review

Fucolipids

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Fucolipids are complex glycosphingolipids found in human erythrocytes and in certain secretory tissues of several mammalian species. They are of special interest because of their antigenic activity in the ABO and Lewis blood group systems and are mainly responsible for this activity in human blood. Although antigenicity was earlier recognized in glycolipid preparations of red blood cells (1, 2), fucose was not identified as a component sugar of glycolipids until 1963 (3, 4). It was not specifically associated with the blood group-active glycolipids until Yamakawa, Nishimura, and Kamimura (5) reported the separation of two glycolipids that were immunologically active and relatively rich in fucose. The first pure fucolipid was isolated from human gastric adenocarcinoma and human bronchogenic carcinoma tissue by Hakomori and Jeanloz in 1964 (6). Since this time much has been discovered about the chemistry and biology of these substances. Research has been stimulated by interest in the immunology of biohaptens that could be obtained in pure form, by interest in cell membrane chemotopography, and by the role of membrane components in normal cell and in tumor cell biology. This review will consider briefly the chemical nature, the methods and techniques for isolation and identification, the biosynthesis, and some aspects of the biology of the fucolipids. Several excellent reviews are available on subjects involving fucolipids: one on blood group antigens by Hakomori and Kobata (7), one on blood group glycolipids in normal and tumor tissue by Hakomori (8), and one on glycolipids of tumor cell membranes by Hakomori (9). The reader is also referred to a general review of the biochemistry of glycosphingolipids by Wiegandt (10). A 1975 symposium on glycolipids, *Glycolipid Methodology*, has been published in book form by the American Oil Chemists' Society Press (11), and Volume 28 part B of *Methods in Enzymology* contains many valuable references relating to the complex glycolipids and fucolipids (12).

I. NATURE OF THE FUCOLIPIDS

All of the fucolipids thus far isolated are ceramide oligosaccharides containing five or more sugars, with the single exception of a fucosyl ceramide (13). Besides the fucose, one glucose, two or more galactoses, and *N*-acetylglucosamine are usually present, and *N*-acetylgalactosamine is present in the fucolipids with blood group A-activity. Only two types of fucolipids containing sialic acid have been reported. All of the sugars are in glycosyl linkage in the pyranosyl form and all are of the D series except fucose. Most of the known fucolipids are glycosyl derivatives of lacto-*N*-tetraosyl ceramide or lacto-*N*-neotetraosyl ceramide, giving rise to the "type 1" and "type 2" (14) oligosaccharide chains, respectively.

Lacto-*N*-tetraosylceramide; Type 1 chain

Gal(β1 → 3)GlcNAc(β1 → 3)Gal(β1 → 4)Glc(β1 → 1)ceramide

Lacto-*N*-neotetraosylceramide, or "paragloboside"; Type 2 chain

Gal(β1 → 4)GlcNAc(β1 → 3)Gal(β1 → 4)Glc(β1 → 1) ceramide

Substitution of fucose, galactose, and *N*-acetylgalactosamine residues at the appropriate positions of these precursors gives rise to all of the ABO and Lewis blood group antigens of the simplest kind. A few examples of these are given in **Table 1** and the complete structure of one is shown in **Fig. 1.** There are a number of variants that include an extra galactose in the precursor chain or deletion of the *N*-acetylglucosamine. Several larger fucolipids contain a -Gal(β1 → 4)-GlcNAc(β1 → 3)-disaccharide inserted between the

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

TABLE 1. Structure of some simple representative fucolipids

Name or Designation of Fucolipid	Structure	Immunological Determinant Group
Blood group H-active type 2 chain	Fuc(α1 → 2)Gal(β1 → 4)GlcNAc(β1 → 3)Gal(β1 → 4)Glc Ceramide	Fuc(α1 → 2)Gal(β1 → 4)GlcNAc-
Blood group H-active type 1 chain	Fuc(α1 → 2)Gal(β1 → 3)GlcNAc(β1 → 3)Gal(β1 → 4)Glc Ceramide	Fuc(α1 → 2)Gal(β1 → 3)GlcNAc-
Blood group A-active type 2 chain	GalNAc(α1 → 3) \ Gal(β1 → 4)GlcNAc(β1 → 3)Gal(β1 → 4)Glc Ceramide / Fuc(α1 → 2)	GalNAc(α1 → 3) \ Gal(β1 → 4)GlcNAc- / Fuc(α1 → 2)
Blood group B-active type 1 chain	Gal(α1 → 3) \ Gal(β1 → 3)GlcNAc(β1 → 3)Gal(β1 → 4)Glc Ceramide / Fuc(α1 → 2)	Gal(α1 → 3) \ Gal(β1 → 3)GlcNAc- / Fuc(α1 → 2)
Blood group Lewis [a]-active type 1 chain	Gal(β1 → 3) \ GlcNAc(β1 → 3)Gal(β1 → 4)Glc Ceramide / Fuc(α1 → 4)	Gal(β1 → 3) \ GlcNAc- / Fuc(α1 → 4)
Blood group Lewis [b]-active type 1 chain	Fuc(α1 → 2)Gal(β1 → 3) \ GlcNAc(β1 → 3)Gal(β1 → 4)Glc Ceramide / Fuc(α1 → 4)	Fuc(α1 → 2)Gal(β1 → 3) \ GlcNAc- / Fuc(α1 → 4)

N-acetylglucosamine and the midchain galactose of the tetraosylceramide precursor. A number of more complex fucolipids have been identified that contain 8–18 sugar residues and have oligosaccharide rather than monosaccharide branches along the carbohydrate chain. Structures of each of the fucolipids that have been characterized will be given in Section IV.

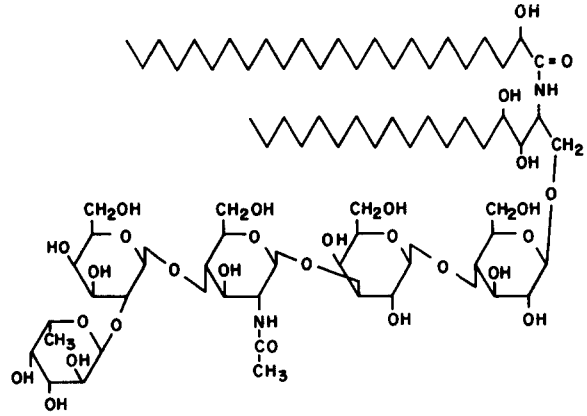

Fig. 1. Structure of a simple blood group H fucolipid with type 2 chain. Abbreviated linear structure: Fuc(α1 → 2)Gal(βl → 4)-GlcNAc(β1 → 3)Gal(β1 → 4)Glc(β1 → 1)ceramide or Fuc(α1 → 2)-Gal(β1 → 4)GlcNAc(β1 → 3)Lac(β1 → 1)ceramide. It is a ceramide of lacto-*N*-fucopentaose IV. The ceramide is cerebronyl phytosphingosine.

No universal nomenclature system exists for this complicated array of glycolipids. A fucolipid is usually given a class designation according to the terminus of the oligosaccharide moiety which is the determinant of its immunological properties. For example, fucolipid 1 (Fig. 1) is designated "blood group H-active fucolipid". A few may be identified specifically by the trivial name of the component oligosaccharide, as "ceramide of lacto-*N*-fucopentaose IV". Nevertheless, each fucolipid so designated exists as a family of individuals with variations in the ceramide portion of the molecule. These variations include both those of the fatty acid residue and of the long chain base and both of these include variations in hydroxylation.

It is apparent that even the simplest fucolipid contains more carbohydrate than ceramide by weight and that the carbohydrate must make a major contribution to the physical and chemical properties of these substances. The fucolipids are all soluble in water and have the chromatographic properties of the more polar lipids. In silicic acid column chromatography or on silica gel thin-layer plates they have lower mobilities than all other neutral lipids, including those glycolipids with shorter oligosaccharide chains, and most of the phospholipids. However they do show lipid properties as well; they are soluble in many organic

solvent systems, they form aggregates or micelles in aqueous solutions, and they are apparently incorporated into cell membranes from such aqueous solutions.

II. ISOLATION OF THE FUCOLIPIDS

The isolation of fucolipids is a process of several stages: a preliminary extraction of the tissue lipids, removal of other lipids from the ceramide oligosaccharide fraction, and resolution of the latter into its pure glycolipid components. Since the fucolipids are very minor components of animal tissues, isolation of useful amounts requires extraction of large quantities of tissue. The techniques for accomplishing the several stages of isolation are listed below. More detailed summaries of these procedures are given elsewhere by Hakomori and Watanabe (15), Slomiany, Slomiany, and Horowitz (16), and the author (17).

Extraction of tissues

Homogenized or minced fresh tissues may be extracted with solvent systems generally suitable for the recovery of lipids: chloroform–methanol 2:1 at ambient temperatures (18–20) or ethanol–ethyl ether 3:1 under reflux (17). The lipids of hexane-washed hog stomach mucosa powder were also extracted with chloroform–methanol 2:1 (21). Preparations of erythrocyte stroma may be extracted *a*) with hot ethanol (15, 22, 23), *b*) with 95% ethanol followed by 90% ethanol at ambient temperature (24), *c*) with ethyl ether–methanol 1:1 at ambient temperature followed by chloroform–methanol 1:1 under reflux (25), or *d*) by shaking an aqueous suspension with toluene (26).

Because of the water solubility of the fucolipids there has been concern about the completeness of extraction with the usual lipid solvent systems. The water of fresh tissue may contribute to the effectiveness of an extraction with these solvents and water might well be included in the system for extraction of lyophilized tissue preparations (27). A system of aqueous buffer–tetrahydrofuran 1:8 and 1:4 used for the extraction of fresh hog stomach mucosa gave better yields of the large complex glycolipids than extraction of dry mucosal powder with anhydrous solvents (16). It is difficult to evaluate extraction procedures for the fucolipids since the assay of both tissue residue and whole lipid extracts for these substances is subject to much chemical and immunological interference (2).

Separation of the ceramide oligosaccharide fraction

Precipitation of the glycolipids and other polar lipids with acetone is an easy and effective way to remove neutral lipids (17, 21, 25, 26). Silicic acid or silica gel column chromatography can be used to remove neutral lipids including ceramide mono- and dihexosides and most of the phospholipids (17, 19, 20, 24–26). Column chromatography using Florisil (magnesium silicate) with (16, 21) or without (17) prior acetylation of the lipids is useful in removing persistent phospholipids, especially after removal of most of the bulk lipids. Phospholipids and other contaminant lipids containing ester groups can be removed by hydrolysis with methanolic KOH (16, 26). Hot alcohol extracts of erythrocyte membranes can be freed of some lipid by precipitation of the glycolipids at low temperatures (15, 23, 24, 26).

Partition of chloroform–methanol 2:1 extracts of tissues with dilute salt solutions after the method of Folch, Lees, and Sloane Stanley (18) tends to divide the fucolipids (15, 20, 23); however, repeated extraction of the lower chloroform phase with equal volumes of 0.1% aqueous NaCl–methanol–chloroform 10:10:1 gives satisfactory yields of fucolipids in the combined upper phases (15, 19, 23), leaving most of the other lipids in the lower phase. Fucolipids in chloroform–methanol 2:1 (18) extracts of hog stomach mucosa powder treated with a higher concentration of salt (0.74% KCl) partitioned entirely to the lower phase and were separated from water-soluble impurities including small amounts of blood group-active glycoprotein contaminant in the upper phase (16). Partition of lipid fractions in benzene–petroleum ether 7:3 vs. methanol–acetone–water 6:2:2 confines the fucolipids to the lower polar phase, leaving most of the phospholipid in the nonpolar upper phase (17).

Resolution of the ceramide oligosaccharide fraction

The composition of this fraction varies with the tissue source and the methods used in its preparation, but it may contain all types of glycosphingolipids. Chromatography of the dialyzed lipid on a columm of DEAE–cellulose removes anionic lipids (19) and permits a preliminary resolution of the neutral glycolipid mixture (15–17, 20). Elution is usually carried out with chloroform–methanol or chloroform–methanol–water systems of increasing methanol and water content. The glycolipids are eluted in a general sequence of increasing carbohydrate chain length. These fractions are then resolved on preparative TLC using silica gels as adsorbant and chloroform–methanol–water systems for development. A 65:25:4 mixture separates smaller ceramide oligosaccharides from the relatively immobile fucolipid mixture; a 65:30:8 mixture permits resolution of the fucolipids (16). Concentrated NH_4OH or glacial acetic acid may also be added to these systems. The system chloroform–methanol–concentrated NH_4OH 40:

80:25 markedly increases the mobility of the ceramide pentasaccharide having Forssman hapten activity, moving it well above the fucolipids (17, 28). The lipid bands on preparative thin-layer plates can be detected by exposure to iodine vapor or by fluorescence in ultraviolet light after spraying with a dilute aqueous solution of Rhodamine 6-G.

Resolution of mixtures of fucolipids with longer oligosaccharide chains is more difficult and frequently requires more than one TLC step. Hakomori and his associates (15) have usually acetylated the TLC fractions for a final resolution of the fucolipids as peracetylated derivatives. The derivatives are then deacetylated in chloroform and methanolic sodium methoxide. Silica gel plates containing sodium tetraborate may reduce the mobility of a fucolipid with a terminal galactose enough to permit separation (24). For purification of the large blood group H-active fucolipid "H3", a decaglycosyl ceramide, DEAE–cellulose fractions were resolved on a long silicic acid column (15), giving a better yield of the pure fucolipid than was obtained from thin-layer plates (29). Losses of fucolipids in TLC is a common problem and the necessity for sequential passes on thin-layer plates for purification is usually expensive.

III. CHARACTERIZATION OF THE FUCOLIPIDS

The techniques for the characterization of the fatty acids and long chain bases of the ceramide portion of the fucolipids are those in general use for all sphingolipids and need not be considered here. The methods used for the characterization of the distinctive oligosaccharide portion have been borrowed or adapted from those used in carbohydrate and glycoprotein analysis and are reviewed below. These provide for the determination of the constituent sugars, their sequence, and the anomeric configuration and position of each linkage.

Determination of constituent sugars

Analysis of fucolipids for constituent sugars involves hydrolysis followed either by preparation of sugar derivatives and analysis by GLC, or by direct identification and analysis by quantitative paper chromatography or by chemical methods.

Hydrolysis is carried out under controlled conditions with *a*) H_2SO_4–acetic acid–water systems (19, 20, 30, 31), *b*) aqueous HCl (32), and *c*) methanolic-HCl (33). Fucose can be removed preferentially with very mild acid hydrolysis (21) prior to hydrolysis in the above systems.

For analysis by GLC the sugars may be converted to the trimethylsilyl derivatives by the method of Sweeley and Walker (33) or reduced with sodium borohydride to the alditol derivatives and then peracetylated (19, 32). The alditol acetates give single peaks for each sugar and are separated with a variety of liquid phases including 3% "ECNSS-M" (cyanoethyl silicone–ethylene glycol succinate) (Applied Science Labs, State College, PA), a mixed phase consisting of 0.2% ethylene glycol succinate + 0.2% ethylene glycol acetate + 1.4% "XE-60" (Applied Science Labs.), and 3% "OV-225" (Supelco, Bellefonte, PA). Recovery of the neutral sugars is generally good, but that of the amino sugars is often low and variable. The economy of a single or double microanalysis for all the sugars has made the GLC methods the ones of choice for fucolipids.

Constituent sugars have also been determined by quantitative paper chromatography and by chemical methods (26). The latter have included the method of Rondle and Morgan (34) for hexosamines, the method of Dische and Shettles (35) for fucose, and commercially available glucose and galactose oxidase systems for these sugars.

Determination of the sequence of sugars

The sequence of the fucolipid sugars has been determined by stepwise hydrolysis with specific enzymes, by mass spectrometry, by periodate oxidation and the Smith degradation, and by partial acid hydrolysis.

1. Stepwise specific enzyme hydrolysis. This is accomplished by incubating the fucolipid with a single glycosidase and fractionating the digest by solvent extraction. The liberated sugar can be identified from the aqueous phase and the lipid product recovered from the solvent phase. The lipid is usually isolated by TLC and analyzed for residual sugars. A portion of the lipid product is then incubated with a different glycosidase and the process is repeated until all of the sugars have been removed, at least to the glucocerebroside product.

This technique requires enzyme preparations that have been purified from other hydrolase enzymes, because incubation times are usually long (12–36 hr) and excess enzyme is used for complete conversion to product. The enzymes also provide information on the anomeric configuration of each linkage and sometimes on the linkage position. They are also useful in degrading more abundant or accessible glycolipids to smaller precursors for biosynthetic studies. Enzymes in common use are the α-fucosidases, the α- and β-galactosidases, β-*N*-acetylhexosaminidase, and α-*N*-acetylgalactosaminidase.

a. Fucosidases. An enzyme from *Aspergillus niger*

(36, 37), highly specific for α-fucosyl(1 → 2)galactose linkages, hydrolyzed blood group H oligosaccharide and three different blood group A-active glycolipids of hog gastric mucosa (38, 39). α-Fucosidases from *Charonia lampas* (40) and bovine kidney (Boehringer, Mannheim, Germany) removed fucose from both simple and complex blood group H (15, 23, 29) and group B (41) fucolipids of human erythrocytes, respectively. Two fucosidases from *Trichomonas foetus*, with α1 → 2 and α1 → 3,4 specificity, hydrolyzed type 2 H-active and Lewis^c fucolipids respectively (42).

b. β-Galactosidase (EC 3.2.1.23) and β-*N*-acetylhexosaminidase (EC 3.2.1.52). These enzymes have been prepared free of other glycosidase activity by Li and Li (43, 44) from jack bean meal. They are widely used in sequence analysis of glycolipids because of the general occurrence of these sugars and because of the specificity of the enzymes. They do not hydrolyze fucolipids but do hydrolyze the product remaining after fucose has been removed.

c. α-Galactosidase (EC 3.2.1.22). An α-galactosidase prepared from ficin (45, 46) removed the terminal galactosyl residue from blood group B fucolipid from human pancreas (47) without prior removal of fucose. An enzyme from coffee beans (Boehringer, Mannheim) hydrolyzed native group B fucolipids from human erythrocytes (41).

d. α-*N*-Acetylgalactosaminidase (EC 3.2.1.49). This enzyme has been partially purified from pig and beef liver (48) and has little α- or β-*N*-acetylglucosaminidase activity. The pig liver enzyme hydrolyzed the native blood group A fucolipid from human erythrocytes, converting it to the blood group H-active fucolipid (15).

2. *Mass spectrometry*. Application of mass spectrometry to the characterization of glycolipids has evolved from its use in the identification of derivatives of component sugars, long chain bases, fatty acids, and ceramides to use with intact glycosphingolipids. Much important structural information can be gathered from mass spectrometry of derivatives of glycolipids, ranging from mono- to hexaglycosyl ceramides, using just conventional scanning ranges, i.e., up to *m/e* 1000 (49–51).

Karlsson and his associates (52) have improved these techniques by extension of the ion scanning range beyond *m/e* 2500 with a high resolution instrument, and by the use of permethylated glycolipids and permethylated glycolipids reduced with $LiAlH_4$. In the latter, the amide groups of ceramide and acetylated amino sugars are converted to the amines, increasing the stability and volatility of the glycolipids. These reduced derivatives produce, in abundance, a series of heavy ions containing the fatty acid residue linked to the entire oligosaccharide through the remaining carbons 1 and 2 of the long chain base. From the masses of these ions may be calculated the molecular weight of the oligosaccharide, the number and mass type of the constituent sugars, and a semiquantitative determination of fatty acids. Comparison of the spectra given by the two derivatives provides information on the kinds and amounts of both fatty acids and long chain bases and on the sequence of sugar types (e.g., hexoses, deoxyhexoses, and amino sugars) including branching and, in a few instances, linkage positions. These workers have applied the techniques to the globoside series, including Forssman hapten, to gangliosides, and to a series of fucolipids. The largest glycolipid analyzed to date is a nonahexosyl ceramide with a derivative mass of 2534. These remarkable developments have been discussed and summarized by Karlsson and coworkers (52, 53).

3. *Periodate oxidation and Smith degradation*. Treatment of fucolipids with 0.6 M $NaIO_4$ for 100 hr (32, 54) or with 0.1 M $NaIO_4$ in aqueous chloroform–methanol 1:5 for 60 hr (38) at ambient temperatures decomposes all sugars with vicinal hydroxyl groups exposed, although traces of glucose may persist. Hakomori and associates (15, 46) have extended the use of this technique for sequencing by altering the conditions of oxidation to achieve oxidation of only the nonreducing terminal sugar.

4. *Partial acid hydrolysis*. Hydrolysis of glycolipids with dilute HCl in chloroform–methanol was introduced by Sweeley and Klionsky (55) for determination of the sugar sequence. Although hydrolysis is not random (56) most glycosidic bonds other than the glucosyl ceramide bond may be hydrolyzed and a series of glycosyl ceramides of various carbohydrate chain lengths are recovered from the hydrolysate by extraction and TLC. Analysis of these glycosyl ceramides for constituent sugars provides the sequence. This method has been applied with modification to the blood group A-active fucolipids of hog gastric mucosa (21, 39, 57).

Determination of sugar linkages

1. *Sugar linkage positions*. Methods for the identification of the linkage positions of the fucolipid sugars include permethylation analysis, specific enzyme hydrolysis, and, in a few instances, mass spectrometry.

a. Permethylation analysis. Permethylation of native fucolipids is usually carried out by the methyl sulfinyl carbanion method of Hakomori (58), although the dimethyl formamide method of Adams and Gray (59) has also been used (25, 26). Hydrolysis of the permethylated derivatives is usually accomplished with one of the acetic acid systems noted under "Deter-

mination of constituent sugars" (above). The sugar derivatives are reduced with sodium borohydride, peracetylated, and the partially methylated alditol acetates are analyzed by GLC using the liquid phases listed above for the determination of sugars (16, 19, 30, 54). The effluent from the gas chromatograph is, ideally, monitored with mass spectrometry (29, 41). These techniques permit generally adequate resolution of the partially methylated alditol acetates of neutral sugars occurring in the fucolipids, although some combinations of interest are poorly resolved. Stellner, Saito, and Hakomori (30) prepared and identified a series of partially methylated alditol acetates from *N*-acetyl glucosamine and *N*-acetyl galactosamine and published their GLC retention times on columns containing ECNSS-M. Good separation of the 3,6 and 4,6 di-*O*-methyl glucosamine derivatives enabled the identification of the type 1 and type 2 chains in the ABO series and in all four of the Lewis antigens.

A comprehensive review by Dutton on carbohydrate analysis by GLC, published in two parts (60, 61), and a review by Lindberg, Lonngren, and Svensson (62) contain much pertinent data on the GLC of sugars.

b. Specific enzyme hydrolysis. The position-specific α-fucosidases discussed above give information on the fucose linkage positions, but the other glycosidases are evidently less specific or nonspecific for position. However, the β-galactosidase of jack bean (44) hydrolyzed gal $\beta 1 \rightarrow 4$ linkages at 25–50 times the rate of gal $\beta 1 \rightarrow 3$ linkages (63, 64).

c. Mass spectrometry. The identification by mass spectrometry of sugar derivatives formed by hydrolysis of permethylated or trimethylsilylated glycolipids has been very helpful in the determination of linkage positions. Spectra of permethylated intact fucolipids may provide linkage position information directly. Karlsson (52) has found an abundant ion—usually the base peak—of *m/e* 182, ($C_9H_{12}O_3N$) originating from hexosamine, in spectra of permethylated blood group A, B, and H fucolipids with the type 2 chain (gal $\beta 1 \rightarrow 4$ glcNAc). The ion is absent in spectra from type 1 chain (gal $\beta 1 \rightarrow 3$ glcNAc) blood group A or Lewis[a] fucolipids (52, 54). Hanfland and Egge (65) found a fairly abundant ion of *m/e* 129 in the spectra of two blood group H-active fucolipids with type 2 chain which they attributed to glucosamine linked in the 4 position.

2. *Anomeric configuration of the sugar linkages.* The α or β linkages are determined with the glycosidases used in sequencing (see above) since these enzymes are highly specific for configuration. Optical rotation (26) and infra red spectra (38) have been used to provide supporting evidence for fucolipid configurations.

IV. BLOOD AND TISSUE FUCOLIPIDS

All of the fucolipids for which structures have been proposed are listed in Tables 2–4. The structure given is the most complete for each fucolipid listed and sometimes more complete in terms of anomeric linkages than that given by other authors reporting on a fucolipid with identical sugars, sugar sequence, and linkage positions.

Blood group A-active fucolipids

All of the blood group A-active fucolipids have the terminal trisaccharide GalNAc($\alpha 1 \rightarrow 3$)[Fuc-($\alpha 1 \rightarrow 2$)]Gal- characteristic of the blood group A glycoproteins and milk oligosaccharides except compounds 17 and 18, where fucose is linked to glucosamine rather than to galactose. These latter structures have not been confirmed and are of interest in terms of the structural requirements for blood group A activity. The recently discovered difucosyl-A glycolipids (7, 8, and 9, **Table 2**) are counterparts of the ovarian cyst glycoproteins, having the same pentasaccharide terminus reported earlier by Lloyd et al. (74).

Dog and human intestine fucolipids contained only hydroxylated fatty acids with phytosphingosine and sphingosine, the major long chain bases (68, 69). The erythrocyte (24) fucolipids, on the other hand, contained predominantly sphingosine with little or no phytosphingosine and only minor amounts of hydroxylated acids. The fatty acids were typically long chain with 63% in the C_{22}–C_{24} group.

The ceramides of the major A-active fucolipids of hog gastric mucosa (3 and 4, Table 2) were different. Fucolipid No. 3 with the type 2 chain had 35.4% hydroxylated fatty acids and 44.4% of all fatty acids were in the C_{22}–C_{24} group. Fucolipid 4 with the type 1 chain had only 11.4% hydroxylated acids and 15.7% of all acids in the C_{22}–C_{24} group. These differences in the ceramides must account for the differences in chromatographic mobility that permitted their separation and might account for their chain types.

Immunological activity of the A-active fucolipids may be determined qualitatively by incubating a 0.1% solution of the lipid with blood type O erythrocytes. The fucolipids are absorbed or incorporated into the membranes and the cells may then be agglutinated by A-antiserum (75). Solutions of the fucolipids preincubated with A-antiserum will inhibit agglutination of A erythrocytes, and this system has been used as a quantitative assay for the antigenic activity of the lipid (21, 57, 67, 76). Fucolipid solutions will also precipitate A-antiserum (75) and a lectin from *Dolichos biflorus* (66) on Ouchterlony plates.

TABLE 2. Proposed structures of blood group A-active fucolipids

No.	Structure	Tissue Source	Yield mg per kg.[b]	Ref.
1	A-(β1 → 4)GlcNAc(β1 → 3)Lac-Ceramide[a]	Human erythrocytes Hog gastric mucosa Dog small intestine	0.15 f 46. f	66 67 68, 69
2	A-(β1 → 3)GlcNAc(β1 → 3)Lac-Ceramide	Hog gastric mucosa Human small intestine		67 68, 69
3	A-(β1 → 4)GlcNAc(β1 → 3)Gal(β1 → 4)Lac-Ceramide	Hog gastric mucosa	98.4 d	38, 21
4	A-(β1 → 3)GlcNAc(β1 → 3)Gal(β1 → 4)Lac-Ceramide	Hog gastric mucosa	111. d	38, 21
5	A-(1 → 3)Lac-Ceramide	Hog gastric mucosa	17.1 f	67
6	A-(β1 → 3)Gal(β1 → 4)Lac-Ceramide	Hog gastric mucosa	19.6 d	39
7	A-(β1 → 4)[Fuc(1 → 3)]GlcNAc(β1 → 3)Lac-Ceramide	Hog gastric mucosa Dog small intestine[1]	82.9 f	57 70
8	A-(β1 → 3)[Fuc(1 → 4)]GlcNAc(β1 → 3)Lac-Ceramide	Human small intestine[1]		
9	A-(β1 → 3)[Fuc(α1 → 4)]GlcNAc(β1 → 3)Gal(β1 → 4)GlcNAc(β1 → 3)-Lac-Ceramide	Human A_1 erythrocytes[2]		52
10	A-(1 → 4)GlcNAc(1 → 3)Gal(1 → 4)GlcNAc(1 → 3)Lac-Ceramide	Human A_1 erythrocytes		66
11	A-(β1 → 4)GlcNAc(β1 → 3) Gal(β1 → 4)GlcNAc(β1 → 3)Lac-Ceramide A-(β1 → 4)GlcNAc(β1 → 6)	Human A_1 erythrocytes		66, 15
12	A-(β1 → 4)Gal(β1 → 3) Lac-Ceramide GalNAc(α1 → 3)Gal(β1 → 6)	Hog gastric mucosa	4.6	71
13	A-(β1 → 3) Gal(β1 → 4)Lac-Ceramide GalNAc(α1 → 3)Gal(β1 → 6)	Hog gastric mucosa	7.80	71
14	A-(β1 → 4)Gal(β1 → 3) Lac-Ceramide GlcNAc(β1 → 4)Gal(β1 → 6)	Hog gastric mucosa	6.25	71
15	A-(1 → 4)GlcNAc(1 → 3) A-(1 → 4)GlcNAc(1 → 6)— Gal(1 → 4)GlcNAc(1 → 4)GlcNAc(1 → 3)- Lac-Ceramide GlcNAc(1 → 4)GlcNAc(1 → 4) GlcNAc(1 → 4)Gal(1 → 4)GlcNAc(1 → 6)	Hog gastric mucosa	10.7	72
16	A-(1 → 4)GlcNAc(1 → 3) Gal(1 → 4)GlcNAc(1 → 4)GlcNAc(1 → 3)- Lac-Ceramide GlcNAc(1 → 4)GlcNAc(1 → 6) GlcNAc(1 → 6)	Hog gastric mucosa	8.8 mg	72
17	GalNAc(1 → 3)Gal(1 → 3 or 4)[Fuc 1 → ?]GlcNAc(1 → 3)Lac-Ceramide	Human A erythrocytes		73
18	GalNAc(1 → 3)Gal(1 → 3 or 4)[Fuc 1 → ?]GlcNAc(1 → 3)Gal(1 → 3)-Lac-Ceramide	Human A erythrocytes		73

[a] A = GalNAc(α1 → 3)[Fuc(α1 → 2)]Gal—The characteristic terminal trisaccharide of Blood group A fucolipids.
[b] Yield, mg fucolipid per kg. tissue: f, fresh or wet weight; d, dry weight.
[1] Spencer, W. A., J. M. McKibbin, E. L. Smith, K-A. Karlsson, I. Pascher, and B. E. Samuelsson. Unpublished data.
[2] Karlsson, K-A. Personal communication.

The hemagglutination inhibition assay is subject to variations due to enhancement of activity by other lipids (2, 67, 77) but is adequate to show substantial differences in activity. The relationships between structure and immunologic activity have been determined for the A-active fucolipid variants of hog gastric mucosa (16). In the absence of auxiliary lipid, fucolipids 3 and 4 (Table 2) were equally active and the most active of the series. They were, respectively, 70 times and 400 times as active as fucolipids 5 and 6 which lacked glucosamine, and 60 times as active as fucolipid 7 which contained two fucose residues. They were 17 times more active than fucolipids 1 and 2 which contained one less mid-chain galactose.

Since erythrocyte membranes contain both blood group-active glycoprotein and fucolipid it has been of some interest to identify the contribution of each to the antigenicity of the membranes. Yamato, Handa, and Yamakawa (78) isolated the glycolipid and glycoprotein fractions from pooled human A erythrocytes. The ratio of the A-antigenic activities of the glycolipid to glycoprotein fractions was about 6:1. Kent et al. (77) isolated the glycolipid and glycoprotein fractions of individual human A erythrocyte samples by a different procedure. The overall average of 15 samples gave a glycolipid to glycoprotein activity ratio of 4.4:1 although there was much variation in the samples. It seems probable that in both studies the glycolipid fractions measured were not significantly contaminated with glycoprotein and that the major fraction of antigenicity was due to glycolipid.

A point of interest raised by the identification of most of the erythrocyte ABO antigenicity with glycolipids is the molecular basis for blood groups A_1 and A_2. This is obviously a complicated problem which might involve differences in concentration or distribution of the A-active fucolipid variants in the membrane and differences in antigenic activity or accessibility of the variants in the membrane as compared to their activity in solution. Hakomori and Watanabe (15) have observed that the very large and uncharacterized A-active fucolipid variant "A_4", or "A_d", (66) is present in greater amounts in A_1 erythrocytes than in A_2, whereas the large uncharacterized H-active fucolipid counterpart (29) has the opposite distribution in these types.

The blood group-active fucolipids, although present in dog intestine in relatively large amounts, are probably not present in canine erythrocytes and are not involved in the dog blood group systems (76). They are identified with two antigens of the canine secretory alloantigen alloantibody (CSA) system described by Zweibaum and et al. (79) as the blood group A and Le^d fucolipids corresponding to the A and Y antigens respectively (27). In a CSA typing survey of 250 mongrel dogs, the A antigen was present in 45.2% of the dogs (79), corresponding roughly to the 13 of 25 dogs in the author's series from which the A-active fucolipid has been isolated (17).

Blood group B-active fucolipids

Blood group B-active fucolipids (**Table 3**) all have the terminal trisaccharide Gal(α1 → 3)[Fuc(α1 → 2)]-Gal- characteristic of blood group glycoproteins. The pancreas fucolipid accumulated in a blood group B patient with Fabry's disease, a glycosphingolipidosis characterized by deficiency of α-galactosidase and accumulation of glycolipids with an α-galactosyl terminus. The tissue contained over 50 times the amount of any fucolipid found thus far in any whole tissue. The isolate was a mixture of about 80% type 1 chain and 20% type 2 chain. The ceramides contained 73% sphingosine and 16% phytosphingosine and all non-hydroxylated fatty acids, over half of which were C_{22} and above (47).

All of the erythrocyte fucolipids isolated had the type 2 chain with ceramide characteristics (41, 65) similar to those of the pancreas lipid. Fucolipids 20 and 21 (Table 3) with different carbohydrate chain lengths were equally active in hemagglutination inhibition of human anti-B serum and about five times as active as a blood group B glycoprotein preparation from ovarian cyst fluid (41).

Blood group B fucolipids have not been found in either hog gastric mucosa or in dog intestine. B-active materials were not detected in canine gastric secretions (80) nor were the CSA antigens cross reactive with human blood group B antigens (79).

Blood group H-active fucolipids

Blood group H-active fucolipids (Table 3) all contained the terminal disaccharide Fuc(α1 → 2) Gal characteristic of blood group H-active glycoproteins and human milk oligosaccharides. The large branched decaglycosyl ceramide "H_3" (No. 26) also had blood group I activity, whereas the others did not (29). The ceramides of the erythrocyte fucolipids were very similar in both base and fatty acid content to the group A and B erythrocyte lipids. In hemagglutination inhibition studies the relative activity of the erythrocyte fucolipids varied greatly depending on the use of *Ulex europeous* lectin, eel serum, or Bombay serum as anti-H reagents (26, 29).

Dog intestinal fucolipid was identical with the H-active erythrocyte fucolipids in all respects except that all the fatty acids were hydroxylated and phytosphingosine was the major long chain base.

The hog gastric mucosal H fucolipid (No. 24, Table

TABLE 3. Proposed structures of group B and H-active fucolipids

No.	Structure	Tissue Source	Yield mg per kg.	Ref.
19	B-(β1 → 3 and 4)GlcNAc(β1 → 3)Lac-Ceramide[a]	Human pancreas	5460. f[c]	47
20	B-(β1 → 4)GlcNAc(β1 → 3)Lac-Ceramide	Human erythrocytes	0.13–1.78 f	26, 41
21	B-(1 → 4)GlcNAc(1 → 3)Gal(1 → 4)GlcNAc(1 → 3)Lac-Ceramide	Human erythrocytes	2.56 f	41
22	B-(β1 → 3)[Fuc(α1 → 4)]GlcNAc(β1 → 3)Lac-Ceramide	Human small intestine[2]		
23	H-(β1 → 4)GlcNAc(β1 → 3)Lac-Ceramide[b]	Human erythrocytes	0.14–0.30 f	26, 41 23
		Dog small intestine	25. f	32
24	H-(α1 → 3)Lac-Ceramide	Hog gastric mucosa	20.4 d	81
25	H-(β1 → 4)GlcNAc(β1 → 3)Gal(β1 → 4)GlcNAc(β1 → 3)Lac-Ceramide	Human erythrocytes	0.25–0.40 f	26, 29
26	H-(β1 → 4)GlcNAc(β1 → 3) Gal(β1 → 4)GlcNAc(β1 → 3)Lac-Ceramide H-(β1 → 4)GlcNAc(β1 → 6)	Human erythrocytes	0.54 f	29

[a] B = Gal(α1 → 3)[Fuc(α1 → 2)]Gal-
[b] H = Fuc(α1 → 2)Gal-
[c] f, fresh, or wet, weight; d, dry weight.
[2] Karlsson, K-A. Personal communication.

3) is another variant from this tissue that lacks *N*-acetyl glucosamine. As in the case of the A-active variants (Nos. 5 and 12, Table 2) the *N*-acetylglucosamine is simply omitted and the penultimate galactose is linked (1 → 3) to the lactosyl residue. Hemagglutination inhibition activity against *Ulex* lectin was only about 1/250 that of hog gastric mucosal glycoprotein.

The Lewis blood group fucolipids

The Lewis blood group fucolipids (Nos. 27–31, Table 4) have the same carbohydrate termini as the corresponding blood group glycoproteins and oligosaccharides of human milk, with fucosyl substitution on the 3 or 4 position of *N*-acetylglucosamine. The common human Lewis fucolipids, Le^a and Le^b, have the type 1 chain with fucose linked to the 4 position of glucosamine, whereas the rare Le^c and Le^d fucolipids have the type 2 chain with fucose linked to the 3 position of glucosamine. These substances have only been isolated and characterized from human or dog intestine, hog gastric mucosa, and human adenocarcinoma. They are present in very small but immunologically significant amounts in erythrocytes, having been absorbed or transferred from plasma lipoproteins (85). They are all characterized by polar ceramides, with phytosphingosine as the major long chain base and from 65% to 100% of the fatty acids hydroxylated.

On the basis of methylation analysis the Le^a-like fucolipids isolated from three different human intestines contained only type 1 chain, whereas the isomers isolated from two different dog intestines contained only type 2 chains. Agglutination by human anti-Le^a serum of human $Le^{(a-b-)}$ erythrocytes coated with these fucolipids was also specific; those treated with the human intestine fucolipids were agglutinated, whereas those treated with the dog intestine fucolipids were not (75). The dog intestine and human adenocarcinoma fucolipids (No. 27, **Table 4**) are apparently identical and are probably the Lewis[c] antigens (86, 87).

In a series of isolated difucosyl Lewis-type glycolipids, four from individual dog intestines and three from human intestines contained all type 2 and type 1 chains, respectively, on the basis of methylation analysis of the native and defucosylated lipids. Of the difucosyl-A lipids, two from dog intestines (No. 7, Table 2) and two from human intestines (No. 8, Table 2) had just type 2 and type 1 chains, respectively (84). Evidently the common human intestinal Lewis fucolipids are Le^a, Le^b, and A-Le^b (type 1 chain), as is the case with human erythrocytes, whereas the common canine fucolipids are Le^c, Le^d, and A-Le^d (type 2 chain). The human intestine contents of Le^a and Le^b (Table 4) are the highest for any fucolipid yet reported in normal tissue.

Other fucolipids

Gangliosides containing fucose have been reported by Suzuki, Ishizuka, and Yamakawa (88) from boar

TABLE 4. The Lewis blood group and other fucolipids

No.	Structure	Tissue Source	Yield mg per kg.	Ref.
27	Gal(β1 → 4)[Fuc(α1 → 3)]GlcNAc(β1 → 3)Lac-Ceramide Lewis^c fucolipid, "X-hapten", or "tumor glycolipid" Ceramide of lacto-*N*-fucopentaose III	Human adenocarcinoma Hog gastric mucosa Dog small intestine[3]	42. f[a] 29.7 f 21.3 f	19 67
28	Gal(β1 → 3)[Fuc(α1 → 4)]GlcNAc(β1 → 3)Lac-Ceramide Lewis^a fucolipid Ceramide of lacto-*N*-fucopentaose II	Human adenocarcinoma Human small intestine	 65.4 f	82 54
29	Fuc(1 → 2)Gal(β1 → 4)[Fuc 1 → 3]GlcNAc(β1 → 3)Lac-Ceramide Lewis^d fucolipid	Dog small intestine[3]	19.8 f	83
30	Fuc(α1 → 2)Gal(β1 → 3)[Fuc(α1 → 4)]GlcNAc(β1 → 3)Lac-Ceramide Lewis^b fucolipid Ceramide of lacto-*N*-difucohexaose I	Human small intestine[3] Human adenocarcinoma	100. f	84 82
31	Fuc(1 → 2)Gal(1 → 3)GlcNAc(1 → 3)Gal → GlcNAc(1 → 3)Lac-Ceramide ↑ Fuc(1 → 4)	Human adenocarcinoma		82
32	Fuc(α1 → 2)Gal(1 → 3)GalNAc(1 → 4)Gal(1 → 4)Glc-Ceramide ↑ [NANA(2 → 3)]	Boar testis	53. f	88
33	Fuc(α1 → 2)Gal(β1 → 3)GalNAc(β1 → 4)Gal(β1 → 4)Glc-Ceramide ↑ [NGNA(2 → 3)]	Bovine liver		89
34	NANA(α2 → 3)Gal(β1 → 4)[Fuc(α1 → 3)]GlcNAc(β1 → 3)Lac-Ceramide	Human kidney		90
35	αFuc-Ceramide	Human colon tumors	0.0–70. d	13

[a] f, fresh or wet weight; d, dry weight.
[3] Spencer, W. A., E. L. Smith, J. M. McKibbin, K-A. Karlsson, I. Pascher, B. E. Samuelsson, J. E. Månsson, and Y-T. Li. Unpublished data.

testis, and by Wiegandt (89) from bovine liver (No. 32 and 33, Table 4). These substances are identical except for *N*-acetyl and *N*-glycolyl groups, respectively, on the neuraminic acid. The sugar sequence is of the ganglioside series and these substances may be regarded as fucosyl derivatives of GM1.

A fucoganglioside of the paragloboside series was isolated by Rauvala (90) (No. 34, Table 4) from human kidney and has the composition of the Le^c fucolipid with *N*-acetyl neuraminic acid substituted on the terminal galactose.

The simplest of all the fucolipids is a fucosyl ceramide (No. 35) isolated by Watanabe, Matsubara, and Hakomori (13) from human colon carcinomas and is the fourth sugar variant of ceramide monohexosides identified thus far.

V. BIOSYNTHESIS OF THE FUCOLIPIDS

Although our knowledge of the biosynthesis of fucolipids is fragmentary, it appears to be a process of stepwise addition by specific glycosyl transferases of single sugar units starting from the ceramide (7, 91). The model for such a process was proposed for gangliosides by Roseman (92) and confirmed by extensive work in a number of laboratories, and it appears appropriate for all the families of glycolipids that do not have repeating carbohydrate sequences. Much of this work was guided and assisted by completed or ongoing biosynthetic studies on the blood group-active glycoproteins and on the oligosaccharides of human milk. The nature of these processes and the properties of the glycosyl transferases involved have been reviewed by Watkins (14), Ginsburg and Neufeld (93), and Hakomori and Kobata (7). The biosynthesis of gangliosides has been reviewed by Fishman and Brady (94).

One interesting aspect of glycolipid biosynthesis relates to distribution of the glycolipids in normal and tumor cell membranes. It has been shown that the glycolipids with the longer oligosaccharide chains are often reduced or deleted in the latter and that the changes are often correlated with the degree of malignancy, with the lack of contact inhibition, and with changes in cellular morphology. Present evidence indicates that the activities of the glycosyl transferases involved in the biosynthesis of fucolipids and other com-

plex lipids are not maintained in tumor tissue or in virus-transformed cells. This aspect of glycolipid metabolism and function has been the subject of two comprehensive reviews by Hakomori (8, 9).

Proposed routes for the biosynthesis of the simpler and more abundant fucolipids are summarized in **Fig. 2.** Each of the numbered reactions involves the addition of a single hexose to a glycolipid acceptor in the type reaction:

hexosyl nucleotide + acceptor

$$\xrightarrow[\text{transferase}]{\text{specific glycosyl}} \text{hexosyl acceptor} + \text{nucleotide}$$

The appropriate sugar nucleotides are indicated for each reaction.

Biosynthesis of the precursor glycolipids

An *N*-acetylglucosaminyl transferase preparation from rabbit bone marrow catalyzed the formation of *N*-acetylglucosaminyl lactosyl ceramide from lactosyl ceramide and UDP-*N*-acetylglucosamine (Reaction 1, Fig. 2). The system appeared to be specific for both sugar nucleotide and glycolipid acceptor (95). This ceramide trihexoside product occurs in only trace amounts in normal tissues (96, 97) and serves as substrate for the synthesis of the next members of the sequence, lacto-*N*-tetraosyl and lacto-*N*-neotetraosyl ceramides (98) (Reaction 2, Fig. 2). This conversion was attained with a Triton X-100-solubilized β-galactosyl transferase from rabbit bone marrow (99). The transferase was most active with the detergent Triton X-100 and was specific for UDP-galactose, Mn^{2+} ions, and the acceptor. It had no activity for other sugar nucleotides or for other sphingolipids that accept galactose in other circumstances, such as ceramide, glucocerebroside, lactosyl ceramide, *N*-acetylgalactosaminyl lactosyl ceramide, or Tay Sach's ganglioside. A similar transferase has been prepared from monkey bone marrow (91).

Biosynthesis of H-active fucolipids

Basu, Basu, and Chien (100) isolated a Golgi-rich membrane preparation from beef spleen homogenate that catalyzed the synthesis of H-active fucolipid from GDP-L-[^{14}C]fucose and lacto-*N*-neotetraosyl ceramide

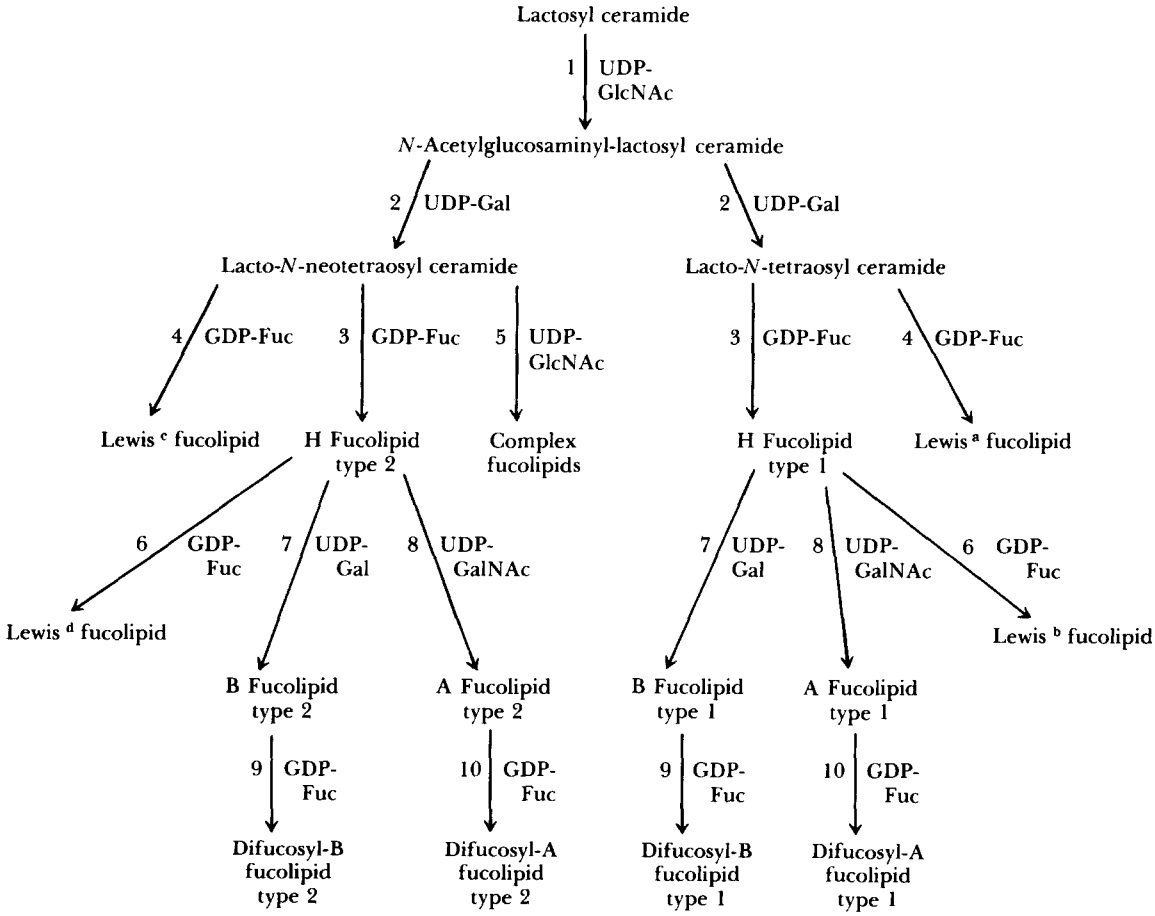

Fig. 2. Proposed biosynthetic pathways for the simple fucolipids

(Reaction 3, Fig. 2); in addition, the system required Mn^{2+} or Mg^{2+} ions and a cationic detergent for maximum activity. The reaction was specific for GDP-L-fucose and more specific for glycosyl ceramide acceptors than for oligosaccharide and glycoprotein acceptors (91). No significant amounts of [^{14}C]fucose were transferred to ganglioside GM1, to ganglio-trihexosyl ceramide, or to other glycolipids in the fucolipid sequence below lacto-*N*-tetraosyl ceramide. Transfer of fucose to both lacto-*N*-neotetraosyl ceramide and rabbit erythrocyte pentahexosyl ceramide (101) was rapid, with K_m values for the acceptors of 0.6 mM and 1.5 mM, respectively. Substrate competition experiments indicated that activity was due to the same enzyme. The product had the chromatographic and immunologic properties of H glycolipid.

Pacuszka and Koscielak (42) demonstrated synthesis of both H and Le^c glycolipids using human serum as a source of fucosyl transferases and lacto-*N*-neotetraosyl ceramide and GDP-L-[^{14}C]fucose as substrates (Reactions 3 and 4, Fig. 2). All normal sera produced both fucolipids, but Bombay sera produced only the Le^c.

Incubation of Bombay-type erythrocytes with GDP-L-fucose and a transferase preparation from human (Group O) gastric mucosa produced phenotypically O-type cells after the cells were preincubated with neuraminidase (102).

Biosynthesis of A-active and B-active fucolipids

A highly purified *N*-acetylgalactosaminyl transferase from porcine submaxillary glands which was very active for glycoprotein substrates (103) catalyzed the conversion of dog intestine H fucolipid to A fucolipid and H megaloglycolipid (104) to its A-like product (Reaction 8, Fig. 2). The K_m value for the H fucolipid substrate was about 0.8 mM, about twice that for glycoprotein and oligosaccharide acceptors. The enzyme had no activity for human intestine Le^a or Le^b fucolipids as acceptors, indicating that the A glycolipid is the immediate precursor of the difucosyl A glycolipid. This is consistent with the strict substrate specificity of the *N*-acetylgalactosaminyl transferase of human milk, which did not transfer *N*-acetylgalactosamine to lacto-*N*-difucohexaose I, the oligosaccharide of Le^b glycolipid (105).

Biosynthesis of A-active from H-active fucolipids in intact erythrocytes incubated with gastric mucosal transferase preparations and UDP-*N*-acetylgalactosamine has been followed with immunological assays (106, 107). Blood group O erythrocytes incubated with preparations from blood group A_1 individuals first acquired A_2 and then A_1 specificity, while group H specificity disappeared. A_2 erythrocytes in this system required a shorter incubation time for appearance of A_1 specificity than did O erythrocytes. A gastric mucosal preparation from a blood group A_2 individual had much less transferase activity than those from A_1 individuals and was much less active in converting type O erythrocytes to A specificity. Using glycoprotein and H-active milk oligosaccharide as acceptors, Schachter et al. (108) found 5–10 times as much transferase activity in the serum of A_1 subjects as in A_2 subjects.

Stellner, Hakomori, and Warner (109) converted H glycolipid to A and B glycolipids with glycosyl transferases present in the 100,000 *g* pellets from homogenates of human cecal and gastric mucosal epithelia. In addition to pure H glycolipid and labeled sugar nucleotide substrates, their system contained a detergent, Mn^{2+} ions, and ATP. *N*-Acetylgalactosamine transferase activity per mg of protein from the cecal mucosa was 120 times greater than that from the serum. The transferase activity of the adenocarcinoma tissue from blood group A_1 and AB subjects averaged only one-fifth that of the normal mucosa from the same subjects. α-Galactosyl transferase activity (Reaction 7, Fig. 2) in the tumor of the AB subject was one-sixth that of the normal mucosa. There was no significant difference in *N*-acetylgalactosaminidase activity between the normal and tumor tissue preparations.

A Triton X-100-solubilized rabbit bone marrow preparation catalyzed the transfer of galactose to lacto-*N*-tetraosyl ceramide, forming the blood group B-active rabbit erythrocyte pentahexosyl ceramide (110). However, this α-galactosyl transferase system is not involved in the biosynthesis of a fucolipid and had low activity for lacto-*N*-fucopentaose I (H-active oligosaccharide) as acceptor. The transferases that produce blood group B-active glycoproteins in primate stomach mucosa or submaxillary glands (111) and in human ovarian cyst linings and fluids (112) require an H-substance or H-like carbohydrate terminus as an acceptor.

Biosynthesis of other fucolipids

Except for synthesis of a Le^c-like fucolipid from lacto-*N*-neotetraosyl ceramide by a fucosyl transferase in human serum (42) (Reaction 4, Fig. 2), there have been no biosynthetic studies on the Lewis glycolipids in systems with purified substrate or enzyme. From the foregoing it is anticipated that the transfer of fucose to the *N*-acetylglucosamine of lacto-*N*-tetraosyl ceramide and lacto-*N*-neotetraosyl ceramide (Reaction 4, Fig. 2) and of the types 1 and 2 H-active glycolipids (Reaction 6, Fig. 2) should be the last step in the biosynthesis of the four Lewis blood group fucolipids. This should require two fucosyl transferases, one with

$\alpha 1 \rightarrow 3$ and the other with $\alpha 1 \rightarrow 4$ specificity for the glucosamine residue. The same transferases might convert the type 1 and 2 A-active and B-active fucolipids to the type 1 and 2 difucosyl A and B glycolipids, respectively (Reactions 9 and 10, Fig. 2). The control of these transferases is of interest in human adenocarcinoma tissue because two abnormalities exist there in the distribution of the Lewis fucolipids: the copresence of Lewis^a and Lewis^b fucolipids (82), and the presence of Lewis^c-like fucolipid (19).

Steiner, Brennan, and Melnick (113) have followed labeled fucose incorporation into lipids of mammalian embryo cell cultures and in cultures of these cells that were infected and transformed by oncornaviruses. The lipids were separated on TLC and the fucolipids were separated on radioautograms. The transformed cells differed from normal cells in that they incorporated much less fucose in the least mobile lipid and more fucose in the more mobile ones. Cultured human cancer cells (114) revealed the same difference in labeling pattern in four of five lines examined when compared to normal human embryonic lung cells. Cells infected with temperature-sensitive (115) or sodium butyrate-sensitive (116) viruses had normal fucolipid labeling patterns and normal morphological appearance at nonpermissive temperature or in the presence of sodium butyrate, respectively, whereas both were abnormal at a permissive temperature or in the absence of butyrate. The changes produced by the temperature-sensitive virus were reversed when nonpermissive culture conditions were restored. Although the fucolipids have not been identified (117), pulse–chase experiments with labeled and unlabeled fucose indicated that the least mobile fucolipid is the stable end product of the normal cell and is formed from more mobile precursors (116). Normal cell cultures at low density did not synthesize this fucolipid as rapidly as cells at higher density, but synthesis rates in transformed cells were not influenced by density. These studies are especially significant because they clearly identify cell lines in which there are close relationships between fucolipid metabolism and the transformation process.

Little is known of the nature of control of fucolipid synthesis. By use of very sensitive labeling (118, 119) and immunologic techniques, Watanabe and Hakomori (120) have shown that the complex blood group A and H glycolipids are present in greater quantities in adult erythrocytes than in the newborn, and are absent in fetal erythrocytes. They suggest that during ontogenesis there is a progressive change in the pattern of the fucolipids toward longer, arborized carbohydrate chains and that this is interrupted in oncogenesis (8, 120).

The possible role of the ceramide in directing or influencing the formation of particular fucolipids is obscure. The more polar ceramides containing hydroxylated fatty acids and phytosphingosine abound in the intestinal fucolipids but either are absent from or are minor components of fucolipids with the same carbohydrate structure from other tissues. The ceramide might relate to the chain type of the A-active fucolipids of hog gastric mucosa, and to the formation of fucosyl ceramide instead of other cerebrosides in human colon carcinoma (13).

The biochemical function of the fucolipids is unknown although their biological properties and distribution patterns in different cellular situations suggest some functions. First, the fucolipids are generally present in rapidly proliferating tissues, such as gastrointestinal epithelium, salivary glands, bone marrow, testis, and pancreatic acini, and are generally absent in tissues that do not undergo cell division or do so at lower rates, such as brain, myocardium, and kidney. The correlation, however, is by no means perfect. In addition, fucolipid distribution patterns change in oncogenesis (8) where they may be deleted, and in ontogenesis (120) where they are extended in chain length. As has been found with certain other glycolipids (9), they may not be synthesized as rapidly at low cell densities as at high densities (116). Many normal cells with presumably normal fucolipid content are subject to contact inhibition whereas transformed cells are opposite in these respects. The stimulating effects on cell division produced by lectins and other carbohydrate-directed reagents, such as $NaIO_4$ (121, 122), and by neuraminidase with galactose oxidase (121) suggest that the cell membrane carbohydrate termini of glycoproteins or glycolipids must play some regulatory role, and presumably a suppressive one, in cell division of tissues capable of rapid proliferation. From the limited data available on the fucolipids, it would appear that they are particularly involved in this role and that the control of their total synthesis, whether in the Golgi apparatus (91) or in the final stages by ectoglycosyl transferases (123), is of significance to the biology of cells and tissues.

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