Reprinted from
ANNUAL REVIEW OF BIOCHEMISTRY
Vol. 40, 1971

SPHINGOLIPIDS

WILHELM STOFFEL
Institut für Physiologische Chemie der Universität Köln
Köln, West Germany

CONTENTS

Glycolipids, including sphingolipids, were comprehensively reviewed in 1965 by Carter (1) and briefly discussed in subsequent chapters of the series (2–4). Essential contributions since then will be referred to in this critical review of the current status of research in this field.

The nomenclature of lipids, and in particular of the complex glycosphingolipids, remains of serious concern despite the semisystematic nomenclature recommended by the IUPAC-IUB (5) and followed in this review. However, more detailed information about the compounds to be discussed can be expressed by *chemical shorthand nomenclature.* In the future this may prove especially valuable because of the discovery of new glycosphingolipids with greater structural complexities in both the ceramide and carbohydrate moities.

ANALYTICAL METHODS

New methods have been introduced for analysis of complex glycosphingolipids which are applicable to either the whole molecule or its respective components.

The separation of neutral (ceramide mono- and polyhexosides) and acidic glycolipids (gangliosides) can in general be achieved by a partition between water and chloroform/methanol as refined by Hakomori (6) and Saito & Hakomori (7). These authors observed that higher ceramide polyhexosides with more than four hexose residues appear together with the gangliosides in the upper aqueous phase. The separation of these two classes

can be carried out on DEAE-cellulose. Higher ceramide polyhexosides such as ceramide penta- to nonahexosides are successfully separated by thin-layer chromatography on silica gel plates after peracetylation (solvent system: 1.2-dichloroethane/methanol/water 97:3:0.5).

An improved procedure for quantitative isolation of ceramide-containing glycolipids from mammalian tissues by two-dimensional thin-layer chromatography has been described by Gray (8) (solvent system: direction 1, chloroform/methanol/water 65:25:4; direction 2, tetrahydrofuran/dimethoxymethane/methanol/water 10:6:4:1) and by Skipski, Smolowe & Barclay (9). Modified procedures for the extraction (10) and the quantitative analysis of ganglioside mixtures (11) were reported, the latter based on the procedure of Suzuki (12).

A microdetermination of gangliosides from total lipid extracts after thin-layer chromatography, based on the densitometry of the charred lipid spots, has been reported by Sandhoff, Harzer & Jatzkewitz (13).

A new tool for the quantitative detection of solutes in thin-layer chromatograms has been designed by Haahti & Jaakonmäki (14). The bands are charred in the presence of cupric oxide in the silica gel layer and the resulting CO_2 is monitored by a thermal conductivity cell.

A spectrophotometric determination of molar amounts of glycosphingolipids (cerebrosides, ceramide polyhexosides, and gangliosides) and ceramides by hydrolysis and reaction of the long-chain base with trinitrobenzenesulfonic acid was described by Yamamoto & Rouser (15). 4*t*-Sphingenine can also be determined fluorimetrically in the μmole range after complex formation with 1-naphthylamine-4-sulfonic acid (16). The method has also been used for the estimation of complex glycosphingolipids.

Vance & Sweeley (17) described a procedure for the quantitative determination of the neutral ceramide hexosides applied to human blood plasma and erythrocytes. The total lipid extract is separated by silicic acid chromatography into the neutral lipids (chloroform), neutral mixed glycolipids (acetone/methanol 9:1), and phospholipids (methanol). Mild alkaline hydrolysis and preparative thin-layer chromatography removed contaminants. Acid hydrolysis and subsequent gas-liquid chromatography of the trimethylsilyl (TMS) derivatives made possible the determination of the composition of both the carbohydrate portion and the long-chain base in the temperature-programed run. With mannitol as internal standard the quantitation was rather satisfactory. This procedure was adapted to a microscale for the analysis of biological specimens such as urine sediment by Desnick, Sweeley & Krivit (18).

The separation of 4*t*-sphingenine, sphinganine, and 4D-hydroxysphinganine on a preparative scale by chromatography on silica gel columns has been described by Barenholz & Gatt (19).

Dinitrophenyl derivatives of long-chain bases can be easily prepared and fractionated either by reversed-phase paper chromatography (20) or according to their degree of unsaturation on silver-nitrate-impregnated silica

gel G (21). *Erythro* and *threo* isomers of N-acetyltrimethylsilyl derivatives of long-chain bases can be separated by gas-liquid chromatography on SE 30 columns (22) or as their DNP derivatives by two-dimensional chromatography on borate-impregnated silica gel G or paper (23). The separation of ceramides from sphingomyelin or any sphingolipid according to the number of double bonds can be achieved after acetylation by argentation thin-layer chromatography of the diacetylceramides (solvent system: chloroform /water 95:5) (24).

Ceramide galactosides and ceramide glucosides can be separated on borate-impregnated silica gel G (25). Sulfatides can be rapidly determined quantitatively in a sensitive spectrophotometric assay of the complex between the cationic dye azure A and the anionic sulfolipids (26).

Attempts to use quantitative enzymatic degradation by glycosidases for the stepwise degradation of the oligosaccharide portion of sphingolipids have been unsuccessful because of the insolubility of the substrates. Coating the substrate to lecithin-impregnated filter paper proved to be partially successful with the trihexosylceramide galactosyl hydrolase (27).

Wiegandt & Baschang (28) investigated the release of the carbohydrate moiety of glycolipids by ozonolysis, a method which leaves the neuraminic acid molecule linked to the sugars. Releasing the carbohydrate from glycosphingolipids by osmium-catalyzed periodate oxidation followed by alkali treatment destroys the N-acetylneuraminic acid linkage (29), but is very useful for the microanalysis of ceramide polyhexosides. A new method for obtaining oligosaccharides in high yield by partial acid hydrolysis of the polysaccharides has been used for structural studies on Gram-negative O antigens, and might prove very useful for analytical studies in the glycolipid field (30).

The isotope-dilution method has been applied to the microanalytical determination of five classes of glycosphingolipids (31). The test substances were tritiated by catalytic reduction of the double bond in the long-chain base. Since unsaturated and hydrogenated ceramide polyhexosides differ in their thin-layer chromatographic behavior, the unkown sample must be reduced before the dilution experiment.

The glycosphingolipid field has been recently enriched by the combined use of gas-liquid chromatography and mass spectrometry (GLC-MS). Karlsson (32) and Gaver & Sweeley (33) used this technique to obtain detailed structural information concerning the TMS ethers or the N-acetylated derivatives, respectively, of the long-chain bases.

Improvements in the preparation of the long-chain bases for gas chromatography have been made by Carter & Gaver (22). Usually the TMS derivatives are prepared with a mixture of pyridine/hexamethyl-disilazane/ trimethylchlorosilane 10:2:1. Attention should be drawn to the convenient silylation reagent, N-methyl-N-trimethylsilyltrifluoracetamide (or formamide), frequently used for amino acid gas-liquid chromatography (34).

Polito, Naworal & Sweeley made use of the observation (35) that TMS

derivatives of vicinal diols exhibit strong fragmentation ions between C atoms carrying these functional groups, and therefore transformed unsaturated compounds into diols by osmium tetroxide oxidation and silylation. This method allows the assignment of the double bond positions from the characteristic fragmentation (36, 37). Vance & Sweeley (17) have developed a quantitative analysis of four glycolipids in small samples of human plasma and erythrocytes using gas chromatography of the methylglycosides obtained by methanolysis of the sphingolipids. The combined GLC-MS analysis has been extended to the analysis of the ceramide and carbohydrate part of the glycosphingolipids, using their trimethylsilyl (TMS) derivatives or their acetates.

Samuelsson & Samuelsson (38, 39) studied a number of synthetic ceramides. Casparrini, Hörning & Horning (40) described the gas-chromatographic separation and identification of TMS derivatives of synthetic ceramides as well as those from natural sources (such as blood plasma or after enzymatic hydrolysis of sphingomyelin from plasma). Cerebrosides (41) have been separated into molecular species by gas-liquid chromatography of the fully silylated compounds at elevated temperatures (320°C). Characteristic fingerprints of the fragmentation mode within the scan limits 1 to 850 m/e were obtained. Karlsson et al (42) preferred to first acetylate then silylate sulfatides, which replace the sulfate group for mass spectroscopy. Sweeley & Dawson (43) attempted to make structural deductions from mass spectra of TMS derivatives of glucosylceramide (Cer-Glc), lactosylceramide (Cer-Glc-Gal), galactosyl-galactosyl-glucosylceramide (Cer-Glc-Gal-Gal), globoside, monosialoganglioside, asialoganglioside, and Tay-Sachs ganglioside. All significant ions which were necessary to deduce the position of glycosidic linkages in the oligosaccharide unit were found. The mass spectrometer however cannot distinguish among the aldohexoses.

These studies were aided by mass spectroscopic studies of TMS derivatives of carbohydrates (44). Björndal et al (45, 46) described the analysis of polysaccharides after exhaustive methylation (47), and the GLC-MS of the alditol acetates of the methylated sugars after hydrolysis, a method used so far only for structural studies of the O antigens of Salmonella. It offers promising perspectives also for structural analysis, including the position of the glycosidic linkages, in the glycolipid field.

STRUCTURES

The chemical transformation of 4*t*-sphingenine led to ribo-2-amino-1,3,4-octadecanetriol, which proved to be identical with 4D-hydroxysphinganine (phytosphingosine) isolated from natural sources (48, 49). Morrison (50) isolated 31 long-chain bases from milk sphingomyelin. These included saturated dihydroxy bases with *n*-C_{12} to C_{20}, iso-C_{17} to C_{20}, and anteiso-C_{17} to C_{19} structures, and unsaturated dihydroxy bases with *n*-C_{12} to C_{20}, iso-C_{14} to C_{19}, and anteiso-C_{15}, C_{17}, and C_{19} structures. The long-chain base pattern was similar in milk ceramide glucoside and ceramide lactoside, three classes

also containing large amounts of long-chain C_{18} to C_{25} *trans*-monoenoic acids (51). Carter, Gaver & Yu (52) found 19-methyl-C_{20}-4D-hydroxysphinganine in the flagellate *Crithidia fasciculata*. Branched long-chain bases are also present in protozoa (53) and ceramide-containing phospholipids were observed in bacteria. The anaerobic bacterium *Bacteroides melaninogenicus* contains the following branched saturated long-chain bases: 17-methylsphinganine, 15-methylhexadecasphinganine, and 16-methylhexadecasphinganine (54). Substantial amounts of 4D-hydroxysphinganine and of a Δ^4-16-methyl-C_{17}-4*t*-sphingenine has been identified in the cerebroside and the sphingomyelin fraction of beef and rat kidney respectively (55, 56).

Several laboratories (57–59) have isolated dienoic long-chain bases, found to be present in plasma sphingomyelin and recently also in human plasma ceramide monohexosides (59); the structures proved to be *erythro*-1,3-dihydroxy-2-amino-4*trans*-14*cis*-octadecadiene (sphinga-4,11-dienine. Plasma sphingomyelin and cerebrosides also contain *trans*- monoenoic fatty acids, e.g. 22:1, 23:1, 24:1 (60, 63). 14-Carbon homologues of 4*t*-sphingenine are present in sphingolipids of crayfish (64), human aorta (61), insects (62, 65), and honeybee (66). Tetradecasphinganine and hexadecasphinganine are the principal long-chain bases in invertebrates (*Musca domestica*) (65), and eicosasphinga-4,11-dienine and eicosasphing-11-enine were found in scorpion (62). The geometry of the double bonds was not reported. The chemistry and occurrence of sphingolipid long-chain bases have been summarized by Karlsson (67, 68).

Ceramides isolated from whole brain tissue contain eicosasphingenine with stearic acid as the main fatty acid. The same holds for gray matter ceramides and sphingomyelin, whereas in white matter ceramides and sphingomyelin the C_{20}-long-chain base is missing and 4*t*-sphingenine is acylated mainly with C_{24} and C_{26} acids (69, 70). The changes in the long-chain base and fatty acid patterns of the gangliosides in the developing rat and human brain were followed: 4*t*-sphingenine, the only long-chain base at birth, exchanges with increasing age with the C_{20} homologue to equal level, while the fatty acid remains stearic acid (71). A study of serum sphingomyelins by argentation chromatography revealed that sphingenine was acylated with saturated *trans*- or *cis*-monoenoic fatty acids (nervonic acid); the same was observed for sphingadienine. This species analysis of sphingomyelin is possible after phospholipase C hydrolysis and fractionation of the ceramides, according to Renkonen (24, 72). By this procedure the occurrence of 2-hydroxy fatty acids predominantly with 16, 22, and 24 C atoms in sphingomyelin of the bovine rennet stomach has been established (73).

Ceramide aminoethyl phosphonates and ceramide phosphorylethanolamines are present in marine invertebrates (74, 75), and have also been isolated from rumen protozoa (76) and the blowfly *Calliphora erythrocephala* (77).

White et al (78–80) described the presence of three unusual phosphosphingolipids in *Bacteroides melaninogenicus* which proved to be ceramide

phosphorylethanolamine, ceramide phosphorylglycerol, and ceramide phosphorylglycerol phosphate. These accounted for 50 to 70% of the lipid phosphorus and also contained iso-long-chain saturated bases.

Klenk & Schorsch (81) isolated the individual galactocerebrosides by a combination of silica gel chromatography, mercuric acetate addition to double bonds, and countercurrent distribution, procedures which made possible the separation of kerasine and nervon from cerebron and oxynervon fractions. The kerasine fraction was separated into the cerebrosides with the homologous C_{23}, C_{24}, and C_{25} acids, and the nervon fraction into those with homologous C_{24}, C_{25}, and C_{26} acids. The cerebron fraction of brain cerebrosides also was separated by countercurrent distribution of the acetylated cerebroside mixture according to the chain length of the 2-hydroxy fatty acids. The main cerebron component contained 2-hydroxy-*n*-tetracosanoic acid, while cerebrosides with C_{22}, C_{23}, and C_{25}-2-hydroxy acids were present in smaller amounts (82). The chain lengths and structures of the fatty acids of cerebrosides change with age (83, 84).

Ceramide glucose has been isolated from brain tissue, human liver, serum, and spleen (85–87). Nishimura & Yamakawa (88) found that palmitic and stearic acids are the acyl groups of the glucosylceramide and suggested that their precursor function in ganglioside biosynthesis is due to the similarity of the fatty acid pattern of this kerasine and the Tay-Sachs ganglioside (89, 90).

Cerebroside esters, in which C_3 or C_6 or both, of the galactose moiety, or C_3 of sphingenine, is esterified with long-chain fatty acids (mainly palmitic, stearic, palmitoleic, and oleic) have been isolated from human brain by Klenk et al (91, 92), Kishimoto, Wajda & Radin (93), and Tamai (94, 95). Karlsson (96, 97) studied the distribution of sphingolipids in bovine kidney and observed a high sulfatide concentration in the outer part of the medulla where the corticosteroid-dependent Na^+-transport system is located.

A relationship between sulfatide content and Na^+, K^+-activated ATPase activity is also apparent in the avian salt gland (herring gull). The ratio of enzyme to sulfatide correlates well in this and other organs of the animal.

Digalactosyl ceramide was isolated from normal human kidney (98). A new sulfatide has been isolated and its structure determinated by methylation studies and chemical degradation by Stoffyn, Stoffyn & Mårtensson (99), who proved it to be the 3′-sulfate ester of Gal-(β1→4)-D-Glc-(β1→1)-Cer. It comprises about 25% of the sulfatide fraction of patients with metachromatic leucodystrophy (100, 101).

Sweeley et al (102, 103) characterized the ceramide trihexoside accumulating in patients with Fabry's disease as Gal-(1→4)-Gal-(1→4)-Glc-(1→1)-Cer. Another glycolipid had the partial structure of Gal-(1→4)-Gal-(1→1)-Cer. These two lipids accumulate in the kidneys; they are also present in blood plasma, but not in the erythrocytes. Miyatake (104) confirmed the structure of ceramide trihexoside, and in addition determined the distribution of the ceramide polyhexosides in the different organs. Sweeley, Snyder

& Griffin (105) established the β-glycosidic linkages in the ceramide trihexoside by NMR spectroscopy.

Miyatake, Handa & Yamakawa (106) studied the chemical structure of the main glycolipid of hog erythrocytes, a ceramide tetrahexoside which has the same oligosaccharide sequence as the Forssman active glycolipid except for the β configuration of the glycosidic linkage of the terminal N-acetylgalactosamine. The following structure was proposed: N-Ac-Gal-(β1→3)-Gal-(β1→4)-Gal-(β1→4)-Glc-(β1→1)-Cer.

The chemical structure of the Forssman hapten has been elucidated by Makita, Suzuki & Yosizawa (107). It differs from globoside only in the α-type glycosidic linkage of the terminal N-acetylgalactosamine residue: N-Ac-Gal-(α1→3)-Gal-(β1→4)-Gal-(β1→4)-Glc-(β1→1)-Cer. Recently glycosphingolipids with even more complex carbohydrate chains have been isolated; these are of increasing importance with regard to their immunochemical properties and membrane functions.

Eto et al (108) described the isolation and characterization of a ceramide pentahexoside from rabbit erythrocyte ghosts and reticulocytes. The structure proved to be Gal-(α1→3)-Gal-(β1→3)-N-Ac-Glc-(β1→3)-Gal-(β1→4)-Glc-(β1→1)-Cer. Since this ceramide pentahexoside inhibits the agglutination of human B erythrocytes with the corresponding antibody, it is concluded that the terminal galactose is bound α-glycosidically.

The animal cell possesses three classes of characteristic glycolipids: (*a*) the neutral glycosphingolipids containing glucose, galactose, N-acetylglucosamine, and N-acetylgalactosamine, (*b*) fucose-containing glycosphingolipids characterized by terminally linked fucose, a "hydrophobic" residue, and (*c*) acid glycosphingolipids (gangliosides) with the typical N-acetylneuraminic acid residues again in terminal positions. Like the gangliosides, fucose-containing glycosphingolipids are present on the cell surface and exert highly specific functions.

Yamakawa (109) isolated the first blood group active glycosphingolipids of the globoside type; however, analysis showed substantial substitution by fucose. Globosides II and III appeared to be specific for blood groups A and B, respectively. Structures for these compounds have not been proposed. Hakomori & Strycharz (110) fractionated and purified the A,B,H, and Lewis a and b haptens of erythrocytes for structural studies, separating them as acetylated compounds by thin-layer chromatography. Component I was deemed to be a ceramide hexahexoside, II was a ceramide heptahexoside, and III was heterogeneous. These haptens contained Gal, Glc, Fuc, N-Ac-Glc, and the blood group A substance N-Ac-Gal in ratio of 2-4:1:1:1:1. So far it can be concluded that these 5, out of about 65 known blood group substances of glycosphingolipid structure, have a common ceramide tetrahexoside backbone with the following structure: Gal-(1→4)-N-Ac-Glc-(1→3)-Gal-(1→4)-Glc-(1→1)-Cer. A, B, H, Lewis a (Le^a), and Lewis b (Le^b) can be converted to this ceramide tetrahexoside by acid treatment or Smith degradation (111, 112). The structural studies on the A and B glyco-

lipid have not yet been completed, but a ceramide octahexoside with a branched oligosaccharide chain has been proposed (S. Hakomori, personal communication). The Le^a hapten is a ceramide pentahexoside and the Le^b hapten is a ceramide hexahexoside. The oligosaccharide chains are in the linear arrangement:

Le^a Gal-(1→3)-N-Ac-Glc-(1→3)-Gal-(1→4)-Glc-(1→1)-Cer

4
↑
1
Fuc

Le^b Fuc-(1→2)-Gal-(1→3)-N-Ac-Glc-(1→3)-Gal-(1→4)-Glc-(1→1)-Cer

4
↑
1
Fuc

Another Le^b active glycolipid turned out to be a ceramide octahexoside:

Fuc-(1→2)-Gal-(1→3)-N-Ac-Glc-(1→3)-Gal→N-Ac-Glc-(1→3)Gal-(1→4)-Glc-(1→1)-Cer

4
↑
1
Fuc

A fucose-containing sphingolipid with a novel type of ceramide and unique carbohydrate moiety but missing blood group A, B, H, and Lewis specifities has been isolated from erythrocytes and chemically characterized by Yang & Hakomori (113):

Gal-(1→4)-N-Ac-Glc-(1→3)-Gal-(1→4)-Glc-(1→1)-Cer

3
↑
1
Fuc

The ceramide is mainly composed of 4-hydroxysphinganine and of long-chain 2-hydroxy fatty acids.

Le^a and Le^b glycolipids were first isolated from adenocarcinoma tissue (114, 115). The presence of fucose-containing glycosphingolipids has been demonstrated in different tissues: epithelial glandular tissues such as hog (116) and dog (117) small intestine, pancreas adenocarcinoma of colon, and metastatic lesions in the liver originating from these tumors (112). Marcus & Cass (118) found that Le^a-and Le^b-active glycosphingolipids are associated with the high- and low-density lipoproteins of plasma, and become integrated into the erythrocyte surface membranes. The site of their biosynthesis is not known.

Blood group A and B glycolipids are absent in tumor tissues, whereas Le^a, Le^b, and H fucose-containing glycosphingolipids have been isolated from these sources as typical constituents. On the other hand Kawanami & Tsuji (119) were not able to find the fucose-containing ceramide polyhexosides described by Hakomori (113) in human gastric carcinoma. Instead ceramide mono-, di-, tri-, and tetrahexosides and cerebrosides were the main components, with 3′-sulfate ester (sulfatides) and hematosides present as minor components.

Wiegandt (120) reported on the occurrence of gangliosides in bovine red cells and spleen, and referred to a N-Ac-glucosamine-containing ganglioside with Gal- (1→3)-N-Ac-Glc-(1→4)-Gal-(1→4)-Glc-(1→1)-Cer as the backbone. Spleen contained a number of other N-acetyl- and N-glycolylneuraminic acid-containing ceramide polyhexosides with N-acetylglucosamine and N-acetylgalactosamine, e.g. Gal-(1→3)-N-Ac-Gal-(1→4)-Gal-(1→4)-Glc-(1→1)-Cer, and Gal-(1→3)-N-Ac-Glc-(1→4)-Gal-(1→4)-Glc-(1→1)-Cer. The neuraminic acids were bound to the 3 position of the terminal galactose residue. A glycosphingolipid with a new sialic acid, O-acetyl-(N-glycolyl)-neuraminic acid, has been isolated from equine erythrocyte ghosts (121). It is a hematoside except that the neuraminic acid carries both an N-glycolyl and an O-acetyl group, the latter linked either to C_4 or to the glycolic acid group. Ishizuka, Kloppenburg & Wiegandt (122) in a characterization of gangliosides from fish brain refer to the same fundamental tetrahexoside structure of gangliosides, Glc-(4→1)-Gal-(4→1)-N-Ac-Gal-(3→1)-Gal in iso- and poikilotherm animals. The difference in the gangliosides is, however, the occurrence of 8–O,N-diacetylneuraminic acid and, in addition, the large quantities (ca 50% of the total gangliosides), of tri-, tetra-, and penta-N-acetylneuraminyl gangliosides. The absolute content of brain gangliosides in fish and amphibians is only 0.3 to 0.5 times that in mammalian brain. A similar comparative study has been reported by Avrova (123). Two review articles comprehensively summarize the chemistry of gangliosides (124, 125).

Carter et al (126, 127) continued their studies of the chemical structure of phytoglycosphingolipids. Oligosaccharides referring to tetra, penta-, hexa-, hepta-, and octahexosides with and without a phosphate ester group were isolated. The complete structure of a phytoglycolipid was established as: N-acetyl-4D-hydroxysphinganine-1-phosphoryl-1′-O-(2′-O-mannosido-6′-O-[(fucosylarabinosyl-galactosyl)-D-glucosamido-1-(1→4)-D-glucoronido)]-myoinositol. Wagner & Zofcsik (128, 129) isolated two glycosphingolipids from the yeasts *Candida utilis* and *Saccharomyces cerevisiae*. One turned out to be dihydrogalactocerebroside, and the other contained C_{18} and C_{20}-4-hydroxysphinganine and sphinganine acylated with long-chain saturated and monoenoic acids (C_{24}) linked via a phosphodiester to myoinositol and D-mannose.

CHEMICAL SYNTHESIS

Weiss (130) and Prostenik, Majhofer-Orescanin & Ries-Lesic (131) established the stereochemical and structural relationship between 4*t*-sphin-

genine and 4D-hydroxysphinganine and proved that it is D-ribo-2-amino-1,3,4,-octadecanetriol. Two elegant synthetic routes for 4D-hydroxysphinganine were developed by Gigg & Gigg (132, 133) with D-galactosamine and 4-galactose as starting material. Gaver & Sweeley (134) and Mendershausen & Sweeley (135) prepared the 3-oxo derivatives (= 3-dehydro-) of N-acetyl-4*t*-sphingenine, sphinganine, and N-carbobenzoxysphinganine, which was transformed to 3-dehydrosphinganine. A simple procedure for the synthesis of the hydrochloride of this and homologous aminohydroxyketones was described by Stoffel & Sticht (136). The same authors (137) carried out chemical syntheses of ^{14}C- and ^{3}H-labeled sphinganines and sphingenines required for the biochemical studies, and of the 1- and 3-phosphate esters of sphinganine (138).

Flowers (139) described a two-step synthesis of lactosylceramide (cytolipin H). Hay & Gray (140) outlined the chemical preparation of D-glucosyl-(β1→1)-ceramide, D-lactosyl-(β1→1)-ceramide, and D-galactosyl-(β1→4)-D-galactosyl-(β1→1)-ceramide. Ceramides obtained from natural sphingomyelin by phospholipase C hydrolysis were tritylated and the 3-O-benzoyl ester was formed. After detritylation the 3-O-benzoylceramide was condensed with the relevant acetobromo sugar in satisfactory yield (12–30%).

D-Galactose-3-sulfate was synthesized by Jatzkewitz & Nowoczek (141) and Stoffyn & Stoffyn (142), and proved to be identical with galactose sulfate liberated from brain sulfatides.

Shapiro has summarized the synthetic work in the sphingolipid field (143, 144, 144a), outlined the advances toward the synthesis of gangliosides (145, 146), and described the synthesis of 4*t*-sphingenine phosphorylcholine (147).

METABOLISM OF GLYCOSPHINGOLIPIDS

Biosynthesis of Glycosphingolipids

Biosynthesis of long-chain bases.—On the basis of experiments carried out independently by Snell et al (148–150) and Stoffel et al (151–153) the mechanism of the sphinganine biosynthesis hitherto proposed (154, 155) can no longer be accepted. In both laboratories the condensation product of palmitoyl CoA and serine has been isolated and chemically identified as 3-dehydrosphinganine. The condensing enzyme requires pyridoxal phosphate. NADPH and a microsomal reductase stereospecifically reduces 3-dehydrosphinganine to D-sphinganine (153), a reaction that has been studied with enzyme preparations from *Hansenula ciferri* and from different rat tissues (Figure 1). The condensing enzyme and reductase exhibit chain length specificities for CoA esters of C_{14} to C_{18} acids and C_{14} to C_{20}-3-dehydrosphinganines (156, 157). The mechanism of formation of the 4-*trans* double bond is still obscure.

In analogy the condensation reaction has also been carried out with mouse brain enzyme preparations leading to C_{18}- and C_{20}-sphinganines

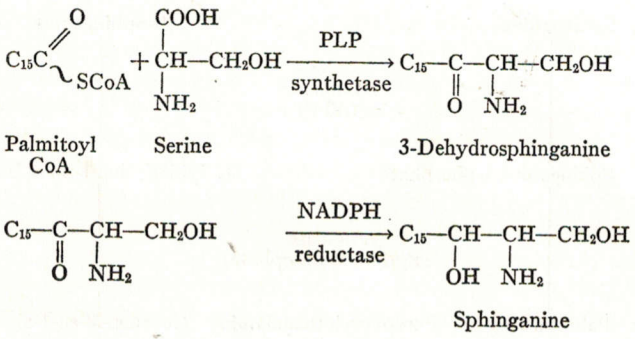

FIGURE 1. Biosynthesis of sphinganine (148–153).

(158). Greene, Kaneshiro & Law (159) found that 3-^{14}C-serine and ^{3}H-palmitate were incorporated into tetraacetyl-4D-hydroxysphinganine by *Hansenula ciferri*. Weiss & Stiller (160) and Stoffel, Sticht & LeKim (161) demonstrated the direct transformation of 1-^{14}C or 3-^{14}C sphinganine into 4-hydroxysphinganine by the yeast. The origin of the hydroxyl group on C_4 is still obscure, although Thorpe & Sweeley (162) conclude from $^{18}O_2$ experiments that it is derived from neither molecular oxygen nor water, but from an unknown donor.

Degradation of long-chain bases.—Stoffel and collaborators studied the degradation of specifically labeled sphinganine, 4*t*-sphingenine, and 4D-hydroxysphinganine in vivo (163–166) and in vitro (167, 168). The long-chain bases sphinganine, 4*t*-sphingenine, and 4D-hydroxysphinganine, independent of their chain length or configuration, are cleaved into a C_2 fragment corresponding to C_1 and C_2 and a long-chain fragment (C_3 to the terminal CH_3 group). The primary fragments of these long-chain bases were identified as phosphoryl-ethanolamine and palmitaldehyde, hexadec-2*t*-enal and 2-hydroxypalmitaldehyde respectively (Figure 2). The degradation is initiated by an ATP-dependent kinase (167–170). The phosphorylation of all long-chain bases has been demonstrated with human erythrocytes (170) and that of sphinganine with an enzyme preparation from bovine kidney (171). A pyridoxal phosphate-dependent, microsomal lyase catalyzed the aldolase-type reaction which has been characterized with synthetic phosphate esters of long-chain bases (168). The results of the studies in vivo have been confirmed in a number of laboratories (172–176). The long-chain aldehydes are utilized after oxidation to palmitate to supply the acyl group of ester lipids or for the formation of the vinyl ether linkage of plasmalogens (177). Phosphorylethanolamine from the sphingolipid is incorporated into phosphatidylethanolamine (166–168).

Ceramides are rapidly formed from *erythro* and *threo* long-chain bases (178). Kanfer & Gal (179) suggest that *threo*-N-acyl-4*t*-sphingenines also are utilized in vivo for sphingomyelin biosynthesis. Their results are at var-

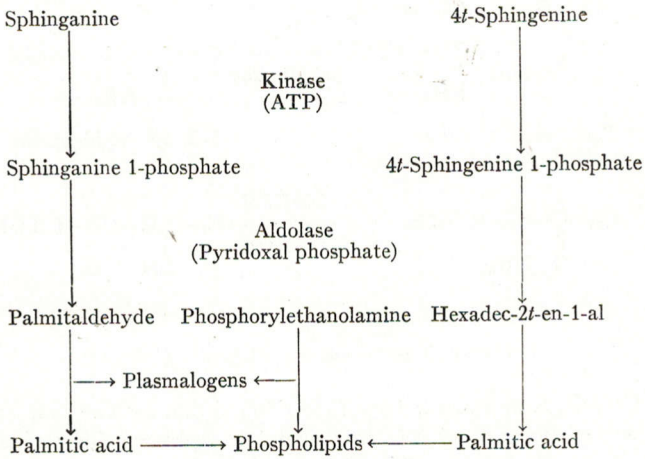

FIGURE 2. Sequence in the degradation of long-chain bases (156).

iance with those of Stoffel, Därr & Assmann (180), who demonstrated that only *erythro*-sphinganine is introduced in the sphingomyelin molecule either as such or after desaturation as *erythro*-4*t*-sphingenine.

The specificity of the acylation of long-chain bases by acyl-CoA esters has been studied by Morell & Radin (181) with brain microsomes of young mice. It has been well established that ceramides with nonhydroxy-*n*-fatty acids are the precursors of gangliosides and sphingomyelin. The hydroxy-fatty acid containing ceramides accept predominantly galactose with the formation of cerebrosides of the cerebron and oxynervon types (182–185).

Fujino & Nakano (186) reported that both the *erythro* and *threo* isomers of N-acylsphingenine and -sphinganine are substrates for the enzymic synthesis of cerebrosides from ceramide and UDP-galactose with enzyme preparations from rat liver and rat brain, the *erythro* form being a somewhat better acceptor. It remains unclear whether the biosynthesis of cerebrosides occurs via the acylation of psychosine or the glycosidation of ceramide.

The incorporation of labeled glucose and galactose into cerebrosides was studied with slices of guinea pig brain cortex by Nishimura, Ueta & Yamakawa (187). A small amount of glucose was found in a kerasine-type glucocerebroside, whereas the phrenosine was labeled with galactose.

McKhann, Levy & Ho (188), and McKhann & Ho (189) in reinvestigating the biosynthesis of sulfatides succeeded in solubilizing the galactocerebroside sulfotransferase from the microsomal fraction of rat brain. Galactosylceramide and lactosylceramide proved to be acceptors for the sulfate group from phosphoadenosyl phosphosulfate (PAPS). These results were confirmed by Stoffyn, Stoffyn & Hauser (190), who carefully characterized the product using ^{14}C-galactose-labeled phrenosine and a microsomal frac-

tion of rat brain. Sulfatides labeled with $^{35}SO_4^{2-}$ were used to determine the synthesis and turnover of myelin (191, 192). Sulfatide synthesis is most rapid between 20 and 25 days after birth.

Dawson & Sweeley (193) studied the metabolism of glycosylceramide, lactosylceramide, galactosyl-galactosyl-glucosylceramide, and globoside of porcine plasma and erythrocytes in vivo over a 3month period with ^{14}C-glucose. Their results suggest that lactosylceramide, galactosyl-galactosyl-glucosylceramide, and globoside of the erythrocyte are synthesized in the bone marrow and are not exchanged with their plasma counterparts. Glucosylceramide not synthesized in the bone marrow was freely exchanged between erythrocyte and plasma. The labeled ceramide oligohexosides occur only at the onset of the erythrocyte catabolism in the plasma. The globoside of the erythrocyte seems to be the source of all four plasma glycosphingolipids.

Fujino, Negishi & Ito (194, 195) reported that *threo* and *erythro*-4*t*-sphingenine form sphingosylphosphorylcholine in the presence of CDP-^{14}C-choline with mitochondria and microsomal enzyme preparations. They also reported (195) the acylation of sphingosylphosphorylcholine to yield sphingomyelin. Recalculation of their data shows surprisingly low radioactivities. They suggest that the *threo* and *erythro* isomers of sphingosylphosphorylcholine can be acceptors for the acyl group.

Gangliosides are synthesized by a stepwise elongation of the oligosaccharide chains, by specific glycosyltransferases (196). These enzymic reactions have been elucidated in the last years step by step. Basu, Kaufman & Roseman (197) achieved the enzymic synthesis of glucosylceramide by a particulate enzyme preparation from embryonic chicken brain starting from ceramide and UDP-glucose, and of lactosylceramide from glucosylceramide and UDP-galactose. A galactosyltransferase catalyzes the transfer of galactose from UDP-galactose to the terminal N-acetylgalactosamine residue of the Tay-Sachs ganglioside, N-Ac-Gal-(β1→4) [NANA-(2→3)]-Gal-(β1→4)-Glc-(1→1)-Cer (198).

The galactosyltransferases from rat spleen and brain, and from mouse kidney which catalyze these reactions together with the glycosidation of sphingenine to psychosine (galactosylsphingenine), of glucosylceramide to lactosylceramide, of lactosylceramide to galactosyl-galactosyl-glucosylceramide, and of Tay-Sachs ganglioside to monosialoganglioside have been studied by Hauser et al (199–202). In agreement with the results of Roseman et al (196) they conclude that these transferases are all different (in heat stability, stimulation by Mg^{++} and Mn^{++}, and inhibition by various sphingolipids), and specific for the sphingenine-containing lipid acceptor. Basu & Kaufman (203) have demonstrated that specific sialyltransferases from chicken embryonic brain transfer N-acetylneuraminic acid from its CMP derivative to lactosylceramide, and Kaufman, Basu & Roseman (204) succeeded in the biosynthesis of the disialogangliosides NANA→NANA→Gal→Glc→Cer, and NANA→Gal→N-Ac-Gal-[NANA]-Gal→Glc→Cer with hematoside and monosialoganglioside as substrates. The transfer of N-

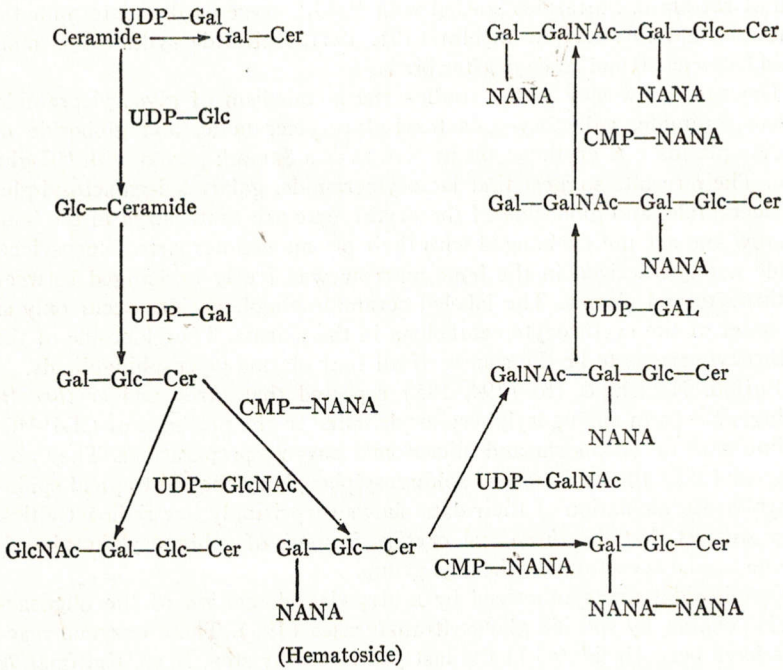

FIGURE 3. Proposed pathway for biosynthesis of gangliosides and blood group glycolipids (206).

Ac-Gal from UDP-N-Ac-Gal to hematoside to yield the Tay-Sachs ganglioside was reported by Steigerwald et al (205). Basu, Kaufman & Roseman (206), on the basis of their enzymatic studies, proposed the pathway shown in Figure 3 for the biosynthesis of gangliosides, which can be extended to blood group substances. At present it appears that complexes of glucosyltransferases are required for the synthesis of glycosphingolipids, glycoproteins, and mucins. Each transferase of the complexes is specific for the acceptor and its analogues, and different transferases catalyze the subsequent reactions. The sugar moiety is added as a monosaccharide unit at the nonreducing end of the carbohydrate chain from the nucleotide precursor.

Glycolipid biosynthesis has also been studied by Coles & Gray (207) and Hay & Gray (208, 209) with kidney homogenates of genetically characterized mouse strains (C57/BL and CBH/He) and those with BP8 ascites tumors. UDP-Gal is transferred to ceramides with hydroxy fatty acids, glucosylceramide, lactosylceramide, and digalactosyl glucosylceramide. The authors noted that the galactosyltransferases have higher activity in the male than in the female and that testosterone controls in part the synthesis of these glucosylceramides. The presence of the ascites tumor in the host leads to a depressed synthesis of lactosyl and digalactosyl glucosylceramide in the

kidney of both sexes. The transfer of galactose of UDP-galactose to the Tay-Sachs' ganglioside with rat and frog liver mitochondrial and microsomal fractions has also been demonstrated by Yip & Dain (210, 211). Although nucleotide sugars are almost certainly substrates of the transferase systems in glycosphingolipid biosynthesis, a recent report by Behrens & Leloir (212) indicates that a polyisoprenylphosphoryl sugar, presumably dolichylphosphoryl sugar, may serve as substrate. It is premature to decide whether the latter substance also provides an alternative mode of sugar transfer in mammalian systems.

The developmental pattern of gangliosides in rat brain has been studied by De Maccioni & Caputto (213), Spence & Wolfe (214), and Suzuki (215). Dukes (216) observed a stimulation of 1-^{14}C glucosamine incorporation into glycolipids of bone marrow cells from rat tibia in tissue cultures. The labeled component has not been identified.

Degradation of Glycosphingolipids

Glycosphingolipids are degraded by the stepwise removal of the sugar units by glycosylceramide hydrolases. These enzymes occur in all organs of the body (217), and apparently are all located in lysosomes (218), where they participate in the hetero- and autophagocytosis of macromolecules. All the hydrolases are characterized by their acidic pH optimum.

A soluble ceramidase which catalyzes the hydrolysis of ceramides to long-chain base and fatty acid has been further purified (219, 220), and has been postulated to catalyze synthesis of ceramides from long-chain bases and free fatty acids.

The enzymatic hydrolysis of sphingomyelin in rat liver has been studied by Heller & Shapiro (221), while Barenholz, Roitman & Gatt (222) purified a sphingomyelinase from rat brain particles. Both enzymes exhibit a phospholipase C-type activity, but are specific for sphingomyelin. Studies in vivo regarding the complete degradation of sphingomyelin have been reported by Nilsson (176).

Ceramide glucoside is hydrolyzed by a β-glucosidase to ceramide and glucose. The enzyme has been purified from spleen (223), intestine (224), and ox brain (225). A β-galactosidase, which has been isolated from rat and calf brain (226–228), and intestinal mucosa (229), hydrolyzes ceramide galactosides (cerebrosides). This enzyme also hydrolytically cleaves ceramide lactoside to ceramide glucoside and galactose (230).

Cerebrosides are completely degraded by intestinal hydrolysis (231). The body pool of cerebrosides is not influenced by dietary cerebrosides directly; however, an active cerebroside synthesis reutilizing the long-chain base and the fatty acids was noted in mucosal cells.

Three β-N-acetylhexosaminidases have been isolated from rat and calf brain by Frohwein & Gatt (232–234). One particulate enzyme (800–20,000 $\times$ g sediment) was purified 65-fold, and was effective with N-acetylgalactosamine and N-acetylglucosamine bound as terminal glycosyl residues. The two supernatant enzymes, an N-acetylglucosaminidase and an N-acetyl-

galactosaminidase, did not hydrolyze glycosphingolipids such as N-AcGal-Gal-Glc-Cer or N-AcGal-Gal-Glc-Cer (globoside). When N-acetylneuraminim acid is linked to the ceramide trihexoside in Tay-Sachs' ganglioside, N-AcGal-(NANA 2→3)-Gal-Glc-Cer, enzymic attack is sluggish due to steric hindrance. A neuraminidase, which attacks gangliosides has been isolated and purified from pig brain by Zambotti et al (235, 236), Sandhoff & Jatzkewitz (237), and by Leibowitz & Gatt (238). This enzyme cleaves the ketosidic bond of N-acetylneuraminic acid linked to a terminal hexose or to another N-acetylneuraminic acid in terminal positions, e.g. in certain di- and trisialogangliosides NANA-(2→3)-Gal-(1→3)-N-AcGal-(1→4)-[NANA 2→3]-Gal-(1→4)-Glc-(1→1)-Cer or NANA- and NGNA-(2→3)-Gal-(1→4)-Glc-(1→1)-Cer (hematosides). Glycoproteins are not attacked.

On the basis of available information about the hydrolytic enzymes of mammalian cells which act on complex glycosphingolipids and gangliosides, all of which are presumably of lysosomal origin, the following stepwise degradation has been devised (238):

di- and trisialogangliosides $\xrightarrow{\text{neuraminidase}}$ monosialoganglioside $\xrightarrow{\beta\text{-galactosidase}}$ N-AcGal-NANA-lactosylceramide $\xrightarrow{\text{N-acetylgalactosaminidase}}$ NANA-lactosylceramide $\xrightarrow{\text{neuraminidase}}$ lactosylceramide $\xrightarrow{\beta\text{-galactosidase}}$ glucosylceramide $\xrightarrow{\beta\text{-glucosidase}}$ ceramide $\xrightarrow{\text{ceramidase}}$ long-chain base and fatty acid

These hydrolytic enzymes have elicited considerable interest in view of deficiencies of specific glycosyl ceramide hydrolases and sphingomyelinase (239–241). Due to the intracellular distribution of the enzymes the following storage diseases are regarded as lysosomal diseases.

The enzymic lesion of the inherited syndrome in the different forms of Niemann-Pick disease, in which sphingomyelin accumulates throughout the body, appears to result from a deficiency of the sphingomyelinase in liver and kidney (242, 243). Application of an elegant combination of the isotope and tissue culture techniques to skin fibroblasts and bone marrow cells of the patients also revealed the very low sphingomyelinase activity in these cells (244, 245, 245a).

Accumulation of glucosylceramide in the spleen, liver, and bone marrow cells of Gaucher patients has been attributed to a deficiency of a glucocerebrosidase. Leucocytes have been identified as a source of the increased glucosylceramide level (246). The enzyme assay can therefore be made with circulating leucocytes and labeled glucocerebroside (247). Cells obtained by amniocentesis and subsequently grown in tissue cultures also can be used for the glucocerebrosidase assay and the detection of this disease in utero (239, 248). Dawson & Stein (248a) recently described a lactosylceramidosis in a 3 year old Negro female. It occurred as a neurovisceral storage disorder with specific elevation of lactosylceramide in erythrocytes, plasma, bone

marrow, liver biopsy, brain biopsy, and urine sediment. A galactosylhydrolase deficiency was demonstrated.

Jatzkewitz, Mehl & Sandhoff (249, 250) have shown that the demyelinating disease, metachromatic leucodystrophy, characterized by a marked excess of 3′-sulfate esters of galacto- and lactocerebrosides, results from deficiency of the sulfatase which hydrolyzes sulfatides to galactocerebrosides and sulfate. The enzymic activity against arylsulfatase has been correlated with the same enzyme (251). Here again the diagnosis can be made with samples of venous blood (252). The accumulation of cerebroside sulfate also has been observed in skin fibroblasts in cultures from patients with infantile metachromatic leucodystrophy (253).

Sweeley et al (254, 255) studied the enzymatic defect in Fabry's disease in which ceramide di- and trihexosides identified as Gal-(1→4)-Gal-(1→4)-Glc-(1→1)-Cer, and Gal-(1→4)-Glc-(1→1)-Cer accumulate in kidney, heart, and blood vessels. Cells in tissue cultures of skin fibroblasts from patients with Fabry's disease showed an accumulation of this ceramide trihexoside and also of an acid mucopolysaccharide. The ceramide trihexosidase was absent in the blood plasma of these patients. This enzyme is also deficient in extracts of biopsies of small intestine from Fabry's patients (256). Kint (257) demonstrated that a nonspecific α-galactosidase is absent in Fabry's disease. The diagnosis can be made by measurement of the α-galactosidase in leucocytes of the patient.

Generalized gangliosidosis, a rare genetic aberration with a progressive accumulation of gangliosides in brain and other tissues, is caused by a β-galactosidase deficiency (258–261). The exact nature of the metabolic abnormality in two forms of Tay-Sachs disease with its accumulation of N-AcGal-(1→4)-[NANA(2→3)]-Gal-(1→4)-Glc-(1→1)-Cer has also been investigated recently (262). The conventional and an exceptional form of Tay-Sachs disease, the latter also with visceral involvement and additional storage of kidney globoside, show a similar but quantitatively different pattern of glycosphingolipid depositions in organs. A deficiency of a β-N-acetylhexosaminidase is the biochemical basis of this storage disease. This enzyme is present cn two forms as isoenzymes A and B (263, 264). Both were absent in the exceptional case (type O), but only the A isoenzyme was absent in the normal type B of Tay-Sachs disease (265, 266). The partial or total lack of β-N-acetylhexosaminidase in Tay-Sachs disease has also been demonstrated in tissue culture from skin fibroblasts (265) and muscle tissue, whereas the N-acetylneuraminidase proved to be normal (267). A comprehensive review on gangliosidoses by Jatzkewitz (268) has appeared.

BIOLOGICAL FUNCTIONS

Our increased understanding of the chemistry and biochemistry of glycosphingolipids, aided by many new microanalytical tools, has brought renewed interest in the biological functions of these heterogeneous groups of

lipids. There is common agreement that glycosphingolipids are mainly constituents of the plasma membrane lipids of mammalian cells (269–275). The importance of lipids, and particularly of glycosphingolipids, as immunological determinants has been largely neglected. However, in recent years the relationship between chemical structure and immunological activity of glycosphingolipids has been intensively studied (for reviews see Rapport & Graf 273 and Brady 274). Because of their localization in the surface membrane, glycosphingolipid structures may permit investigations of immunological properties of cell membrane in areas such as organ specificity of antitissue sera, Forssman-hapten, a heterogenetic antigen randomly distributed in animal species, and blood group substances of the ABH and Lewis groups. The immunochemistry of malignant cell membranes and their difference from normal cell membranes, the problem of contact inhibition, and finally the relation of glycosphingolipids of the plasma membrane to the formation of the virus envelope during the maturation process are areas in which glycosphingolipids have gained considerable importance.

It is not yet clear whether the ceramide part of glycosphingolipids is involved in the immune reaction. Arnon, Sela & Rachaman (275) found that lactosylceramide is a much better inhibitor of the immune reaction to a chemically prepared lactosyl-4*t*-sphingenine polypeptide conjugate than is the nonacylated lactosyl-4*t*-sphingenine. The chain length of the fatty acid seems to be important for the requisite complementary character which may be related to the hydrophobic nature of the inhibitor. The immunological activity of galactocerebrosides is well established. By use of a stable aqueous solution containing cerebroside-lecithin-cholesterol (2:1:4), precipitation reactions in agar gel have been carried out and antibodies detected in sera of experimental allergic encephalomyelitis and experimental allergic neuritis (276). Cerebroside had a protective effect when injected intradermally (277). Absorption studies of anticerebroside antibodies with cerebroside-lecithin (lecithin serves as auxiliary lipid) were reported by Rapport, Cavanna & Graf (278).

Taketomi & Yamakawa (279, 280) described the antigenic properties of synthetic protein complexes with glycolipids, e.g. sphingenine-protein, ceramide-protein, and sphingosylphosphorylcholine-protein conjugates.

Lactosylceramide (=cytolipin H) was the first glycosphingolipid hapten characterized (273), and its gel diffusion analysis with antitissue and antilactose sera has been studied (281). The identity of cytolipin K and globosides (N-AcGal-(β1→3)-Gal-(β1→4)-gal-(β1→4)-Glc-(1→1-Cer) isolated from human kidney and erythrocytes was established immunologically by agar diffusion experiments (282, 283) and subsequently by chemical techniques (107). Antibodies against the pure globoside have been prepared in rabbits and measured by quantitative precipitation, hemagglutination, immune hemolysis, and immune electrophoresis (284). The effect of auxiliary lipids such as lecithin, cholesterol, cerebrosides, and gangliosides on the aggregation of the immunologically active glycolipid was also studied. Globoside is

present in adult human erythrocytes as cryptic antigen, which can be uncovered by trypsin treatment. Fetal erythrocytes strongly react with antiglobo-side serum without prior trypsin treatment of the cell surface (285). Pascal, Saifer & Gitlin (286, 287) produced antibodies against gangliosides of normal brain and Tay-Sachs gangliosides. Such gangliosides proved to be very weak antigens.

The controversy about whether the blood group substances are glycopeptides or glycosphingolipids is not yet settled (288). Several detailed studies, however, support the idea that the cellular ABH and Lewis blood group substances have glycosphingolipid structures. Progress in the elucidation of their structures has been discussed earlier in this review. (p. 63–65).

The intercellular interaction of normal cells has been referred to *recognition sites* on their surface membranes which contain carbohydrate groups (289, 290). Specific carbohydrates are present in the plasma membrane of transformed cells (291, 292). Changes in the glycosphingolipid pattern of a malignant cell have been observed in human adenocarcinoma cells, in which Le^a and Le^b active glycolipids appear; however, the A and B antigens are lost. Cells in tissue culture transformed spontaneously or by polyoma virus and SV 40 were investigated for their changes in the glycosphingolipid pattern by Hakomori & Murakami (293). Polyoma-transformed hamster kidney fibroblasts (BHK 21-C 13) show a decrease in hematoside content and a corresponding increase in lactosyl- and glucosylceramides. Brady et al (294–296) on the other hand noticed a decrease of the disialogangliosides and an increase in hematoside and monosialogangliosides in spontaneously and SV 40-transformed line of mouse fibroblasts (3T3). Chemically transformed rat hepatoma cells (Morris H 5123 cell line) were supposed to undergo the same change.

Hakomori, Teather & Andrews (297) point out that the cell surface hematoside is in a different organizational state in normal and virally transformed cells. The glycolipid is supposedly masked in normal cells but unmasked in transformed cells. The absence of certain carbohydrate residues in certain glycolipids together with a concomitant increase of their precursor glycolipids suggests an "incomplete synthesis."

The presence of glycolipids in myxovirus envelope has been noticed by Blough & Lawson (298); however, a component analysis was not carried out. Glycosphingolipids have been studied in BHK 21-F, MK, MDBK, and HaK cell lines in relation to the production of the paramyxovirus SV 5 and the envelope of this virus (Klenk & Choppin 271). The plasma membranes of MK and MDBK cells contain only small amounts of gangliosides but appreciable amounts of neutral glycosphingolipids; membranes of HaK and BKH on the other hand are rich in gangliosides and contain only traces of neutral glycolipids. The glycolipid pattern of the virus envelope resembles closely that of the plasma membrane except that gangliosides are missing, presumably because of the neuraminidase localized in the virus envelope. Cells such as BHK 21-F, which are rich in gangliosides, are very sensi-

tive to virus-induced cell fusion with subsequent cell disintegration, whereas MK cells with a low ganglioside content are resistant to cell fusion after virus infection. The virus maturation seems to be favored at the surface of cells with little or no ganglioside content and high phosphatidylethanolamine content.

LITERATURE CITED

1. Carter, H. E., Johnson, P., Weber, E. J. 1965. *Ann. Rev. Biochem.* 34:109
2. Olson, J. A. 1966. *Ann. Rev. Biochem.* 35:559
3. Shapiro, B. 1967. *Ann. Rev. Biochem.* 36:247
4. Kates, M., Wassef, M. K. 1970. *Ann. Rev. Biochem.* 39:323
5. IUPAC-IUB Commission on Biochemical Nomenclature; The Nomenclature of Lipids. 1968. *Biochim. Biophys. Acta* 152:1
6. Hakomori, S., Murakami, W. T. 1968. *Proc. Nat. Acad. Sci. USA* 59:254
7. Saito, T., Hakomori, S. *J. Lipid Res.* In press
8. Gray, G. M. 1967. *Biochim. Biophys. Acta* 144:511, 519
9. Skipski, V. P., Smolowe, A. F., Barclay, M. 1967. *J. Lipid Res.* 8:295
10. Spence, M. W. 1969. *Can. J. Biochem.* 47:735
11. MacMillan, V. H., Wherrett, J. R. 1969. *J. Neurochem.* 16:1621
12. Suzuki, K. 1965. *J. Neurochem.* 12: 629, 969
13. Sandhoff, K., Harzer, K., Jatzkewitz, H. 1968. *Z. Physiol. Chem.* 349: 283
14. Haahti, E., Jaakonmäki, I. 1969. *Ann. Med. Exp. Biol. Fenn.* 47:1
15. Yamamoto, A., Rouser, G. 1970. *Lipids* 5:442
16. Coles, L., Gray, G. M. 1970. *J. Lipid Res.* 11:164
17. Vance, D. E., Sweeley, C. C. 1967. *J. Lipid Res.* 8:621
18. Desnick, R. J., Sweeley, C. C., Krivit, W; 1970. *J. Lipid Res.* 11:31
19. Barenholz, Y., Gatt, S. 1968. *Biochim. Biophys. Acta* 152:790
20. Michalec, C. 1967. *J. Chromatogr.* 28:489
21. Karlsson, K. A. 1967. *Acta Chem. Scand.* 21:2577
22. Carter, H. E., Gaver, R. C. 1967. *J. Lipid Res.* 8:391
23. Michalec, C. 1966. *J. Chromatogr.* 24:228; 1967. 31:643
24. Renkonen, O. 1965. *J. Am. Oil Chem. Soc.* 42:298
25. Young, O. M., Kanfer, J. N. 1965. *J. Chromatogr.* 19:611
26. Kean, E. L. 1968. *J. Lipid Res.* 9:319
27. Dawson, G., Sweeley, C. C. 1969. *J. Lipid Res.* 10:402
28. Wiegandt, H., Baschang, G. 1965. *Z. Naturforsch.* 20:164
29. Hakomori, S. 1966. *J. Lipid Res.* 7: 789
30. Galanos, C., Lüderitz, O., Himmelspach, K. 1969. *Eur. J. Biochem.* 8:332
31. Seyama, Y., Yamakawa, T., Komai, T. 1968. *J. Biochem. Tokyo* 64: 487
32. Karlsson, K. A. 1965. *Acta Chem. Scand.* 19:2425
33. Gaver, R. C., Sweeley, C. C. 1966. *J. Am. Chem. Soc.* 88:3643
34. Donike, M. 1969. *J. Chromatogr.* 42: 103
35. Polito, A. J., Naworal, J., Sweeley, C. C. 1969. *Biochemistry* 8:1811
36. Capella, P., Zorzut, C. M. 1968. *Anal. Chem.* 40:1458
37. Eglinton, G., Hunneman, D. H., McCormick, A. 1968. *Org. Mass Spectrom.* 1:593
38. Samuelsson, B., Samuelsson, K. 1968. *Biochim. Biophys. Acta* 164:421
39. Samuelsson, B., Samuelsson, K. 1969. *J. Lipid Res.* 10:41
40. Casparrini, G., Horning, E. C., Horning, M. G. 1969. *Chem. Phys. Lipids* 3:1
41. Samuelsson, K., Samuelsson, B. 1969. *Biochem. Biophys. Res. Commun.* 37:15
42. Karlsson, K. A., Samuelsson, B., Steen, G. O. 1969. *Biochem. Biophys. Res. Commun.* 37:22
43. Sweeley, C. C., Dawson, G. 1969. *Biochem. Biophys. Res. Commun.* 37:6
44. DeJongh, D. C. et al 1969. *J. Am. Chem. Soc.* 91:1728
45. Björndal, H., Hellerqvist, C. G., Lindberg, B., Svensson, S. 1970. *Angew. Chem.* 82:643
46. Björndal, H., Lindberg, B., Svensson, S. 1967. *Acta Chem. Scand.* 21: 1801; *Carbohyd. Res.* 5:433
47. Hakomori, S. 1964. *J. Biochem. Tokyo* 55:205
48. Weiss, B. 1965. *Biochemistry* 4:686
49. Prostenik, M., Majhofer-Orescanin, B., Ries-Lesic, B. 1965. *Tetrahedron* 21:651
50. Morrison, W. R. 1969. *Biochim. Biophys. Acta* 176:537
51. Morrison, W. R., Hay, J. D. 1970. *Biochim. Biophys. Acta* 202:460
52. Carter, H. E., Gaver, R. C., Yu,

R. K. 1966. *Biochem. Biophys. Res. Commun.* 22:316
53. Carter, H. E., Gaver, R. C. 1967. *Biochem. Biophys. Res. Commun.* 29:886
54. White, D. C., Tucker, A. N., Sweeley, C. C. 1969. *Biochim. Biophys. Acta* 187:527
55. Carter, H. E., Hirschberg, C. B. 1968. *Biochemistry* 7:2296
56. Karlsson, K. A. 1966. *Acta Chem. Scand.* 20:2884
57. Renkonen, O., Hirvisalo, E. L. 1969. *J. Lipid Res.* 10:687
58. Hayashi, A., Matsubara, T. 1970. *Biochim. Biophys. Acta* 202:228
59. Polito, A. J., Akita, T., Sweeley, C. C. 1968. *Biochemistry* 7:2609
60. Renkonen, O. 1970. *Biochim. Biophys. Acta* 210:190
61. Panganamala, R. V., Geer, J. C., Cornwell, D. G. 1969. *J. Lipid Res.* 10:445
62. O'Connor, J. D., Polito, A. J., Monroe, R. E., Sweeley, C. C., Bieber, L. L. 1970. *Biochim. Biophys. Acta* 202:195
63. Hirvisalo, E. L., Renkonen, O. 1970. *J. Lipid Res.* 11:54
64. Moscatelli, E. A., Gilliland, K. M. 1969. *Lipids* 4:244
65. Bieber, L. L., O'Connor, J. D., Sweeley, C. C. 1969. *Biochim. Biophys. Acta* 187:157
66. Karlander, S. G., Karlsson, K. A., Samuelsson, B., Steen, G. O. 1969. *Acta Chem. Scand.* 23:3597
67. Karlsson, K. A. 1970. *Chem. Phys. Lipids* 5:6
68. Karlsson, K. A. *J. Lipid Res.* In press
69. Klenk, E., Huang, R. T. C. 1968. *Z. Physiol. Chem.* 349:451
70. Klenk, E., Huang, R. T. C. 1969. *Z. Physiol. Chem.* 350:373
71. Rosenberg, A., Stern, N. 1966. *J. Lipid Res.* 7:122
72. Karlsson, K. A. 1968. *Acta Chem. Scand.* 22:3050
73. Karlsson, K. A., Nilsson, K., Samuelsson, B., Steen, G. O. 1969. *Biochim. Biophys. Acta* 176:660
74. Hori, T., Itasaka, O., Inoue, H. 1966. *J. Biochem. Tokyo* 59:570
75. Simon, G., Rouser, G. 1967. *Lipids* 2:55
76. Dawson, R. M. C., Kemp, P. 1967. *Biochem. J.* 105:837
77. Dawson, R. M. C., Kemp, P. 1968. *Biochem. J.* 106:319
78. LaBach, J. P., White, D. C. 1969. *J. Lipid Res.* 10:528
79. Rizza, V., Tucker, A. N., White, D. C. 1970. *J. Bacteriol.* 101:84
80. White, D. C., Tucker, A. N. 1970. *Lipids* 5:56
81. Klenk, E., Schorsch, E. U. 1967. *Z. Physiol. Chem.* 348:1061
82. Klenk, E., Rivera, M. E. 1969. *Z. Physiol. Chem.* 350:1589
83. Lesch, P., Meier, S., Bernhard, K. 1966. *Helv. Chim. Acta* 49:791
84. Svennerholm, L., Ställberg-Stenhagen, S. 1968. *J. Lipid Res.* 9:215
85. Suzuki, K., Chen, G. C. 1967. *J. Lipid Res.* 8:105
86. Kean, E. L. 1966. *J. Lipid Res.* 7:449
87. Pilz, H., Sandhoff, K., Jatzkewitz, H. 1966. *J. Neurochem.* 13:1273
88. Nishimura, K., Yamakawa, T. 1968. *Lipids* 3:262
89. Tamai, Y., Yamakawa, T. 1969. *Jap. J. Exp. Med.* 39:85
90. Tamai, Y., Yamakawa, T. 1968. *Jap. J. Exp. Med.* 38:143
91. Klenk, E., Doss, M. 1966. *Z. Physiol. Chem.* 346:296
92. Klenk, E., Löhr, J. P. 1967. *Z. Physiol. Chem.* 348:1712
93. Kishimoto, Y., Wajda, M., Radin, N. S. 1968. *J. Lipid Res.* 9:27
94. Tamai, Y., Taketomi, T., Yamakawa, T. 1967. *Jap. J. Exp. Med.* 37:79
95. Tamai, Y. 1968. *Jap. J. Exp. Med.* 38:65
96. Karlsson, K. A., Samuelsson, B. E., Steen, G. O. 1968. *Acta Chem. Scand.* 22:2723
97. Karlsson, K. A., Samuelsson, B. E., Steen, G. O. 1969. *Biochim. Biophys. Acta* 176:429
98. Mårtensson, E. 1966. *Biochim. Biophys. Acta* 116:296
99. Stoffyn, A., Stoffyn, P., Mårtensson, E. 1968. *Biochim. Biophys. Acta* 152:353
100. Mårtensson, E. 1966. *Biochim. Biophys. Acta* 116:521
101. Malone, M., Stoffyn, P. 1965. *Biochim. Biophys. Acta* 98:219
102. Sweeley, C. C., Klionsky, B. 1963. *J. Biol. Chem.* 238:PC 3148
103. Vance, D. E., Krivit, W., Sweeley, C. C. 1969. *J. Lipid Res.* 10:188
104. Miyatake, T. 1969. *Jap. J. Exp. Med.* 39:35
105. Sweeley, C. C., Snyder, P. D., Griffin, C. E. Submitted for publication
106. Miyatake, T., Handa, S., Yamakawa, T. 1968. *Jap. J. Exp. Med.* 38:135
107. Makita, A., Suzuki, C., Yosizawa, Z.

1966. *J. Biochem. Tokyo* 60 :502
108. Eto, T., Ichikawa, Y., Nishimura, K., Ando, S., Yamakawa, T. 1968. *J. Biochem. Tokyo* 64 :205
109. Yamakawa, T., Nishimura, S., Kamimura, M. 1965. *Jap. J. Exp. Med.* 35 :201
110. Hakomori, S., Strycharz, G. D. 1968. *Biochemistry* 7 :1279
111. Hakomori, S. 1970. *Chem. Phys. Lipids* 5 :96
112. Hakomori, S., Andrews, H. 1970. *Biochim. Biophys. Acta* 202 :225
113. Yang, H., Hakomori, S. *J. Biol. Chem.* In press
114. Hakomori, S., Jeanloz, R. W. 1964. *J. Biol. Chem.* 239 :PC3606
115. Hakomori, S., Koscielak, J., Bloch, K. J., Jeanloz, R. W. 1967. *J. Immunol.* 98 :31
116. Suzuki, C., Makita, A., Yosizawa, Z. 1968. *Arch. Biochem. Biophys.* 127 :140
117. McKibbin, J. M. 1969. *Biochemistry* 8 :679
118. Marcus, D. M., Cass, L. E. 1969. *Science* 164 :553
119. Kawanami, J., Tsuji, T. 1968. *Jap. J. Exp. Med.* 38 :123
120. Wiegandt, H. 1969. *Chemistry and Metabolism of Sphingolipids.* New York: Dekker. In press
121. Hakomori, S. Saito, T. 1969. *Biochemistry* 8 :5082
122. Ishizuka, I., Kloppenburg, M., Wiegandt, H. 1970. *Biochim. Biophys. Acta* 210 :299
123. Avrova, N. F. 1968. *Zh. Evol. Biokhim. Fiziol.* 4 :128
124. Ledeen, R. 1966. *J. Am. Oil Chem. Soc.* 43 :57
125. Wiegandt, H. 1968. *Angew. Chem.* 80 :89
126. Carter, H. E., Strobach, D. R., Hawthorne, J. N. 1969. *Biochemistry* 8 :383
127. Carter, H. E., Kisic, A., Koob, J. L., Martin, J. A. 1969. *Biochemistry* 8 :389
128. Wagner, H., Zofcsik, W. 1966. *Biochem. Z.* 346 :343
129. Wagner, H., Zofcsik, W. 1966. *Biochem. Z.* 346 :333
130. Weiss, B. 1965. *Biochemistry* 4 :686
131. Prostenik, M., Majhofer-Orescanin, B., Ries-Lesic, B. 1965. *Tetrahedron* 21 :651
132. Gigg, J., Gigg, R., Warren, C. D. 1966. *J. Chem. Soc.* 1872
133. Gigg, J., Gigg, R. 1966. *J. Chem. Soc.* 1876, 1879
134. Gaver, R. C., Sweeley, C. C. 1966. *J. Am. Chem. Soc.* 88 :3643
135. Mendershausen, P. B., Sweeley, C. C. 1969. *Biochemistry* 8 :2633
136. Stoffel, W., Sticht, G. *Z. Physiol. Chem.* In press
137. Stoffel, W., Sticht, G. 1967. *Z. Physiol. Chem.* 348 :1561
138. Stoffel, W., Tschung, T. *Z. Physiol. Chem.* In press
139. Flowers, H. M. 1967. *Carbohydr. Res.* 4 :42
140. Hay, J. B., Gray, G. M. 1969. *Chem. Phys. Lipids* 3 :59
141. Jatzkewitz, H., Nowoczek, G. 1967. *Chem. Ber.* 100 :1667
142. Stoffyn, A., Stoffyn, P. 1967. *J. Org. Chem.* 32 :4001
143. Shapiro, D. 1965. *J. Am. Oil Chem. Soc.* 42 :267
144. Shapiro, D. 1969. *Chemistry of Sphingolipids.* Paris: Hermann
144a. Shapiro, D. 1970. *Chem. Phys. Lipids* 5 :80
145. Shapiro, D., Acher, A. J., Rachaman, E. S. 1967. *J. Org. Chem.* 32 :3767
146. Acher, A. J., Shapiro, D. 1969. *J. Org. Chem.* 34 :2652
147. Shapiro, D., Rachaman, E. S., Rabinsohn, Y., Diver-Haber, A. 1967. *Chem. Phys. Lipids* 1 :183
148. Braun, P. E., Snell, E. E. 1967. *Proc. Nat. Acad. Sci. USA* 58 :298
149. Braun, P. E., Snell, E. E. 1968. *J. Biol. Chem.* 243 :3775
150. Brady, R. N., Di Mari, S. J., Snell, E. E. 1969. *J. Biol. Chem* 244 : 491
151. Stoffel, W., LeKim, D., Sticht, G. 1967. *Z. Physiol. Chem.* 348 :1570
152. Stoffel, W., LeKim, D., Sticht, G. 1968. *Z. Physiol. Chem.* 349 :664
153. Stoffel, W., LeKim, D., Sticht, G. 1968. *Z. Physiol. Chem.* 349 :1637
154. Brady, R. O., Koval, G. J. 1958. *J. Biol. Chem.* 233 :26
155. Brady, R. O., Formica, J. V., Koval, G. J. 1958. *J. Biol. Chem.* 233 : 1072
156. Stoffel, W. 1970. *Chem. Phys. Lipids* 5 :139
157. Snell, E. E., Di Mari, S. J., Brady, R. N. 1970. *Chem. Phys. Lipids* 5 :116
158. Braun, P. E., Morell, P., Radin, N. S. 1970. *J. Biol. Chem.* 245 : 335
159. Greene, M. L., Kaneshiro, T., Law, J. H. 1965. *Biochim. Biophys. Acta* 98 :582

160. Weiss, B., Stiller, R. L. 1967. *J. Biol. Chem.* 242:2903
161. Stoffel, W., Sticht, G., LeKim, D. 1968. *Z. Physiol. Chem.* 349:1149
162. Thorpe, S. R., Sweeley, C. C. 1967. *Biochemistry* 6:887
163. Stoffel, W., Sticht, G. 1967. *Z. Physiol. Chem.* 348:941
164. Stoffel, W., Sticht, G. 1967. *Z. Physiol. Chem.* 348:1345
165. Stoffel, W., LeKim, D., Sticht, G. 1969. *Z. Physiol. Chem.* 349:1233
166. Stoffel, W., Henning, R. 1968. *Z. Physiol. Chem.* 349:1400
167. Stoffel, W., Sticht, G., LeKim, D. 1968. *Z. Physiol. Chem.* 349:1745
168. Stoffel, W., Assmann, G. 1970. *Z. Physiol. Chem.* 351:1041
169. Keenan, R. W., Maxam, A. 1969. *Biochim. Biophys. Acta* 176:348
170. Stoffel, W., Assmann, G., Binczek, E. 1970. *Z. Physiol. Chem.* 351: 635
171. Keenan, R. W., Haegelin, B. 1969. *Biochem. Biophys. Res. Commun.* 37:888
172. Keenan, R. W., Okabe, K. 1968. *Biochemistry* 7:2696
173. Barenholz, Y., Gatt, S. 1968. *Biochemistry* 7:2603
174. Gatt, S., Barenholz, Y. 1968. *Biochem. Biophys. Res. Commun.* 32: 588
175. Karlsson, K. A., Samuelsson, B. E., Steen, G. O. 1967. *Acta Chem. Scand.* 21:2566
176. Nilsson, A. 1968. *Biochim. Biophys. Acta* 164:575
177. Stoffel, W., LeKim, D., Heyn, G. 1970. *Z. Physiol. Chem.* 351:875
178. Sribney, M. 1966. *Biochim. Biophys. Acta* 125:542
179. Kanfer, J. N., Gal, A. E. 1966. *Biochem. Biophys. Res. Commun.* 22:442
180. Stoffel, W., Därr, W., Assmann, G. 1971. *Z. Physiol. Chem.* In press
181. Morell, P., Radin, N. S. 1970. *J. Biol Chem.* 245:342
182. Kopaczyk, K. C., Radin, N. S. 1965. *J. Lipid Res.* 6:140
183. Fujino, Y., Ito, S. 1968. *Biochim. Biophys. Acta* 152:627
184. Basu, S., Schultz, A., Basu, M. 1969. *Fed. Proc.* 28:540
185. Morell, P., Radin, N. S. 1969. *Biochemistry* 8:506
186. Fujino, Y., Nakano, M. 1969. *Biochem. J.* 113:573
187. Nishimura, K., Ueta, N., Yamakawa, T. 1966. *Jap. J. Exp. Med.* 36:91
188. McKhann, G. M., Levy, R., Ho, W. 1965. *Biochem. Biophys. Res. Commun.* 20:109
189. McKhann, G. M., Ho, W. 1967. *J. Neurochem.* 14:717
190. Stoffyn, P., Stoffyn, A., Hauser, G. 1970. *Trans. Am. Soc. Neurochem.* 1:114
191. Davison, A. N., Gregson, N. A. 1966. *Biochem. J.* 98:915
192. Pritchard, E. T. 1966. *J. Neurochem.* 13:13
193. Dawson, G., Sweeley, C. C. 1970. *J. Biol. Chem.* 245:410
194. Fujino, Y., Negishi, T., Ito, S. 1968. *Biochem. J.* 109:310
195. Fujino, Y., Negishi, T. 1968. *Biochim. Biophys. Acta* 152:428
196. Roseman, S. 1970. *Chem. Phys. Lipids* 5:270
197. Basu, S., Kaufman, B., Roseman, S. 1968. *J. Biol. Chem.* 243:5802
198. Basu, S., Kaufman, B., Roseman, S. 1965. *J. Biol. Chem.* 240:PC4115
199. Hauser, G. 1967. *Biochem. Biophys. Res. Commun.* 28:502
200. Hildebrand, J., Stoffyn, P., Hauser, G. 1970. *J. Neurochem.* 17:403
201. Hildebrand, J., Hauser, G. 1969. *J. Biol. Chem.* 244:5170
202. Hauser, G., Hildebrand, J. 1969. *Fed. Proc.* 28:595
203. Basu, S., Kaufman, B. 1965. *Fed. Proc.* 24:479
204. Kaufman, B., Basu, S., Roseman, S. 1968. *J. Biol. Chem.* 243:5804
205. Steigerwald, J. C., Kaufman, B., Basu, S., Roseman, S. 1966. *Fed. Proc.* 25:587
206. Basu, S., Kaufman, B., Roseman, S. *J. Biol. Chem.* In press
207. Coles, L., Gray, G. M. 1970. *Biochem. Biophys. Res. Commun.* 38: 520
208. Hay, J. B., Gray, G. M. 1970. *Biochem. Biophys. Res. Commun.* 38: 527
209. Hay, J. B., Gray, G. M. 1970. *Biochim. Biophys. Acta* 202:566
210. Yip, M. C. M., Dain, J. A. 1970. *Biochim. Biophys. Acta* 206:252
211. Yip, M. C. M., Dain, J. A. 1970. *Biochem. J.* 118:247
212. Behrens, N. H., Leloir, L. F. 1970. *Proc. Nat. Acad. Sci. USA* 66: 153
213. De Maccioni, A. H. R., Caputto, R. 1968. *J. Neurochem.* 15:1257
214. Spence, W. M., Wolfe, L. S. 1967. *Can. J. Biochem.* 45:671

215. Suzuki, K. 1965. *J. Neurochem.* 12: 969
216. Dukes, P. P. 1968. *Biochem. Biophys. Res. Commun.* 31:345
217. Gatt, S. 1966. In *Inborn Disorders in Sphingolipid Metabolism,* ed. S. M. Aronson, B. Volk. New York: Pergamon. 261 pp.
218. Weinreb, N. J., Brady, R. O., Tappel, A. L. 1968. *Biochim. Biophys. Acta* 159:141
219. Gatt, S. 1963. *J. Biol. Chem.* 238: PC3131
220. Yavin, E., Gatt, S. 1969. *Biochemistry* 8:1692
221. Heller, M., Shapiro, B. 1966. *Biochem. J.* 98:763
222. Barenholz, Y., Roitman, A., Gatt, S. 1966. *J. Biol. Chem.* 241:3731
223. Brady, R. O., Kanfer, J. N., Shapiro, D. 1965. *J. Biol. Chem.* 240:39
224. Brady, R. O., Gal, A. E., Kanfer, J. N., Bradley, R. M. 1965. *J. Biol Chem.* 240:3766
225. Gatt, S. 1966. *Biochem. J.* 101:687
226. Gatt, S., Rapport, M. M. 1966. *Biochim. Biophys. Acta* 113:567
227. Bowen, D. M., Radin, N. S. 1968. *Biochim. Biophys. Acta* 152:587, 599
228. Bowen, D. M., Radin, N. S. 1969. *J. Neurochem.* 16:501
229. Brady, R. O., Gal, A. E., Bradley, R. M., Mårtensson, E. 1967. *J. Biol. Chem.* 242:1021
230. Gatt, S., Rapport, M. M. 1966. *Biochem. J.* 101:680
231. Nilsson, A. 1969. *Biochim. Biophys. Acta* 187:113
232. Frohwein, Y. Z., Gatt, S. 1966. *Biochim. Biophys. Acta* 128:216
233. Frohwein, Y. Z., Gatt, S. 1967. *Biochemistry* 6:2783
234. Frohwein, Y. Z., Gatt, S. 1967. *Biochemistry* 6:2775
235. Zambotti, V., Tettamanti, G., Berra, B. 1965. *Proc. Fed. Eur. Biochem. Soc., Vienna,* A 236
236. Sassi, A., Di Donato, S., Tettamanti, G., Zambotti, V. 1966. *Proc. Fed. Eur. Biochem. Soc., Warsaw,* F 12
237. Sandhoff, K., Jatzkewitz, H. 1967. *Biochim. Biophys. Acta* 141:442
238. Leibovitz, Z., Gatt, S. 1968. *Biochim. Biophys. Acta* 152:136
239. Brady, R. O. 1969. *Med. Clin. N. Am.* 53:827
240. Brady, R. O. 1970. *Ann. Rev. Med.* 21:317
241. Fredrickson, D. S. 1966. In *The Metabolic Basis of Inherited Disease,* ed. I. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson. New York: McGraw. 586 pp.
242. Brady, R. O., Kanfer, J. N., Mock, M. B., Fredrickson, D. S. 1966. *Proc. Nat. Acad. Sci. USA* 55: 366
243. Schneider, P. B., Kennedy, E. P. 1967. *J. Lipid Res.* 8:202
244. Danes, B. S., Bearn, A. G. 1966. *J. Exp. Med.* 1:123
245. Matalon, R., Dorfman, A. 1966. *Proc. Nat. Acad. Sci. USA* 56: 1310
245a. Sloan, H. R. 1970. *Chem. Phys. Lipids* 5:250
246. Kattlove, H. E. et al 1969. *Blood* 33:379
247. Kampine, J. P., Brady, R. O., Kanfer, J. N., Feld, M., Shapiro, D. 1967. *Science* 155:86
248. Sloan, H. R., Uhlendorf, B. W., Kanfer, J. N., Brady, R. O., Fredrickson, D. S. 1969. *Biochem. Biophys. Res. Commun.* 34:582
248a. Dawson, G., Stein, A. D. 1970. *Science* 170:556
249. Jatzkewitz, H., Mehl, E., Sandhoff, K. 1970. *Biochem. J.* 117:6P
250. Mehl, E., Jatzkewitz, H. 1968. *Biochim. Biophys. Acta* 151:619
251. Mehl, E., Jatzkewitz, H. 1965. *Biochem. Biophys. Res. Commun.* 19:407
252. Percy, A. K., Brady, R. O. 1968. *Science* 161:594
253. Porter, M. T., Fluherty, A. L., Harris, S. E., Kihara, H. 1970. *Arch. Biochem. Biophys.* 138:646
254. Matalon, R., Dorfman, A., Dawson, G., Sweeley, C. C. 1969. *Science* 164:1522
255. Mapes, C. A., Anderson, R. L., Sweeley, C. C. 1970. *FEBS Lett.* 7:180
256. Brady, R. O. et al 1967. *N. Engl. J. Med.* 276:1163
257. Kint, J. A. 1970. *Science* 167:1268
258. Okada, S., O'Brien, J. S. 1968. *Science* 160:1002
259. Brady, R. O., O'Brien, J. S., Bradley, R. M., Gal, A. E. 1970. *Biochim. Biophys. Acta* 210:193
260. Dacremont, G., Kint, J. A. 1968. *Clin. Chim. Acta* 21:421
261. van Hoof, F., Hers, H. G. 1968. *Eur. J. Biochem.* 7:34
262. Sandhoff, K., Andreae, U., Jatzkewitz, H. 1968. *Life Sci.* 7:283
263. Robinson, D., Stirling, J. L. 1968. *Biochem. J.* 107:321

264. Sandhoff, K. 1968. *Z. Physiol. Chem.* 349:1095
265. Okada, S., O'Brien, J. S. 1969. *Science* 165:698
266. Sandhoff, K. 1969. *FEBS Lett.* 4:351
267. Kolodny, E. H., Brady, R. O., Volk, B. 1969. *Biochem. Biophys. Res. Commun.* 37:526
268. Sandhoff, K., Jatzkewitz, H., Peters, G. 1969. *Naturwissenschaften* 56: 356
269. Weinstein, D. B., Warren, L., Marsh, J. B. 1967. *Fed. Proc.* 28:3654
270. Dod, B. J., Gray, G. M. 1968. *Biochem. J.* 110:50P
271. Klenk, H. D., Choppin, P. W. 1970. *Proc. Nat. Acad. Sci. USA* 66:57
272. Renkonen, O., Gahmberg, C. G., Simons, K., Kääriäinen, L. *Acta Chem. Scand.* In press
273. Rapport, M. M., Graf, L. 1969. *Progr. Allergy* 13:273
274. Brady, R. O. 1966. *J. Am. Oil Chem. Soc.* 43:67
275. Arnon, R., Sela, M., Rachaman, E. S., Shapiro, D. 1967. *Eur. J. Biochem.* 2:79
276. Niedieck, B., Kuwert, E., Polacios, C., Drees, O. 1965. *Ann. NY Acad. Sci.* 122:266
277. Niedieck, B., Kuck, U. 1967. *Z. Immunitaetsforsch. Allerg. Klin. Immunol.* 133:43
278. Rapport, M. M., Cavanna, R., Graf, L. 1967. *J. Neurochem.* 14:9
279. Taketomi, T., Yamakawa, T. 1966. *Lipids* 1:31
280. Taketomi, T., Yamakawa, T. 1967. *Jap. J. Exp. Med.* 37:423
281. Graf, L., Rapport, M. M. 1966. *Fed. Proc.* 25:474
282. Rapport, M. M., Graf, L. 1965. *Fed. Proc.* 24:176
283. Graf, L., Rapport, M. M. 1965. *Int. Arch. Allergy* 28:171
284. Koscielak, J., Hakomori, S., Jeanloz, R. W. 1968. *Immunochemistry* 5: 441
285. Hakomori, S. 1969. *Vox Sang.* 16: 478
286. Pascal, T. A., Saifer, A., Gitlin, J. 1966. *Proc. Soc. Exp. Biol. Med.* 121:739
287. Pascal, T. A., Saifer, A., Gitlin, J. See Ref. 217, 289
288. Poulik, M. D. 1969. In *Red Cell Membrane Structure and Function,* ed. G. A. Jamieson, T. F. Greewalt. Philadelphia: Lippincott
289. Kalckar, H. M. 1965. *Science* 150: 305
290. Shen, L., Ginsburg, V. 1968. In *Biological Properties of the Mammalian Surface Membrane,* ed. L. A. Mason, Monogr. 8:6. Philadelphia: Wistar Inst. Press
291. Cox, R. P., Gesner, B. M. 1965. *Proc. Nat. Acad. Sci. USA* 54: 1571
292. Burger, M. M., Goldberg, A. R. 1967. *Proc. Nat. Acad. Sci. USA* 57: 359
293. Hakomori, S., Murakami, W. T. 1968. *Proc. Nat. Acad. Sci. USA* 59:254
294. Mora, P. T., Brady, R. O., Bradley, R. M., McFarland, V. W. 1969. *Proc. Nat. Acad. Sci. USA* 63: 1290
295. Brady, R. O., Borek, C., Bradley, R. M. 1969. *J. Biol. Chem.* 244: 6552
296. Brady, R. O., Mora, P. T., Kolodny, E. H., Borek, C. 1970. *Fed. Proc.* 29:410
297. Hakomori, S., Teather, C., Andrews, H. 1968. *Biochem. Biophys. Res. Commun.* 33:563
298. Blough, H. A., Lawson, D. E. M. 1968. *Virology* 36:286

215. Suzuki, K. 1965. *J. Neurochem.* 12: 969
216. Dukes, P. P. 1968. *Biochem. Biophys. Res. Commun.* 31:345
217. Gatt, S. 1966. In *Inborn Disorders in Sphingolipid Metabolism,* ed. S. M. Aronson, B. Volk. New York: Pergamon. 261 pp.
218. Weinreb, N. J., Brady, R. O., Tappel, A. L. 1968. *Biochim. Biophys. Acta* 159:141
219. Gatt, S. 1963. *J. Biol. Chem.* 238: PC3131
220. Yavin, E., Gatt, S. 1969. *Biochemistry* 8:1692
221. Heller, M., Shapiro, B. 1966. *Biochem. J.* 98:763
222. Barenholz, Y., Roitman, A., Gatt, S. 1966. *J. Biol. Chem.* 241:3731
223. Brady, R. O., Kanfer, J. N., Shapiro, D. 1965. *J. Biol. Chem.* 240:39
224. Brady, R. O., Gal, A. E., Kanfer, J. N., Bradley, R. M. 1965. *J. Biol Chem.* 240:3766
225. Gatt, S. 1966. *Biochem. J.* 101:687
226. Gatt, S., Rapport, M. M. 1966. *Biochim. Biophys. Acta* 113:567
227. Bowen, D. M., Radin, N. S. 1968. *Biochim. Biophys. Acta* 152:587, 599
228. Bowen, D. M., Radin, N. S. 1969. *J. Neurochem.* 16:501
229. Brady, R. O., Gal, A. E., Bradley, R. M., Mårtensson, E. 1967. *J. Biol. Chem.* 242:1021
230. Gatt, S., Rapport, M. M. 1966. *Biochem. J.* 101:680
231. Nilsson, A. 1969. *Biochim. Biophys. Acta* 187:113
232. Frohwein, Y. Z., Gatt, S. 1966. *Biochim. Biophys. Acta* 128:216
233. Frohwein, Y. Z., Gatt, S. 1967. *Biochemistry* 6:2783
234. Frohwein, Y. Z., Gatt, S. 1967. *Biochemistry* 6:2775
235. Zambotti, V., Tettamanti, G., Berra, B. 1965. *Proc. Fed. Eur. Biochem. Soc., Vienna,* A 236
236. Sassi, A., Di Donato, S., Tettamanti, G., Zambotti, V. 1966. *Proc. Fed. Eur. Biochem. Soc., Warsaw,* F 12
237. Sandhoff, K., Jatzkewitz, H. 1967. *Biochim. Biophys. Acta* 141:442
238. Leibovitz, Z., Gatt, S. 1968. *Biochim. Biophys. Acta* 152:136
239. Brady, R. O. 1969. *Med. Clin. N. Am.* 53:827
240. Brady, R. O. 1970. *Ann. Rev. Med.* 21:317
241. Fredrickson, D. S. 1966. In *The Metabolic Basis of Inherited Disease,* ed. I. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson. New York: McGraw. 586 pp.
242. Brady, R. O., Kanfer, J. N., Mock, M. B., Fredrickson, D. S. 1966. *Proc. Nat. Acad. Sci. USA* 55: 366
243. Schneider, P. B., Kennedy, E. P. 1967. *J. Lipid Res.* 8:202
244. Danes, B. S., Bearn, A. G. 1966. *J. Exp. Med.* 1:123
245. Matalon, R., Dorfman, A. 1966. *Proc. Nat. Acad. Sci. USA* 56: 1310
245a. Sloan, H. R. 1970. *Chem. Phys. Lipids* 5:250
246. Kattlove, H. E. et al 1969. *Blood* 33:379
247. Kampine, J. P., Brady, R. O., Kanfer, J. N., Feld, M., Shapiro, D. 1967. *Science* 155:86
248. Sloan, H. R., Uhlendorf, B. W., Kanfer, J. N., Brady, R. O., Fredrickson, D. S. 1969. *Biochem. Biophys. Res. Commun.* 34:582
248a. Dawson, G., Stein, A. D. 1970. *Science* 170:556
249. Jatzkewitz, H., Mehl, E., Sandhoff, K. 1970. *Biochem. J.* 117:6P
250. Mehl, E., Jatzkewitz, H. 1968. *Biochim. Biophys. Acta* 151:619
251. Mehl, E., Jatzkewitz, H. 1965. *Biochem. Biophys. Res. Commun.* 19:407
252. Percy, A. K., Brady, R. O. 1968. *Science* 161:594
253. Porter, M. T., Fluherty, A. L., Harris, S. E., Kihara, H. 1970. *Arch. Biochem. Biophys.* 138:646
254. Matalon, R., Dorfman, A., Dawson, G., Sweeley, C. C. 1969. *Science* 164:1522
255. Mapes, C. A., Anderson, R. L., Sweeley, C. C. 1970. *FEBS Lett.* 7:180
256. Brady, R. O. et al 1967. *N. Engl. J. Med.* 276:1163
257. Kint, J. A. 1970. *Science* 167:1268
258. Okada, S., O'Brien, J. S. 1968. *Science* 160:1002
259. Brady, R. O., O'Brien, J. S., Bradley, R. M., Gal, A. E. 1970. *Biochim. Biophys. Acta* 210:193
260. Dacremont, G., Kint, J. A. 1968. *Clin. Chim. Acta* 21:421
261. van Hoof, F., Hers, H. G. 1968. *Eur. J. Biochem.* 7:34
262. Sandhoff, K., Andreae, U., Jatzkewitz, H. 1968. *Life Sci.* 7:283
263. Robinson, D., Stirling, J. L. 1968. *Biochem. J.* 107:321

264. Sandhoff, K. 1968. *Z. Physiol. Chem.* 349:1095
265. Okada, S., O'Brien, J. S. 1969. *Science* 165:698
266. Sandhoff, K. 1969. *FEBS Lett.* 4:351
267. Kolodny, E. H., Brady, R. O., Volk, B. 1969. *Biochem. Biophys. Res. Commun.* 37:526
268. Sandhoff, K., Jatzkewitz, H., Peters, G. 1969. *Naturwissenschaften* 56: 356
269. Weinstein, D. B., Warren, L., Marsh, J. B. 1967. *Fed. Proc.* 28:3654
270. Dod, B. J., Gray, G. M. 1968. *Biochem. J.* 110:50P
271. Klenk, H. D., Choppin, P. W. 1970. *Proc. Nat. Acad. Sci. USA* 66:57
272. Renkonen, O., Gahmberg, C. G., Simons, K., Kääriäinen, L. *Acta Chem. Scand.* In press
273. Rapport, M. M., Graf, L. 1969. *Progr. Allergy* 13:273
274. Brady, R. O. 1966. *J. Am. Oil Chem. Soc.* 43:67
275. Arnon, R., Sela, M., Rachaman, E. S., Shapiro, D. 1967. *Eur. J. Biochem.* 2:79
276. Niedieck, B., Kuwert, E., Polacios, C., Drees, O. 1965. *Ann. NY Acad. Sci.* 122:266
277. Niedieck, B., Kuck, U. 1967. *Z. Immunitaetsforsch. Allerg. Klin. Immunol.* 133:43
278. Rapport, M. M., Cavanna, R., Graf, L. 1967. *J. Neurochem.* 14:9
279. Taketomi, T., Yamakawa, T. 1966. *Lipids* 1:31
280. Taketomi, T., Yamakawa, T. 1967. *Jap. J. Exp. Med.* 37:423
281. Graf, L., Rapport, M. M. 1966. *Fed. Proc.* 25:474
282. Rapport, M. M., Graf, L. 1965. *Fed. Proc.* 24:176
283. Graf, L., Rapport, M. M. 1965. *Int. Arch. Allergy* 28:171
284. Koscielak, J., Hakomori, S., Jeanloz, R. W. 1968. *Immunochemistry* 5: 441
285. Hakomori, S. 1969. *Vox Sang.* 16: 478
286. Pascal, T. A., Saifer, A., Gitlin, J. 1966. *Proc. Soc. Exp. Biol. Med.* 121:739
287. Pascal, T. A., Saifer, A., Gitlin, J. See Ref. 217, 289
288. Poulik, M. D. 1969. In *Red Cell Membrane Structure and Function,* ed. G. A. Jamieson, T. F. Greewalt. Philadelphia: Lippincott
289. Kalckar, H. M. 1965. *Science* 150: 305
290. Shen, L., Ginsburg, V. 1968. In *Biological Properties of the Mammalian Surface Membrane,* ed. L. A. Mason, Monogr. 8:6. Philadelphia: Wistar Inst. Press
291. Cox, R. P., Gesner, B. M. 1965. *Proc. Nat. Acad. Sci. USA* 54: 1571
292. Burger, M. M., Goldberg, A. R. 1967. *Proc. Nat. Acad. Sci. USA* 57: 359
293. Hakomori, S., Murakami, W. T. 1968. *Proc. Nat. Acad. Sci. USA* 59:254
294. Mora, P. T., Brady, R. O., Bradley, R. M., McFarland, V. W. 1969. *Proc. Nat. Acad. Sci. USA* 63: 1290
295. Brady, R. O., Borek, C., Bradley, R. M. 1969. *J. Biol. Chem.* 244: 6552
296. Brady, R. O., Mora, P. T., Kolodny, E. H., Borek, C. 1970. *Fed. Proc.* 29:410
297. Hakomori, S., Teather, C., Andrews, H. 1968. *Biochem. Biophys. Res. Commun.* 33:563
298. Blough, H. A., Lawson, D. E. M. 1968. *Virology* 36:286