review

Biosynthesis and metabolic degradation of sphingolipids not containing sialic acid

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Abstract Interest in sphingolipid metabolism has increased rapidly during the past decade, and many of the steps involved in the biosynthesis and metabolic degradation of sphingolipids are now known. In this review these studies are critically examined. Emphasis has been placed on the in vitro studies with cell-free systems, since these represent the groundwork for further purification and characterization of the enzyme systems involved. Experimental problems specific to this field of study, and the manner in which these may affect interpretation of experimental results, are discussed.

Supplementary key words long-chain bases · glycosphingolipids · inborn errors of sphingolipid metabolism · ceramide · sphingomyelin · cerebroside · sulfatide · globoside · blood groups

THE SPHINGOLIPIDS have for over a century attracted the attention of neurochemists and, more recently, of a growing number of investigators in many other areas of biochemical research. This group of compounds, composed of many chemical species, is now known to be represented by one or more of its members in almost all eukaryotes investigated. Of particular interest are recent reports dealing with the presence of sphingolipids in bacteria (1, 2). Recent reviews by Mårtensson (3), Karlsson (4), and Stoffel (5) contain references to sphingolipid distribution. As new sphingolipids are discovered, their chemistry and metabolism continue to fascinate and challenge investigators who are seeking to define the role which these compounds play in the dynamics of cellular function and in the structural aspects of cell membranes.

Since the discovery in 1958 by Rapport et al. of the haptenic properties of cytolipin H (6), the relationships between the chemical structure and immunological activity of glycosphingolipids (7) and sphingolipid blood-group substances (8) have become subjects of intensive research. Other observations on the biological function of sphingolipids may be cited: (*a*) changes in glycosphingolipid patterns in malignant cells as well as in virus-transformed cells grown in culture (9–11) suggest that these lipids are immunological determinants in surface membranes; (*b*) studies of sulfatide distribution indicate a possible functional role for these acidic lipids in sodium-transporting systems (12); (*c*) Stoffel and coworkers have observed that the 2-carbon product of biodegradation of sphingolipid base, phosphorylethanolamine, is readily incorporated into phosphoglycerides (13), while the long-chain aldehyde, which is the other direct degradation product, is preferentially incorporated into plasmalogens (14). This suggests that sphingolipid–glycerolipid interrelationships are more

Abbreviations and terminology: The trivial nomenclature, instead of the IUPAC-IUB terminology, has been used. LCB, long-chain bases; dihydrosphingosine, sphinganine or D-*erythro*-2-amino-1,3-dihydroxyoctadecane; sphingosine, sphingenine or D-*erythro*-2-amino-1,3-dihydroxyoctadecene-4; phytosphingosine, 4-hydroxysphinganine. Keto, as a prefix to an LCB, indicates the 3-dehydro derivative. The 20-carbon homologs of the LCB are indicated by the appropriate prefix (e.g., C_{20}). Cer, ceramide, is an *N*-acyl amide of sphingosine or dihydrosphingosine. The type of fatty acid in the *N*-acyl position of ceramide or cerebrosides may be indicated by the prefixes nonhydroxy FA for nonhydroxy fatty acid, or hydroxy FA for 2-hydroxy fatty acid. Cerebrosides are identified as either galactosylceramide or glucosylceramide. Sulfatide is galactosylceramide sulfate. Sugar moieties of more complex glycolipids are abbreviated as follows: Glc, glucose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine.

extensive and important than heretofore realized.

In view of current interests in the biological functions of sphingolipids and their metabolic relationships to other cellular lipids, it is appropriate to review progress in studies pertaining to the biological synthesis and degradation of these compounds. Emphasis will be on in vitro studies with cell-free systems. Chemistry and biological distribution of sphingolipids will be mentioned only to the extent that it aids in the interpretation of metabolic studies. Metabolism of glycosphingolipids containing sialic acid is not included here, as it has been recently reviewed (15, 16). The chemistry of sphingolipids was reviewed in 1965 by Carter, Johnson, and Weber (17) and in 1969 by Shapiro (18).

Experimental difficulties specific to this field of study relate to the insolubility of substrates and products in aqueous solutions. In addition, most of the enzymatic activities involved in sphingolipid biosynthesis are particulate. In vitro systems for assay of enzymes of sphingolipid metabolism usually contain large amounts of one or more detergents to emulsify the substrate. The detergent, substrate, and endogenous lipid present in the enzyme source form a mixed micelle. The use of detergents may influence the results of in vitro assays in a nonphysiological manner:

(*a*) The solubility of substrates of a particular enzyme in the presence of a detergent may differ from solubility in vivo. Orientations of the potential substrates within the micelle may also differ. It is likely that studies of enzyme specificity for various substrates in the presence of detergents may reflect not so much the true physiological specificity of the enzyme but rather the relative ease of mixed micelle formation of each substrate with the detergents being studied.

(*b*) The lipid end product of a reaction may be more or less soluble than the original substrate. An extreme case would involve driving an enzymatic reaction in the wrong (nonphysiological) direction by precipitation of an insoluble product from two relatively soluble precursors.

(*c*) The protein-denaturing activity of detergents is well known. Thus, there is the possibility that partial denaturation of the enzyme might affect its activity and specificity.

(*d*) Enzymes involved in sphingolipid synthesis are particulate and, in general, appear to resist solubilization by standard procedures. This difficulty may be an artifact of the assay conditions; it is possible that the proteins possessing the enzymatic activity are released but, upon solubilization, become susceptible to the protein-denaturing effects of detergents present in the assay mixture and are inactivated during the incubation.

Another source of ambiguity, to be discussed in greater detail in following sections, results from the common use of nonphysiological substrates.

These points are not to be interpreted as a general criticism of in vitro studies involving detergents or synthetic substrates, since in many cases this is the only way to study the reaction. However, these factors must be considered in evaluating the physiological significance of in vitro results.

Some of the potential problems arising from the presence of detergents in incubation mixtures may be eliminated by use of a solid support for the substrate. Lipid substrate is evaporated on an inert substance such as Celite or paper (19, 20), and this solid phase is incubated with a particulate enzyme preparation. An ingenious adaptation of this principle was utilized by Wenger, Petitpas, and Pieringer (21), who evaporated a lipid substrate directly from benzene solution on a particulate enzyme preparation. In one case where use of solid media has been compared with use of detergents, the support method promoted higher enzyme activity for a given amount of substrate (19). A potential drawback of this approach is that kinetic data that can be obtained are limited, since the effective concentration of the substrate cannot be calculated.

One other caution with respect to interpretation of biosynthetic studies should be mentioned. In order to demonstrate conclusively that an exogenous lipid substrate is being utilized by a particle-bound enzyme preparation, it is not enough to show that it stimulates the reaction being studied. Incorporation of the exogenous substrate should be unequivocally demonstrated in order to offset the criticism that the added exogenous lipid is merely stimulating incorporation of endogenous substrate by alternate pathways. The most straightforward, and most difficult, way of demonstrating this is to show incorporation of a synthetic, radioactively labeled substrate. The necessity for such proof is emphasized by recent work which demonstrates that many enzymes, including some involved in lipid metabolism, have a requirement for structural lipid to maintain enzymatic activity (see Ref. 22 for discussion).

BIOSYNTHESIS

Long-chain bases

The initial experiments on the biological origin of sphingolipid bases established that ^{14}C-labeled acetate and serine were incorporated into the sphingolipids of animal tissues (23–25). Brady and coworkers (26, 27) were the first to achieve the biosynthesis of a long-chain base in a cell-free preparation.

As a result of reports that vitamin B_6 deficiency results in decreased levels of phytosphingosine in the yeast *Hanseniaspora valbyensis* (28) and that another yeast, *Hansenula ciferri*, can utilize palmitate and serine to synthesize dihydrosphingosine and phytosphingosine (29),

the mechanism of sphingolipid biosynthesis was reinvestigated. Braun and Snell (30, 31), using particulate preparations of *H. ciferri*, demonstrated the synthesis of dihydrosphingosine from palmitoyl CoA, serine, and NADPH. A requirement for pyridoxal phosphate was established by treating the enzyme preparation with cysteine to deplete it of this cofactor. Sphingolipid-synthesizing activity was lost but could be restored by addition of pyridoxal phosphate. Palmitaldehyde was incorporated into dihydrosphingosine only under conditions which permitted its conversion to the preferred substrate, palmitoyl CoA. An additional biosynthetic product, sphingosine, was also found to accumulate in this system. Contrary to previous reports (26, 27), the biosynthesis of these two bases is not stimulated by added metal ions; indeed, divalent metal ions may interfere. Under certain assay conditions (26, 27), pyridoxal phosphate and Mn^{2+} catalyze a nonenzymatic decarboxylation of serine (30, 32). Further studies (31, 33) disclosed that, in the absence of NADPH, the initial condensation product of palmitoyl CoA and serine is ketodihydrosphingosine. Identical results were obtained in independent studies by Stoffel, LeKim, and Sticht (34), who worked with particulate preparations of *H. ciferri* or rat liver. The mechanism of dihydrosphingosine biosynthesis, as presently visualized, is presented in Fig. 1. Displacement of CO_2 from the Schiff base intermediate (*I*) during condensation with palmitoyl CoA (reactions *b* and *c*) is thought to occur in a concerted fashion. An alternative route (not shown) to the aminoketone (*IV*), which might proceed by labilization of the α-hydrogen atom of intermediate *I*, is eliminated by the experimental results of Weiss (35); tritium on the α-carbon of serine is retained during sphingosine biosynthesis in rats. The NADPH-dependent reduction (reaction *e*) of ketodihydrosphingosine (*IV*) occurs stereospecifically to form the D-*erythro* isomer of dihydrosphingosine (*V*) (31, 36). The reductase has recently been studied in some detail with respect to its substrate specificity and stereochemistry of hydrogen transfer (37).

The synthesis of sphingosine by the particulate enzyme fraction from yeast also occurs by way of the aminoketone intermediate (31), which has been isolated and characterized as ketosphingosine (33). It first appeared that ketodihydrosphingosine was a common precursor for both dihydrosphingosine and sphingosine in yeast, but that the pathways diverged prior to reduction of the carbonyl intermediates. However, subsequent investigations (38) revealed that sphingosine arises by an independent route from hexadecenoyl CoA and serine in a manner analogous to the formation of dihydrosphingosine. The yeast cells employed in these studies contain enzymes which rapidly interconvert palmitoyl CoA and *trans*-2-hexadecenoyl CoA (37, 38). The failure of attempts to demonstrate sphingosine synthesis from 2-hexadecanoic acid (32) is probably attributable to the lack of an enzyme in yeast for the activation of the free fatty acid.

The observation by Stoffel, LeKim, and Sticht (39) of sphingosine and dihydrosphingosine synthesis in rats from intravenously administered [3-^{14}C]ketodihydrosphingosine is not inconsistent with this pathway for sphingosine biosynthesis, if the supposition is made that the exogenous aminoketone was rapidly degraded in such a manner as to give palmitoyl CoA, which then served as the actual precursor in those experiments. A

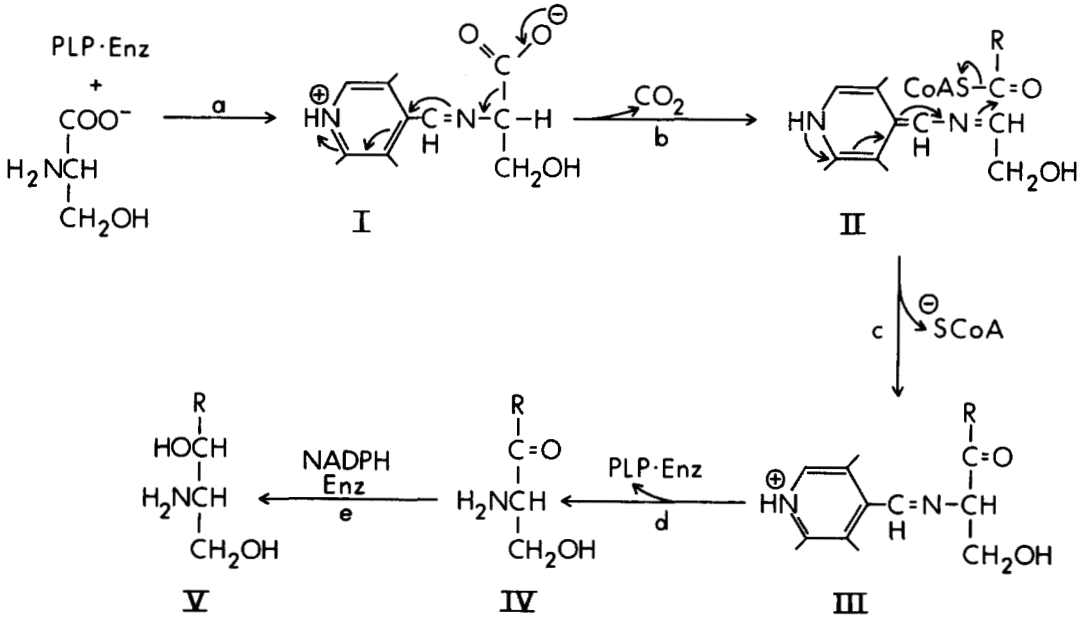


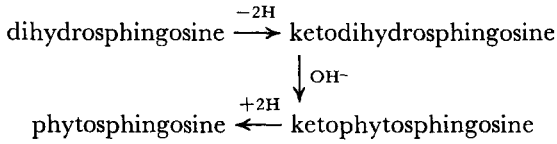
FIG. 1. Mechanism of dihydrosphingosine biosynthesis. PLP, pyridoxal phosphate; Enz, enzyme; R, $CH_3(CH_2)_{13}CH_2$-.

similar explanation may apply to the demonstration of conversion of [4,5-^{3}H]dihydrosphingosine to sphingosine in rats (40). An earlier report by Brady, Formica, and Koval (27) that dihydrosphingosine is converted in vitro to sphingosine by desaturation was contested by Fujino (41), who also had reported the synthesis of free sphingosine by brain homogenates (42). The methods utilized for separation of sphingosine and dihydrosphingosine appear to have been inadequate in these earlier studies. The use of a more extensive array of chromatographic systems to characterize the reaction products from a cell-free system derived from *H. ciferri* (36, 43) has failed to substantiate the biosynthesis of the free unsaturated base by either of these proposed routes. Studies by Polito and Sweeley (44) on the incorporation of specifically deuterated palmitate into sphingosine by cell-free extracts of *H. ciferri* showed that the pro-R hydrogens of palmitate are removed in the formation of the *trans* double bond. It was suggested that the mechanism of desaturation involves an antiperiplanar elimination reaction.

The biosynthesis of sphingolipid bases by microsomal preparations from mouse brain has recently been reexamined by Braun, Morell, and Radin (43). These studies demonstrated that dihydrosphingosine is formed in neural tissue in a manner analogous to the yeast pathway depicted in Fig. 1, and its synthesis does not require any additional cofactors. The data rule out palmitaldehyde as a direct precursor of LCB in the nervous system. Synthesis of the C_{20} homologs of dihydrosphingosine and sphingosine occurs by similar routes in both yeast (36) and brain (43). This pathway is of special significance in the nervous system because the long-chain base composition of some brain sphingolipids changes markedly during development. Rosenberg and Stern (45) demonstrated that gangliosides of fetal rat brains contain only C_{18} bases, whereas postnatal development is accompanied by an increase of C_{20} bases; both bases are present in nearly equal amounts in the gangliosides of adult rats.

Brain microsomes also contain an enzymatic acylating system which converts part of the biosynthetically produced dihydrosphingosine to acyldihydrosphingosine (ceramide) and which also gives rise to acylated sphingosine (43, 46). Sphingosine does not accumulate as the free base in this system (43), possibly because it is acylated more rapidly than dihydrosphingosine. In this regard, the cell-free yeast preparations have been very useful, since sphingosine accumulates and may be isolated. Evidently, acylation occurs at a slower rate in yeast than in brain. Although the precursor of sphingosine, 2-hexadecenoyl CoA, is probably the same in brain as in yeast, the interconversion of this compound with palmitoyl CoA by enzymes of neural origin remains to be determined. A recent report on attempts to separate and purify the enzymatic components of sphingolipid synthesis indicates that this approach may provide some details of the biosynthetic mechanisms (47).

Efforts to establish the biosynthetic route to phytosphingosine in vitro have thus far been only partially successful (32, 33, 44). In vivo experiments have suggested that synthesis of this base may occur via an intermediate common to dihydrosphingosine formation. Stoffel, Sticht, and LeKim (48) showed that cells of *H. ciferri* grown in a medium containing [1-^{14}C, 3-^{3}H]dihydrosphingosine can utilize this base to form phytosphingosine with loss of most of the tritium at C-3. It appears, therefore, that dihydrosphingosine is first transformed to ketodihydrosphingosine, with subsequent hydroxylation occurring at C-4 to form a carbonyl analog of phytosphingosine:



These results contradict an earlier report of Weiss and Stiller (49), who noted the incorporation of [4,5-^{3}H]dihydrosphingosine into phytosphingosine in the same organism and concluded from the ratio of isotope in substrate and product that dihydrosphingosine was hydroxylated intact. Other suggested pathways, such as the hydration of sphingosine, appear to be untenable in the light of available experimental data (48). Polito and Sweeley (44) followed the incorporation of perdeuterated palmitate into phytosphingosine by cells of *H. ciferri*. The phytosphingosine was found to be labeled with 30 deuterium atoms, thereby establishing that the hydroxylation of ketodihydrosphingosine (or possibly dihydrosphingosine) occurred directly. An alternative pathway, involving dehydrogenation of ketodihydrosphingosine and hydration of the resulting ketosphingosine, was excluded by these results since, in this case, an additional deuterium atom would be displaced. A similar approach was used to determine that the introduction of the hydroxyl group at C-4 occurs stereospecifically with retention of configuration (44). The mechanism by which hydroxylation might occur is particularly intriguing in view of the studies by Thorpe and Sweeley (50). They used a combined gas–liquid chromatography–mass spectrometry procedure and showed that none of the oxygen atoms of phytosphingosine was derived from molecular oxygen. Since the hydroxyl group on C-4 of phytosphingosine is only slightly labeled with ^{18}O from water, it is likely derived from some unknown hydroxyl donor in the medium. Clarification of this problem may have to await the development of an in vitro biosynthetic system.

Ceramide

Early work by Zabin (51) demonstrated that a rat brain microsomal fraction, in the presence of palmitoyl CoA and an NADPH-generating system, could bring about the incorporation of radioactive serine into ceramide. The interpretation of this result was that a long-chain base was formed initially and then acylated to form ceramide. This hypothesis was supported by the observation that exogenous LCB reduced the amount of radioactivity incorporated into ceramide. Later, Sribney (52) demonstrated the in vitro acylation of LCB to form ceramide. This reaction was dependent on acyl CoA or an acyl CoA-generating system.

Since ceramide is probably an intermediate in the formation of most sphingolipids, it is likely that the initial *N*-acyl fatty acid distribution of sphingolipids is determined when ceramides are synthesized. The distribution of *N*-acyl fatty acids of brain sphingolipids (45, 53–57) exhibits specificity with respect to the type of sphingolipid, its location in the brain, age of the animal, etc. Morell and Radin (46) have examined the mouse brain acyl CoA:LCB acyltransferase activity in detail with respect to its specificity for acyl CoAs and LCBs. The rate and extent of conversions of stearoyl, lignoceroyl, palmitoyl, and oleoyl CoAs to ceramide were in the ratio of about 60:12:3:1. This ratio might be expected from the relative distribution of fatty acid moieties of brain sphingolipids, taking into account the relatively rapid turnover of gangliosides compared with whole brain sphingolipids (58, 59). Gangliosides contain mostly stearic acid in the *N*-acyl position, and this could account for the bias in stearic acid utilization. There is also evidence that the stearic acid-containing galactosylceramides turn over more rapidly than longer-chain homologs (60). Ullman and Radin[1] have studied the incorporation of stearoyl CoA and lignoceroyl CoA into ceramides, using brain microsomal preparations from mice of several ages. Enzymatic activity for synthesis of lignoceroyl-sphingosine rose rapidly, during myelinogenesis, compared with activity for formation of stearoyl-sphingosine. This is tentative evidence for the involvement of at least two enzyme systems in the synthesis of ceramide. In any case, the data demonstrating relatively low amounts of palmitic and oleic acids in *N*-acyl linkages of brain sphingolipids are readily explained by the observed specificity in vitro. This specificity is highlighted by the observation that in young mice roughly 40% of the total fatty acid moieties consist of palmitic and oleic acids in ester linkages (61). Since total brain phospholipids turn over relatively rapidly, and since their synthesis involves acyl CoA derivatives (see Eichberg, Hauser, and Karnovsky [62] for a review), a large proportion of the acyl CoAs synthesized must be palmitoyl and oleoyl CoAs. Thus, the ceramide synthetases must exhibit considerable specificity to exclude these acyl CoAs. There is little difference between utilization of *erythro-* and *threo*-sphingosines either in vitro (46) or in vivo (63). In vitro experiments with exogenous acceptor did not reveal any marked specificity for sphingosine over dihydrosphingosine (46). However, as indicated previously, experiments with *in situ* formation of LCB indicated that sphingosine was preferentially acylated to form ceramide.

Yavin and Gatt (64) have proposed that ceramide is biosynthesized by a reversal of the ceramidase reaction (see below) which hydrolyzes ceramide to free fatty acid and LCB. They explain Sribney's (52) results by suggesting that the acyl CoA is hydrolyzed to free fatty acid before the formation of ceramide. However, the synthetic activity of ceramidase isolated from brain is effective with fatty acids that occur in brain sphingolipids only to a negligible extent. Indeed, the efficacy of fatty acids as substrates for ceramidase correlates with water solubility, and it appears possible that the synthetic capability of ceramidase results from conversion of a relatively soluble substrate to an insoluble ceramide. This result is in contrast to the physiological specificity of the acyltransferase (46). Further evidence against a biosynthetic role for ceramidase was obtained in studies of subcellular fractions when it was demonstrated that enzymatic activity for ceramide synthesis is concentrated in the microsomal fraction while ceramidase has its highest specific activity in lysosome-rich fractions (46). However, the possibility that ceramidase is involved in turnover of sphingolipids in membranes cannot be ruled out.

The above discussion of ceramide biosynthesis relates only to the formation of nonhydroxy FA-ceramide. It has been tacitly assumed that biosynthesis of hydroxy FA-ceramide involves a hydroxy fatty-acyl CoA derivative. In the case of brain sphingolipids, galactosylceramides containing hydroxyl fatty acids in the *N*-acyl position account for almost 70% of the total (53, 55, 56), and hydroxy fatty acids are also found in sulfatides. Sphingolipids containing hydroxy fatty acids are also of importance in extraneural tissues (3, 65).

Ullman and Radin[1] have synthesized hydroxy fatty acyl CoAs and have demonstrated in vitro biosynthesis of hydroxy FA-ceramide by a rat brain preparation. They have also obtained kinetic evidence for independent utilization of stearoyl CoA and its hydroxy analog in formation of ceramide, implying the existence of separate enzymes.

Sphingomyelin

The enzymatic transfer of phosphorylcholine from CDP-choline to ceramide was first demonstrated by

[1] Ullman, D., and N. S. Radin. Personal communication.

Sribney and Kennedy (66), who used a particulate preparation from chicken liver (Fig. 2). However, under the assay conditions utilized, only ceramides with relatively short chain (C_2–C_{12}) *N*-acyl fatty acids were active as acceptors. It was also shown that only the nonphysiological isomer *threo*-ceramide was active as an acceptor, although naturally occurring sphingolipids contain the *erythro* isomer. In a later study, Sribney (67) studied the ability of microsomal and mitochondrial fractions to effect the transfer of phosphorylcholine from CDP-choline to *N*-acetyl-*threo*- or *N*-acetyl-*erythro*-sphingosine. The results indicated that mitochondria were able to transfer phosphorylcholine to either the *threo*- or *erythro*-ceramide analogs. Microsomes utilized only the unnatural *threo* isomer, and furthermore, the presence of microsomes inhibited utilization of the *erythro* analog by mitochondria.

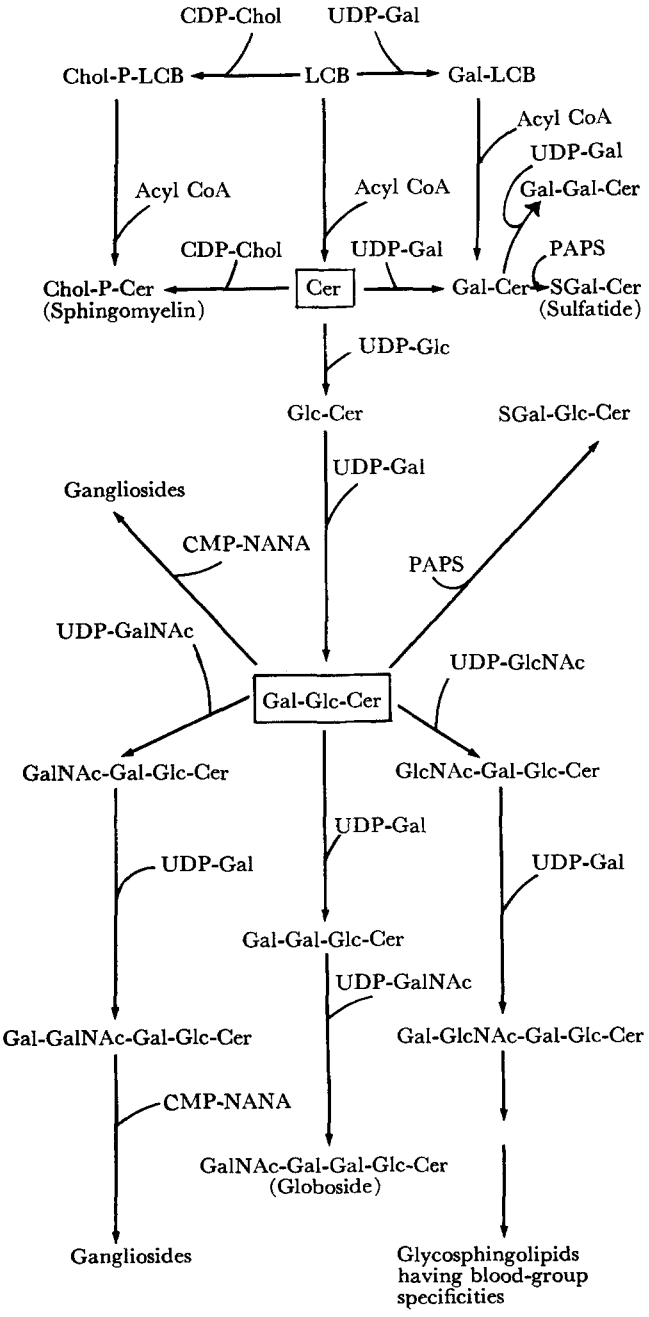


FIG. 2. Biosynthesis of complex sphingolipids. Abbreviations: Chol, choline (Chol-P-Cer is sphingomyelin); S, sulfate on position 3 of galactose; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; and NANA, *N*-acetylneuraminic acid.

It is likely that the specificity for the *threo*-ceramide derivatives observed in the above studies is artifactual in nature arising from the use of detergents and nonphysiological substrates, a possibility discussed by Rosenberg (68) and Radin (69). Fujino et al. (70), using a different detergent combination, have reported that microsomal and mitochondrial fractions can utilize either *erythro*- or *threo*-ceramides as substrates for sphingomyelin formation. The same lack of *threo–erythro* specificity for sphingomyelin biosynthesis has been observed in vivo. The in vitro work, and the in vivo studies by Kopaczyk and Radin (71), indicate that ceramide is the major metabolic precursor of sphingomyelin.

Another pathway for sphingomyelin formation, acylation of sphingosylphosphorylcholine, was reported by Brady et al. (72). Mitochondrial or microsomal fractions were shown to contain activity for acylation (by [^{14}C]-stearoyl CoA) of exogenous sphingosylphosphorylcholine (Fig. 2). The enzymatic activity in the mitochondrial fraction could be solubilized with sodium cholate. This pathway was confirmed by Fujino and Negishi (73), who also demonstrated that either *erythro*- or *threo*-sphingosylphosphorylcholine could be utilized as substrates. The obligatory initial step for sphingomyelin biosynthesis, formation of sphingosylphosphorylcholine, via this pathway was reported by Fujino, Negishi, and Ito (74). Either *threo*- or *erythro*-sphingosines serve as acceptors for the choline moiety of CDP-choline. This reaction is catalyzed by preparations of microsomes or mitochondria. Possibly these enzymes are responsible for the turnover of sphingomyelin in mitochondrial membranes.

Galactosylceramide

Most work on the metabolism of galactosylceramide has been done with brain tissue extracts because young mammals synthesize large amounts of this lipid during myelin formation. Brain cerebrosides are very heterogeneous with respect to their *N*-acyl fatty acids; both saturated and unsaturated hydroxy and nonhydroxy fatty acids with 16 to 28 carbons are found (75). In vitro studies by Burton, Sodd, and Brady (76) demonstrated the formation of galactosylceramide from the galactose moiety of UDP-Gal and an endogenous precursor. Two alternative biosynthetic routes were proposed:

(*a*) Acylation of a long-chain base followed by galactosylation:

long-chain base + acyl CoA → ceramide + CoA (I)

ceramide + UDP-Gal →

galactosylceramide + UDP (II)

(*b*) Galactosylation of a long-chain base to form psychosine, followed by acylation:

long-chain base + UDP-Gal → psychosine + UDP (III)

psychosine + acyl CoA →

galactosylceramide + CoA (IV)

The fatty acyl CoA moiety above is assumed to contain either a hydroxy fatty acid or a nonhydroxy fatty acid, respectively. Both pathways can be demonstrated in vitro (see Fig. 2). The galactosylation of hydroxy FA-ceramide to form hydroxy FA-galactosylceramide (reaction II) has been demonstrated by Morell and Radin (19) and Basu et al. (77), who used particulate fractions from developing brain. This reaction has been confirmed both in brain tissue (78–80) and in kidney (81). Enzymatic activity is fivefold higher in brain white matter than it is in gray matter (80). Further evidence that exogenous hydroxy FA-ceramide is incorporated intact into galactosylceramide was obtained by use of synthetic substrates and by mass spectrometric identification of the product (79).

It has been demonstrated that nonhydroxy FA-galactosylceramide is also synthesized by galactosylation of ceramide. Morell, Costantino-Ceccarini, and Radin (82) reported that galactosylation of exogenous nonhydroxy FA-ceramide by a crude microsomal system is stimulated by the addition of crude lecithin to the incubation mixture; this unusual result has recently been confirmed (83). Assay of nonhydroxy FA-galactosylceramide synthesis is complicated by relatively low activity of this enzyme; activity for hydroxy FA-galactosylceramide synthesis is three times higher.[2] There is also considerable incorporation of galactose into nonhydroxy FA-galactosylceramide in the absence of exogenous substrate (82), presumably due to relatively high levels of endogenous nonhydroxy FA-ceramide present in brain (84, 85). Specificity in the utilization of the exogenous ceramide was demonstrated (82) by showing that the chain length of the fatty acid in the biosynthesized galactosylceramide was the same as that of the synthetic precursor ceramide. Incorporation of the nonhydroxy FA-ceramide was also shown directly by using radioactive ceramide (82) and by mass spectrometric techniques (83). Enzymatic activity for nonhydroxy FA-ceramide galactosylation is relatively easily inactivated (compared with activity for hydroxy FA-ceramide galactosylation) by treatment of microsomes with acetone or detergents.[3] It is not clear whether this is due to the existence of separate enzymes for nonhydroxy FA- and hydroxy FA-galactosylceramide biosynthesis or whether the availability of the two substrates to a common enzyme system is differentially affected by these treatments.

The alternative pathway for nonhydroxy FA-galactosylceramide biosynthesis must also be considered, since reaction III, formation of psychosine, has been demonstrated by Cleland and Kennedy (86) and confirmed by a number of authors (19, 87, 88). Acylation of psychosine by acyl CoA to form nonhydroxy FA-galactosylceramide was reported by Brady (89). Although we were unable to reproduce this result under a variety of conditions (82), a recent report confirming the acylation of psychosine has appeared (90). There is a need for further investigation of the psychosine pathway to clarify its role in galactosylceramide biosynthesis; in vitro studies of enzyme kinetics and developmental studies would appear to be feasible at the present time.

Sulfatides

Transfer of radioactive sulfur from 3′-phosphoadenosine-5′-phosphosulfate to galactosylceramide to form galactosylceramide sulfate (sulfatide) has been demonstrated in rat (91) and sheep (92) brain preparations (Fig. 2). Balasubramanian and Bachhawat reported (92) that exogenous galactosylceramide was not sulfated, and they presented evidence that, in their system, only an *in situ* protein-bound substrate could act as acceptor. However, McKhann and coworkers (93, 94) were able to solubilize the enzyme and then show stimulation by exogenous galactosylceramide. The peak of enzymatic activity in rat brain occurs during the period of maximal myelination (93), an expected result since brain sulfatide is found largely in myelin. A sulfated lactosylceramide is found in kidney (95), and the brain enzyme can catalyze the transfer of sulfate to lactosylceramide (94, 96). The incorporation of radioactive galactosylceramide into sulfatide has been demonstrated using rat kidney (97) and brain (94) preparations. The in vitro product has the sulfate on position 3 of galactose (97), as does the natural sulfate.

Glucosylceramide

Glucosylceramide is found in many extraneural tissues (3). Its biosynthesis is also of interest as an initial step in formation of gangliosides. Basu, Kaufman, and Roseman (98) used a particulate preparation from embryonic chicken brains to demonstrate that ceramide

[2] Morell, P., and E. Costantino-Ceccarini. Unpublished results.

[3] Morell, P., and N. S. Radin. Unpublished results.

can be glucosylated, by UDP-Glc, to form glucosylceramide (Fig. 2). The enzyme from mouse brain does not exhibit specificity for chain length; lignoceroyl-sphingosine is as active a substrate as stearoyl-sphingosine (82) and hydroxy FA-ceramides are also utilized[2] (99). This is a somewhat unexpected result because brain gangliosides contain largely stearoyl-LCB as their ceramide component. The enzyme from embryonic chicken brain has been reported to be thermally labile, being inactivated after about 15 min of preincubation at 37°C in the absence of UDP-Glc. This may represent, in part, the denaturing activity of the detergent present. Enzymatic activity from mouse brain is linear with time for several hours when the nonhydroxy FA-ceramide substrate is coated on Celite, although reactivity drops off rapidly if detergent is used to emulsify the substrate.[2]

Oligoglycosylceramides

These neutral oligoglycosylceramides whose biosynthesis has been studied may, with minor exceptions, be classified into three groups (Table 1): (*a*) the tetraglycosylceramide, globoside I (100–102), (*b*) the tetraglycosylceramide backbones of blood-group substances (8), and (*c*) asialogangliosides (103), as well as di- and triglycosylceramides, which share a common sequence with one of the tetraglycosylceramides. Globoside I (GalNAc-Gal-Gal-Glc-Cer) and glycosphingolipids chemically related to it by removal of one or more sugars, Gal-Gal-Glc-Cer, and lactosylceramide are widely distributed in extraneural organs (3). Formation of lactosylceramide has been demonstrated in Hauser's laboratory (104, 105) and by Basu et al. (98). The enzymatic activity from both rat spleen (105) and embryonic chicken brain (98) is particulate and rather heat-sensitive. Incorporation of radioactive glycosylceramide into lactosylceramide has been demonstrated (105). The next step, galactosylation of lactosylceramide by UDP-Gal, can be catalyzed by spleen (105) and kidney (81, 106) homogenates. The two enzymatic activities which transfer galactose to glucosylceramide and lactosylceramide, respectively, can be differentiated by heat inactivation, and they are inhibited by different sphingolipids (105). The final step in globoside synthesis, possibly *N*-acetylgalactosylation by UDP-GalNAc has not yet been studied.

TABLE 1. Anomeric structure of ceramide tetrahexosides

Globoside I	GalNAc(β,1–3)Gal(α,1–4) Gal(β,1–4)Glc(1–1)Cer
Sphingolipids with blood-group specificities (common backbone)	Gal(β,1–3)GlcNAc(β,1–4) Gal(1–4)Glc(1–1)Cer
Asialoganglioside	Gal(β,1–3)GalNAc(β,1–4) Gal(β,1–4)Glc(1–1)Cer

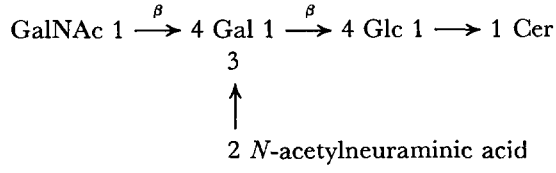
Several glycosphingolipids having blood-group specificities and sharing the common tetrahexosylceramide backbone Gal-GlcNAc-Gal-Glc-Cer have been described (8). The committed step in the synthesis of this compound, *N*-acetylglucosylation of lactosylceramide, has been demonstrated by Basu et al. (107). Recently Basu and Basu (108) have demonstrated another step in this biosynthetic sequence, galactosylation by UDP-Gal of GlcNAc-Gal-Glc-Cer.

The major pathway for biosynthesis of gangliosides probably involves addition of sialic acid to lactosylceramide (109). However, there is some evidence indicating the possibility that the entire tetraglycosylceramide backbone of gangliosides can be assembled before the addition of sialic acid. Handa and Burton (110) used a particulate fraction from young rat brain to demonstrate that lactosylceramide could act as an acceptor for the sugar moiety of UDP-GalNAc. The next step, galactosylation by UDP-Gal of GalNAc-Gal-Glc-Cer, was demonstrated by Yip and Dain (111). Although the enzymatic activity is present in young rat brain, it was found to be most active in frog brain; the enzyme activity from frog brain has been solubilized and characterized (111). A minor component of rat kidney, digalactosylceramide, has been characterized and its synthesis by a kidney homogenate has been studied in Gray's laboratory (81, 106). The galactosylation of galactosylceramide by UDP-Gal was demonstrated. A previously reported failure to demonstrate this reaction (98) may have been due to use of detergents in the incubation mixture. Gray and his coworkers utilized Celite instead of detergents to disperse the substrate. The biosynthetic steps discussed above are summarized in Fig. 2.

CATABOLISM

Oligoglycosylceramides

Globoside I (GalNAc-Gal-Gal-Glc-Cer) can be hydrolyzed by an enzyme isolated from calf brain by Frohwein and Gatt (112). Localization in subcellular fractions before solubilization (113) and the acid pH optimum indicated that the enzyme originated in lysosomes. Further insight into the physiological role of hexosaminidases has been obtained by study of two human inborn errors of metabolism: (*a*) Tay-Sachs disease, which is characterized chemically by storage of a particular ganglioside (114, 115)



and (*b*) a variant of this disease characterized by storage of the Tay-Sachs ganglioside and globoside (Table 1) in visceral organs. It has been observed in many laboratories that human hexosaminidase can be split into two fractions by starch–gel electrophoresis or isoelectric focusing. One of these activities, hexosaminidase A, is missing in Tay-Sachs disease (116, 117), a finding which correlates with the inability of human muscle tissue from Tay-Sachs patients to hydrolyze the terminal *N*-acetylgalactosaminyl moiety of Tay-Sachs ganglioside (118). Sandhoff (117) has shown that in the variant form of Tay-Sachs disease characterized by visceral storage of globoside both hexosaminidase A and B are missing. This correlates with the total lack of hexosaminidase activity associated with these patients (119). On this basis it is assumed that the physiological role of hexosaminidase A is in ganglioside metabolism, whereas hexosaminidase B is involved in globoside degradation.

After removal of the terminal hexosamine from globoside I, the new terminal galactose residue of the triglycosylceramide can be removed by a galactosidase. Such an enzyme, relatively specific for this substrate (since it is inactive against both galactosyl- and lactosylceramides), was purified over 220-fold by Brady et al. (120). Although the enzyme was purified from rat intestinal tissue only, it was shown to be present in brain and extraneural organs. Since in patients with Fabry's disease the enzyme is totally absent in small intestine as well as in other extraneural organs (121) and body fluids (122), it is likely that the enzyme in intestine is the same as that present in other organs. Kint has demonstrated (123) that the missing enzyme in Fabry's disease is an α-galactosidase, and the enzymatic defect can now be assayed specifically by a colorimetric determination with synthetic substrates.

Lactosylceramide galactosidase is present in rat brain preparations (124), and it has been partially purified with respect to its ability to hydrolyze β-galactosides preferentially to β-glucosides (125). The reaction required a low concentration of bile salts (124, 126). A similar activity was characterized in kidney (127). A clinically distinct lipid storage disease, characterized by accumulation of lactosylceramide, has been related to a marked deficiency in lactosylceramide galactosidase (128).

Gangliosides are degraded in a stepwise manner (15, 16) to a monosialolactosylceramide, hematoside. With the removal of the remaining sialic acid to form lactosylceramide, the degradative pathway converges with that of globoside catabolism. It is likely that lactosylceramide from both globoside and gangliosides is metabolized by the same enzymes; this would account for the accumulation of lactosylceramide in extraneural organs as well as in brain in lactosylceramidosis (128). The reactions discussed above are summarized in Fig. 2.

Glucosylceramide

An enzyme which hydrolyzes glucosylceramide to glucose and ceramide (Fig. 3) has been purified 82-fold from a 100,000 *g* supernatant fraction of human spleen tissue (129). A similar enzyme was obtained from a crude mitochondrial fraction of ox brain (130). This brain glucosylceramide glucosidase was purified 10-fold (125). The spleen enzyme is soluble, in contrast with the particulate nature of glucosylceramide glucosidase from brain (125), leukemic myelocytes (131), rat liver, and rat kidney (132).

A deficiency in glucosylceramide glucosidase is observed in patients with Gaucher's disease (133), and there is concomitant accumulation of glucosylceramide in spleen and other visceral organs. It is likely that the glucosylceramide which accumulates originates from the sphingolipids of granulocytes (134) or globoside I of erythrocytes (135). It is of interest that in some cases of the infantile form of this disease there is severe damage of CNS neurons; presumably the glucosylceramide accumulates (136) in these cases as a degradation product of gangliosides. Gaucher's disease can be diagnosed by glucosylceramide glucosidase assay of leukocytes (137) and tissue cultures (138).

An enzyme from rat small intestine, purified 2000-fold in 38% yield, was shown to be active with both glucosylceramide and galactosylceramide (139); the reaction products are free sugar and ceramide. An in vivo study (140) demonstrated the physiological importance of this enzyme and its localization within the mucoidal cells.

Sulfatides

An enzyme which cleaves the sulfate from sulfatide has been purified 6000-fold from pig kidney (141). As is the case with most sphingolipid hydrolases, the activity was present primarily in a particulate fraction and had an acid pH optimum (suggesting that it is probably lysosomal). The purified enzyme showed arylsulfatase A activity but demonstrated relatively low activity against sulfatide until it was recombined with a high molecular weight, heat stable factor. In a further study (142), arylsulfatase A and sulfatide sulfatase activities were compared in various fractions obtained by electrophoresis. Arylsulfatase A and sulfatide sulfatase activities did not migrate together, and the slight difference in migration was assumed to be due to separation of the enzyme from the heat stable "complementary" fraction required for full activity against sulfatide. Further evidence that arylsulfatase A is related to sulfatide sulfatase comes from observations that in cases of metachromatic leukodystrophy, a disease characterized by accumulation of sulfatide (143), both enzymes are depressed (144, 145).

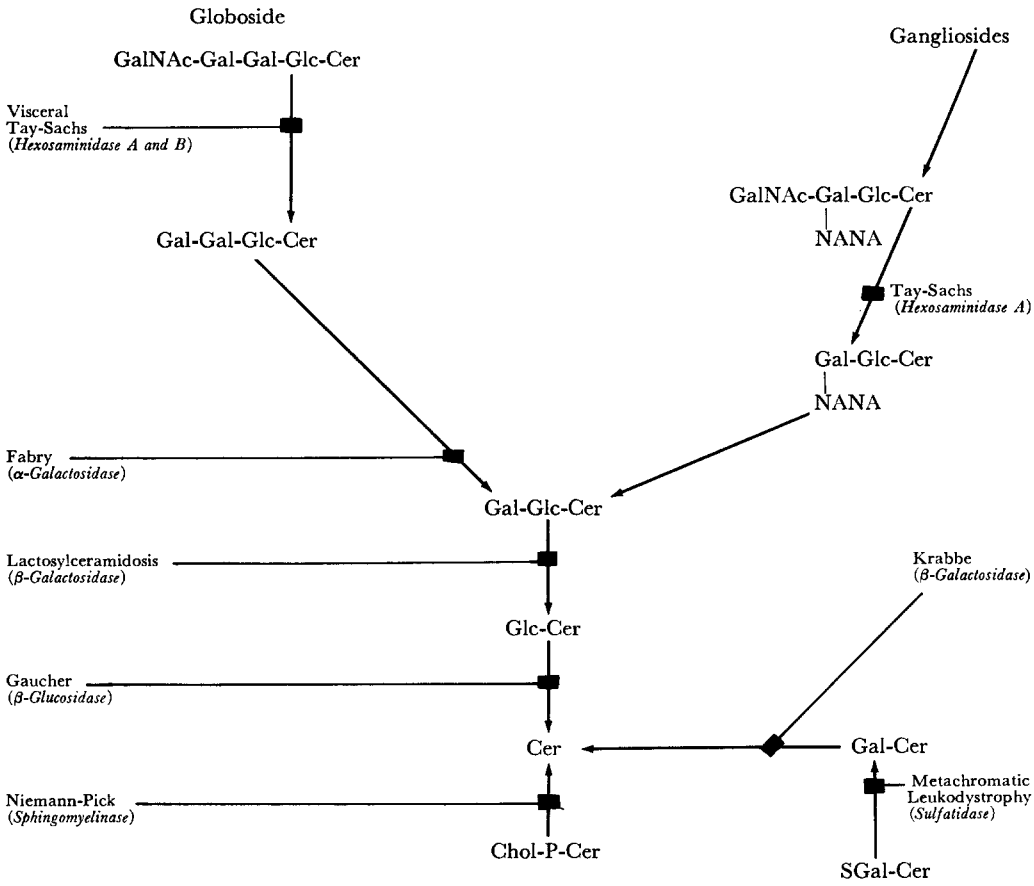


FIG. 3. Reaction sequence for metabolic degradation of complex sphingolipids. The enzymatic deficiencies characteristic of the various human diseases are indicated in italic type (e.g., in Gaucher's disease the β-glucosidase catalyzing the degradation of lactosylceramide to glucosylceramide is depressed). Abbreviations are as in Fig. 2.

Data obtained in developmental studies, and topographical distribution within brain, are consistent with the identity of these two enzymes (146).

Galactosylceramide

In vitro degradation of galactosylceramide to galactose and ceramide (Fig. 3) was demonstrated by Hajra et al. (147). Cerebroside galactosidase from rat brain was purified by Bowen and Radin (148); a 300-fold increase in specific activity with 27% recovery was achieved. One step was an extensive digestion with pancreatic enzymes in the presence of sodium cholate. Hydrolytic activity of the purified galactosidase was largely dependent on the presence of bile salts in the incubation mixture (149). The reason for this bile salt requirement is not known (150). The enzyme is lysosomal, with an apparent molecular weight of 50,000 (149). The cerebroside galactosidase was not completely separated from cerebroside glucosidase activity.

A convenient assay for galactosylceramide galactosidase, applicable to crude homogenates, has been devised by Bowen and Radin (151). This procedure was adapted by Suzuki and Suzuki (152) to demonstrate a marked deficiency of this enzyme in brains of patients with Krabbe's globoid cell leukodystrophy.

Arora and Radin (153, 154) have tested a series of compounds resembling ceramide for their effect on galactosylceramide galactosidase of brain and kidney. Although many of these compounds are inhibitory, one was strongly stimulatory at a concentration as low as 1 mM. These results have been discussed in terms of the mechanism of enzyme action and related to possible in vivo control mechanisms.

Sphingomyelin

Several bacteria are known to contain phospholipase C activity that degrades sphingomyelin to ceramide and phosphorylcholine (see Ref. 155 for a review). The specificities of these enzyme fractions are rather low since they are active against lecithin, phosphatidylethanolamine, and other phospholipids. A sphingomyelinase with a phospholipase D-type specificity, namely, hydrolysis of sphingomyelin to *N*-acylsphingosylphosphate and choline has been studied. This extra-

cellular enzyme of *Corynebacterium ovis* is relatively specific for sphingomyelin (156).

Catabolism of sphingomyelin in mammalian tissue proceeds via a splitting of the phosphodiester bridge to yield ceramide and phosphorylcholine (Fig. 3). Such enzymes have been partially purified from crude mitochondrial fractions of rat liver (157, 158), rat brain (159), and human spleen (160).

Sphingomyelinase from mammalian tissues has been solubilized by sonication (157), treatment with detergents (159), or, in the case of the human spleen enzyme (160), by dialysis under specified conditions. The specificity of these preparations for the natural, *erythro* isomer of sphingomyelin and lack of activity against lecithin have been described (158, 159). Studies of possible substrates and inhibitors (159) are difficult to interpret because of lack of information as to the effect of these compounds on mixed micelles of substrate and detergent and consequent accessibility to the enzyme (68). The acid pH optimum and detergent stimulation of these mammalian sphingomyelinases are indicative of a lysosomal origin. This was confirmed (132) by subcellular fractionation and assay of sphingolipid hydrolase and marker enzymes. In this study, Weinreb, Brady, and Tappel (132) observed also that a significant amount of sphingomyelinase (more than could be accounted for by contamination) was present in mitochondria. This observation, together with evidence that enzymes involved in sphingomyelin biosynthesis are present in mitochondrial fractions (67, 70, 72, 74), supports a hypothesis that sphingomyelin metabolism may take place independently in mitochondria. A comparative study of the enzymes in microsomes and mitochondria would be of considerable interest.

An in vivo study of the catabolism of injected tritiated dihydrosphingomyelin confirms the in vitro studies by providing evidence for degradation in the sequence dihydrosphingomyelin, *N*-acyldihydrosphingosine, dihydrosphingosine (161). A deficiency of sphingomyelinase has been demonstrated in Niemann-Pick disease (160, 162). Lack of this enzyme is responsible for massive accumulation of sphingomyelin in liver and spleen. This enzyme deficiency is detectable in circulating leukocyte preparations (132) and in tissue cultures (163). Dietary sphingomyelin is hydrolyzed by a pancreatic enzyme that, unlike lysosomal enzymes, has a slightly alkaline pH optimum (164).

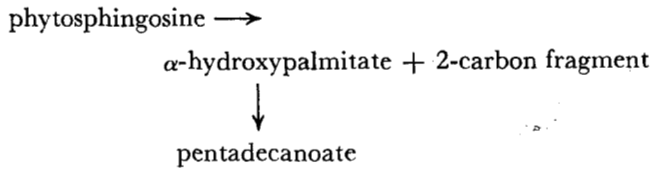
Ceramide

A ceramidase has been purified 200-fold from the brains of 16-day-old rats. To achieve this purification Yavin and Gatt (64) took advantage of the marked resistance of this enzyme to proteolytic digestion, and used trypsin and chymotrypsin to digest other proteins. The purified enzyme preparation required sodium cholate or sodium taurocholate for activity and was stimulated further by Triton X-100. This ceramidase preparation was active in degrading palmitoyl-, stearoyl-, and oleoyl-sphingosine as well as dihydrosphingosine analogs. It was reported that neither acetylsphingosine nor lignoceroyl-sphingosine was hydrolyzed. If this represents a true specificity of the purified enzyme, then other enzymes may exist for the degradation of the very-long-chain ceramides. Another possible explanation is the extreme insolubility of lignoceroyl-sphingosine which might make it inaccessible to the enzyme. The enzyme did not attack galactosylceramide or sphingomyelin.

The question of specificity needs to be further investigated by the use of a different assay in which solubility of substrate is not so important (e.g., by use of a solid support) and/or by synthesis of more soluble long-chain ceramides (e.g., nervonoyl-sphingosine).

Long-chain bases

The metabolic fate of the sphingolipid bases has only recently been investigated. Several investigators (165–168) have described the fate of long-chain bases in rats after parenteral administration of radioactive precursors. Tritium-labeled phytosphingosine was shown to be converted mainly to pentadecanoic acid, whereas dihydrosphingosine and sphingosine were primarily degraded to palmitic acid. The possibility that phytosphingosine might be an intermediate in the degradation of the other two bases was eliminated by these data. Subsequent studies (167) indicated that cleavage between carbon atoms 2 and 3 of phytosphingosine could also occur and that the product, α-hydroxypalmitate, may then give rise to pentadecanoic acid:



In parallel experiments, Stoffel and Sticht (169, 170) investigated the metabolism of isotopically labeled bases and demonstrated that the 2-carbon cleavage product of dihydrosphingosine and sphingosine was ethanolamine, or its derivative, which was subsequently incorporated into phosphatidylethanolamine and phosphatidylcholine. Both *threo* and *erythro* isomers of the administered base were similarly degraded. Similarly, in vivo experiments with C_{20}-dihydrosphingosine revealed a cleavage pathway resulting in a 2-carbon fragment and stearic acid (171). Independently, Keenan and Okabe (172) observed that tritiated dihydrosphingosine was degraded to palmitic acid in rats with retention of tritium at position 2.

This observation led them to conclude that sphingosine is not an intermediate.

Metabolism of phytosphingosine was studied by Karlsson, Samuelsson, and Steen by growing the yeast *Hansenula ciferri* in the presence of the ^{14}C-labeled base (173). Formation of ethanolamine and α-hydroxypalmitate suggested a catabolic mechanism similar to that found in rats. This observation was confirmed by Stoffel et al. (48). It was then established (174) that 3-ketodihydrosphingosine is not an intermediate in the degradation of dihydrosphingosine in vitro, as has been concluded from in vivo experiments (39). Instead, subcellular fractions of rat liver contain a very active reductase which reduces the carbonyl compound to dihydrosphingosine. Catabolism of this base then proceeds by phosphorylation of the primary hydroxyl group, followed by cleavage of a 2-carbon fragment which was identified as phosphorylethanolamine (Fig. 4). The other products of this pathway were found to be a mixture of palmitaldehyde and palmitic acid. The use of a doubly labeled substrate, [3-^{14}C, 3-^{3}H]-dihydrosphingosine, provided evidence that the phosphorylated base is split into palmitaldehyde (with retention of both labels) and phosphorylethanolamine in an aldolase-type reaction, with subsequent oxidation of the palmitaldehyde to palmitic acid (Fig. 4). In vivo studies have provided additional support (175) for this catabolic scheme. The degradation of sphingosine follows a similar route (176). The aldehyde formed is an efficient precursor of the aldehydogenic chain of plasmalogens (14).

Enzymatic catalysis of the cleavage reaction by a rat liver mitochondrial fraction requires ATP and Mg^{2+} and appears to be analogous to the pyridoxal phosphate-dependent cleavage of threonine by threonine aldolase (177). Keenan and Haegelin (178) and Hirschberg, Kisic, and Schroepfer (179) have clearly demonstrated the enzymatic synthesis of labeled dihydrosphingosine phosphate from dihydrosphingosine and ATP. Neither the quantitative relationships between synthetic and degradative pathways for sphingolipid bases nor the regulation of these metabolic events has as yet been investigated.

INTERRELATIONSHIPS OF METABOLIC PATHWAYS INVOLVING SPHINGOLIPIDS

There is a general assumption that pathways of sphingolipid metabolism are controlled by the same type of mechanisms operative for other metabolic pathways. However, experiments which can be discussed in terms of control mechanisms such as induction, repression, or feedback inhibition of enzymes involved in sphingolipid metabolism are rare. Perhaps the most important obstacle to progress in this field is the lack of purified enzyme preparations and the consequent inability to determine quantities of a given enzyme. Indeed, because of the heterogeneity of fatty acid moieties and LCBs within a given class of sphingolipids we are uncertain of the number of different enzymes that may be involved in carrying out related biosynthetic reactions (e.g., is the specificity of ceramide galactosylation accounted for by one enzyme or a group of enzymes?).

Some of the questions with regard to metabolic interrelationships and perhaps the number of enzymes involved in related reactions can be approached by indirect methods. For example, enzymes active in biosynthesis of the sphingolipids of myelin can be characterized through developmental studies. The appearance of enzymatic activity in brain for galactosylation of hydroxy FA-ceramide to form hydroxy FA-galactosylceramide (80, 99) indicates that this enzyme is probably involved in myelination. The dependence of activity on the age of the animal clearly differentiates this enzyme from the enzyme involved in galactosylation of glucosylceramide to form lactosylceramide (88).

Perturbed systems can also be useful in obtaining information as to control of sphingolipid metabolism. For instance, injection of erythrocyte stroma into rat causes a twofold increase in several of the sphingolipid hydrolase activities of liver and spleen, indicating that possibly these enzymes are inducible to meet increased demand for their activity (180). An interesting bacterial system has recently become available. When grown in a vitamin

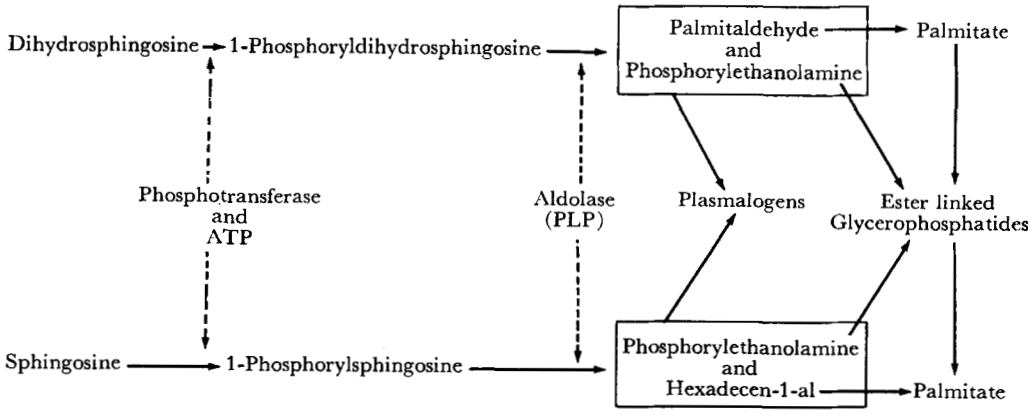


FIG. 4. Reaction sequence for metabolic degradation of sphingolipid bases.

K-supplemented medium, the anaerobic organism *Bacteroides melaninogenicus* contains nearly half of its phospholipids as the phosphosphingolipids ceramide phosphorylethanolamine and ceramide phosphatidylglycerol (2). In the absence of vitamin K, sphingolipid synthesis and growth cease (181). Succinate restores a slow rate of growth, and an aberrant form of elongated cell, with greatly reduced sphingolipid content, is formed. Vitamin K addition restores both normal growth and sphingolipid biosynthesis. Recovery of sphingolipid biosynthesis in depleted cells is very rapid, and this system may provide opportunities for studies of the control of sphingolipid biosynthesis. It is not known whether vitamin K plays a direct or indirect role in control of sphingolipid biosynthesis in this organism. The above observation, as well as the stimulation of CDP-choline:ceramide cholinephosphotransferase (sphingomyelin synthetase) of chicken liver mitochondria by vitamin K (67), suggests that the role of the vitamin in sphingolipid metabolism should be further investigated.

Advantage can be taken of mutations in higher organisms in which the genetic lesion affects either directly or indirectly an aspect of sphingolipid metabolism in a relatively specific fashion. It has been reported that in Krabbe's leukodystrophy the sulfate transferase activity involved in sulfatide biosynthesis is reduced (182). This result bears reinvestigation, since in the assay procedure utilized (182) only endogenous galactosylceramide served as acceptor. Galactosylceramide is present in greatly reduced amounts in Krabbe's brain, and lack of endogenous acceptor could account for the low enzyme activity. However, even if the interpretation of reduced sulfate transferase is correct, this effect is presumably secondary to the almost total lack of galactosylceramide galactosidase (151). A puzzling result has been obtained by Ullman and Radin,[1] who have assayed hydroxy FA-ceramide:galactose UDP-Gal transferase (cerebroside synthetase) and found this activity present at control levels. This observation makes it difficult to explain why galactosylceramide does not accumulate to massive levels in Krabbe's brain, if indeed synthesis of galactosylceramide is normal and catabolism is blocked. An animal model of Krabbe's leukodystrophy is available (183), and it would be of considerable interest to study the metabolism of sphingolipids in this system.

In a murine mutant characterized ultrastructurally as having an early arrest in myelinogenesis (184), there is a reduction of about 60% in enzymatic activity for galactosylation of both hydroxy FA- and nonhydroxy FA-galactosylceramide biosynthesis (185). Other enzymes, such as the one that mediates the glucosylation of ceramide, are at similar levels in the brains of control and mutant mice. This observation suggests that either a single enzyme is involved in galactosylation of different ceramides or that, if more than one enzyme is involved, these are controlled in a coordinate fashion.

Although sphingolipids are present in microorganisms (1, 2) and have been studied in yeast (186), no advantage has been taken of the mutability of these systems in studies of sphingolipid metabolism. Other potentially important fields of research have to do with interrelationships of sphingolipid and glycerophospholipid metabolism, through the degradation products of long-chain bases (13, 14, 187), as well as coordination of sphingolipid metabolism and structural protein metabolism in membrane systems (188–190). Sphingolipid metabolism is a field lagging at least a decade or two behind amino acid and nucleic acid metabolism. The gap may not be closed rapidly, but many exciting problems in this field, as well as the basic tools needed to attack them, are available.

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REFERENCES

1. White, D. C., A. N. Tucker, and C. C. Sweeley. 1969. Characterization of the iso-branched sphinganines from the ceramide phospholipids of *Bacteroides melaninogenicus*. *Biochim. Biophys. Acta.* **187:** 527–532.
2. LaBach, J. P., and D. C. White. 1969. Identification of ceramide phosphorylethanolamine and ceramide phosphorylglycerol in the lipids of an anaerobic bacterium. *J. Lipid Res.* **10:** 528–534.
3. Mårtensson, E. 1969. Glycosphingolipids of animal tissue. *Progr. Chem. Fats Other Lipids.* **10:** 365–407.
4. Karlsson, K.-A. 1970. On the chemistry and occurrence of sphingolipid long-chain bases. *Chem. Phys. Lipids.* **5:** 6–43.
5. Stoffel, W. 1971. Sphingolipids. *Annu. Rev. Biochem.* **40:** 57–82.
6. Rapport, M. M., L. Graf, V. P. Skipski, and N. F. Alonzo. 1958. Cytolipin H, a pure lipid hapten isolated from human carcinoma. *Nature* (London). **181:** 1803–1804.
7. Rapport, M. M., and L. Graf. 1969. Immunochemical reactions of lipids. *Progr. Allergy.* **13:** 273–331.
8. Hakomori, S. I. 1970. Glycosphingolipids having blood-group ABH and Lewis specificities. *Chem. Phys. Lipids.* **5:** 96–115.
9. Hakomori, S. I., and W. T. Murakami. 1967. Glycolipids of hamster fibroblasts and derived malignant-transformed cell lines. *Proc. Nat. Acad. Sci. USA.* **59:** 254–261.
10. Hakomori, S. I., C. Teather, and H. Andrews. 1968.

Organizational difference of cell surface "hematoside" in normal and virally transformed cells. *Biochem. Biophys. Res. Commun.* **33:** 563–568.
11. Brady, R. O., C. Borek, and R. M. Bradley. 1969. Composition and synthesis of gangliosides in rat hepatocyte and hepatoma cell lines. *J. Biol. Chem.* **244:** 6552–6554.
12. Karlsson, K.-A., B. E. Samuelsson, and G. O. Steen. 1969. Sphingolipid composition of the avian salt gland. *Biochim. Biophys. Acta.* **176:** 429–431.
13. Henning, R., and W. Stoffel. 1969. The relationship between phospholipid biosynthesis, sphingosine degradation and serine metabolism. *Hoppe-Seyler's Z. Physiol. Chem.* **350:** 827–835.
14. Stoffel, W., D. LeKim, and G. Heyn. 1970. Metabolism of sphingosine bases. XIV. Sphinganine (dihydrosphingosine), an effective donor of the alk-1′-enyl chain of plasmalogens. *Hoppe-Seyler's Z. Physiol. Chem.* **351:** 875–883.
15. Svennerholm, L. 1970. Ganglioside metabolism. *In* Comprehensive Biochemistry. Vol. 18. Lipid Metabolism. M. Florkin and E. H. Stotz, editors. Elsevier, Amsterdam. 201–227.
16. Svennerholm, L. 1970. Gangliosides. *In* Handbook of Neurochemistry. Vol. 3. A. Lajtha, editor. Plenum Press, New York. 425–452.
17. Carter, H. E., P. Johnson, and E. J. Weber. 1965. Glycolipids. *Annu. Rev. Biochem.* **34:** 109–142.
18. Shapiro, D. 1969. Chemistry of Sphingolipids. Hermann, Paris.
19. Morell, P., and N. S. Radin. 1969. Synthesis of cerebroside by brain from uridine diphosphate galactose and ceramide containing hydroxy fatty acid. *Biochemistry.* **8:** 506–512.
20. Dawson, G., and C. C. Sweeley. 1969. New technique for enzymic hydrolysis of glycosphingolipids. *J. Lipid Res.* **10:** 402–405.
21. Wenger, D. A., J. W. Petitpas, and R. A. Pieringer. 1968. The metabolism of glyceride glycolipids. II. Biosynthesis of monogalactosyl diglyceride from uridine diphosphate galactose and diglyceride in brain. *Biochemistry.* **7:** 3700–3707.
22. Lennarz, W. J. 1970. Lipid metabolism. *Annu. Rev. Biochem.* **39:** 359–388.
23. Zabin, I., and J. F. Mead. 1953. The biosynthesis of sphingosine. I. The utilization of carboxyl-labeled acetate. *J. Biol. Chem.* **205:** 271–277.
24. Sprinson, D. B., and A. Coulon. 1954. The precursors of sphingosine in brain tissue. *J. Biol. Chem.* **207:** 585–592.
25. Zabin, I., and J. F. Mead. 1954. The biosynthesis of sphingosine. II. The utilization of methyl-labeled acetate, formate and ethanolamine. *J. Biol. Chem.* **211:** 87–93.
26. Brady, R. O., and G. J. Koval. 1958. The enzymatic synthesis of sphingosine. *J. Biol. Chem.* **233:** 26–31.
27. Brady, R. O., J. V. Formica, and G. J. Koval. 1958. The enzymatic synthesis of sphingosine. II. Further studies on the mechanism of the reaction. *J. Biol. Chem.* **233:** 1072–1076.
28. Haskell, B. E., and E. E. Snell. 1965. Effect of Vitamin B_6 deficiency on the composition of yeast lipids. *Arch. Biochem. Biophys.* **112:** 494–555.
29. Greene, M. L., T. Kaneshiro, and J. H. Law. 1965. Studies on the production of sphingolipid bases by the yeast, *Hansenula ciferri*. *Biochim. Biophys. Acta.* **98:** 582–588.
30. Braun, P. E., and E. E. Snell. 1967. The biosynthesis of dihydrosphingosine in cell-free preparations of *Hansenula ciferri*. *Proc. Nat. Acad. Sci. USA.* **58:** 298–303.
31. Braun, P. E., and E. E. Snell. 1968. Biosynthesis of sphingolipid bases. II. Keto intermediates in synthesis of sphingosine and dihydrosphingosine by cell-free extracts of *Hansenula ciferri*. *J. Biol. Chem.* **243:** 3775–3783.
32. Braun, P. E. 1967. Sphingolipid synthesis in extracts of *Hansenula ciferri*. Ph.D. dissertation. University of California, Berkeley.
33. Brady, R. N., S. J. DiMari, and E. E. Snell. 1969. Biosynthesis of sphingolipid bases. III. Isolation and characterization of ketonic intermediates in the synthesis of sphingosine and dihydrosphingosine by cell-free extracts of *Hansenula ciferri*. *J. Biol. Chem.* **244:** 491–496.
34. Stoffel, W., D. LeKim, and G. Sticht. 1968. Biosynthesis of dihydrosphingosine *in vitro*. *Hoppe-Seyler's Z. Physiol. Chem.* **349:** 664–670.
35. Weiss, B. 1963. The biosynthesis of sphingosine. I. A study of the reaction with tritium-labeled serine. *J. Biol. Chem.* **238:** 1953–1959.
36. Snell, E. E., S. J. DiMari, and R. N. Brady. 1970. Biosynthesis of sphingosine and dihydrosphingosine by cell-free systems from *Hansenula ciferri*. *Chem. Phys. Lipids.* **5:** 116–138.
37. Stoffel, W., D. LeKim, and G. Sticht. 1968. Stereospecificity of the NADPH-dependent reduction reaction of 3-oxodihydrosphingosine (2-amino-1-hydroxyoctadecane-3-one). *Hoppe-Seyler's Z. Physiol. Chem.* **349:** 1637–1644.
38. DiMari, S. J., R. N. Brady, and E. E. Snell. 1971. Biosynthesis of sphingolipid bases. IV. The biosynthetic origin of sphingosine in *Hansenula ciferri*. *Arch. Biochem. Biophys.* **143:** 553–565.
39. Stoffel, W., D. LeKim, and G. Sticht. 1967. Metabolism of sphingosine bases. IV. 2-Amino-1-hydroxyoctadecane-3-one (3-oxodihydrosphingosine), the common intermediate in the biosynthesis of dihydrosphingosine and sphingosine and in the degradation of dihydrosphingosine. *Hoppe-Seyler's Z. Physiol. Chem.* **348:** 1570–1574.
40. Weiss, B., and R. L. Stiller. 1968. *In vivo* utilization of dihydrosphingosine-4-5-^{3}H. *Proc. Soc. Exp. Biol. Med.* **128:** 689–693.
41. Fujino, Y. 1964. Studies on conjugated lipids. Part XVIII. Enzymatic synthesis of the sphingosine base. *Agr. Biol. Chem.* **28:** 807–808.
42. Fujino, Y., and I. Zabin. 1962. The configuration of sphingosine synthesized in rat brain homogenates. *J. Biol. Chem.* **237:** 2069–2071.
43. Braun, P. E., P. Morell, and N. S. Radin. 1970. Synthesis of C_{18}- and C_{20}-dihydrosphingosines, ketodihydrosphingosines, and ceramides by microsomal preparations from mouse brain. *J. Biol. Chem.* **245:** 335–341.
44. Polito, A. J., and C. C. Sweeley. 1971. Stereochemistry of the hydroxylation in 4-hydroxysphinganine. Formation and the steric course of hydrogen elimination in sphing-4-enine biosynthesis. *J. Biol. Chem.* **246:** 4178–4187.
45. Rosenberg, A., and N. Stern. 1966. Changes in sphingosine and fatty acid components of the gangliosides in developing rat and human brain. *J. Lipid Res.* **7:** 122–131.
46. Morell, P., and N. S. Radin. 1970. Specificity in ceramide biosynthesis from long chain bases and various fatty acyl coenzyme A's by brain microsomes. *J. Biol. Chem.* **245:** 342–350.

47. Kanfer, J. N., and S. Bates. 1970. Sphingolipid metabolism. II. The biosynthesis of 3-keto-dihydrosphingosine by a partially-purified enzyme from rat brain. *Lipids.* **5:** 718–720.
48. Stoffel, W., G. Sticht, and D. LeKim. 1968. Metabolism of sphingosine bases. VI. Synthesis and degradation of sphingosine bases in *Hansenula ciferri*. *Hoppe-Seyler's Z. Physiol. Chem.* **349:** 1149–1156.
49. Weiss, B., and R. L. Stiller. 1967. Biosynthesis of phytosphingosine. Hydroxylation of dihydrosphingosine. *J. Biol. Chem.* **242:** 2903–2908.
50. Thorpe, S. R., and C. C. Sweeley. 1967. Chemistry and metabolism of sphingolipids. On the biosynthesis of phytosphingosine by yeast. *Biochemistry.* **6:** 887–897.
51. Zabin, I. 1957. Biosynthesis of ceramide by rat brain homogenates. *J. Amer. Chem. Soc.* **79:** 5834–5835.
52. Sribney, M. 1966. Enzymatic synthesis of ceramide. *Biochim. Biophys Acta.* **125:** 542–547.
53. Kishimoto, Y., and N. S. Radin. 1959. Composition of cerebroside acids as a function of age. *J. Lipid Res.* **1:** 79–82.
54. Ställberg-Stenhagen, S., and L. Svennerholm. 1965. Fatty acid composition of human brain sphingomyelins: normal variation with age and changes during myelin disorders. *J. Lipid Res.* **6:** 146–155.
55. O'Brien, J. S., and E. L. Sampson. 1965. Fatty acid and fatty aldehyde composition of the major brain lipids in normal human gray matter, white matter, and myelin. *J. Lipid Res.* **6:** 545–551.
56. Cherayil, G. D. 1968. Fatty acid composition of brain glycolipids in Alzheimer's disease, senile dementia, and cerebrocortical atrophy. *J. Lipid Res.* **9:** 207–214.
57. Svennerholm, L., and S. Ställberg-Stenhagen. 1968. Changes in the fatty acid composition of cerebrosides and sulfatides of human nervous tissue with age. *J. Lipid Res.* **9:** 215–225.
58. Kishimoto, Y., W. E. Davies, and N. S. Radin. 1965. Turnover of the fatty acids of rat brain gangliosides, glycerophosphatides, cerebrosides, and sulfatides as a function of age. *J. Lipid Res.* **6:** 525–531.
59. Suzuki, K. 1967. Formation and turnover of the major brain gangliosides during development. *J. Neurochem.* **14:** 917–925.
60. Hajra, A. K., and N. S. Radin. 1963. Isotopic studies of the biosynthesis of the cerebroside fatty acids in rats. *J. Lipid Res.* **4:** 270–278.
61. Kishimoto, Y., W. E. Davies, and N. S. Radin. 1965. Developing rat brain: changes in cholesterol, galactolipids, and the individual fatty acids of gangliosides and glycerophosphatides. *J. Lipid Res.* **6:** 532–536.
62. Eichberg, J., G. Hauser, and M. L. Karnovsky. 1969. Lipids of nervous tissue. *In* The Structure and Function of Nervous Tissue. G. H. Bourne, editor. Academic Press, New York. 185–287.
63. Kanfer, J. N., and A. E. Gal. 1966. *In vivo* conversion of *erythro* and *threo* DL-sphingosine-^{3}H to ceramide and sphingomyelin. *Biochem. Biophys. Res. Commun.* **22:** 442–446.
64. Yavin, E., and S. Gatt. 1969. Enzymatic hydrolysis of sphingolipids. VIII. Further purification and properties of rat brain ceramidase. *Biochemistry.* **8:** 1692–1698.
65. Coles, E., and J. L. Foote. 1970. Fatty acids of glycosphingolipids from pig blood fractions. *J. Lipid Res.* **11:** 433–438.
66. Sribney, M., and E. P. Kennedy. 1958. The enzymatic synthesis of sphingomyelin. *J. Biol. Chem.* **233:** 1315–1322.
67. Sribney, M. 1968. Stimulation and inhibition of sphingomyelin synthetase. *Arch. Biochem. Biophys.* **126:** 954–957.
68. Rosenberg, A. 1970. Sphingomyelin: enzymatic reactions. *In* Handbook of Neurochemistry. Vol. 3. A. Lajtha, editor. Plenum Press, New York. 453–466.
69. Radin, N. S. 1959. Discussion in The Biology of Myelin. S. R. Korey, editor. Harper & Row, New York. 271.
70. Fujino, Y., M. Nakano, T. Negishi, and S. Ito. 1968. Substrate specificity for ceramide in the enzymatic formation of sphingomyelin. *J. Biol. Chem.* **243:** 4650–4651.
71. Kopaczyk, K. C., and N. S. Radin. 1965. In vivo conversions of cerebroside and ceramide in rat brain. *J. Lipid Res.* **6:** 140–145.
72. Brady, R. O., R. M. Bradley, O. M. Young, and H. Kaller. 1965. An alternative pathway for the enzymatic synthesis of sphingomyelin. *J. Biol. Chem.* **240:** PC3693–PC3694.
73. Fujino, Y., and T. Negishi. 1968. Investigation of the enzymatic synthesis of sphingomyelin. *Biochim. Biophys. Acta.* **152:** 428–430.
74. Fujino, Y., T. Negishi, and S. Ito. 1968. Enzymic synthesis of sphingosylphosphorylcholine. *Biochem. J.* **109:** 310–311.
75. Radin, N. S., and Y. Akahori. 1961. Fatty acids of human brain cerebrosides. *J. Lipid Res.* **2:** 335–341.
76. Burton, R. M., M. A. Sodd, and R. O. Brady. 1958. The incorporation of galactose into galactolipides. *J. Biol. Chem.* **233:** 1053–1060.
77. Basu, S., A. M. Schultz, M. Basu, and S. Roseman. 1971. Enzymatic synthesis of galactocerebroside by a galactosyltransferase from embryonic chicken brain. *J. Biol. Chem.* **246:** 4272–4279.
78. Neskovic, N. M., J. L. Nussbaum, and P. Mandel. 1970. Enzymatic deficiency in neurological mutants. Brain uridine diphosphate galactose:ceramide galactosyltransferase in Jimpy mouse. *FEBS Lett.* **8:** 213–216.
79. Hammarstrom, S., and B. Samuelsson. 1970. On the biosynthesis of cerebrosides from 2-hydroxy acid ceramides: use of deuterium labeled substrate and multiple ion detector. *Biochem. Biophys. Res. Commun.* **41:** 1027–1035.
80. Shah, S. N. 1971. Glycosyl transferases of microsomal fractions from brain: synthesis of glucosyl ceramide and galactosyl ceramide during development and the distribution of glucose and galactose transfer in white and grey matter. *J. Neurochem.* **18:** 395–402.
81. Coles, L., and G. M. Gray. 1970. The biosynthesis of digalactosylceramide in the kidney of the C57/BL mouse. *Biochem. Biophys. Res. Commun.* **38:** 520–526.
82. Morell, P., E. C. Costantino-Ceccarini, and N. S. Radin. 1970. The biosynthesis by brain microsomes of cerebrosides containing nonhydroxy fatty acids. *Arch. Biochem. Biophys.* **141:** 738–748.
83. Hammarstrom, S. 1971. On the biosynthesis of cerebrosides containing nonhydroxy acids. 2. Mass spectrometric evidence for the ceramide pathway. *Biochem. Biophys. Res. Commun.* **45:** 468–475.
84. O'Brien, J. S., and G. Rouser. 1964. The fatty acid composition of brain sphingolipids: sphingomyelin, ceramide, cerebroside, and cerebroside sulfate. *J. Lipid Res.* **5:** 339–342.
85. Klenk, E., and R. T. C. Huang. 1968. Zur Kenntnis der

Gehirnceramide und der darin vorkommenden Sphingosinbasen. *Hoppe-Seyler's Z. Physiol. Chem.* **349:** 451–454.
86. Cleland, W. W., and E. P. Kennedy. 1960. The enzymatic synthesis of psychosine. *J. Biol. Chem.* **235:** 45–51.
87. Neskovic, N. M., J. L. Nussbaum, and P. Mandel. 1969. Enzymatic synthesis of psychosine in "Jimpy" mice brain. *FEBS Lett.* **3:** 199–201.
88. Hildebrand, J., P. Stoffyn, and G. Hauser. 1970. Biosynthesis of lactosyl-ceramide by rat brain preparations and comparison with the formation of ganglioside GM_1 and psychosine during development. *J. Neurochem.* **17:** 403–411.
89. Brady, R. O. 1962. Studies on the total enzymatic synthesis of cerebrosides. *J. Biol. Chem.* **237:** PC2416–PC2417.
90. Hammarstrom, S. 1971. On the biosynthesis of cerebrosides containing non-hydroxy acids. *Biochem. Biophys. Res. Commun.* **45:** 459–467.
91. McKhann, G. M., R. Levy, and W. Ho. 1965. Metabolism of sulfatides. I. The effect of galactocerebrosides on the synthesis of sulfatides. *Biochem. Biophys. Res. Commun.* **20:** 109–113.
92. Balasubramanian, A. S., and B. K. Bachhawat. 1965. Formation of cerebroside sulphate from 3′-phosphoadenosine-5′-phosphosulphate in sheep brain. *Biochim. Biophys. Acta.* **106:** 218–220.
93. McKhann, G. M., and W. Ho. 1967. The *in vivo* and *in vitro* synthesis of sulphatides during development. *J. Neurochem.* **14:** 717–724.
94. Farrell, D. F., and G. M. McKhann. 1971. Characterization of cerebroside sulfotransferase from rat brain. *J. Biol. Chem.* **246:** 4694–4702.
95. Stoffyn, A., P. Stoffyn, and E. Mårtensson. 1968. Structure of kidney ceramide dihexoside sulfate. *Biochim. Biophys. Acta.* **152:** 353–357.
96. Cumar, F. A., H. S. Barra, H. J. Maccioni, and R. Caputto. 1968. Sulfation of glycosphingolipids and related carbohydrates by brain preparations from young rats. *J. Biol. Chem.* **243:** 3807–3816.
97. Stoffyn, P., A. Stoffyn, and G. Hauser. 1971. Structure of sulfatides biosynthesized in vitro. *J. Lipid Res.* **12:** 318–323.
98. Basu, S., B. Kaufman, and S. Roseman. 1968. Enzymatic synthesis of ceramide-glucose and ceramide-lactose by glycosyltransferases from embryonic chicken brain. *J. Biol. Chem.* **243:** 5802–5804.
99. Brenkert, A., and N. S. Radin. 1972. Synthesis of galactocerebroside and glucocerebroside by rat brain: assay procedures and changes with age. *Brain Res.* **36:** 183–193.
100. Yamakawa, T., S. Nichimura, and M. Kamimura. 1965. The chemistry of the lipids of posthemolytic residue or stroma of erythrocytes. XIII. Further studies on human red cell glycolipids. *Jap. J. Exp. Med.* **35:** 201–207.
101. Rapport, M. M., and L. Graf. 1965. Immunochemical identity of cytolipin K and globoside. *Federation Proc.* **24:** 176. (Abstr.)
102. Hakomori, S. I., and B. Siddiqui. 1971. Anomeric structures of globoside and ceramide trihexoside of human erythrocytes and hamster fibroblasts. *J. Biol. Chem.* **246:** 2271–2277.
103. Klenk, E. 1969. On cerebrosides and gangliosides. *Progr. Chem. Fats Other Lipids.* **10:** 409–431.
104. Hauser, G. 1967. The enzymatic synthesis of ceramide lactoside from ceramide glucoside and UDP-galactose. *Biochem. Biophys Res. Commun.* **28:** 502–509.
105. Hildebrand, J., and G. Hauser. 1969. Biosynthesis of lactosylceramide and triglycosylceramide by galactosyltransferases from rat spleen. *J. Biol. Chem.* **244:** 5170–5180.
106. Hay, J. B., and G. M. Gray. 1970. Glycosphingolipid biosynthesis in kidneys of normal C3H/He mice and of those with BP8 ascites tumours. *Biochem. Biophys. Res. Commun.* **38:** 527–532.
107. Basu, S., M. Basu, H. Den, and S. Roseman. 1970. Enzymatic synthesis of a triglycosylceramide related to blood group glycosphingolipids. *Federation Proc.* **29:** 410. (Abstr.)
108. Basu, M., and S. Basu. 1971. Biosynthesis of a tetraglycosylceramide related to blood group glycosphingolipids. *Federation Proc.* **30:** 1133. (Abstr.)
109. Kaufman, B., S. Basu, and S. Roseman. 1967. Studies on the biosynthesis of gangliosides. *In* Inborn Disorders of Sphingolipid Metabolism. S. M. Aronson and B. W. Volk, editors. Pergamon Press, New York. 193–213.
110. Handa, S., and R. M. Burton. 1969. Biosynthesis of glycolipids: incorporation of *N*-acetyl galactosamine by a rat brain particulate preparation. *Lipids.* **4:** 589–598.
111. Yip, M. C. M., and J. A. Dain. 1969. The enzymic synthesis of ganglioside: 1. Brain uridine diphosphate D-galactose: *N*-acetyl-galactosaminyl-galactosyl-glucosyl-ceramide galactosyl transferase. *Lipids.* **4:** 270–277.
112. Frohwein, Y. Z., and S. Gatt. 1967. Enzymatic hydrolysis of sphingolipids. VI. Hydrolysis of ceramide glycosides by calf brain β-*N*-acetylhexosaminidase. *Biochemistry.* **6:** 2783–2787.
113. Frohwein, Y. Z., and S. Gatt. 1967. Isolation of β-*N*-acetylhexosaminidase, β-*N*-acetylglucosaminidase, and β-*N*-acetylgalactosaminidase from calf brain. *Biochemistry.* **6:** 2775–2782.
114. Svennerholm, L. 1962. The chemical structure of normal human brain and Tay-Sachs gangliosides. *Biochem. Biophys. Res. Commun.* **9:** 436–441.
115. Ledeen, R., and K. Salsman. 1965. Structure of the Tay-Sachs ganglioside. *Biochemistry.* **4:** 2225–2233.
116. Okada, S., and J. S. O'Brien. 1969. Tay-Sachs disease: generalized absence of a β-D-*N*-acetylhexosaminidase component. *Science.* **165:** 698–700.
117. Sandhoff, K. 1969. Variation of β-*N*-acetylhexosaminidase-pattern in Tay-Sachs disease. *FEBS Lett.* **4:** 351–354.
118. Kolodny, E. H., R. O. Brady, and B. W. Volk. 1969. Demonstration of an alteration of ganglioside metabolism in Tay-Sachs disease. *Biochem. Biophys. Res. Commun.* **37:** 526–531.
119. Suzuki, Y., J. C. Jacob, K. Suzuki, K. M. Kutty, and K. Suzuki. 1971. G_{M2}-gangliosidosis with total hexosaminidase deficiency. *Neurology.* **21:** 313–328.
120. Brady, R. O., A. E. Gal, R. M. Bradley, and E. Mårtensson. 1967. The metabolism of ceramide trihexosides. I. Purification and properties of an enzyme that cleaves the terminal galactose molecule of galactosylgalactosylglucosylceramide. *J. Biol. Chem.* **242:** 1021–1026.
121. Brady, R. O., A. E. Gal, R. M. Bradley, E. Mårtensson, A. L. Warshaw, and L. Laster. 1967. Enzymatic defect in Fabry's disease. Ceramidetrihexosidase deficiency. *New Engl. J. Med.* **276:** 1163–1167.
122. Mapes, C. A., R. L. Anderson, and C. C. Sweeley. 1970. Trihexosyl ceramide: galactosyl hydrolase in normal

human serum and plasma and its absence in patients with Fabry's disease. *Federation Proc.* **29:** 409. (Abstr.)
123. Kint, J. A. 1970. Fabry's disease: α-galactosidase deficiency. *Science.* **167:** 1268–1269.
124. Gatt, S., and M. M. Rapport. 1966. Enzymic hydrolysis of sphingolipids. Hydrolysis of ceramide lactoside by an enzyme from rat brain. *Biochem. J.* **101:** 680–686.
125. Gatt, S., and M. M. Rapport. 1966. Isolation of β-galactosidase and β-glucosidase from brain. *Biochim. Biophys. Acta.* **113:** 567–576.
126. Radin, N. S., L. Hof, R. M. Bradley, and R. O. Brady. 1969. Lactosylceramide galactosidase: comparison with other sphingolipid hydrolases in developing rat brain. *Brain Res.* **14:** 497–505.
127. Sandhoff, K., H. Pilz, and H. Jatzkewitz. 1964. Über den enzymatischen Abbau von *N*-Acetylneuraminsäure-freien Gangliosidresten (Ceramid-oligosaccharide). *Hoppe-Seyler's Z. Physiol. Chem.* **338:** 281–285.
128. Dawson, G., and A. O. Stein. 1970. Lactosyl ceramidosis: catabolic enzyme defect of glycosphingolipid metabolism. *Science.* **170:** 556–558.
129. Brady, R. O., J. Kanfer, and D. Shapiro. 1965. The metabolism of glucocerebrosides. I. Purification and properties of glucocerebroside-cleaving enzyme from spleen tissue. *J. Biol. Chem.* **240:** 39–43.
130. Gatt, S. 1966. Enzymatic hydrolysis of sphingolipids. Hydrolysis of ceramide glucoside by an enzyme from ox brain. *Biochem. J.* **101:** 687–691.
131. Kampine, J. P., R. O. Brady, R. A. Yankee, J. N. Kanfer, D. Shapiro, and A. E. Gal. 1967. Sphingolipid metabolism in leukemic leukocytes. *Cancer Res.* **27:** 1312–1315.
132. Weinreb, N. J., R. O. Brady, and A. L. Tappel. 1968. The lysosomal localization of sphingolipid hydrolases. *Biochim. Biophys. Acta.* **159:** 141–146.
133. Brady, R. O., J. N. Kanfer, and D. Shapiro. 1965. Metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher's disease. *Biochem. Biophys. Res. Commun.* **18:** 221–225.
134. Kattlove, H. E., J. C. Williams, E. Gaynor, M. Spivack, R. M. Bradley, and R. O. Brady. 1969. Gaucher cells in chronic myelocytic leukemia: an acquired abnormality. *Blood.* **33:** 379–390.
135. Fredrickson, D. S. 1966. Cerebroside lipidosis: Gaucher's disease. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill, New York. 565–586.
136. Svennerholm, L. The metabolism of gangliosides in cerebral lipidoses. *In* Inborn Disorders of Sphingolipid Metabolism. S. M. Aronson and B. W. Volk, editors. Pergamon Press, New York. 169–186.
137. Kampine, J. P., R. O. Brady, and J. N. Kanfer. 1967. Diagnosis of Gaucher's disease and Niemann-Pick disease with small samples of venous blood. *Science.* **155:** 86–88.
138. Brady, R. O. 1970. Cerebral lipidoses. *Annu. Rev. Med.* **21:** 317–334.
139. Brady, R. O., A. E. Gal, J. N. Kanfer, and R. M. Bradley. 1965. The metabolism of glucocerebrosides. III. Purification and properties of a glucosyl- and galactosylceramide-cleaving enzyme from rat intestinal tissue. *J. Biol. Chem.* **240:** 3766–3770.
140. Nilsson, A. 1969. Metabolism of cerebroside in the intestinal tract of the rat. *Biochim. Biophys. Acta.* **187:** 113–121.
141. Mehl, E., and H. Jatzkewitz. 1964. Eine Cerebrosidsulfatase aus Schweineniere. *Hoppe-Seyler's Z. Physiol. Chem.* **339:** 260–276.
142. Mehl, E., and H. Jatzkewitz. 1968. Cerebroside 3-sulfate as a physiological substrate of arylsulfatase A. *Biochim. Biophys. Acta.* **151:** 619–627.
143. Jatzkewitz, H. 1960. Die Leukodystrophie, Typ Scholz, (metachromatische Form der diffusen Sklerose) als Sphingolipidose (Cerebrosid-schwefelsäureester-Speicherkrankheit). *Hoppe-Seyler's Z. Physiol. Chem.* **318:** 265–277.
144. Austin, J. H., A. S. Balasubramanian, T. N. Pattabiraman, S. Saraswathi, D. K. Basu, and B. K. Bachhawat. 1963. A controlled study of enzymic activities in three human disorders of glycolipid metabolism. *J. Neurochem.* **10:** 805–816.
145. Mehl, E., and H. Jatzkewitz. 1965. Evidence for the genetic block in metachromatic leucodystrophy (ML). *Biochem. Biophys. Res. Commun.* **19:** 407–411.
146. Farooqui, A. A., and B. K. Bachhawat. 1971. The regional distribution, age dependent variation and species differences of brain arylsulphatases. *J. Neurochem.* **18:** 635–646.
147. Hajra, A. K., D. M. Bowen, Y. Kishimoto, and N. S. Radin. 1966. Cerebroside galactosidase of brain. *J. Lipid Res.* **7:** 379–386.
148. Bowen, D. M., and N. S. Radin. 1968. Purification of cerebroside galactosidase from rat brain. *Biochim. Biophys. Acta.* **152:** 587–598.
149. Bowen, D. M., and N. S. Radin. 1968. Properties of cerebroside galactosidase. *Biochim. Biophys. Acta.* **152:** 599–610.
150. Radin, N. S. 1970. Cerebrosides and sulfatides. *In* Handbook of Neurochemistry. Vol. 3. A. Lajtha, editor. Plenum Press, New York. 415–424.
151. Bowen, D. M., and N. S. Radin. 1969. Cerebroside galactosidase: a method for determination and a comparison with other lysosomal enzymes in developing rat brain. *J. Neurochem.* **16:** 501–511.
152. Suzuki, K., and Y. Suzuki. 1970. Globoid cell leucodystrophy (Krabbe's disease): deficiency of galactocerebroside β-galactosidase. *Proc. Nat. Acad. Sci. USA.* **66:** 302–309.
153. Arora, R. C., and N. S. Radin. 1972. Ceramide-like synthetic amides that inhibit cerebroside galactosidase. *J. Lipid Res.* **13:** 86–91.
154. Arora, R. C., and N. S. Radin. Stimulation *in vitro* of cerebroside galactosidase by *N*-acyl-2-amino-2-methyl-propanol. *Lipids.* In press.
155. Kates, M. 1963. Lipolytic enzymes. *In* Lipid Metabolism. K. Block, editor. Wiley, New York. 165–237.
156. Souček, A., C. Michalec, and A. Součková. 1967. Enzymic hydrolysis of sphingomyelins by a toxin of *Corynebacterium ovis*. *Biochim. Biophys. Acta.* **144:** 180–182.
157. Heller, M., and B. Shapiro. 1966. Enzymic hydrolysis of sphingomyelin by rat liver. *Biochem. J.* **98:** 763–769.
158. Kanfer, J. N., O. M. Young, D. Shapiro, and R. O. Brady. 1966. The metabolism of sphingomyelin. I. Purification and properties of a sphingomyelin-cleaving enzyme from rat liver tissue. *J. Biol. Chem.* **241:** 1081–1084.
159. Barenholz, Y., A. Roitman, and S. Gatt. 1966. Enzymatic hydrolysis of sphingolipids. II. Hydrolysis of sphingomyelin by an enzyme from rat brain. *J. Biol. Chem.* **241:** 3731–3737.
160. Schneider, P. B., and E. P. Kennedy. 1967. Sphingomyelinase in normal human spleens and in spleens from sub-

jects with Niemann-Pick disease. *J. Lipid Res.* **8:** 202–209.
161. Schneider, P. B., and E. P. Kennedy. 1968. Metabolism of labeled dihydrosphingomyelin in vivo. *J. Lipid Res.* **9:** 58–64.
162. Brady, R. O., J. N. Kanfer, M. B. Mock, and D. S. Fredrickson. 1966. The metabolism of sphingomyelin. II. Evidence of an enzymatic deficiency in Niemann-Pick disease. *Proc. Nat. Acad. Sci. USA.* **55:** 366–369.
163. Sloan, H. R., B. W. Uhlendorf, J. N. Kanfer, R. O. Brady, and D. S. Fredrickson. 1969. Deficiency of sphingomyelin-cleaving enzyme activity in tissue cultures derived from patients with Niemann-Pick disease. *Biochem. Biophys. Res. Commun.* **34:** 582–588.
164. Nilsson, A. 1968. The presence of sphingomyelin and ceramide-cleaving enzymes in the small intestinal tract. *Biochim. Biophys. Acta.* **176:** 339–347.
165. Barenholz, Y., and S. Gatt. 1967. The utilization and degradation of phytosphingosine by rat liver. *Biochem. Biophys. Res. Commun.* **27:** 319–330.
166. Gatt, S., and Y. Barenholz. 1968. Degradation of sphingosine bases by cell-free preparations. α-Hydroxy palmitic acid, an intermediate of phytosphingosine degradation. *Biochem. Biophys. Res. Commun.* **32:** 588–594.
167. Barenholz, Y., and S. Gatt. 1968. Degradation of sphingosine, dihydrosphingosine, and phytosphingosine in rats. *Biochemistry.* **7:** 2603–2609.
168. Roitman, A., Y. Barenholz, and S. Gatt. 1967. Utilization and degradation of dihydrosphingosine and phytosphingosine by intact rat brain. *Israel J. Chem.* **5:** 134.
169. Stoffel, W., and G. Sticht. 1967. Metabolism of sphingosine bases. I. Degradation and incorporation of (3-^{14}C)-*erythro*-DL-dihydrosphingosine and (7-$^{3}H_2$)*erythro*-DL-sphingosine into sphingolipids of rat liver. *Hoppe-Seyler's Z. Physiol. Chem.* **348:** 941–943.
170. Stoffel, W., and G. Sticht. 1967. Metabolism of sphingosine bases. II. Studies on the degradation and transformation of [3-^{14}C]*erythro*-DL-dihydrosphingosine, [7-^{3}H]*erythro*-DL-sphingosine, [5-^{3}H]*threo*-L-dihydrosphingosine and [3-^{14}C;1-^{3}H]*erythro*-DL-dihydrosphingosine in rat liver. *Hoppe-Seyler's Z. Physiol. Chem.* **348:** 1345–1351.
171. Stoffel, W., and A. Scheid. 1969. Metabolism of sphingosine bases. XII. Eicosasphinganine (eicosadihydrosphingosine) and 3-dehydroeicosasphinganine (3-dehydroeicosadihydrosphingosine). *Hoppe-Seyler's Z. Physiol. Chem.* **350:** 1593–1604.
172. Keenan, R. W., and K. Okabe. 1968. The degradation of tritiated dihydrosphingosine in the intact rat. *Biochemistry.* **7:** 2696–2701.
173. Karlsson, K.-A., B. E. Samuelsson, and G. O. Steen. 1967. Studies on sphingosines. 15. Degradation of phytosphingosine to hydroxy fatty acid and ethanolamine by the yeast *Hansenula ciferri*. *Acta Chem. Scand.* **21:** 2566–2567.
174. Stoffel, W., G. Sticht, and D. LeKim. 1968. Degradation *in vitro* of dihydrosphingosine and dihydrosphingosine phosphate to palmitaldehyde and ethanolamine phosphate. *Hoppe-Seyler's Z. Physiol. Chem.* **349:** 1745–1748.
175. Stoffel, W., G. Sticht, and D. LeKim. 1969. Degradation of (1-^{14}C)dihydrosphingosine (sphinganine), (1-^{14}C)2-amino-1,3-dihydroxyheptane and (1-^{14}C)dihydrosphingosine phosphate in rat liver. *Hoppe-Seyler's Z. Physiol. Chem.* **350:** 63–68.
176. Stoffel, W., and G. Assmann. 1970. Metabolism of sphingosine bases. XV: Enzymatic degradation of 4-*t*-sphingenine 1-phosphate (sphingosine 1-phosphate) to 2-*t*-hexadecen-1-al and ethanolamine phosphate. *Hoppe-Seyler's Z. Physiol. Chem.* **351:** 1041–1049.
177. Keenan, R. W., and A. Maxam. 1969. The *in vitro* degradation of dihydrosphingosine. *Biochim. Biophys. Acta.* **176:** 348–356.
178. Keenan, R. W., and B. Haegelin. 1969. The enzymatic phosphorylation of sphinganine. *Biochem. Biophys. Res. Commun.* **37:** 888–894.
179. Hirschberg, C. B., A. Kisic, and G. J. Schroepfer, Jr. 1970. Enzymatic formation of dihydrosphingosine 1-phosphate. *J. Biol. Chem.* **245:** 3084–3090.
180. Kampine, J. P., J. N. Kanfer, A. E. Gal, R. M. Bradley, and R. O. Brady. 1967. Response of sphingolipid hydrolases in spleen and liver to increased erythrocytorhexis. *Biochim. Biophys. Acta.* **137:** 135–139.
181. Lev, M., and A. F. Milford. 1971. Vitamin K stimulation of sphingolipid synthesis. *Biochem. Biophys. Res. Commun.* **45:** 358–362.
182. Bachhawat, B. K., J. Austin, and D. Armstrong. 1967. A cerebroside sulphotransferase deficiency in a human disorder of myelin. *Biochem. J.* **104:** 15c–17c.
183. Suzuki, Y., J. Austin, D. Armstrong, K. Suzuki, J. Schlenker, and T. Fletcher. 1970. Studies in globoid leukodystrophy: enzymatic and lipid findings in the canine form. *Exp. Neurol.* **29:** 65–75.
184. Wisniewski, H., and P. Morell. 1971. Quaking mouse: ultrastructural evidence for arrest of myelinogenesis. *Brain Res.* **29:** 63–73.
185. Costantino-Ceccarini, E., and P. Morell. 1971. Quaking mouse: *in vitro* studies of brain sphingolipid biosynthesis. *Brain Res.* **29:** 75–84.
186. Stodola, F. H., M. H. Deinema, and J. F. T. Spencer. 1967. Extracellular lipids of yeasts. *Bacteriol. Rev.* **31:** 194–213.
187. Stoffel, W., and D. LeKim. 1971. On the stereospecificity of the biochemical dehydrogenation of the 1-*O*-alkyl glyceryl to the 1-*O*-alk-1′-enyl glyceryl ether bond. *Hoppe-Seyler's Z. Physiol. Chem.* **352:** 501–511.
188. Greenfield, S., W. T. Norton, and P. Morell. 1971. Quaking mouse: isolation and characterization of myelin protein. *J. Neurochem.* **18:** 2119–2128.
189. Smith, M. E., and C. M. Hasinoff. 1971. Biosynthesis of myelin proteins *in vitro*. *J. Neurochem.* **18:** 739–747.
190. Benjamins, J. A., N. Herschkowitz, J. Robinson, and G. M. McKhann. 1971. The effects of inhibitors of protein synthesis on incorporation of lipids into myelin. *J. Neurochem.* **18:** 729–738.