Chem.Abs., 35, 772[2] (1941).
140. T. Yabuta and T. Hayashi, Agric. Hort. (Tokyo), 15, 1991 (1940).
141. H. Munekata and S. Kato, Bull. Agric. Chem. Soc. Japan, 21, 71 (1957); Chem. Abs., 51, 18137i (1957).
142. E. Sandegren and H. Beling, Brauerei, 11, 231 (1958).
143. Y. Harugoro and J. Hironobu, Agric. Biol. Chem. (Tokyo), 27, 76 (1963); Ref. Zhur. Khim., 23F647 (1963).
144. M. S. Moro, Y. Pomeranz, and J. A. Shellenberger, Phyton, 20, 59 (1963); Ref. Zhur. Biol., 4G75 (1964).
145. D. E. Briggs, J. Inst. Brewing, 69, 13 (1963); Ref. Zhur. Biokhim., 20F791 (1963).
146. B. I. S. Srivastava and W. O. S. Meredith, Canad. J. Botany, 40, 1257 (1962).
147. R. F. Bawden, R. V. Dahlstrom, and M. R. Sfat, Amer. Soc. Brewing Chem. Proc., 137 (1959).
148. L. E. Weller, S. H. Wittewr, M. J. Bucovac, and H. M. Sell, Plant Physiol., 32, 371 (1957).
149. L. G. Paleg, Plant Physiol., 35, 293 (1960).
150. L. G. Paleg, D. H. B. Sparrow, and A. Jennings, Plant Physiol., 37, 579 (1962).
151. M. Bouillenna-Walrand, Bull. Soc. roy. Sci. Liege, 28, 21 (1959); Ref. Zhur. Biokhim., 22722 (1959).
152. S. Kuraishi and R. M. Muir, Naturwiss., 50, 337 (1963).
153. K. S. Sastry and R. M. Muir, Science, 140, 494 (1963).
154. P. E. Pilet, Compt. rend., 245, 1327 (1957).
155. P. E. Pilet and W. Wurglen, Ber. Schweiz. Botan. Ges., 68, 54 (1958); Ref. Zhur. Biokhim., 16955 (1959).
156. S. Kuraishi and R. M. Muir, Science, 137, 760 (1962).
157. P. W. Brian and H. G. Hemming, Ann. Bot., [N.S.], 22, 1 (1958).
158. T. Hayashi and Y. Murakami, Bull. Nat. Inst. Agric. Sci. Japan, 159 (1958).
159. A. W. Galston and D. C. McCune, "Plant Growth Regulation", Iowa Univ. Press, Iowa City, 1961, p. 611.
160. A. J. Vlitos and W. Meudt, Nature, 180, 284 (1957).
161. G. Kuse, Bot. Mag. (Tokyo), 71, 151 (1958).
162. A. W. Galston and H. Warburg, Plant Physiol., 34, 16 (1959).
163. T. Kato, Mem. Coll. Sci., Kyoto Univ., Ser. B., 20, 189 (1951); Chem. Abs., 48, 88861 (1954).
164. P. W. Brian and H. G. Hemming, Ann. Appl. Biol., 45, 489 (1957).
165. G. J. M. Van der Kerk, G. W. van Eyk, and J. A. Weber, Chem. Weekblad, 60, 185 (1964).
166. Y. Richard, H. Borel, and F. Peretti, Compt. rend., 255, 1470 (1962).
167. M. Kh. Chailakhyan and N. P. Aksenova, Fiziol. Rast., 6, 699 (1959).
168. N. P. Aksenova, Fiziol. Rast., 10, 166 (1963).
169. H. D. Klämbst, Adv. Hort. Sci. Appl., 2, 156 (1962); Ref. Zhur. Biol., 2G38 (1964).
170. K. Koshimizu, H. Iwamura, T. Mitsui, and Y. Ogawa, Nature, 198, 1306 (1963).
171. B. H. Most and A. J. Vlitos, "Regulateurs naturels de la croissance végétale", CNRS, Paris, 1964, p. 287.
172. F. Hayashi, S. Blumenthal-Goldschmidt, and L. Rappaport, Plant Physiol., 37, 774 (1962).
173. P. J. Keay, J. S. Moffat, and T. P. S. Mulholland, J. Chem. Soc., 1605 (1965).
174. D. C. Aldridge, J. P. Hanson, and T. P. S. Mulholland, J. Chem. Soc., 3539 (1965).
175. J. R. Hanson and T. P. S. Mulholland, J. Chem. Soc., 3550 (1965).
176. I. A. Gurvich, I. M. Mil'shtein, and V. F. Kucherov, Izv. Akad. Nauk SSSR, Ser. Khim., 184 (1966).

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GLYCOSPHINGOLIPIDS: ESTABLISHMENT OF STRUCTURE, SYNTHESIS, BIOLOGICAL ROLE

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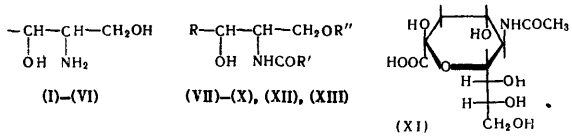
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The present review considers lipids that are grouped together under the name glycosphingolipids. In addition to structural similarities, these compounds have at least one property in common in that they are capable of taking part in specific immunological reactions, the study of which may open a new approach to the understanding of the fine structural differences in animal and human tissues in normal and pathological states.

I. NOMENCLATURE

The term "sphingolipoids" was proposed by Carter to denote those lipids that contain the aliphatic amino-glycol sphingosine (I).[1] This term may now be extended to natural derivatives of dihydrosphingosine (II), C_{20}-sphingosine (III), and C_{20}-dihydrosphingosine (IV), which are found together with derivatives of sphingosine (I) in animal tissue, and also to the compounds phytosphingosine (V) and dehydrophytosphingosine (VI) and their C_{20}-homologues, which have been isolated from plants.



(I)–(VI) (VII)–(X), (XII), (XIII) (XI)

I, R = $CH_3[CH_2]_{12}CH{=}CH-$
II, R = $CH_3[CH_2]_{14}-$
III, R = $CH_3[CH_2]_{14}CH{=}CH-$
IV, R = $CH_3[CH_2]_{16}-$
V, R = $CH_3[CH_2]_{13}CH(OH)-$
VI, R = $CH_3[CH_2]_{11}CH{=}CHCH(OH)-$

VII, R″ = hexose
VIII, R″ = hexose–SO_3H
IX, R″ = oligosaccharide
X, R″ = oligosaccharide–hexosamine
XII, R″ = oligosaccharide–sialic acids
XIII, R″ = oligosaccharide–hexosamines–sialic acids
R as in compounds (I)–(VI)
R′ fatty acid residue

We shall apply the term "glycosphingolipids" to the group of sphingolipids that contain carbohydrates in addition to the sphingosines, (I)–(VI). In the present review we shall consider the glycosphingolipids of animal origin, that is, mainly derivatives of the sphingolipids (I)–(IV)† (Scheme 1).

The simplest representatives of this group, the cerebrosides (VIII), are monoglycosides of *N*-acetylsphingosines (ceramides).

The sulphatides or cerebroside sulphates (VIII) are sulphate esters of the cerebrosides.

The ceramide-oligohexosides (IX) are derivatives of the ceramides that contain oligosaccharides with simple hexoses as components. The name "cytosides" has also been proposed for ceramide disaccharides.

The globosides (X) are hetero-oligosaccharides of the ceramides containing hexosamines in addition to the simplest monoses; if the hetero-oligosaccharides contain sialic acids (XI) instead of hexosamines, they are termed "hematosides" (XII).

The sialic acids (XI) include all the acylated neuraminic acids[3], being, as it were, products of the aldol condensation of *N*-acylmannosamine and pyruvic acid.

The term "gangliosides" (XIII) is applied to derivatives of sphingosine containing fatty acids, monoses, hexosamines, and sialic acids.

II. STRUCTURE AND DISTRIBUTION

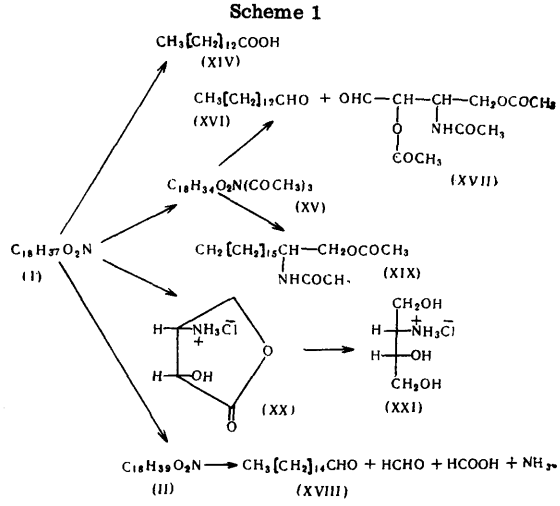
1. Sphingosines

Sphingosine (I) was first isolated from brain lipids by Thudichum in 1901.[4] It was soon shown that it was an amino-diol with a double bond at the 4–5-position in a long hydrocarbon chain[5,6].

Oxidation of sphingosine (I) with chromic acid gave myristic acid (XIV),[7] and ozonolysis of triacetylsphingosine (XV) gave myristaldehyde (XVI) and a triacetylaminotetrose (XVII),[8] the structure of which was established by Cater[9,10] (Scheme 1)

† Recently Karlsson reported for the first time that derivatives of phytosphingosines (V) occur in animal and human tissues[2].

Scheme 1



(I) → $CH_3[CH_2]_{12}COOH$ (XIV); (I) → $CH_3[CH_2]_{12}CHO$ (XVI) + $OHC-CH(O)-CH(NHCOCH_3)-CH_2OCOCH_3$ (XVII); (I) → $C_{18}H_{34}O_2N(COCH_3)_3$ (XV); (I) → $CH_3[CH_2]_{15}CH(NHCOCH_3)-CH_2OCOCH_3$ (XIX); (I) → (XX) → (XXI); (II) → $CH_3[CH_2]_{14}CHO$ (XVIII) + HCHO + HCOOH + NH_3

Two molecules of periodic acid are required for the oxidation of dihydrosphingosine (II) to palmitaldehyde (XVIII), formaldehyde, ammonia, and formic acid. When the triacetate (XV) was hydrogenated in the presence of platinum, the diacetate of 1-hydroxy-2-amino-octadecane (sphingine) (XIX) was formed as a result of hydrogenolysis of the 3-acetoxy-group, which indicates that one of the hydroxyl groups of sphingosine (I) is in the allyl position[11].

The D-configuration at the $C_{(2)}$ atom of sphingosine was established almost simultaneously by two methods. Carter and Humiston[11] made a comparative study of changes in optical rotation on dilution with water of dioxan or acetic acid solutions of *N*-benzoyl-α-aminostearic acid; the latter compound is isolated in the oxidation of *N*-benzoylsphingine, which is obtained from sphingosine triacetate (XV), and of *N*-acyl derivatives of known L- and D-amino-acids.

Kiss[12] converted sphingosine (I) by ozonisation into 3-amino-2-hydroxybutyrolactone hydrochloride (XX), which he then reduced to laevorotatory D-*erythro*-2-aminobutane-1,3,4-triol (XXI) (see Scheme 1).

The configuration at the $C_{(3)}$ atom was established by synthesis of the *threo*- and *erythro*-isomers of dihydrosphingosine (II)[13] (see pp. 868–869). The infrared spectrum of sphingosine (I) contains a band at 10.3 μm, indicating a *trans* double bond[14,15]. Total synthesis finally confirmed the structure of sphingosine (I) as D-(+)-*erythro*-*trans*-2-amino-1,3-dihydroxyoctadec-4-ene.

Dihydrosphingosine (II), which is identical with the hydrogenation product of sphingosine (I), was first discovered in the larvae of cat tapeworms[16], and it was soon also isolated[17,18] from the cerebroside fraction of the spinal cord and, in smaller amounts, from cow brain.

In recent years new amino-diols of animal origin, C_{20}-sphingosine (III) and C_{20}-dihydrosphingosine (IV), have been isolated. C_{20}-sphingosine (III) was first discovered by a group of Yugoslav biochemists in the brains of cows and horses[19,20]. The mixture of natural amino-diols was oxidised with chromic acid, and the methyl esters of the acids obtained were subjected to gas–liquid chromatography; it was shown that these contained methyl myristate and methyl palmitate, while methyl stearate was found in the products of oxidation and methylation of the dihydro-compounds. Recently, a rapid method was proposed for

the determination of sphingolipid bases by gas–liquid chromatography of their trimethylsilyl derivatives[21].

The C_{20}-sphingosine (III), termed "icosisphingosine"[22], has also been found as the main component in a mixture of bases obtained by acid hydrolysis of a mucolipid[23,24] from ox brain and also of gangliosides from human brain[25].

Gas–liquid chromatography of a mixture of methyl esters of fatty acids obtained by periodate cleavage at the $C_{(2)}$–$C_{(3)}$ bond of the sphingosine fraction of human-brain gangliosides showed the presence of C_{20}-dihydrosphingosine (IV).[25] It was isolated by preparative paper chromatography of the mixture of dinitrophenyl derivatives of sphingosines obtained from human brain, cow brain, or human hair[26]. The ganglioside fraction of the brain consists of two-thirds C_{20}-sphingosines (III, IV) and one-third C_{18}-sphingosines (I, II) with about 10% of dihydro-compounds (II, IV).[27]

2. Cerebrosides

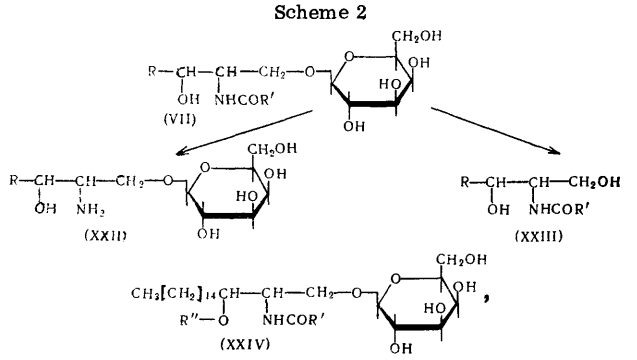
The first two cerebrosides — phrenosin and cerasine — were discovered by Thudichum in his study of the composition of the brain[28]. Later Klenk isolated two more cerebrosides, nervon and oxynervon[29,30]. These four cerebrosides differ from one another in their fatty acid components. Phrenosin contains cerebronic acid, $CH_3[CH_2]_{21}$–CHOH–COOH, cerasine contains lignocerinic acid, $CH_3[CH_2]_{22}COOH$, nervon contains nervonic (*cis*-tetracos-15-enoic) acid, and oxynervon contains hydroxynervonic acid. Cerebrosides containing other fatty acids are also known[31,32].

Repeated thin-layer chromatography has been used to separate a mixture of cerebrosides isolated from human spinal cords into 12 components containing different fatty acid residues[33]. Gas–liquid chromatography of the methyl esters of acids obtained by the hydrolytic cleavage of human brain cerebrosides indicates that these contain even-numbered and odd-numbered, saturated and unsaturated acids, as well as α-hydroxy-acids, with an unbranched chain and with from 14 to 26 carbon atoms[34].

The cerebrosides from normal human and animal brains contain galactose. In some anomalous conditions, in particular in lipidosis (Gaucher's disease), glucocerebroside accumulates in the human spleen[35,36]; it is also present, though in much smaller amounts, in the healthy human spleen[37].

The structure of cerebrosides has been established. When selectively hydrolysed, they are converted into sphingosine galactoside (psychosine) (XXII), and, under other conditions, into an *N*-acylsphingosine (ceramide) (XXIII)[28,29] (Scheme 2).

Scheme 2



where R = $CH_3[CH_2]_{12}CH{=}CH-$, R′ is a fatty acid residue, and R″ = $C_nH_{n+1}CH{=}CH-$.

Psychosine (XXII) is not reduced by the usual reagents for the aldehyde group; it is therefore a glycoside. Exhaustive methylation of cerebrosides (VII) and subsequent hydrolysis give 2,3,4,6-tetramethylgalactose, which indicates that these compounds contain a 1,5-pyranose ring[38,39].

When hexa-acetylphrenosin, or triacetylsphingosine (XV), is subjected to hydrogenolysis on platinum followed by hydrolysis, sphingine and acetic acid are formed[40], which means that the galactose is linked through the primary hydroxyl group of the ceramide. The β-configuration of the glycoside bond has been demonstrated by total stereospecific synthesis of some cerebrosides (see p. 872).

Using thin-layer chromatography on the cerebroside fraction of brain, Kochetkov and coworkers isolated a small quantity of a galactocerebroside with a greater R_f value than that of the classical cerebrosides[41]. The infrared spectrum and its behaviour on alkaline and acid hydrolysis indicated that this compound contains more than 90% of dihydrosphingosine, and that an alkenyl ether group is linked to the second hydroxyl group. The new compound was named sphingoplasmalogen (XXIV).[42]

3. Sulphatides

Thudichum detected sulphur in brain cerebrosides[28]. In 1933 Blix isolated the sulphatide (VIII) as the potassium salt from normal human brain (20–25% of total cerebrosides)[43]. The sulphatide contained fatty acids (mainly cerebronic), galactose, sphingosine, and a sulphuric acid residue. The sulphatides (VIII) can be separated from the cerebrosides (VII) by chromatography on diethylaminoethylcellulose[44].

Gas–liquid chromatography has shown that the composition of the sulphatides from grey and white matter of the human brain is similar to the fatty acid composition of the cerebrosides isolated from the same tissues, but the sulphatides contain smaller amounts of hydroxy-acids[45].

Yatzkewitz[46], using column chromatography on paper, separated sulphatides from human brain containing cerebronic, lignocerinic, and nervonic acids. Two sulphatides of rat brain isolated by Bakke and Cornatzer probably correspond to the sulphate esters of cerasine and phrenosin[47,48].

Thannhauser and Boncoddo[49], who methylated the cerebroside sulphate of cow brain and studied the products of subsequent hydrolysis by paper chromatography, showed that in this compound the sulpho-group is linked to the first hydroxyl group of galactose at $C_{(6)}$. However, re-investigation of the same sulphatide by exhaustive methylation in dimethylformamide, followed by methanolysis and gas–liquid chromatography of the methylated sugars, showed that methyl-2,4,6-trimethylgalactoside[50] was present, and not 2,3,4-trimethylgalactoside[49]. The absorption at 820 cm^{-1} (C–O–S vibrations) corresponds to the equatorial position of the $-OSO_3H$ group[51], which for the $C_{(1)}$ conformation of galactopyranose occurs when the $-OSO_3H$ group is in the 6-position or the 3-position. Obviously the sulphato-group is linked to the hydroxyl group at the $C_{(3)}$ atom of galactose, and Thannhauser and Boncoddo[49] were misled by the closeness of the R_f values obtained in the paper chromatography of the trimethylgalactoses.

On the basis of infrared spectra and a study of the methylation products, Hakomori and coworkers[52] confirmed the cerebroside 3′-sulphate structure; they also

isolated a complex sulphatide consisting of cerebroside 3′- and 6′-sulphates, which may be linked together as a diester of sulphuric acid.

4. Ceramide-Oligohexosides

Using chromatography on silicic acid of chloroform–methanol extracts, Rapport and coworkers[53] isolated the simplest ceramide-oligohexoside (IX) from human epidermal carcinoma, and they named it cytolipin H.[54,55] Compounds of similar structure have been separated from ox spleen[56,57], human spleen[37], horse erythrocytes[58], human erythrocytes[50], and human kidney tissue[59,60].

Cytolipin H is a mixture of ceramide β-lactosides in which sphingosine is linked by an amide bond with lignoceric [tetracosanoic], palmitic, behenic [docosanoic], or (to a lesser degree) another higher fatty acid[61]. The fatty acid composition of the compound was elucidated by paper chromatography and gas–liquid chromatography.

Analysis of the products of acid hydrolysis of cytolipin H with the aid of paper chromatography showed that the carbohydrate portion of the molecule contains glucose and galactose in equimolecular amounts. The configuration of the carbohydrate residue was established by an immunochemical method in which the reaction of cytolipin H with antibodies is inhibited by the addition of simple carbohydrates[54] (see p. 873). Total synthesis confirmed its structure (see p. 873) as a D-*erythro*-*N*-acylsphingosine 1-*O*-β-lactoside.

Other ceramide oligohexosides (IX), galactosyl-(1→4)-galactosylceramide and galactosyl-(1→4)-galactosyl-(1→4)-glucosylceramide[60], have been isolated from human spleens[37], kidneys[59], and erythrocytes[62], and from cow kidneys, cow's milk[63], and horse kidneys.

5. Globosides

It has been shown by a study of the products of moderate acid hydrolysis, oxidation with periodic acid, and exhaustive methylation in dimethylformamide that globoside, the main glycolipid of human erythrocytes[58,64], is *N*-acetylgalactosaminyl-(1→6)-galactosyl-(1→4)-galactosyl-(1→4)-glucosyl-(1→1)-*N*-lignocerylsphingosine.

A compound similar in structure to the globoside from erythrocytes has been isolated from human kidneys by Rapport and coworkers[66] and also by Makita and coworkers[67], and shown to be *N*-acetylgalactosaminyl)-(1→3)-galactosyl-(1→4)-galactosyl-(1→4)-glucosyl-(1→1)-ceramide[59]. Globosides have also been obtained by silica-gel fractionation of methanol–ether extracts of sheep and guinea-pig erythrocytes[62].

Recently a glycosphingolipid was isolated[68] from cancer tissue which appears to be formally a globoside, but which differs from known compounds of this type in that it has a high content of glucosamine (9–10%) and a fucose 6-desoxyhexoside (8.5–9.5%). The ratio glucose : galactose : fucose is 1 : 2.3 : 1.2. This is the main component of water-soluble glycolipids of human adenocarcinomate. Attempts to obtain the same globoside containing fucose from normal tissues were unsuccessful.

6. Hematosides

The hematoside *N*-acetylneuraminyl-(2→3)-galactosyl-(1→4)-glucosyl-(1→1)-*N*-acylsphingosine was isolated from the erythrocyte stroma of dogs, cats, and horses by Yamakawa and Suzsuki[69], and later by Klenk and Padberg[70]. Its sialic acid[71,72] is easily eliminated[73] by the action of a specific hydrolytic enzyme, neuraminidase (or sialidase), isolated from *Vibrio cholerae*.

A hematoside that probably contains two molecules of galactose and no glucose has been separated from human brain[74–76]. A more complex hematoside containing four molecules of neutral hexose and two molecules of sialic acids per molecule of ceramide has recently been isolated from hog brain[77].

7. Gangliosides

Gangliosides were first discovered in 1925 by Landsteiner and Levene[78] in extract of horse kidney, but it was not until 1942 that Klenk[79] isolated the relatively pure compound from the human cerebral cortex; it contains sphingosine, glucose, galactose, galactosamine, *N*-acetylneuraminic acid, and higher fatty acids (mainly stearic acid) in equimolecular proportions[80,81].

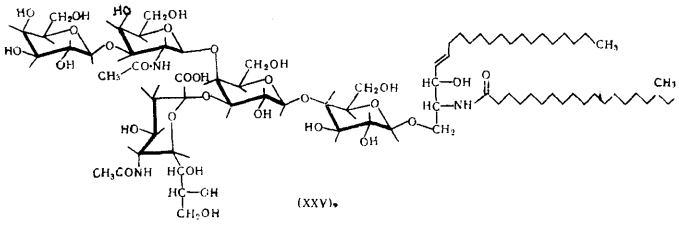
It was later shown that many gangliosides differing in composition and structure occur in nature. The fatty acid composition of brain gangliosides was studied by gas–liquid chromatography of the methyl esters obtained by saponification of the gangliosides[82–86]. Stearic acid was present to the extent of 72 to 96%; apart from this the other main acids in gangliosides are linear saturated C_{20}, C_{22}, and C_{24} acids.

The structure of the carbohydrate portion of the monosialoganglioside isolated from human brain (which in Svennerholm's nomenclature is denoted G_{M1}[87]) was determined by the collaborative work of several laboratories[88–91]. Moderate acid hydrolysis of G_{M1} gives the two disaccharides *O*-α-*N*-acetyl-D-galactosaminyl-(1→3)-galactose and *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-desoxy-D-galactose[92].

On acetolysis with a mixture of acetic acid and acetic anhydride containing a little sulphuric acid[93], this ganglioside gives a ganglio-*N*-tetrose: galactosyl-(1→3)-*N*-acetylgalactosaminyl-(1→4)-galactosyl-(1→4)-glucose; two ganglio-*N*-trioses: galactosyl-(1→3)-*N*-acetylgalactosaminyl-(1→4)-galactose and *N*-acetylgalactosaminyl-(1→4)-galactosyl-(1→4)-glucose; two ganglio-*N*-bioses: galactosyl-(1→3)-*N*-acetylgalactosamine and *N*-acetylgalactosaminyl-(1→4)-galactose; and a lactose: galactosyl-(1→4)-glucose, the structure of which was established by periodate oxidation followed by reduction with potassium tetrahydroborate. The following saccharides containing *N*-acetylneuraminic acid were also isolated: sialyl-(2→3)-galactose, sialyl-(2→3)-galactosyl-(1→4)-glucose, sialylganglio-*N*-trioses, and a sialylganglio-*N*-tetrose. The structure of these compounds was determined by the enzyme method, which is based on the known relationship between the rate of enzymatic elimination of sialic acid and its location.

As has been shown by the periodate oxidation of methylsphingosine, obtained by exhaustive methylation of the ganglioside G_{M1}, the carbohydrate portion of this compound is linked through the primary hydroxyl group of sphingosine[88].

The structure of the ganglioside G_{M1} thus corresponds to formula (XXV); this structure was recently re-confirmed by periodate oxidation[94].



(XXV).

Another monosialoganglioside, G_{M2},[90] has also been isolated; normal human brain contains 3–5% of this compound, but much larger amounts are found in the brains of persons that have died of the infantile form of the hereditary "Tay-Sachs'" disease. For structure determination, the ganglioside G_{M2} was cleaved by acid hydrolysis to acylsphingosine saccharides and free sugars, which were then analysed quantitatively and characterised[90,95] (see Scheme 3).

Two disialogangliosides (G_{D1a} and G_{D1b}) and a trisialoganglioside (G_{T1a})[88,90,95,96] isolated from human brain are quantitatively cleaved by sialidase to give the monosialoganglioside G_{M1}. G_{D1a} is hydrolysed much more rapidly than G_{D1b}; G_{T1a} is first converted into G_{D1b} (with a little G_{D1a}), and then to G_{M1}. Hydrolysis of G_{T1a} with 0.01 *N* hydrochloric acid at 100° gives equal amounts of G_{D1a} and G_{D1b}. Since it is known that sialyl-(2→6)-lactose is hydrolysed more slowly by sialidase than sialyl-(2→3)-lactose, it may be supposed[95] that in G_{D1a} the second sialic acid molecule is linked with the $C_{(3)}$ atom of the end galactose, in G_{D1b} it is linked with the $C_{(6)}$ atom of the same galactose residue, and in G_{T1a} both hydroxyl groups are replaced.

Johnson and McCluer[94], using the method of periodate oxidation, studied the structure of yet another group of polysialogangliosides similar in structure to G_{M1} (see Scheme 3).

Oxidation of one galactose residue in the disialoganglioside G_{D1c} (according to Svennerholm's nomenclature; 3-G according to the present authors) shows that the end galactose residue is not replaced; destruction of only one molecule of *N*-acetylneuraminic acid and the stability of the second molecule indicate that the two sialic acid molecules are linked by a (2→8) bond[98–100]. Kuhn and Wiegandt isolated such a disialoganglioside[101].

The requirement of 6 moles of periodic acid for the complete oxidation of the trisialoganglioside G_{T1b}[94] is explained by the elimination of 2 moles of *N*-acetylneuraminic acid and 1 mole of galactose, and the formation of the disialoganglioside G_{D1c} as an intermediate under the action of sialidase shows that G_{T1b} also contains a (2→8)-sialic acid dimer. It can be shown by cleavage with 2 moles of periodic acid that the end galactose is not replaced, and the third mole of *N*-acetylneuraminic acid is not linked to this galactose (as in G_{D1a}), but possibly to the $C_{(6)}$ atom of *N*-acetylgalactosamine, but further evidence is needed to confirm this.

The trisialoganglioside G_{T1c}, isolated by Kuhn and Wiegandt[101] (G_{IV} according to the authors), differs in the position of the third *N*-acetylneuraminic acid residue. It is possible that the fourth sialic acid residue in $G_{Tet.1}$ (G_V according to Kuhn), which was isolated with G_{T1c}, is linked to the $C_{(6)}$ atom of the *N*-acetylgalactosamine in the same way as the third residue in G_{T1b} (see Scheme 3).

Scheme 3 *

G_{M1}–	gal[1→3]galNHAc[1→4]gal[1→4]glu[1→1]–R
G_{M2}–	galNHAc[1→4]gal[1→4]glu[1→1]–R [3 ↑ 2] NAcHa
G_{D1a}	NAcHa[2→3]gal[1→3]galNHAc[1→4]gal[1→4]glu[1→1]–R [3 ↑ 2] NAcHa
G_{D1b}	NAcHa[2→6]gal[1→3]galNHAc[1→4]gal[1→4]glu[1→1]–R [3 ↑ 2] NAcHa
G_{D1c}	gal[1→3]galNHAc[1→4]gal[1→4]glu[1→1]–R [3 ↑ 2] NAcHa[2→8]NAcHa
G_{T1a}	NAcHa[2→6]gal[1→3]galNHAc[1→4]gal[1→4]glu[1→1]–R [3 ↑ 2] [3 ↑ 2] NAcHa NAcHa
G_{T1b}	NAcHa [2 ↑ 6] gal[1→3]galNHAc[1→4]gal[1→4]glu[1→1]–R [3 ↑ 2] NAcHa[2→8]NAcHa
G_{T1c}	NAcHa[2→3]gal[1→3]galNHAc[1→4]gal[1→4]glu[1→1]–R [3 ↑ 2] NAcHa[2→8]NAcHa
$G_{Tet.1}$	NAcHa [2 ↑ 6] NAcHa[2→3]gal[1→3]galNHAc[1→4]gal[1→4]glu[1→1]–R [3 ↑ 2] NAcHa[2→8]NAcHa

* gal = galactose; galNHAc = *N*-acetylgalactosamine; glu = = glucose; NAcHa = *N*-acetylneuraminic acid; R = ceramide.

Recently preparative thin-layer chromatography has been used to separate brain gangliosides, and a mixture of trisialogangliosides was obtained in which the ratios hexose : ceramide and galactose : glucose were 3 : 1, which could correspond to a content of 25% of galactose ganglioside with no glucose in this mixture[102].

Kuhn and Wiegandt[103], using chromatography on kieselguhr, isolated a new ganglioside from erythrocytes and milk of long-horned cattle; this contains glucosamine instead of galactosamine and is readily cleaved by employing neuraminidase[103]. Examination of enzymatic and acid hydrolysis and by ozonisation[104] showed that it may be similar to the ganglioside isolated previously from erythrocyte stroma[105].

In a study of substances that determine blood-group specificity[106] (see p. 873), two gangliosides were separated that contain galactosamine, glucosamine, galactose, glucose, sialic acid, fucose, and sphingosine residues; they differ in fatty acid composition and show a sphingosine : glucose : : sialic acid ratio of 2 : 1 : 1.

The molecular weight of gangliosides varies between 1500 and 3000. However, in aqueous solutions they may form micelles [74] with an aggregate weight of 250 000; some workers [107] consider these to be high-molecular compounds in which hundreds of structural components of ordinary gangliosides are combined [108].

In an aqueous medium, the micelle must be oriented in such a way that the neuraminic acid residues are on its surface so as to be able to associate with water molecules, but the lipid part of the molecule is screened inside. According to Klenk and Gielen [74], in organic solutions the micelle must be, as it were, turned inside out, with the hydrophilic neuraminic acid residues concealed behind a barrier of sphingosine carbohydrate residues and fatty acids.

Howard and Burton [109] put forward the hypothesis of van der Waals interaction between the hydrophobic residues in the micelle. Thus, for example, sphingosine ganglioside No. 1 may be drawn into association with the fatty acid chain of ganglioside No. 2, the sphingosine of which will be associated with the fatty acid chain of ganglioside No. 3, and so on. This means that kinetic liberation of one hydrophobic chain need not lead to dissolution of the ganglioside molecule, since the other hydrophobic chain will still be "entrapped" in the micelle.

Non-ionic and non-covalent bonds of this kind may occur in such heterogeneous micelle-like structure as cell membranes and subcellular particles consisting of lipids and proteins.

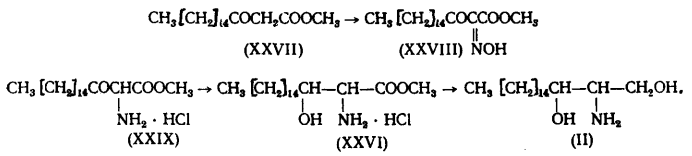
III. SYNTHETIC STUDIES

1. Synthesis of Dihydrosphingosine

Syntheses of sphingosine (I), dihydrosphingosine (II), and some sphingolipids, which have been carried out to finally establish the structure of these compounds, have advanced the development of biological studies, which require considerable quantities of the individual compounds.

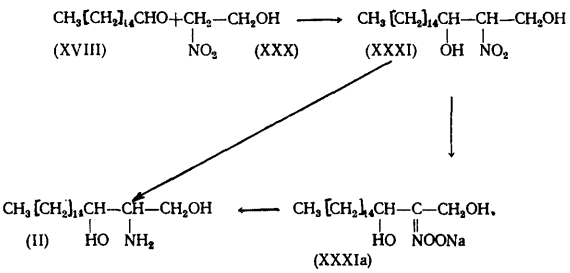
In 1947, Carter and coworkers [110] proposed a synthesis of dihydrosphingosine through methyl α-amino-β-hydroxystearate (XXVI), and in 1951 Gregory and Malkin [111] synthesised a mixture of racemates of dihydrosphingosine (II) from methyl 3-ketostearate (XXVII) by nitrosation [112] followed by stepwise reduction of the oxime (XXVIII), at first with palladium on charcoal in the presence of 2 moles of hydrochloric acid, then on platinum oxide, and finally with lithium tetrahydroaluminate (XXVIII) → (XXIX) → (XXVI) → → (II) (Scheme 4).

Scheme 4



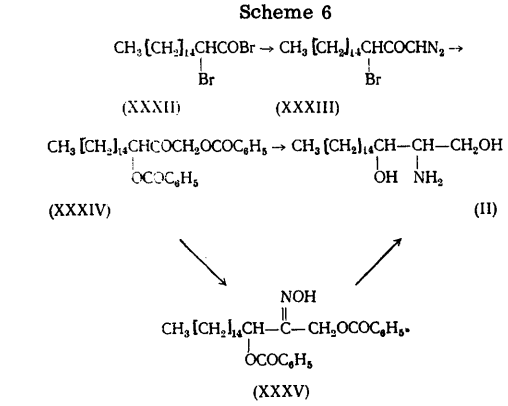
In the same year, Grob and coworkers [113] described a synthesis of a mixture of racemates of dihydrosphingosine (II): palmitaldehyde (XVIII) was condensed with nitroethanol (XXX) in the presence of sodium hydroxide, with subsequent reduction of the nitro-diol (XXXI) on Raney nickel (see Scheme 5); the *threo*-isomer (IIb) predominated in the mixture obtained. The total yield of dihydrosphingosine (IIa, b) was increased from 10.5 to 44% by using sodium methoxide as a condensing agent [114], separating the intermediate salt of the *aci*-form of the nitro-diol (XXXIa), and carrying out the reduction on platinum oxide.

Scheme 5



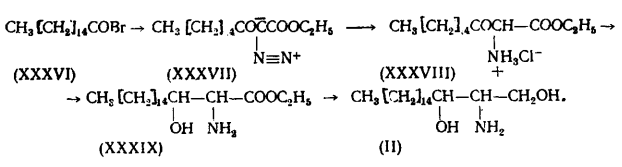
A group of Yugoslav investigators [115] has described a synthesis of a mixture of racemates of (II) in which 2-bromoheptadecanoyl bromide (XXXII) is condensed with diazomethane; the 3-bromo-1-diazodecan-2-one (XXXIII) is treated with silver benzoate and benzoic acid and the keto-diester (XXXIV) obtained is subjected to reductive amination on Raney nickel; alternatively, the oxime (XXXV), obtained from (XXXIV), is reduced with lithium tetrahydroaluminate (see Scheme 6).

Scheme 6



A synthesis of dihydrosphingosine (II) from the more accessible palmitic acid has also been described [116]: palmitoyl bromide (XXXVI) is condensed with diazoacetoacetic ester, and the 2-diazopalmitoylacetic ester (XXXVII) is reduced in steps, first by hydrogenation on palladium and then with sodium tetrahydroborate and lithium tetrahydroaluminate (XXXVII) → (XXXVIII) → (XXXIX) → (IIa, b) (see Scheme 7).

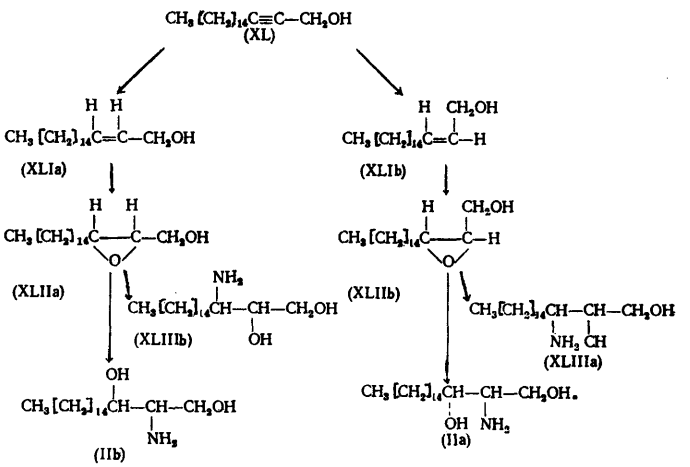
Scheme 7



A general defect of the syntheses described above is that they are not stereospecific; moreover, no methods of separating the diastereoisomers of (II) which are formed have been worked out.

The problem of the stereochemistry of sphingosine (I) at the carbon atoms $C_{(2)}$ and $C_{(3)}$ has been solved by stereospecific synthesis of the *erythro*- (IIa) and *threo*- (IIb) isomers of dihydrosphingosine [117,118]. Catalytic hydrogenation of 1-hydroxyoctadec-2-yne (XL) gives *cis*-1-hydroxyoctadecene (XLIa), but chemical reduction gives the *trans*-compound (XLIb); oxidation of the product with peroxyphthalic acid gives the corresponding *cis*- or *trans*-epoxide (XLIIa, b) Ring opening of the epoxides (XLII) with ammonia is accompanied by inversion of configuration and leads to *threo*- (IIb) and *erythro*- (IIa) dihydrosphingosines. Small quantities of the isomeric amino-diols (XLIIIa, b) are formed at the same time (see Scheme 8).

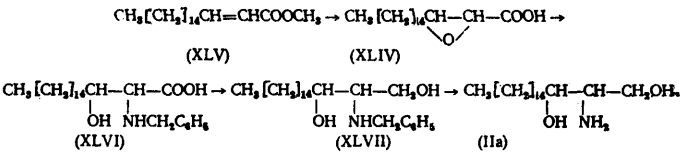
Scheme 8



At the same time, Carter and coworkers [13] synthesised D-(+)-, L-(-)-*erythro*, and DL-*threo*-dihydrosphingosines (IIa, b) by reduction of the corresponding *erythro*- and *threo*-α-amino-β-hydroxystearates [119] (XXVa, b) and separation of DL-*erythro*-dihydrosphingosine (IIa) into the antipodes as L-glutamates [120]. This work completed investivations to establish the structure of sphingosine (I).

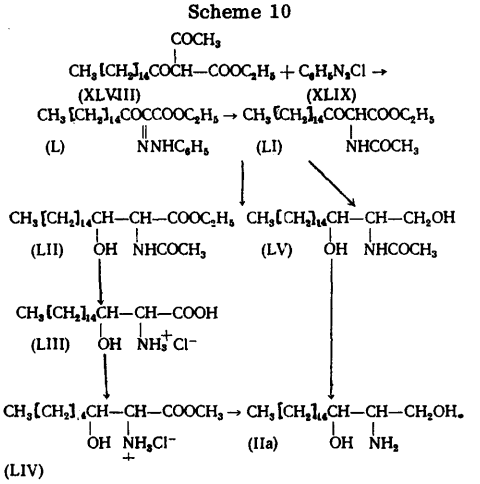
The great diversity of methods already proposed did not reduce the interest of chemists in developing new and more effective methods for the synthesis of dihydrosphingosine (II). Still another stereospecific synthesis of *erythro*-dihydrosphingosine (IIa) was described in 1964.[121] The reaction of *trans*-2,3-epoxystearic acid (XLIV), [122,123] obtained by epoxidation and subsequent saponification of methyl *trans*-octadec-2-enoate (XLV), with benzylamine leads to the formation of DL-*erythro*-2-benzylamino-3-hydroxystearic acid (XLVI). When the methyl ester (XLVI) was reduced with lithium tetrahydroaluminate and then hydrogenolysed, DL-*erythro*-dihydrosphingosine (IIa) was formed, (XLVI) → (XLVII) → (IIa) (see Scheme 9).

Scheme 9



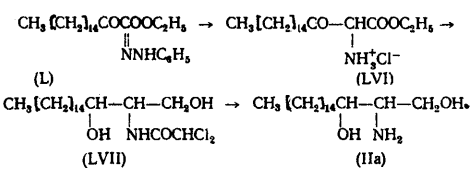
In developing a synthesis of sphingosine (see this page), Shapiro and coworkers also worked out a synthesis of (IIa). [124] Ethyl palimitoylacetoacetate (XLVIII) was condensed by Japp and Klingemann's method [125] with phenyldiazonium chloride (XLIX), and the product was hydrolysed to give the phenylhydrazone (L), which was then reduced with zinc and acetic acid in the presence of acetic anhydride to give ethyl 2-acetamido-3-ketostearate (LI). Reduction of (LI) with sodium tetrahydroborate gave an unseparable mixture of *erythro*- and *threo*-racemates (LII), which were converted into dihydrosphingosine (II) by saponification followed by esterification and reduction with lithium tetrahydroaluminate: (LII) → (LIII) → (LIV) → (IIa); the *erythro*-isomer of (II) was separated as the tribenzoate. It is more convenient to prepare (IIa) in two stages by reduction of (LI) with lithium tetrahydroaluminate to give *erythro*-*N*-acetyl-2-amino-1,3-dihydroxyoctadecane (LV), which is then hydrolysed (see Scheme 10).

Scheme 10



However, when the scale of the synthesis is increased, the yield of (IIa) decreases considerably because of the resistance of the amide (LV) to hydrolysis. For this reason, Shapiro and Sheradsky [126] later modified the method. Reduction of the phenylhydrazone (L) with zinc and aqueous 97% acetic acid leads to the keto-ester (LVI), which is separated as the hydrochloride. After this latter compound has been reduced with lithium tetrahydroaluminate, the mixture of isomers obtained is treated with methyl dichloroacetate [127], and *erythro*-*N*-dichloroacetyldihydrosphingosine (LVII) is separated; this is easily hydrolysed to *erythro*-dihydrosphingosine (IIa) (see Scheme 11). The second synthesis of dihydrosphingosine appears to be very efficient.

Scheme 11



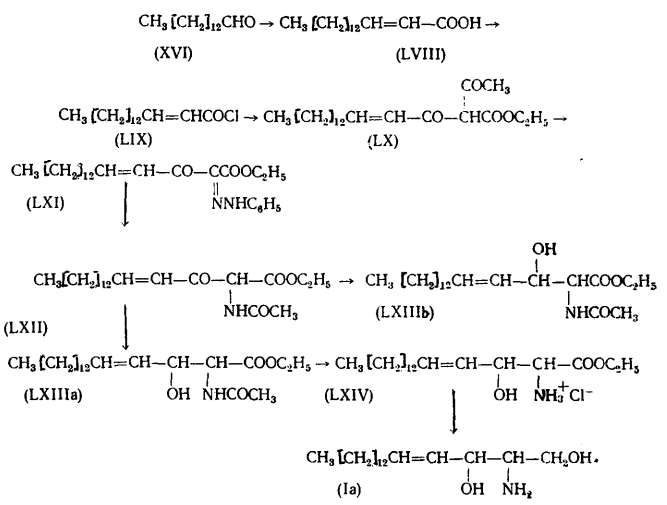
2. Synthesis of Sphingosine

There are two main schemes for the synthesis of sphingosine; these were first developed at almost the same time in the laboratories of Shapiro [128] and Grob [129].

In Shapiro's synthesis (Scheme 12), Knoevenagel–Doebner condensation of myristaldehyde (XVI) with malonic acid in the presence of piperidine gives *trans*-hexadec-2-enoic

acid (LVIII), from which the unstable *trans*-hexadec-2--enoyl chloride (LIX) is obtained by reaction with thionyl chloride.

Scheme 12



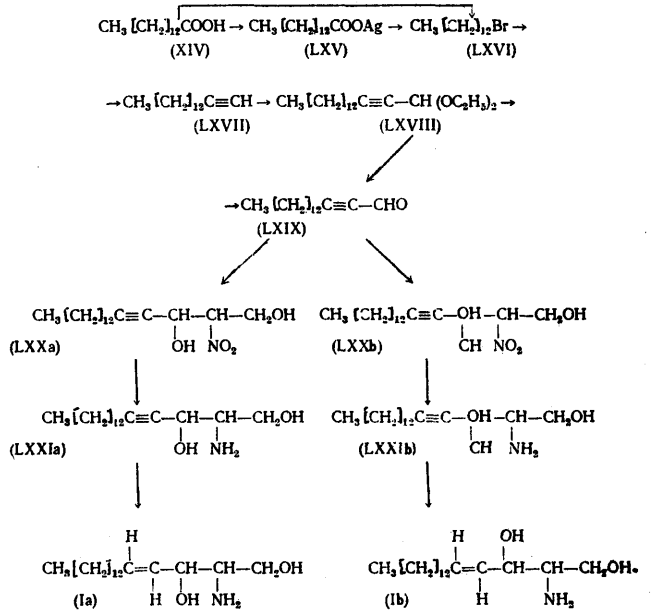
Condensation of the acid chloride (LIX) with ethyl acetoacetate gives ethyl 2-acetyl-3-keto-octadec-4-enoate (LX), which undergoes the Japp–Klingemann reaction with phenyldiazonium chloride under rigorously controlled conditions to give the phenylhydrazone (LXI). Reduction of the latter to the acetamidoester (LXII) with zinc and acetic acid, followed by selective reduction with sodium tetrahydroborate leads to a mixture of diastereomeric alcohols (LXIIIa, b), from which only the *erythro*-isomer (LXIIIa) can be crystallised. When (LXIIIa) is deacetylated with dilute hydrochloric acid in dioxan, the hydrochloride of *erythro*-ethyl 2-amino-3-hydroxyoctadec-4-enoate (LXIV) is formed in relatively low yield, which is due to partial conversion of (LXIV) to the *threo*-isomer. Reduction of (LXIV) with lithium tetrahydroaluminate leads to racemic *erythro*--sphingosine (Ia). The total yield of the latter is about 4%, calculated on the myristaldehyde (XVI).

The relatively accessible myristic acid (XIV) is the starting material in Grob and Gadient's synthesis (Scheme 13) of sphingosine, in which silver myristate (LXV) is converted into tridecyl bromide (LXVI) by the Hunsdiecker–Borodine reaction, or by direct bromination in the presence of red mercury(II) oxide [130] according to the method worked out by the present authors. Condensation of tridecyl bromide with sodium acetylide in liquid ammonia gives pentadec-1--yne (LXVII); this is converted by Iotsich's reaction with orthoformic acid into the diethylacetal of hexadec-2-yn-1--al (LXVIII), which is then hydrolysed to the aldehyde (LXIX). When this is condensed with nitroethanol in the presence of potassium carbonate, a mixture of *erythro*- and *threo*--1,3-dihydroxy-2-nitro-octadec-4-ynes (LXXa, b) is formed; the pure *threo*- (LXXb) and *erythro*- (LXXa) isomers are separated by fractional crystallisation.

Reduction of the nitrodiols (LXX) with zinc and hydrochloric acid leads to a mixture of *erythro*- and *threo*--1,3-dihydroxy-2-amino-octadec-4-ynes (LXXIa, b); *erythro*- and *threo*-sphingosines (Ia, b) are obtained by subsequent reduction of the triple bond with lithium tetrahydroaluminate. The overall yield of *erythro*-sphingosine, calculated on myristic acid (XIV) is about 7%; *threo*--sphingosine (Ib) is obtained at the same time in a yield of about 9%. The *cis*-isomers of sphingosine can be obtained by reducing the aminodiols (LXXI) on $Pd/CaCO_3$.

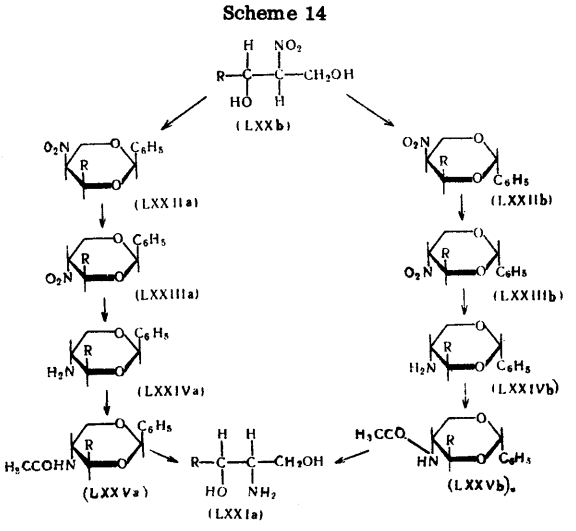
Thus, the more efficient synthesis of Grob opens the way to the preparation of all the possible spatial isomers of sphingosine, which are of great interest in biological studies.

Scheme 13



Because the unnatural *threo*-isomer (Ib) predominates in the latter synthesis, Grob and Gadient proposed a method for the *threo–erythro*-isomerisation of sphingosine [129]. For this purpose a mixture of two intermediate cyclic benzal derivatives (LXXIIa, b) is prepared, these being formed in a ratio of 5 : 1 in the reaction of *threo*-1,3-dihydroxy-2--nitro-octadec-4-yne (LXXb) with benzaldehyde in the presence of zinc chloride (Scheme 14; R = $CH_3[CH_2]_{12}C{\equiv}C-$).

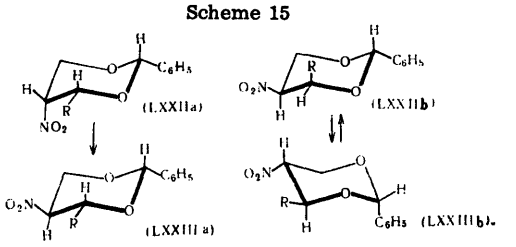
Scheme 14



The cyclic acetals (LXXIIa, b) differ in the orientation of the phenyl group in the 2-position of the 1,3-dioxan ring. Treatment of (LXXIIa) with sodium ethoxide in ethanol results in virtually complete conversion into the epimer (LXXIIIa), and when this reaction is carried out with (LXXIIb), an equilibrium mixture is formed containing the epimer (LXXIIIb) and the starting (LXXIIb) in the ratio 3 : 2.

This kind of difference in the equilibrium position is explained by the greater stability of the most favoured completely equatorial conformation (LXXIIIa) in comparison with (LXXIIa) and the approximately identical stabilities of (LXXIIb) and (LXXIIIb) (Scheme 15).

Scheme 15



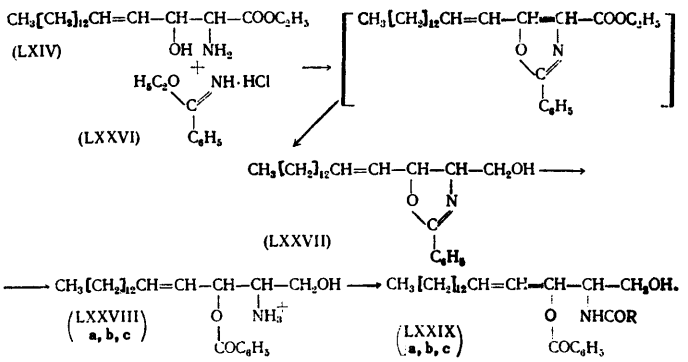
When (LXXIII) is reduced with aluminium amalgam, the amines (LXXIV) are formed, and on acetylation these give *erythro*-5-acetamido-4-(pentadec-1-ynyl)-2-phenyl-*m*--dioxans (LXXVa, b) (see Scheme 14); hydrolysis of the latter gives *erythro*-2-amino-1,3-dihydroxyoctadec-4--yne (LXXIa). The large number of stages and the long time required for some stages are disadvantages of the method. Because of these disadvantages, the present authors have worked out a new method for the *threo–erythro*-isomerisation of sphingosine, the main features of which are described in the following Section.

Grob's method has been successfully used for the synthesis of C_{20}-*erythro*-sphingosine (III) [131] and C_{20}-*erythro*--dihydrosphingosine (IV) [132] and their stereoisomers, which have been isolated in recent years; it has also been used for the synthesis of a number of long-chain amino-diols, homologues of sphingosine [133].

3. Methods of Synthesis of Glycosphingolipids

Natural sphingolipids, in particular glycosphingolipids, contain a free allyl hydroxyl group, and it is desirable that this group should be temporarily blocked during the synthesis [134-137]. With sphingosines (I) as starting materials, this blocking is difficult because of the presence in these bases of a complex system of active centres of similar reactivity in conjunction with an ethylene bond, which makes allyl rearrangements possible. The first important advance was made by Shapiro and coworkers on the basis of their sphingosine synthesis, in which the intermediate *erythro*--ester (LXIV) has a potential primary sphingosine hydroxyl group in a latent form as an ester grouping. Condensation of the *erythro*-ester (LXIV) with ethyl iminobenzoate hydrochloride (LXXVI)‡ and subsequent reduction of the ester group with lithium tetrahydroaluminate gives the oxazoline (LXXVII)† (Scheme 16).

Scheme 16



When the oxazoline (LXXVII) is treated with dilute sulphuric acid in tetrahydrofuran, *erythro*-3-*O*-benzoylsphingosine (LXXVIIIa) is formed.

Using *L*- and *D*-tartaric acids, Shapiro [137] separated the sulphate (LXXVIIIa) into antipodes by way of L-*erythro*--3-*O*-benzoylsphingosine *L*-tartrate (LXXVIIIb) and D-*erythro*-3-*O*-benzoylsphingosine *D*-tartrate (LXXVIIIc). Acylation of the sulphate (LXXVIIIa) or the tartrates (LXXVIIIb, c) with a fatty-acid chloride in the presence of sodium acetate–acetic acid buffer leads to DL-, L-, and D-*erythro*-*N*-acyl-3-*O*-benzoylsphingosines (LXXIXa, b, c) respectively; these are then used for the direct synthesis of sphingolipids.

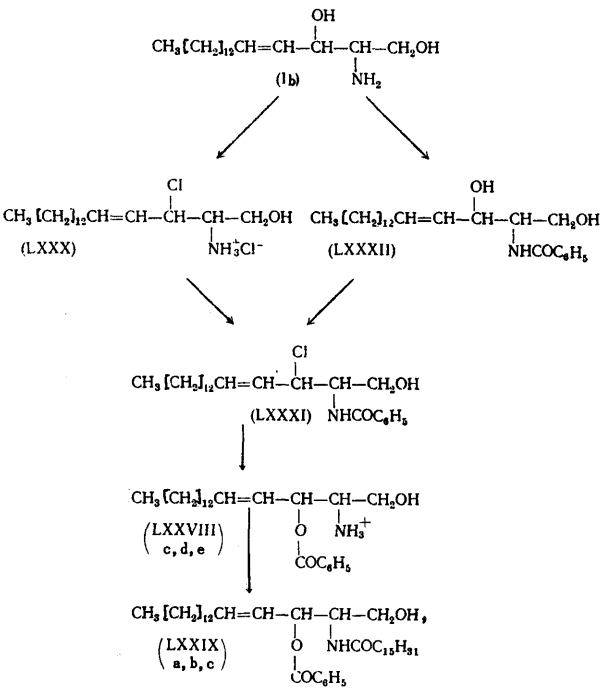
We have developed another method [138] of protecting the allyl hydroxyl group with simultaneous *threo–erythro*-isomerisation; this enables natural derivatives of *erythro*--sphingosine to be prepared from its threo-form (Ib), which is preferentially formed in Grob's synthesis. When a salt of *threo*-sphingosine (Ib) (Scheme 17) reacts with thionyl chloride in chloroform at 20°, *threo-trans*-2-amino-3--chloro-1-hydroxyoctadec-4-ene (LXXX) is formed, and on *N*-benzoylation with benzoic anhydride in the presence of sodium hydrogen carbonate this gives *threo-trans*-2--benzamido-3-chloro-1-hydroxyoctadec-4-ene (LXXXI).

The latter can also be obtained by chlorinating *threo*-*N*--benzoylsphingosine (LXXXII), that is, by changing the order of the stages. It is interesting to note that appreciable quantities of the oxazaline (LXXVII), which is an intermediate in the syntheses of sphingolipids by the Shapiro method (see Scheme 16), is not formed here. Apparently its formation is not favoured energetically [139].

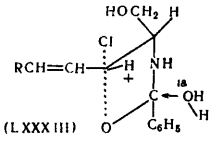
When the β-chloroamide (LXXXI) was heated in aqueous tetrahydrofuran, *erythro*-3-*O*-benzoylsphingosine (LXXVIIId) was obtained; this compound can be used in directed syntheses of sphingolipids. Measurement by amperometric titration of the amount of chloride ions liberated has shown that the reaction is an equilibrium process. It was shown by a special experiment with water containing heavy oxygen [140] that the reaction probably proceeds through a hypothetical transition state (LXXXIII); the regenerated oxygen function of *erythro*-3-*O*-benzoylsphingosinium (LXXVIIId) chloride is formed from the oxygen of the carbonyl group,

‡ Better termed α-ethoxybenzylideniminium chloride (Ed. of (Translation).

Scheme 17



but the carbonyl function of the ester grouping in (LXXIIId) is built up with the participation of oxygen from the water.

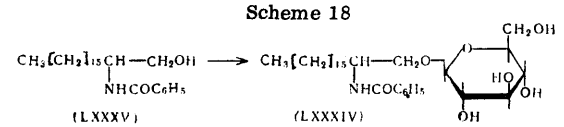


In this example [141,142], separation into antipodes is effected by allowing the chloride (LXXVIIId) to react with the mono-silver salt of *D*-tartaric acid [143], followed by fractional crystallisation of the *D*-tartrates of D- and L-*erythro*-3-*O*-benzoylsphingosines (LXXVIIIc, e) formed. Palmitoylation of the chloride (LXXVIIId) or the tartrates (LXXVIIIc, e) leads to DL-, D-, and L-*erythro*-3-*O*-benzoyl-*N*-palmitoylsphingosines (LXXIXa, b, c), identical with the corresponding compounds obtained by Shapiro's method.

4. Synthesis of Cerebrosides and Psychosine

The history of synthetic studies in the glycosphingolipid field begins in 1957, when Prostenik and Krvavica [144] described the synthesis of 1-β-D-glucopyranosyl-*DL*- and 1-β-D-glucopyranosyl-*L*-benzoylsphingine (LXXXIV), in which *N*-benzoyl-*DL*-sphingine or *N*-benzoyl-*L*-sphingine (LXXXV) reacts with α-benzobromoglucose in the presence of mercury cyanide in nitromethane, the reaction product being subsequently saponified with sodium methoxide [145] (Scheme 18).

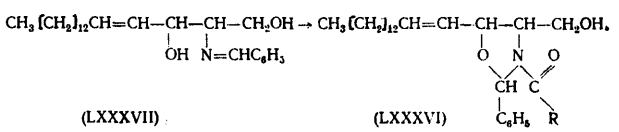
Scheme 18



Kiss was the first to synthesise the dihydrocerebroside [146,147]; he treated dihydro-*N*-tetracosanoylsphingosine with α-acetobromogalactose, and the mixture of α- and β-galactosides, formed as penta-acetates, was subsequently anomerised with titanium tetrachloride. On the basis of this synthesis it was concluded that dihydrocerasine, obtained by hydrogenation of natural cerasine or neurone, has the α-configuration, since it was found to be identical with the synthetic α-galactoside of dihydroceramide (m.p. 186°, $[\alpha]_D$ = +5.31°). In subsequent studies, Shapiro and Flowers [135] showed that this result was incorrect.

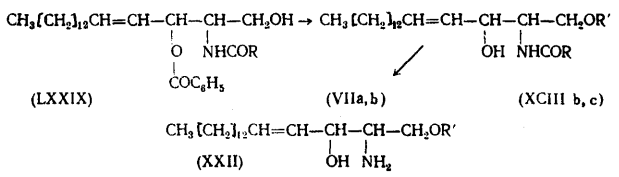
Attempts by Weiss [136] to prepare glycosides of the substituted oxazolidine (LXXXVI), which is obtained by alkylation of *N*-benzylidenesphingosine (LXXXVII) (see Scheme 19), were unsuccessful, as were the attempts of Shapiro to prepare a glycoside of the oxazolidine (LXXVII) (see Scheme 16); these failures were because of the extremely low reactivity of the hydroxyl group in these compounds, and also because of the sensitivity to alkali of the small amounts of glycoside formed.

Scheme 19



Shapiro and Flowers [135] were the first to synthesise cerebrosides (VII) from protected ceramides (LXXIX) obtained by the method described in the previous Section. Condensation of benzoylceramides (LXXIX) with α-acetobromogalactose or α-acetobromoglucose in the presence of mercury cyanide and subsequent saponification of the protecting groups leads to β-D-galacto- and β-D-gluco-cerebrosides (VII) (Scheme 20), identical in physical properties (infrared spectrum and $[\alpha]_D$) with natural cerasine and phrenosine, and with the cerebroside that is stored in the human spleen in Gaucher's disease.

Scheme 20

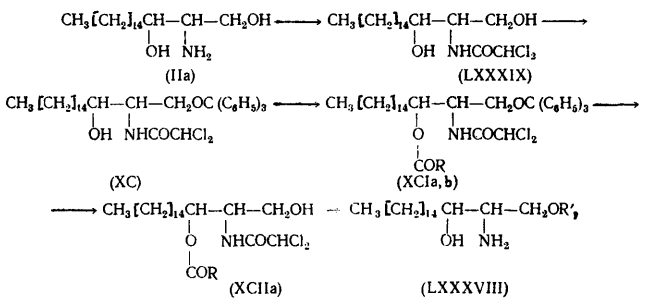


VIIa, R′ = $CH_3[CH_2]_{22}$, R″ = glucose; VIIb, R = Cl_2CH, R″ = galactose;
XXII, R″ = galactose; XCIIIb, R = $CH_3[CH_2]_{22}$, R″ = lactose;
XCIIIc, R = $CH_3[CH_2]_{14}$, R″ = lactose.

Shapiro and coworkers [137] have also described the preparation of psychosine (XXII) — a precursor of cerebrosides in biosynthesis [148] — and its dihydro-derivative (LXXXVIII), which is of interest in biosynthetic studies, particularly the synthesis of cerebrosides labelled in the fatty acid residue. The dichloroacetyl group was found to be the best group for the temporary protection of the amine function in psychosine (XXII) and dihydropsychosine (LXXXVIII). Psychosine (XXII) is obtained in the same way as the cerebrosides (VII) (see Scheme 20), the dichloroacetamide (VIIb) formed being hydrolysed by boiling with barium hydroxide.

A new method was worked out for the synthesis of dihydropsychosine (LXXXVIII) (see Scheme 21).

Scheme 21



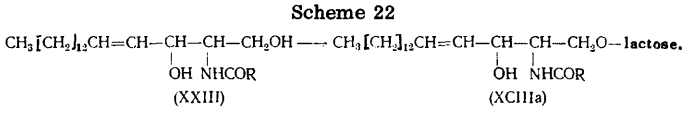
where (a) R = $-C_6H_5NO_2$-*p*; (b) R = C_6H_5; (c) R′ = glucose.

When dihydrosphingosine (IIa) reacts with methyldichloroacetate, the amide (LXXXIX) is formed; the primary hydroxyl group of this compound is temporarily tritylated and the secondary hydroxyl group is then *p*-nitrobenzoylated, (LXXXIX) → (XC) → (XCIa), since the benzoyl derivative (XCIb) is difficult to purify. Removal of the trityl protecting group with dilute acetic acid leads to the dichloroacetamido-ester (XCIIa), which is converted into dihydropsychosine (LXXXVIII) in the usual way. Later it was pointed out [149] that natural derivatives of *erythro*-sphingosine could be synthesised by this new method (see Scheme 21). This is particularly interesting since it provides a shorter synthetic route from natural *erythro*-sphingosine to the sphingolipids.

5. Synthesis of Cytolipin H

Schram and coworkers [150] synthesised the lactoside (XCIIIa), which is similar in structure to cytolipin H, using as a starting material ceramide (XXIII) obtained by enzymatic hydrolysis [151] of sphingomyelin from ox heart (Scheme 22). The infrared spectra of the lactoside (XCIIIa) and cytolipin H are almost identical, but their specific rotations differ ($[\alpha]_D$ = +2.1° and -10.8° respectively).

Scheme 22



In 1964 a synthesis of 1-*O*-β-D- and 1-*O*-β-DL-lactosyl-*N*-lignocerylsphingosines (XCIIIb) was described [152], but experimental details were not published; the method used was that developed by Shapiro for the preparation of cerebrosides (see Scheme 20). The lactoside (XCIIIb) was found to be identical in physicochemical and serological properties with cytolipin H. The work of Schram [150] has been subjected to critical examination. In particular, the probable ambiguity in lactoside formation by the unprotected ceramide (XXIII) has been pointed out; this has been related to the increased angle of rotation of the lactoside (XCIIIa).

The lactosides of *erythro*-*N*-palmitoylsphingosine (XCIIIc) and its *threo*-isomer, which are similar in structure to cytolipin H, have been prepared independently by the present authors [141,142], using the new method of synthesising sphingolipids described above [138] (see Scheme 20).

IV. BIOLOGICAL PROPERTIES

1. Immunological Properties

Recent studies in connection with the cancer problem have shown that there are significant differences in structure and biochemical composition between normal cells and tumour cells; these differences result from malignant degeneration of the tissues [153].

It has now been shown that all glycosphingolipids have the properties of organ-specific and tissue-specific haptens [154]. It is possible that glycosphingolipids, occurring as they do in membranes, participate in immunological control of cell growth [155].

Rapport and Graf made an extensive study of glycosphingolipid haptens. Using the method of complement fixation, they were the first to show that the individual lipid hapten isolated from human epidermoid carcinoma, cytolipin H, is a tissue-specific substance in a number of malignant neoplasms, such as carcinoma of the lungs, spinal cord, kidneys, and throat; Hodgkin's granuloma; bone sarcoma; lymphosarcoma; myelogenic leukaemia; and malignant melanoma. On the other hand, cytolipin H does not react with antiserum against carcinoma of the rectum or colon or epidermoid carcinoma [53,156]. Large quantities of this compound occur in the spleen of cancer patients, and small quantities occur in their livers, but none has been detected in the kidney tissues.

Repeated attempts to prepare antibodies that react with lipid extracts of cancer tissue by injecting tissue fractions of normal organs have been unsuccessful. This indicates that cytolipin H is present in only insignificant amounts, if at all, in normal organs [157]. However, Japanese biochemists [37,50,59,60] have isolated a number of glycosphingolipids, especially lactosylceramides, from various organs, including kidneys from healthy human subjects.

Immunological examination of dihydrocytolipin H, [57] and also of synthetic D- and DL-*erythro*-lactosyl-*N*-lignocerylsphingosines [158], has shown that the presence of a double bond and optical activity in the lipid part of the molecule are not essential for the immunological activity of cytolipin H. However, a change in the structure of the carbohydrate part of the molecule leads to loss of activity [159]. Rapport therefore concluded that the immunological specificity of glycosphingolipid haptens, and particularly of cytolipin H, depends, largely if not entirely, on the structure of the carbohydrate part of the molecule [61], and he has recently confirmed this conclusion [66]. The lipid residues only play a part in the localisation of the hapten in the cell membrane.

Schram and coworkers [150] accepted this point of view too readily; they thought that lactosides of any aliphatic or aromatic alcohols must behave in the same way as cytolipin H in the complement fixation reaction.

Attempts to immunise rabbits with cytolipin H, even in the presence of pure auxiliary lipids — lecithin and cholesterol — proved unsuccessful. Specific antibodies were obtained when immunisation was carried out with a mixture of cytolipin H and total tissue lipids.

The simplest glycosphingolipid, galactocerebroside [159,160], is an organ-specific lipid of brain [161]. The antiserum obtained by immunisation of rabbits with fractions of human and cow brain is active in the complement-binding reaction with galactocerebroside [160] in the presence of auxiliary

lipids, but it does not react with glucocerebroside or with cytolipin H.

The globoside isolated from human kidney and named cytolipin K is an organ-specific kidney hapten[66]. Forssman's hapten from sheep erythrocytes also contains sugar, fatty acid, and sphingosine residues characteristic of glycosphingolipids[61].

Yokoyama and coworkers[162] have proposed a useful method for the preparation of antiganglioside sera. When immunisation was effected with gangliosides deposited on the surface of erythrocytes, a high antibody titre was obtained. The specificity of the antiganglioside antibodies was demonstrated by the haemagglutination reaction, which is scarcely observed with erythrocytes covered with asialogangliosides.

In common with some other compounds, brain gangliosides inhibit the haemagglutination reaction with antibodies against Rhesus-positive erythrocytes[163], which indicates that gangliosides and blood-group antigen are immunologically similar[164]. However, antiganglioside serum does not react with Rhesus-positive erythrocytes in the haemagglutination test. This appears to be a reflection of fine structural differences between the ganglioside and the antigen determinant (possibly a complex lipopolysaccharide[165]) of Rhesus-positive human erythrocytes.

Two ganglioside-like compounds have also been separated from human erythrocytes[105], which, in addition to mucopolysaccharides[166], are substances characteristic of blood group A. These substances differ in physicochemical properties and in composition from the human erythrocyte globosides to which the group activity of the human blood groups A, B, O, and AB is ascribed[167]. Here the activity appears to be due to the presence of active mucopolysaccharides as impurities in the preparations.

In accordance with the classical method of diazo-fixation of Landsteiner[169], the reaction of diazotised *N*-*p*-aminobenzoyldihydropsychosine with proteins[168] gives artificial antigens. Immunochemical analysis by the precipitation method showed that the antiserum obtained by immunisation with such artificial albuminolipid antigens contains at least two types of antibody, antidihydropsychosine and antialbumin antibodies. However, the question to what extent antibodies obtained in this way will be complementary to natural glycosphingolipid haptens remains open.

2. Biological Activity of Sphingosine and Cerebrosides

The high-molecular-weight lipoprotein enzyme thromboplastin catalyses one of the stages in the complex process of blood coagulation[170]. The anticoagulant activity of sphingosine[171,172] is associated with its antithromboplastic[173] (that is, antienzyme) activity. This was first demonstrated in chicken plasma[174].

The use of comparatively small quantities of sphingosine in the presence of a lipid blood-coagulation activator has no inhibiting effect, but, if sphingosine is first incubated with chick blood plasma and the lipid activator is then added, the rate of coagulation is greatly increased compared with that using the lipid activator alone.

Thus, the way in which sphingosine acts depends on its concentration, on the concentration of lipid activator, and on the individual properties of the plasma. Synthetic *erythro*- and *threo*-dihydrosphingosines were found to be weaker coagulation inhibitors than sphingosine; *N*-benzoyldihydrosphingosine and triacetylsphingosine are entirely inactive. Synthetic homologues of sphingosine show the greatest activity when the chain contains 17–18 carbon atoms[133]. The presence of a double bond, free hydroxy- and amino-groups, and a long hydrocarbon chain are therefore necessary for anticoagulant activity[174].

When cerebrosides are introduced into the human organism or into experimental animals, more cholesterol is excreted with the faeces and the level of cholesterol in the blood is reduced[175]. The mechanism of this process is still unknown.

3. Neurophysiological Role of Gangliosides

Recently a number of workers have noted the important part played by the sialic acid components of gangliosides.

Van Heyningen showed in vitro experiments[176] that the gangliosides of ox brain are receptors for tetanus toxin[177], and also for such drugs as strychnine, brucine, and thebaine, which have similar neurophysiological activity[178]. The fixing power of the gangliosides is proportional to the number of sialic acid residues present in the molecule, but it also depends on the structure of the whole carbohydrate part of the molecule[179]. For example, hematosides isolated from horse brain or horse erythrocytes, which do not contain hexosamine, and the ganglioside that accumulates in the spleens of patients with "Tay-Sachs'" disease are virtually inactive[178].

The fixation of tetanus toxin by gangliosides is strictly specific for this toxin, and the non-specific inactivation of diphtheria and staphylococcal toxins by these compounds, which has been noted by North and Doery[180,181] has nothing in common with the fixation of tetanus toxin; this toxin is not inactivated at all[176]. Gangliosides also interact with serotonin, tryptophan, and related compounds, but not with γ-aminobutyric acid, β-hydroxybutyric acid, histamine, adrenaline, or noradrenaline[178].

The process by which the tetanus toxin is fixed by the ganglioside receptor in nerve tissue is directly related to the mechanism of action of the toxin. This is confirmed by the high specificity, and also by the fact that the gangliosides and the synapses inhibited by the toxin are found in the grey matter of the human brain.

The reaction of gangliosides with bacterial endotoxin that produces fever leading to disorders of the heat-regulating function of the body has also been described[182].

It may be that the reaction of the ganglioside with the toxin or with the drugs mentioned above is the first step that leads to the biochemical attack on which the pharmacological action of these compounds is based. On the other hand, the receptor may be responsible for adsorption of the toxin at the infection sites, and for its localisation in the nerve cells with subsequent centripetal transfer along the nerve columns to the site of activity in the brain or spinal cord. However, none of these hypotheses can be accepted without reservation.

Brain gangliosides are mainly associated with fractions of subcellular particles[183], which have an affinity with basic proteins[184], especially with microsoma[185] and, to a lesser degree, with mitochondria. They are readily extracted from brain tissues, but when added to a suspension of subcellular fractions of basic proteins, for example,

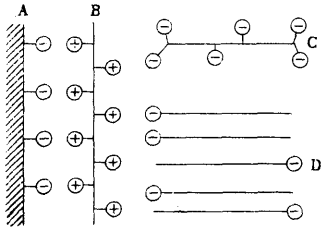
protamine, the gangliosides cannot be separated[142]. Evidently the natural gangliosides, which are acid components of lipid-rich membranes that participate in active cation transport, are linked to the basic proteins by ionic forces. This accounts for the opposite effects of these proteins and gangliosides on the excitability of the brain.

The normal brain exhibits continuous electrical and metabolic activity, which can be stimulated, particularly by electrical impulses[187]. Isolated brain tissue in a suitable nutrient solution can breathe and respond to electrical stimuli by showing increased assimilation of glucose and oxygen[188]. When such a system is cooled to 0°, the respiratory response to electrical stimulation is only 15% of the maximal reaction of normal tissue, but this response increases to 52–80% when 60–120 μg per mg of gangliosides is added. Evidently, when the tissue is cooled, the histones — which are basic proteins linked with ribonucleic acid in cell nuclei — lose their capacity of forming nucleohistone gel[184]. They separate from the RNA and migrate out of the nuclei, blocking the acid sections of the membranes. This stops reversible transfer of potassium ions in cerebral tissue after stimulation. Added gangliosides associate with these basic proteins and neutralise their activity[189-192].

Inhibition of brain excitability by protamines occurs in the same way as when cooling is applied, and it is abolished by gangliosides in exactly the same way.

Ordinary acid reagents cannot replace gangliosides. Neither *N*-acetylneuraminic acid nor L-glutamic acid restores excitability. Monoacid compounds, which cannot form aggregates, can only neutralise the basic groups of protamine, but this is not sufficient to restore excitability, which is associated with the presence of acid groups on the external surface of the membrane (see Scheme 23). On the other hand, polyacid compounds such as sialomucopolysaccharides, chondroitin sulphate, poly-L-glutamic acid, the aromatic hexasulphonic acid suramin, or micellar aggregates of such compounds as lecithin, phosphatidylethanolamine, sphingomyelin, and phosphatidylserine — compounds of widely different structure — all behave like gangliosides[193].

Scheme 23



Hypothetical interaction between acid areas of tissue (A), basic proteins (B), and polyacid molecules (C), or micellar aggregates (D).

This activity of gangliosides is higher than the activity of all other compounds and it increases with the number of sialic acid residues in the ganglioside. It is noteworthy that gangliosides restore the excitability of brain tissue and they are present in concentrations close to the critical concentration for the formation of micelles. Individual molecules of gangliosides probably first react with tissues that bind protamines, and then they combine to form micelles.

A similar explanation has been advanced for the metachromatic properties of gangliosides[194], that is, the capacity to form associations with suitable basic dyes (for example, Toluidine Blue or Pyocyanine[195]) that differ in colour from the dye itself[196]. This effect is due to association of the dye molecules with suitably arranged acid groups of the compounds being dyed[197]. A molecule of thiazine dye appears to combine with the acid groups of micellar gangliosides as a result of ionic forces, and it then undergoes association, due to secondary forces, with the formation of metachromatic dimers and polymers. The effect disappears in organic solvents, apparently because of a change in the structure of the ganglioside micelle, sialic acid residues being removed from the surface of the sphere, and also because of interaction of the dye and the solvent, which destroys the metachromatic aggregate. Because it can react with gangliosides, protamine also inhibits the metachromatic reaction with Toluidine Blue.

Using a histochemical method based on metachromatic reactions, Koenig[198] studied the localisation of the gangliosides in the nerve system, and he showed that the glycolipoprotein cytoplasmprotein granules (the so-called synaptic vesicles) containing acetylcholine[199] are also rich in gangliosides. In this connection, Burton and coworkers[109,200] put forward the hypothesis that gangliosides play a part in the transmission of nerve impulses through synapses in accordance with the concepts of a neuro-humoral mechanism[109,200]. At present, however, these concepts have given way to the idea[201] that acetylcholine functions in a uniform manner in synaptic connections and conducting axon membranes.

From the foregoing it is clear that gangliosides play an essential part in neurophysiological processes, but our knowledge in this field is still very unsatisfactory.

Further extension of our ideas on the significance of glycosphingolipids in biological systems will probably be mainly determined by the development of biochemical, biophysical, and synthetic work in this field. The problems that now have to be solved are concerned not only with the elucidation of the primary structure of new, hitherto unknown, types of glycosphingolipids, but also with establishing the actual shapes of these compounds in three-dimensional space, that is, of the forms in which they carry out their biological functions.

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While this manuscript was being prepared for the press, interesting reviews appeared on the chemistry of gangliosides[202], and sulphatides[203], and on methods of analysis of glycolipids[204], in addition to a review article on the immunochemical properties of glycolipids[205]. Further studies have led to the isolation of a number of new sphingosine bases from animal tissues[206-208]. Thus, twenty compounds of this type are known already; these differ in chain length, number of hydroxyl groups, and degree of unsaturation. A number of new glycosphingolipids have been isolated, including *O*-acylcerebrosides from ox brain[209], and also gangliosides from human brain[210,211], leucocytes[212], and placenta[213].

1. H.Carter, D.Galanos, and G.Fujino, Canad.J.Biochem. Physiol., 34, 320 (1956).
2. K.Karlsson, Acta Chim.Scand., 18, (10), 2397 (1964).
3. F.Blix, A.Gottschalk, and E.Klenk, Nature, 179, 1088 (1957).

4. J. L. W. Thudichum, "Die chemische Konstitution des Gehirns", Pietzecker, Tübingen, 1901.
5. P. Levene and C. West, J. Biol. Chem., 16, 549 (1913).
6. P. Levene and C. West, J. Biol. Chem., 18, 481 (1914).
7. E. Klenk, Z. physiol. Chem., 185, 169 (1929).
8. E. Klenk and W. Diebold, Z. physiol. Chem., 198, 25 (1931).
9. H. Carter, J. Biol. Chem., 142, 449 (1942).
10. H. Carter, F. Glick, W. Norris, and G. Phillips, J. Biol. Chem., 170, 285 (1947).
11. H. Carter and C. Humiston, J. Biol. Chem., 191, 727 (1951).
12. J. Kiss, Helv. Chim. Acta, 37, 1471 (1954).
13. H. Carter and D. Shapiro, J. Amer. Chem. Soc., 75, 1007 (1953).
14. K. Mislow, J. Amer. Chem. Soc., 74, 5155 (1952).
15. G. Marinetti and E. Stotz, J. Amer. Chem. Soc., 76, 1347 (1954).
16. A. Lesuk and R. Anderson, J. Biol. Chem., 139, 457 (1941).
17. H. Carter and W. Norris, J. Biol. Chem., 145, 709 (1942).
18. H. Carter, W. Norris, F. Glick, G. Phillips, and R. Harris, J. Biol. Chem., 170, 269 (1947).
19. M. Prostenik and B. Majhofer-Orescanin, Naturwiss., 47, (17), 399 (1960).
20. B. Majhofer-Orescanin and M. Prostenik, Croat. Chem. Acta, 33, (4), 219 (1961).
21. R. Garver and S. Sweeney, J. Amer. Oil Chemists' Soc., 42, (4), 234 (1965).
22. N. Stanacev and E. Chargaff, Biochim. Biophys. Acta, 59, 733 (1962).
23. A. Rosenberg and E. Chargaff, J. Biol. Chem., 232, 1031 (1958).
24. N. Stanacev and E. Chargaff, Biochim. Biophys. Acta, 98, (1), 168 (1965).
25. K. Sambasivarao and R. McCluer, J. Lipid Res., 5, 103 (1964).
26. K. Karlsson, Nature, 188, 312 (1960).
27. K. Karlsson, Acta Chem. Scand., 18, (2), 565 (1964).
28. J. Thudichum, "A Treatise on the Chemical Composition of Brain", 1884.
29. E. Klenk, Z. physiol. Chem., 153, 74 (1926).
30. E. Klenk and R. Harle, Z. physiol. Chem., 189, 243 (1930).
31. A. Chibnall, S. Piper, and E. Williams, Biochem. J., 55, 707 (1953).
32. A. Chibnall, S. Piper, and E. Williams, Biochem. J., 55, 711 (1953).
33. G. Hooghwinkel, P. Borri, and R. Riemersma, Rec. Trav. chim., 83, 576 (1964).
34. J. O'Brien and G. Rouser, J. Lipid Res., 5, (3), 339 (1964).
35. O. Hillborg and L. Svennerholm, Acta Paediat., 49, 707 (1960).
36. N. Halliday, H. Deuel, L. Tragenman, and W. Ward, J. Biol. Chem., 132, 171 (1940).
37. A. Makita and T. Yamakawa, J. Biochem., 51, 124 (1962).
38. J. Pryde and R. Humphreys, Biochem. J., 18, 661 (1924).
39. J. Pryde and R. Humphreys, Biochem. J., 20, 825 (1926).
40. H. Carter and F. Greenwood, J. Biol. Chem., 199, 283 (1952).
41. N. K. Kochetkov, I. G. Zhukova, and I. S. Glukhoded, Biochim. Biophys. Acta, 70, 716 (1963).
42. N. K. Kochetkov, I. G. Zhukova, and I. S. Glukhoded, Dokl. Akad. Nauk SSSR, 139, 608 (1961).
43. G. Blix, Z. physiol. Chem., 219, 82 (1933).
44. L. Svennerholm and H. Thorin, J. Lipid Res., 3, 483 (1962).
45. J. O'Brien, D. Fillerup, and J. Mead, J. Lipid Res., 5, 109 (1964).
46. H. Yatzkewitz, Z. physiol. Chem., 320, 134 (1960).
47. J. Bakke and W. Cornatzer, Fed. Proc., 19, 224 (1960).
48. J. Bakke and W. Cornatzer, J. Biol. Chem., 236, 653 (1961).
49. S. J. Thannhauser and N. Boncoddo, Fed. Proc., 12, 280 (1953).
50. T. Yamakawa, N. Kiso, S. Nanda, A. Makta, and S. Yokoyama, J. Biochem., 52, 226 (1962).
51. A. Lloyd and K. Dodgson, Biochim. Biophys. Acta, 46, 116 (1961).
52. S. Hakomori, T. Yshimoda, and K. Nakamura, J. Biochem., 52, 468 (1962).
53. M. Rapport, L. Graf, and N. Alonzo, Cancer, 12, 438 (1959).
54. M. Rapport, L. Graf, and J. Yariv, Arch. Biochem. Biophys., 92, 438 (1961).
55. M. Rapport, V. Skipski, and C. Sweeley, J. Lipid Res., 2, 148 (1961).
56. E. Klenk and F. Reinkamp, Z. physiol. Chem., 273, 253 (1942).
57. M. Rapport, H. Schneider, and L. Graf, J. Biol. Chem., 237, 1056 (1962).
58. E. Klenk, Z. physiol. Chem., 291, 259 (1952).
59. A. Makita, J. Biochem., 55, 269 (1964).
60. A. Makita and T. Yamakawa, J. Biochem., 55, 365 (1964).
61. M. Rapport, J. Lipid Res., 2, 25 (1961).
62. T. Yamakawa, R. Yrie, and M. Iwanaga. J. Biochem., 48, 490 (1960).
63. W. Morrison and L. Smith, Biochim. Biophys. Acta, 84, 759 (1964).
64. T. Yamakawa, J. Biochem., 39, 393 (1952).
65. T. Yamakawa, S. Yokoyama, and N. Kiso, J. Biochem., 52, 228 (1962).
66. M. Rapport, L. Graf, and T. Schneider, Arch. Biochem. Biophys., 105, 431 (1964).
67. A. Makita, M. Iwanaga, and T. Yamakawa, J. Biochem., 55, 202 (1964).
68. S. Hakomori and R. Yanloz, J. Biol. Chem., 239, 3606 (1964).
69. T. Yamakawa and S. Suzsuki, J. Biochem., 38, 199 (1951).
70. E. Klenk and G. Padberg, Z. physiol. Chem., 327, 249 (1962).
71. A. Gottschalk, "The Chemistry and Biology of Sialic Acids and Related Substances", Cambridge University Press, London, 1960.
72. A. Gottschalk, Rev. Pure Appl. Chem. (Australia), 12, 46 (1962).
73. L. Svennerholm, Acta Chem. Scand., 17, 860 (1963).
74. E. Klenk and W. Gielen, Z. physiol. Chem., 319, 283 (1960).
75. E. R. Berman and S. Gatt, "Cerebral Sphingolipidoses : A Symposium on Tay-Sachs' Disease and Allied Disorders" (Edited by S. M. Aronson and B. W. Volk), Academic Press, New York, 1962, p. 237.
76. E. Klenk, W. Gielen, and G. Padberg, *ibid.*, p. 301.
77. G. Tettamanti, L. Bertona, and V. Sambotti, Biochim. Biophys. Acta, 84, (6), 756 (1964).
78. K. Landsteiner and P. Levene, J. Immunol., 10, 731 (1925).
79. E. Klenk, Z physiol. Chem., 273, 76 (1942).
80. E. Klenk and W. Gielen, Bull. Soc. Chim. biol., 42, 1395 (1960).
81. L. Svennerholm, Nature, 177, 524 (1956).
82. E. Trams, L. Ginffrida, and A. Karmen, Nature, 193, 680 (1962).
83. L. Svennerholm and A. Raal, Biochim. Biophys. Acta, 53, 422 (1961).
84. R. Kuhn, H. Wiegandt, and H. Egge, Angew. Chem., 73, 580 (1961).
85. E. Klenk and W. Gielen, Z. physiol. Chem., 326, 158 (1961).
86. J. A. Dain, G. Schmidt, and S. J. Thannhauser, Fed. Proc., 20, 269 (1961).
87. L. Svennerholm, J. Lipid Res., 5, 145 (1964).
88. E. Klenk and W. Gielen, Z. physiol. Chem., 326, 144 (1962).
89. R. Cote and W. Morgan, Nature, 178, 1171 (1956).
90. L. Svennerholm, Biochem. Biophys. Res. Comm., 9, 436 (1962).
91. E. Klenk, U. Hendricks, and W. Gielen, Z. physiol. Chem., 330, 140 (1962).
92. T. Painter, J. Cheese, and W. Morgan, Chem. and Ind., 1535 (1962).
93. R. Kuhn and W. Wiegandt, Chem. Ber., 96, 866 (1963).
94. G. Johnson and R. McCluer, Biochim. Biophys. Acta, 84, (5),

587 (1964).
95. L. Svennerholm, J. Neurochem., 10, 613 (1963).
96. E. Klenk and W. Gielen, Z. physiol. Chem., 330, 218 (1963)
97. R. Kuhn, H. Egge, R. Brossmer, A. Gaude, P. Klesse, W. Lochinger, E. Röhm, H. Trischamann, and D. Tschampel, Angew. Chem., 72, 805 (1960).
98. A. Rosenberg and E. Chargaff, Biochim. Biophys. Acta, 42, 357 (1960).
99. E. McGuire and S. Binkley, Fed. Proc., 21, 172 (1962).
100. D. Aminoff, F. Dodyk, and S. Roseman, J. Biol. Chem., 238, 1177 (1963).
101. R. Kuhn and H. Wiegandt, Z. Naturforsch., 18b, 541 (1963).
102. L. Wolfe and J. Lowden, Canad. J. Biochem., 42, (7), 1041, 1057 (1964).
103. R. Kuhn and H. Wiegandt, Z. Naturforsch., 19b, 80 (1964).
104. H. Wiegandt and G. Baschang, Z. Naturforsch., 20b, 164 (1965).
105. G. Uhlenbruck and M. Krüpe, Nature, 199, 1289 (1963).
106. J. Koscielak, Biochim. Biophys. Acta, 78, (2), 313 (1963).
107. A. Rosenberg and E. Chargaff, J. Biol. Chem., 232, 1031 (1958).
108. S. Bogoch, Biochem. J., 68, 319 (1958).
109. R. E. Howard and R. M. Burton, Biochem. Pharmacol., 13, (12), 1677 (1964).
110. H. Carter, W. Norris, and H. Rockwell, J. Biol. Chem., 170, 295 (1947).
111. G. Gregory and T. Malkin, J. Chem. Soc., 2453 (1951).
112. K. Ställberg-Stenhagen, Arkiv Kemi, 20, A (19), (1945).
113. C. Grob, E. Jenny, and H. Utzinger, Helv. Chim. Acta, 34, 2249 (1951).
114. M. Egerton, G. Gregory, and T. Malkin, J. Chem. Soc., 2272 (1952).
115. M. Prostenik and N. Stanacev, J. Org. Chem., 18, 59 (1953).
116. J. Sallay, F. Dutka, and G. Fodor, Helv. Chim. Acta, 37, 778 (1954).
117. E. Jenney and C. Grob, Helv. Chim. Acta, 36, 1454 (1953).
118. C. Grob, Helv. Chim. Acta, 36, 1454 (1953).
119. H. Carter, J. Harrison, amd D. Shapiro, J. Amer. Chem. Soc., 75, 4705 (1953).
120. H. Carter and D. Shapiro, J. Amer. Chem. Soc., 75, 5131 (1953).
121. K. Sisidi, N. Hirowatati, and T. Ysida, J. Org. Chem., 29, (9), 2783 (1964).
122. G. Myers, J. Amer. Chem. Soc., 74, 1390 (1952).
123. V. A. Artamonov, Zhur. Obshch. Khim., 28, 1355 (1958).
124. D. Shapiro, H. Segal, and H. Flowers, J. Amer. Chem. Soc., 80, 2170 (1958).
125. R. Japp and F. Klingemann, Chem. Ber., 20, 2492, 3284, 3398 (1887).
126. D. Shapiro and T. Sheradsky, J. Amer. Chem. Soc., 28, 2157 (1963).
127. J. Controulis, M. Rebstock, and H. Crooks, J. Amer. Chem. Soc., 71, 2463 (1949).
128. D. Shapiro, H. Segal, and H. Flowers, J. Amer. Chem. Soc., 80, (5), 1194 (1958).
129. C. Grob and F. Gadient, Helv. Chim. Acta, 40, 1145 (1957).
130. S. Cristol and W. Frith, Jnr., J. Org. Chem., 26, 280 (1961).
131. B. Majhofer-Orescanin and M. Prostenik, Croat. Chem. Acta, 34, 161 (1962).
132. B. Majhofer-Orescanin and M. Prostenik, Tetrahedron, 12, 56 (1961).
133. E. Jenny and J. Druey, Helv. Chim. Acta, 42, (2), 401 (1959).
134. B. Weiss, J. Org. Chem., 26, 491 (1961).
135. D. Shapiro and H. Flowers, J. Amer. Chem. Soc., 83, 3327 (1961).
136. B. Weiss, J. Org. Chem., 26, 491 (1961).
137. D. Shapiro, E. Rachaman, and T. Sheradsky, J. Amer. Chem. Soc., 86, 4472 (1964).
138. A. Ya. Veinberg, L. A. Vakulova, V. G. Mairanovskii, and G. I. Samokhvalov, Zhur. Obshch. Khim., 34, 3979 (1964).
139. J. Sicher and M. Pankowa, Coll. Czech. Chem. Comm., 20, 1409 (1955).
140. A. Ya. Veinberg, L. A. Vakulova, and G. I. Samokhvalov, Zhur. Org. Khim., 2, 337 (1966).
141. A. Ya. Veinberg, L. A. Vakulova, and G. I. Samokhvalov, Zhur. Vses. Khim. Obshch. im. Mendeleeva, 9, 348 (1964).
142. A. Ya. Veinberg, L. A. Vakulova, and G. I. Samokhvalov, Zhur. Org. Khim., 1, 968 (1965).
143. W. Perkin, J. Chem. Soc., 51, 362 (1887).
144. M. Prostenik and N. Krvavica, Croat. Chem. Acta, 29, (2), 101 (1957).
145. B. Helferich and K. Weis, Chem. Ber., 89, 314 (1956).
146. J. Kiss, Chimia (Switz.), 13, 115 (1959).
147. J. Kiss, Acta Chim. Acad. Sci. Hung., 5, 477 (1955).
148. R. Brady, J. Biol. Chem., 237, 2416 (1962).
149. D. Shapiro, J. Amer. Oil Chemists' Soc., 42, 267 (1965).
150. A. Schram, E. Byers, and R. Wilson, Nature, 197, 1074 (1963).
151. M. Sribney and E. Kennedy, J. Biol. Chem., 233, 1315 (1958).
152. D. Shapiro and E. Rachaman, Nature, 201, 878 (1964).
153. E. Day, Ann. Rev. Biochem., 31, 549 (1962).
154. R. O. Brady and E. G. Trams, Ann. Rev. Biochem., 33, 75 (1964).
155. H. Green, in "Ciba Foundation Symposium on Carcinogenesis", Churchill, London, 1959, p. 131.
156. L. Graf and M. Rapport, Cancer Res., 20, 546 (1960).
157. M. Rapport and L. Graf, Cancer Res., 21, 1225 (1961).
158. M. Rapport and L. Graf, Nature, 201, 879 (1964).
159. S. Joffe, M. Rapport, and L. Graf, Nature, 197, 60 (1963).
160. M. Rapport, L. Graf, L. Autilio, and W. Norton, J. Neurochem., 11, (12), 855 (1964).
161. B. Yankovic, K. Isakovic, and L. Micailovic, Internat. Arch. Allergy Appl. Immunol., 17, 211 (1960).
162. M. Yokoyama, E. Trams, and R. Brady, J. Immunol., 90, 372 (1963).
163. M. Dodd, N. Bigley, and V. Geyer, Science, 132, 1398 (1960).
164. M. Dodd, N. Bigley, and V. Geyer, J. Immunol., 90, 518 (1963).
165. B. Urbaschek, O. Thide, J. Gurndice, and O. Preisler, Naturwiss., 50, 229 (1963).
166. E. A. Kabat, "Blood Group Substances: Their Chemistry and Immunochemistry", Academic Press, New York, 1956.
167. T. Yamakawa, M. Matsumoto, S. Suzuki, and T. Yida, J. Biochem., 43, 41 (1956).
168. T. Taketomi and T. Yamakawa, J. Biochem., 54, 444 (1963).
169. K. Landsteiner, "The Specificity of Serological Reactions", Cambridge, 1947.
170. Ya. V. Byalik and E. L. Khodorova, "Biokhimiya Svertyvaniya Krovi" (Biochemistry of Blood Coagulation), 1957.
171. W. Seegers and R. Landaburn, Fed. Proc., 16, 116 (1957).
172. E. Hecht, Fed. Proc., 16, 194 (1957).
173. L. M. Tocantins and R. Carroll, "Blood Clotting and Allied Problems", New York, 1949.
174. E. Hecht and D. Shapiro, Science, 125, 1041 (1957).
175. K. Carroll, J. Lipid Res., 1, 171 (1960).
176. W. Van Heyningen and P. Miller, J. Gen. Microbiol., 24, 107 (1961).
177. W. Van Heyningen, J. Gen. Microbiol., 20, 310 (1959).
178. W. Van Heyningen, J. Gen. Microbiol., 31, 375 (1963).
179. A. Bernheimen and W. Van Heyningen, J. Gen. Microbiol., 24, 121 (1961).
180. E. North and H. Doery, Brit. J. Exp. Path., 42, 23 (1961).
181. H. Doery and E. North, Austral. J. Exp. Biol. Med. Sci., 39, 333 (1961).
182. O. Westphal, Naturwiss., 45, 60 (1959).

183. L. Wolfe, Biochem. J., 79, 348 (1961).
184. L. Wolfe and H. McIlwain, Biochem. J., 78, 33 (1961).
185. J. Wherett and H. McIlwain, Biochem. J., 84, 232 (1962).
186. L. Wolfe, Biochem. J., 77, 9P (1960).
187. H. McIlwain, "Chemical Exploration of the Brain", Elsevier, Amsterdam, 1963.
188. H. McIlwain, Biochem. J., 49, 382 (1951).
189. C. Thompson and H. McIlwain, Biochem. J., 79, 342 (1961).
190. R. Woodman and H. McIlwain, Biochem. J., 79, 1P (1961).
191. H. McIlwain, J. Physiol., 152, 60P (1960).
192. S. Balakrishnan and H. McIlwain, Biochem. J., 81, 72 (1961).
193. H. McIlwain, Biochem. J., 90, 442 (1964).
194. A. Harris and A. Saifer, in "Cerebral Sphingolipidoses: A Symposium on Tay-Sachs' Disease and Allied Disorders", Academic Press, New York, 1962, p. 271.
195. A. Harris and A. Saifer, J. Neurochem., 5, 218 (1960)
196. D. Booth, J. Neurochem., 9, 265 (1962).
197. B. Sylvan, Quart. J. Microscop. Sci., 95, 327 (1954).
198. K. Koenig, Nature, 195, 782 (1962).
199. D. Nachmansohn, "Chemical and Molecular Basis of Nerve Activity", Academic Press, New York, 1959, p. 235.
200. R. M. Burton, R. E. Howard, S. Baer, and Y. M. Balfour, Biochim. Biophys. Acta, 84, (4), 441 (1964).
201. D. Nachmansohn (Editor), "Molecular Biology: Elementary Processes of Nerve Conduction and Muscle Contraction" (Translated into Russian), Moscow, 1964, p. 282.
202. R. Ledeen, J. Amer. Oil Chemists' Soc., 43, (2), 57 (1966).
203. P. J. Stoffyn, J. Amer. Oil Chemists' Soc., 43, (2), 69 (1966).
204. H. E. Carter, Ann. Rev. Biochem., 34, 109 (1965).
205. R. O. Brady, J. Amer. Oil Chemists' Soc., 43, 67 (1966).
206. K. A. Karlsson, Acta Chem. Scand., 19, 2423, 2425 (1965).
207. C. Michalec, Biochim. Biophys. Acta, 106, 197 (1965); 116, 400 (1966).
208. C. Michalec and Z. Kollman, Clinica Chim. Acta, 13, 529 (1966).
209. I. G. Zhukova, I. S. Glukhoded, and N. K. Kochetkov, Dokl. Akad. Nauk SSSR, 167, 346 (1966).
210. R. J. Penich and R. M. McCluer, Biochem. Biophys. Acta, 106, 435 (1965).
211. R. J. Penich, M. H. Meisler, and R. H. McCluer, Biochem. Biophys. Acta, 116, 279 (1966).
212. C. Miras, J. D. Mantzos, and G. M. Levis, Biochem. J., 38, 782 (1966).
213. L. Svennerholm, Acta Chem. Scand., 19, 1506 (1965).

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