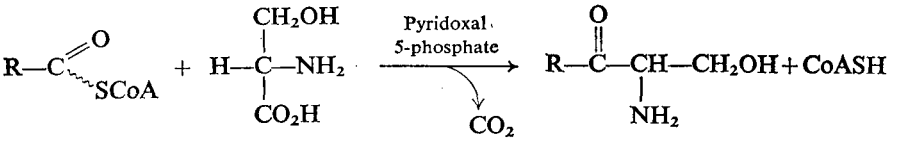
Sphingosine [(2*S*,3*R*)-sphing-4-*trans*-enine]



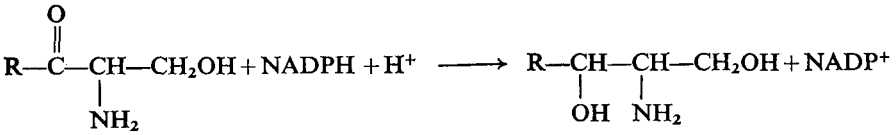
(III)

Phytosphingosine [(2*S*,3*S*,4*R*)-D-4-hydroxysphinganine]

With regard to the biosynthesis of these long-chain bases it has been accepted for a long time that palmitaldehyde and serine condense to form dihydrosphingosine, which is desaturated by an FAD-enzyme (Brady & Koval, 1958; Brady *et al.*, 1958). Today it is clear that this suggested reaction does not occur, but that palmitoyl-CoA and serine condense with decarboxylation to form 3-dehydrosphinganine (3-oxodihydrosphingosine), pyridoxal 5-phosphate being the coenzyme of the synthase (Stoffel *et al.*, 1968*a*; Braun & Snell, 1968; Brady *et al.*, 1969). The structure of this 3-oxo compound has been proved by reduction with $NaBH_4$ to give dihydrosphingosine, *N*-acetylation and comparison with test substances. The first optical centre at C-2 (2*S*) is therefore derived from C-2 of serine (2L = 2*S*) with retention of the configuration:

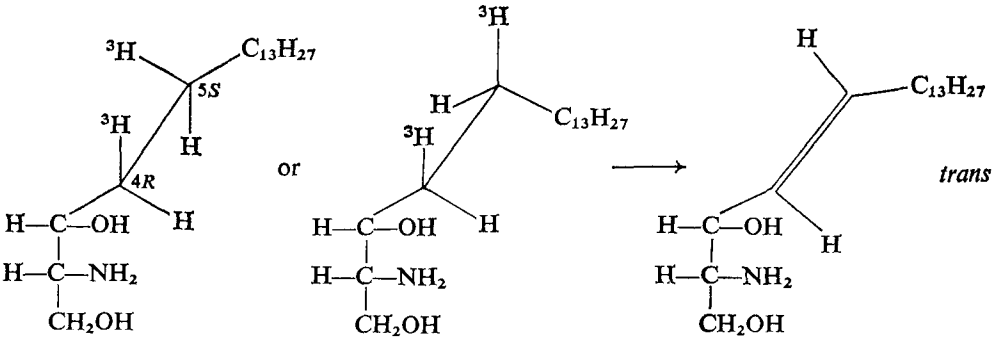


In a subsequent reaction the 3-oxo group is reduced by an NADPH-dependent reductase, yielding dihydrosphingosine (Stoffel *et al.*, 1968*b*):



The hydride ion from the B side of NADPH is transferred and the second optically active centre (3*R*) is formed. The synthase and the reductase are bound to the membranes of the endoplasmic reticulum. Both enzymes posses a chain-length optimum between C_{16} and C_{18}. After the formation of the carbon skeleton and its substituents at C-1, C-2 and C-3 has been clarified, the way by which the 4-*trans*-double bond is formed had to be explained. It had been suggested that a dehydration of phytosphingosine would lead to sphingosine. However, experiments with biosynthetically labelled phytosphingosine and 5-hydroxydihydrosphingosine excluded such a mechanism (W. Stoffel, unpublished work). When dihydro[3-^{14}C, 3-^{3}H]sphingosine was used as substrate the isotope ratio in the sphingosine formed remained constant. This proved that dihydrosphingosine is the immediate precursor of sphingosine (Stoffel *et al.*, 1971). We then attacked the problem of the formation of the 4-*trans*-double bond and its stereospecificity. Our working hypothesis was that the hydrogen atoms at C-4 and C-5 of

dihydrosphingosine must not themselves be stereospecifically labelled. Since young rat brains incorporate palmitate efficiently into the long-chain bases during the myelination period, the four ^{3}H-labelled palmitates, namely [2*R*-^{3}H]-, [2*S*-^{3}H]-, [3*R*-^{3}H]- and [3*S*-^{3}H]-palmitate, would satisfy all requirements. In four parallel experiments these isomeric palmitates, with which [1-^{14}C]palmitate was admixed to establish a defined ^{3}H/^{14}C isotope ratio, were injected intracerebrally and the long-chain bases of brain sphingomyelin were analysed. The ^{3}H marker was eliminated when [2*R*-^{3}H]- or [3*S*-^{3}H]-palmitate served as precursor, whereas [2*S*-^{3}H]- and [3*R*-^{3}H]-palmitate were incorporated into sphingosine with retention of the ^{3}H and the original isotope ratio of the substrate. *cis*-Elimination leads to the formation of the *trans*-double bond:



Hydroxylated intermediates have not been found, and phytosphingosine and the two isomeric 5-hydroxydihydrosphingosines do not serve as precursors. However, this does not exclude the participation of activated oxygen bound by non-haem iron.

A massive kinetic isotope effect was observed when [2*R*-^{3}H]palmitate was administered. This indicates that the intermediarily formed dihydrosphingosine with the 4*R*-^{3}H, which is eliminated in the introduction of the double bond, loses this isotope more slowly than the ^{1}H isotope. The 5*S*-^{3}H isotope, on the other hand, is eliminated without showing an isotope effect. We conclude from this observation that the first step is the elimination of the 4*R*-hydrogen followed by that of the 5*S*-hydrogen (Stoffel *et al.*, 1971). [These results are at variance with those of Polito & Sweeley (1971), who describe an elimination of the 4*R*- and 5*R*-hydrogen atoms.]

For chemical reasons a mechanism involving an FAD-linked oxidoreductase seems unlikely, because C-4 and C-5 are not activated at all. This is the present status of our knowledge of sphingosine biosynthesis.

Similarly we studied the stereospecificity of the introduction of the 4-hydroxyl group into phytosphingosine in the yeast *Hansenula ciferrii*. Only the 4*R*-^{3}H is eliminated. Again a striking kinetic isotope effect has been observed (Stoffel & Binczek, 1971). These results agree with those reported by Polito & Sweeley (1971).

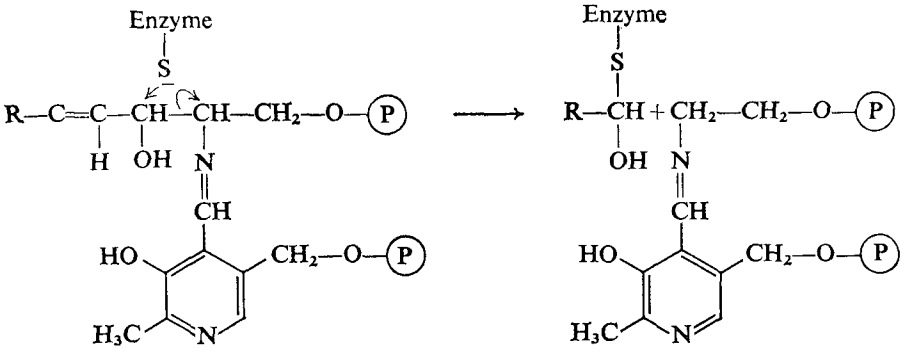
The degradation of sphingosine bases has been intensively studied in my laboratory by using labelled sphingosine, dihydrosphingosine and phytosphingosine. The compounds were labelled in the 1- and the 3-position and in the alkyl chain with either ^{3}H or ^{14}C. This first allowed determination of the mode of the degradation which in general occurs very rapidly, at a rate comparable with that of fatty acids. Studies *in vivo* indicated that the long-chain base molecules are cleaved into a C_{16} fragment, isolated mostly as palmitate, and a C_2 fragment, present as free ethanolamine or its phosphate ester or incorporated into phosphatidylethanolamine. Homologous long-chain bases underwent the same $C_{(n-2)}+C_2$ degradation (Stoffel & Sticht, 1967*a*,*b*; Stoffel & Scheid, 1969).

When we studied this degradation *in vitro* it immediately became apparent that ATP was required:

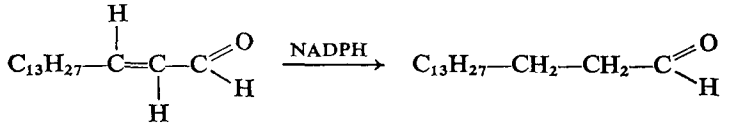
$$\text{Sphingosine} + \text{ATP} \xrightarrow{\text{Kinase}} \text{sphingosine 1-phosphate} + \text{ADP}$$

The primary hydroxyl group is phosphorylated at C-1 (Stoffel *et al.*, 1968*c*, 1969*a*, 1970*a*). The kinase has in the meantime been isolated from thrombocytes (Stoffel *et al.*, 1970*a*, 1973), liver (Hirschberg *et al.*, 1970) and kidney (Keenan & Maxam, 1969).

The further degradation of the 1-phosphate ester requires a microsomal enzyme, which is pyridoxal 5-phosphate-dependent. The reaction products are phosphorylethanolamine, representing C-1 and C-2 of the long-chain bases, and palmitaldehyde, hexadec-2-*trans*-enal and 2-hydroxypalmitaldehyde (Stoffel *et al.*, 1968*c*, 1969*a*,*b*; Stoffel & Assmann, 1970, 1972). The cleavage of the C–C bond mimics the threonine aldolase reaction. The enzyme sphingosine 1-phosphate aldolase attacks the Schiff base of the substrate, presumably in the way indicated below, since we found that thiol groups of the aldolase are involved in the lyase reaction:



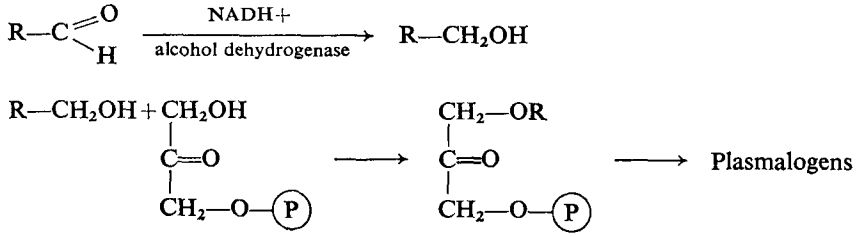
The two fragments of dihydrosphingosine 1-phosphate and sphingosine 1-phosphate, namely palmitaldehyde and hexadec-2-*trans*-enal and phosphorylethanolamine, are excellent precursors for phospholipid biosynthesis. Phosphorylethanolamine originating from the degradation of the long-chain bases is a much better precursor than is serine for phosphatidylethanolamine synthesis (Henning & Stoffel, 1969). We than isolated and characterized the enzyme alk-2-*trans*-enal reductase, which is NADPH-dependent and reduces the 2-*trans*-double bond of hexadec-2-*trans*-enal to palmitaldehyde (Stoffel & Assmann, 1973):



Hexadec-2-*trans*-enal Palmitaldehyde

Palmitaldehyde can now undergo oxidation to palmitate and thence to CO_2 via β-oxidation. On the other hand palmitate may be incorporated as an acyl group into ester lipids (triglycerides, phospholipids) or into the ceramide part of sphingolipids.

Another reaction leads to the reduction of the carbonyl group of palmitaldehyde to hexadecanol. This is incorporated into alkyl ether lipids (Snyder *et al.*, 1969) and the alkenyl ether linkages of plasmalogens in a very efficient way (Stoffel *et al.*, 1970*b*; Stoffel & LeKim, 1971):



The stereochemistry of the hydrogen elimination in the transformation of the alkyl into the alk-1-*cis*-enyl ether linkage in plasmalogen has been studied with [1*S*-^{3}H]-, [1*R*-^{3}H]-, [2*S*-^{3}H]- and [2*R*-^{3}H]-hexadecanol. The formation of this *cis*-olefinic bond of the vinyl ether linkage is based on the elimination of the 1*S*-^{3}H and 2*S*-^{3}H isotope of the labelled alkyl ether (Stoffel & LeKim, 1971).

The combination of the synthesis and the degradation of long-chain sphingosine bases also provides an additional pathway for the incorporation of serine into phospholipids. The studies reported here briefly summarize the close relationship between the metabolism of the long-chain bases and phospholipid synthesis.

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New Concepts in Brain Cholesterol Metabolism

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Although cholesterol biosynthesis has been a matter of intense investigation for at least 20 years, its mode of formation has been approached in a piecemeal manner. The reasons for this happening are many, but the main reason, no doubt, is the very number of biosynthetic steps specific to cholesterol biosynthesis: three steps from acetate to mevalonate, at least nine from mevalonate to squalene, and possibly nine more steps from squalene to cholesterol. This is only a rough estimate, for various postulated intermediates, especially between squalene and cholesterol, could put the number of distinct enzymic steps much higher than 21 individual steps. Indeed, at least 25 sterols that could be precursors of cholesterol have been isolated or have been shown to be