BIOCHEMISTRY OF BRAIN

Editor

S. KUMAR

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PREFACE

Brain has been considered to be the "*master control*" of all human functions. It plays a very important role in human metabolism. In the last quarter century, study of brain metabolism has grown from an obscure science, being explored by a very few scientists, into a major area of scientific discipline attracting a varied talent from a number of fields. This remarkable development in the study of brain biochemistry has provided a wealth of information helping the understanding of its functions and metabolism.

This volume puts together some of the recent developments in the area of brain biochemistry. The contributors are all people who are leading authorities in their fields of study. Together they have provided an expert and critical review of important progress being made in this fascinating and rapidly growing area of science. The volume should prove helpful to the workers in specific areas, as well as to those who are interested in the study of brain biochemistry, as a single source of information. Each chapter reviews a particular subject in detail, giving information on the available knowledge to date, methods of study and results, and guidelines for future work.

I am grateful to the contributors for their excellent work, time, help and above all, patience, and to my associates and the publishers for their continued cooperation and encouragement without which this volume would not have been possible.

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INTRODUCTION

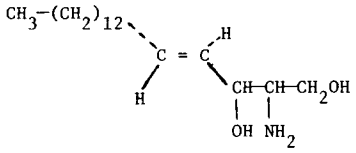
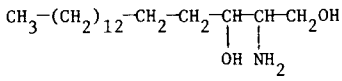
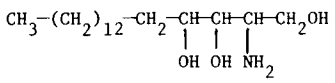
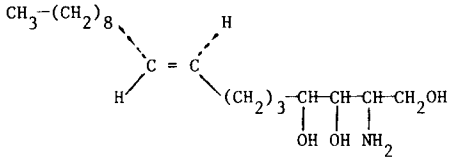
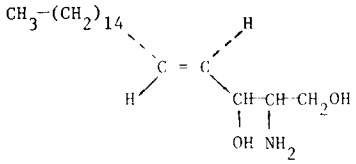
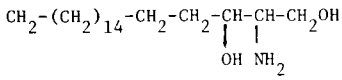
The widely-accepted term sphingolipid is derived from the aliphatic base sphingosine which is present in the structural framework of all these compounds. The isolation of sphingosine from hydrolysates of brain lipids was reported by Thudichum (1882, 1901) who assigned to it the empirical formula $C_{16}H_{35}NO_2$. The molecular formula was corrected to $C_{18}H_{37}NO_2$, by Klenk in 1929 but it was not until the 1950's that the full structure of sphingosine was elucidated (Carter & Humiston, 1951) and confirmed by its total synthesis (Shapiro & Segal, 1954; Shapiro *et al*, 1958). The sudden spurt of interest in the chemistry of sphingosine and related lipids since then is primarily due to interest in the sphingolipid storage diseases which are probably the best understood congenital storage disorders of the nervous system.

STRUCTURE AND NOMENCLATURE OF SPHINGOSINE AND RELATED BASES

Sphingosine is the major naturally occurring base present in sphingolipids. Carter and Humiston (1951) determined its structure (Table I) to be (D+) *erythro*-1, 3-dihydroxy-2-amino-4-transoctadecene. Minor constituents related to sphingosine that have also been isolated from brain tissue include sphingosines with chain lengths either longer or shorter than C_{18}, and branched-chain sphingosines and bases with more than one double bond or more than two hydroxyl groups.

TABLE I

Structure and Nomenclature of Sphingosine Bases

Common Name	Structure	Currently accepted Generic Name
Sphingosine		4-Sphingenine
Dihydro-sphingosine		Sphinganine
Phytosphin-gosine		4-D-Hydroxy-sphinganine
Dehydrophyto-sphingosine		4-D-Hydroxy, 8-sphingenine
C_{20}-Sphingosine		4-Eciosa sphingenine
C_{20}-dihydro-sphingosine		Eciosa-sphinganine

The fully-saturated analogue of sphingosine, dihydrosphingosine, is also almost invariably present along with sphingosine. The names and structures of some of the more frequently-occurring sphingosine bases are given in Table 1.

In the present system of nomenclature the C_{18} saturated base, dihydrosphingosine, is tentatively designated as sphinganine. According to this nomenclature, sphingosine is 4-sphingenine (the prefix 4 indicates the position of the double bond and phytosphingosine is termed 4-hydroxysphinganine. Homologues of C-18 are designated by an appropriate prefix (Table 1).

The primary amino group at C-2 in sphingosine is always *N*-acylated in sphingolipids, whereas the primary hydroxyl group at position 1 is either esterified or glycosylated. The *N*-acylated derivative of sphingosine, ceramide is the precursor of

most of the sphingolipids and it has been isolated in the free state from neuronal and several other tissues (Gatt, 1963; Martensson, 1969; Samuelsson, 1969). Although various fatty acids have been detected in ceramide, the C_{20}-C_{24} fatty acids predominate in neutral glycosphingolipids and sphingomyelin, whereas stearic acid is the major component of gangliosides.

CLASSIFICATION OF SPHINGOLIPIDS

The classification of sphingolipids is primarily based on the substituent groups attached to the hydroxyl group at C-1 of sphingosine or its derivatives. In phosphosphingolipids this hydroxyl group is esterified in a phosphate diester, as with phosphoryl choline in sphingomyelin, whereas in the glycosphingolipids the C-1 hydroxyl group is directly glycosylated by mono-, di-, or oligosaccharides. The glycosphingolipids acquire an anionic nature if the oligosaccharide moiety has acidic groups, as in sulfatide (galactose 3-sulphate) or in the gangliosides which contain sialic acid. Gangliosides are an important group of water-soluble acidic sphingolipids containing 3 or more hexose units attached to the C-1 hydroxyl of ceramide together with one or more sialic acid residues.

Sphingolipids are present in virtually all mammalian tissues and fluids although they are generally less abundant than the glycerides and cholesterol. They were once considered to be confined to the membranes of eukaryotic cells and to be absent from bacteria. Recent studies, however, have shown their occurrence in some of these organisms. In extra-neuronal tissues, the sphingolipids are believed to be localized mainly in plasma membrane and to contribute to the surface properties and to specific membrane functions of the cell.

CHEMICAL STRUCTURES AND OCCURRENCE

Structural studies of glycosphingolipids were mainly carried out in order to characterize the lipids accumulated in the brain and/or other tissues in inborn errors of metabolism such as Gaucher's and Fabry's disease. These diseases will be discussed in detail later in this volume. The structures of some glycosphingolipids are shown in Tables 2 and 3.

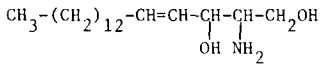
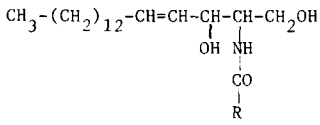
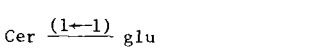
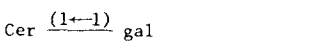
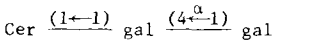
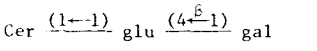
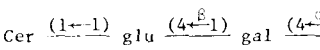
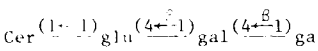
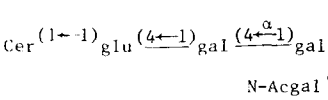
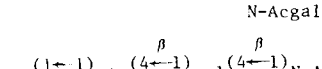
Neutral Glycosphingolipids and Sulfatide

The first sugar residue attached to ceramide is usually glucose or galactose giving rise to the simple members of the series, glucocerebroside and galactocerebroside, respectively. More complex oligosaccharides are formed by the addition of galactose or *N*-acetylgalactosamine residues to the first sugar unit. In sulfatide, the C-3 hydroxyl group of galactosyl ceramide is esterified with sulphuric acid (Yamakawa *et al.*, 1962; Stoffyn & Stoffyn, 1963, 1963a; Stoffyn, 1966).

The stereochemical configurations of different sphingolipids derived from glucocerebroside usually follow a similar pattern (Table 2) (Yamakawa *et al.*, 1965;

TABLE II

Structure of Some of the Neutral Sphingolipids

Structure	Common Name	Remarks
	Sphingosine	Constituent base of all sphingolipids.
	Ceramide	Constituent base of all glycosphingolipids.
	Glucocerebroside or Glucosyl ceramide	Present in serum, spleen, kidney, in traces in almost all tissues, precursor of gangliosides.
	Galactocerebroside or Galactosyl ceramide	Major cerebroside of brain, high concentration in myelin sheath, intermediate metabolite of sulfatide.
	Digalactosyl ceramide	Has α 1→4 linkage minor constituent detected in kidney. Accumulates in Fabry's disease.
	Lactosyl ceramide	Important intermediate in metabolism of ganglioside detected in kidney and brain.
	Digalactosyl glucosyl ceramide	Accumulates in Fabry's disease.
	Globoside	Present in human red cell membrane.
	"Forssman hapten"	Present in red cell membrane and kidney.
	Asialo G_{M1} ganglioside	Intermediate in the metabolism of gangliosides.

Cer = ceramide. glu = glucose. gal = galactose. N-Acgal = N-acetyl galactosamine.

Makita & Yamakawa, 1963; Makita *et al.*, 1966). The sugar moieties exist in pyranoside form and the glycosidic linkages are normally in the β-configuration. Forssman hapten (Makita *et al.*, 1966), a derivative of glucocerebroside, is a well-known exception in which the linkage between the C-1 hydroxyl group of terminal *N*-acetyl galactosamine and the C-3 hydroxyl of galactose is in the α anomeric configuration (Ando & Yamakawa 1970; Siddiqui & Hakamori, 1971; Stellner *et al.*, 1973).

TABLE III

Structure of Major Gangliosides and their Nomenclature

Common Name Svennerholm nomenclature+	Structure	Other Abbreviations Weigandt*	 Klenk**
Monosialo gangliosides			
G_{M3}-gangliosides (Haematoside)	Cer (1←1) glu (4←β1) gal 3 ↑ 2 NANA	G_{Lact}^{1}	B_2
G_{M2}-ganglioside (Tay-Sachs ganglioside)	Cer (1←1) glu (4←β1) gal (4←β1) galNAc 3 ↑ 2 NANA	G_{GNTr}^{II}	A_1
G_{M1}-ganglioside	Cer(1←1)glu (4←β1) gal (4←β1) galNAc (3←β1) gal 3 ↑ 2 NANA	G_{GNT}^{1}	A_2
Disialogangliosides			
G_{D1a}-ganglioside	Cer(1←1) glu (4←β1) gal (4←β1)galNAc (3←β1) gal 3 3 ↑ ↑ 2 2 NANA NANA	G_{GNT}^{II}	B_1
G_{D1b}-ganglioside	Cer(1←1) glu (4←β1) gal (4←β1)galNAc (3←β1) gal 3 ↑ 2 NANA (8←2) NANA	G_{GNT}^{III}	C_1
Trisialogangliosides			
G_{T1}-ganglioside	Cer(1←1) glu (4←β1) gal (4←β1)galNAc (3←β1) gal 3 3 ↑ ↑ 2 2 NANA (8←2) NANA NANA	G_{GNT}^{IV}	C_3

+ Svennerholm (1964)

* Kuhn & Weigandt (1963)

** Klenk & Gielen (1960)

NANA = N-acetylneuraminic acid. Other abbreviations are same as in Table II.

Sphingosine is the major component in all neutral glycosphingolipids and accounts for more than 90% of the basic fraction. Among the other bases, C_{18}-dihydrosphingosine occurs in brain while phytosphingosine occurs in kidney as a minor constituent (Carter & Hirchberg, 1968; Karlsson, 1964; Karlsson & Martensson, 1968; Michalec & Kolman, 1966). Galactocerebroside consists predominantly of C_{18}-sphingosine. The fatty acid compositions of neutral glycosphingo-

lipids are somewhat similar to those of other sphingolipids. Predominantly, C_{18} to C_{24} saturated acids are attached to the free amino group of sphingosine (Martensson, 1966; Suomi & Agranoff, 1965; Miras *et al.*, 1966; Yamakawa, 1966). Galactosylceramide, which is the major glycosphingolipid of human brain has a relatively high content of α-hydroxy fatty acids which comprise about 50% of the total fatty acids (Svennerholm & Stallberg-Stenhagen, 1968). Depending on the nature of the fatty acid attached to sphingosine, the brain galactosylceramide is designated as cerasin (having normal fatty acids) or pherosin (having α-hydroxy fatty acids). Kidney dihexosylceramide and monohexosyl ceramide also have substantial amounts of α-hydroxy fatty acids. Most of the other glycosphingolipids have only trace amounts of these fatty acids.

Neutral glycosphingolipids derived from galactocerebroside predominate in neuronal tissue. Galactocerebroside and sulfatides constitute a significant portion of brain glycosphingolipid, especially in myelin sheath and white matter where cerebrosides and sulfatide make up some 25% of the total lipids compared to only 7% in gray matter.

Neutral glycosphingolipids based on glucocerebroside are in higher concentrations in non-neuronal tissue. Glucosyl ceramide (glucocerebroside) is the major sphingolipid constituent of plasma although the concentration of galactosyl ceramide is higher in liver and spleen. In the kidney and in the red cell membrane tetrahexosylceramides derived from glucocerebroside predominate (Yamakawa *et al.*, 1965).

Gangliosides

The currently accepted name ganglioside was applied by Klenk (1941, 1942) to the lipid material that he isolated from the brain of a Tay-Sachs patient. Because of their structural and corresponding unwieldly nomenclature, several shorthand notations for gangliosides have been suggested (Svennerholm, 1964; Kuhn & Weigandt, 1963; Klenk & Gielen, 1960; Penick *et al.*, 1966). Table 3 gives the structures and shorthand nomenclature of some of the major gangliosides.

Gangliosides contain one or more residues of sialic acid attached to the same or different sugar moieties of the oligosaccharide chain. Sialic acid is the accepted name for compounds derived from neuraminic acid (5-amino-3, 5 dideoxy D-*glycero*-D-*galacto*-nonulosonic acid) (Fig. 1). In sialic acid, the amino group may be *N*-acetylated, as in the case of *N*-acetyl neuraminic acid (NANA), or *N*-glycolylated as in the case of *N*-glycolyl neuraminic acid (NGNA). Human brain gangliosides contain mostly NANA, whereas NGNA occurs as a minor constituent in sheep, pig and bovine brains (Yu & Leeden, 1970).

In most of the gangliosides, the major bases are C_{18} and C_{20} sphingosines which occur in approximately equal proportions (Carter *et al.*, 1947; Sambosivarao & McCluer, 1964) together with minor amounts of their dihydroanalogues. The main fatty acid component of the gangliosides is stearic acid. However, other saturated fatty acids also occur. Lactosyl ceramide (Cer-glu-gal) appears to be the precursor of all gangliosides which usually also contain *N*-acetylgalactosamine.

Gangliosides (mainly G_{M1}, G_{D1a}, G_{D1b} and G_{T1}) are concentrated in the gray mat-

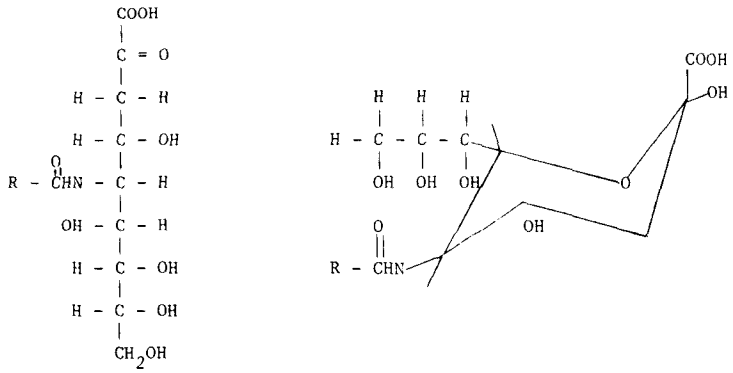


Fig. 1 *Structure of neuraminic acid*
Left-hand side: open form. Right-hand side: ring form
In NANA, $R = CH_3$
In NGNA, $R = CH_2OH$

ter of the brain where they constitute some 5% of the total lipid (Klenk, 1941, 1942; Lapetina *et al.*, 1967; Weigandt, 1967) whereas they comprise only 0.6% of the total lipids of white matter (Leeden & Yu, 1973). They are also present in small amounts in spleen, erythrocytes, liver, kidney and spinal fluid.

ISOLATION OF GLYCOSPHINGOLIPIDS

Gangliosides are soluble in water and form high molecular weight micelles, whereas the solubility of neutral glycosphingolipids in water increases from that of the insoluble cerebrosides with increasing chain length of the oligosaccharide portion of the molecule. Details of procedures for extraction of total lipids and separation of neutral and anionic sphingolipids are documented in reviews by Rouser *et al.* (1967) and Skipsky (1975). Commensurate with the scope of the present chapter, we will briefly outline the principles of isolation and characterization of glycosphingolipids. The ground tissue, homogenate, or acetone powder, is extracted with a mixture of chloroform and methanol. Although solvent mixtures containing different proportions of chloroform have been used (Booth, 1962; Svennerholm, 1963), the original extraction procedure of Folch *et al.* (1957) using chloroform-methanol, 2 : 1, appears to be most effective. The lipids are then partitioned using the Folch procedure (Folch *et al.*, 1957) or modified Folch procedures (Suzuki, 1965; Radin, 1969), when gangliosides separate in the upper layer of aqueous methanol containing KCl. The lower layer of chloroform-methanol contains neutral glycosphingolipids together with phospholipids, glycerides, fatty acids, steroids and their esters. Separation of the glycosphingolipids from the chloroform-methanol fraction can be achieved by thin layer chromatography, or column chromatography using silicic acid or ion exchange resins. In column chromatography neutral lipids are eluted first with chloroform followed by the glycosphingolipids with acetone-methanol, 9 : 1, while the phospholipids are retained on the column and can be eluted only with methanol. Sequential elution of different classes

of lipids from chromatographic columns of various adsorbants using gradually increasing polar solvents for successive elutions is a widely used technique for the separation not only of glycosphingolipids but for almost all lipid classes. Excellent detailed review articles are available in the literature (Rouser *et al.*, 1967; Sweeley, 1969).

Thin layer chromatography (TLC) is probably the most extensively used technique for qualitative and quantitative analyses of both neutral glycosphingolipids and the anionic gangliosides. The lipids are extracted from the tissue and partitioned by the Folch procedure which separates the gangliosides in the aqueous phase and the neutral glycosphingolipids in the organic solvent phase. The neutral glycosphingolipids can then be directly isolated from phospholipids and the neutral lipids by TLC. Using TLC plates containing 80% silica gel and 20% magnesium silicate and a two-step development in acetone-pyridine-chloroform water (40 : 60 : 5 : 4) followed by ethyl ether-pyridine-ethanol and 2N NH_4OH (65 : 30 : 8 : 2) Skipsky *et al.* (1967) have separated these lipids in one step. Gangliosides are largely retained in the aqueous phase during partitioning in the Folch procedure but because of the formation of water-insoluble complexes of calcium and gangliosides, some gangliosides are retained in the chloroform layer (Berh & Lehn, 1973). A modified procedure described by Carter & Kanfer (1975) allows a complete extraction of gangliosides in the aqueous phase. Gangliosides form micelles of very high molecular weight in water solution, and column chromatography over ion-exchange resins of florisil is often performed to remove phospholipids and other contaminants before the sample is ready for analytical or preparative TLC. Contaminating phospholipids may be cleaved by mild alkaline hydrolysis in the crude ganglioside extract at this stage. Acid hydrolysis may be employed to remove sialic acid residues when the asialo derivatives are to be isolated.

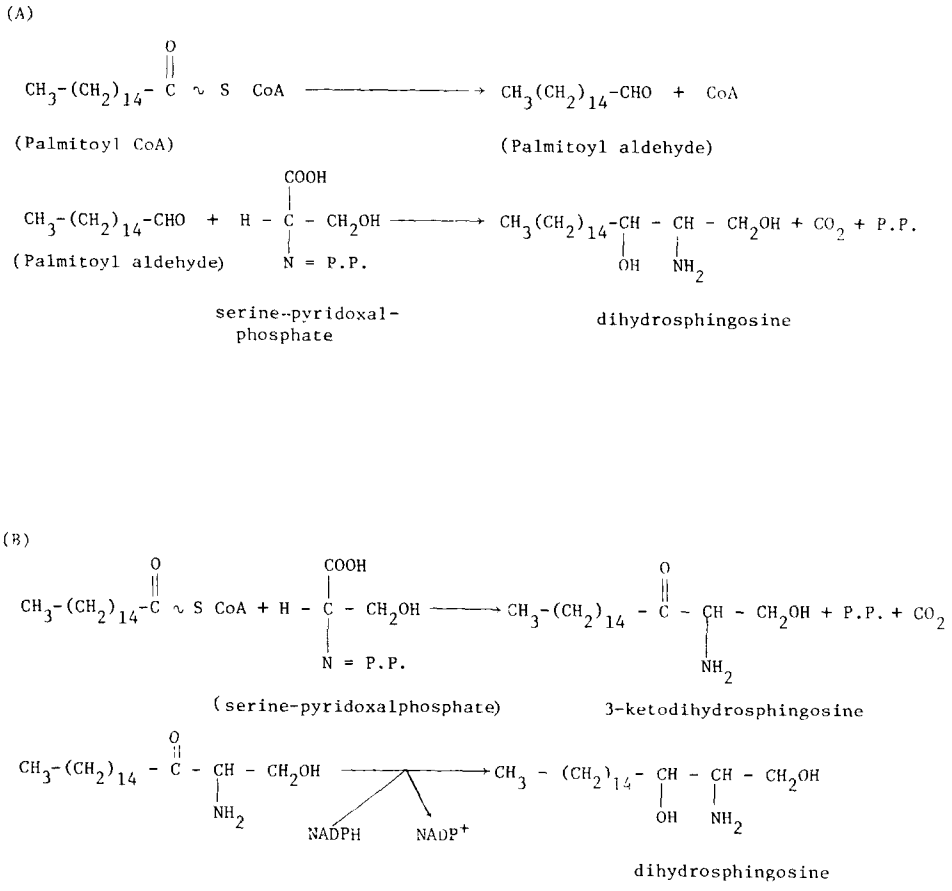
Radioactive Labeling of Glycosphingolipids

Radioactively-labeled gangliosides and neutral glycosphingolipids are used as natural substrates for the assay of specific sphingoglycosidases as discussed later. For radioactive labeling of sphingolipids, two major approaches have been used. In the first, radioactively-labeled [^{14}C] precursors are injected into experimental animals from which the labeled products are subsequently isolated. [^{14}C] sialic acid—and [^{14}C] *N*-acetylgalactosomine—labeled GM_2 ganglioside and several other sphingolipids have been labeled by this technique (Kolodny *et al.*, 1970). The other approach consists of enzymatic oxidation of terminal galactose or *N*-acetyl galactosamine residue by galactose oxidase followed by reduction with tritiated sodium borohydride. Galactosyl ceramide, lactosyl ceramide and G_{M2} ganglioside labeled at terminal galactose residue have been prepared in this way (Radin *et al.*, 1969; Suzuki & Suzuki, 1972). Syntheses of glycosphingolipids radioactively-labeled at the sphingosine (Iwamori *et al.*, 1975) and fatty acid moieties (Mapes *et al.*, 1973) have also been achieved.

BIOSYNTHESIS OF GLYCOSPHINGOLIPIDS

Sphingosines

The biosynthesis of dihydrosphingosine or its analogues can proceed via condensation of the fatty aldehyde (e.g. palmitoyl aldehyde) with serine linked in the form of a Schiff's base to pyridoxal phosphate (Brady & Koval, 1958; Brady *et al.*, 1958; Brady, 1969). Manganese ions are required for this reaction (Fig. 2A). Stoffel *et al.* (1968) however have isolated enzymes from several tissues which seem to favor an alternate biosynthetic pathway as shown in Fig. 2B. This pathway does not require aldehyde as the intermediate and proceeds directly with fatty acyl CoA. Formation of sphingosine from dihydrosphingosine is not well understood.



Figs. 2A & 2B *Biosynthesis of sphingosine*

Neutral Glycosphingolipids and Sulfatide

Addition of fatty acid and hexose to sphingosine leading to the biosynthesis of cerebrosides can proceed either through the addition of the hexose to ceramide or through the direct addition of the sugar residue to sphingosine followed by acylation of the free-NH_2 group of sphingosine (Fig. 3).

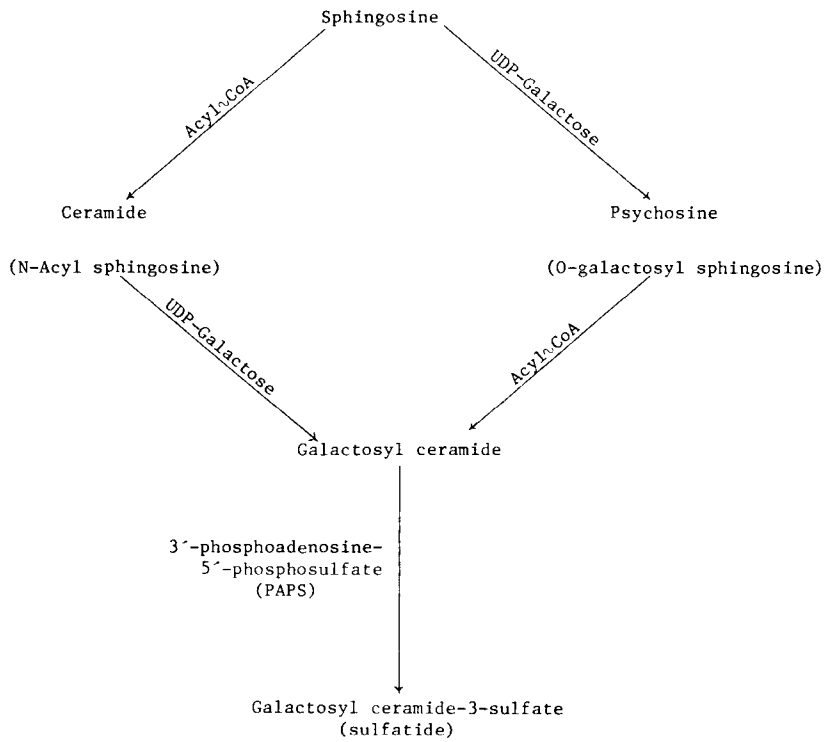


Fig. 3. *Biosynthesis of sulfatide*

Fig. 4. *Possible routes of ganglioside biosynthesis*

abbreviations:

Cer = ceramide
glc = glucose
gal = galactose
galNAc = N-acetylgalactosamine
UDP-glc = uridine diphosphate glucose
UDP-gal = uridine diphosphate galactose
UDP-galNAc = uridine diphosphate N-acetyl-galactosamine
CMP = cytidine monophosphate
NANA = N-acetylneuraminic acid