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Characterization of Dog Small Intestinal Fucolipids with Human Blood Group H Activity†

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ABSTRACT: Fucolipids with human blood group H activity were isolated from several dog small intestines. On the basis of mass spectrometry, periodate oxidation, enzyme degradation, methylation, and immunologic studies the following structure is proposed: Fucα(1→2)Galβ(1→4)Glc-NAcβ(1→3)Galβ(1→4)Glc-ceramide. The ceramide was shown by mass spectrometry to contain hydroxyhexadecanoic acid and phytosphingosine as major constituents.

Fucolipids with human blood group H activity have been isolated from human erythrocytes (Yamakawa et al., 1965; Hakomori and Strycharz, 1968; Hakomori, 1970; Koscielak et al., 1973; Stellner et al., 1973a; Gorniak and Koscielak, 1972; Watanabe et al., 1974), human pancreas (Hakomori, 1970), dog small intestine (Hiramoto et al., 1973), pig stomach mucosa (Slomiany et al., 1974), and adenocarcinoma of the gastrointestinal tract (Hakomori et al., 1967). Complete structures for the H-active fucolipids which have been reported included a ceramide tetraglycoside in pig stomach mucosa (Slomiany et al., 1974), and a ceramide pentaglycoside (Koscielak et al., 1973; Stellner et al., 1973a) and heptaglycoside (Koscielak et al., 1973) in human erythrocytes. H-active fucolipids with eight or more sugar residues per molecule have been described in human erythrocytes (Stellner et al., 1973a; Hakomori et al., 1972; Watanabe et al., 1974), but their complete structures have not been determined. Structure studies on the dog small intestinal H-active fucolipid are reported in this paper.

Experimental Procedures

Materials. Forssman hapten was isolated from dog small intestine by methods in use in this laboratory (Vance et al., 1966; Smith and McKibbin, 1972). Lacto-*N*-fucopentaose I was a gift from Dr. Ginsburg of the National Institutes of Health. Melibiose, lichenin, and laminarin were purchased from K and K Laboratories, Inc., Plainview, N.Y. Sodium taurocholate was purchased from Nutritional Biochemicals Co., Cleveland, Ohio. β-*N*-Acetylhexosaminidase and β-galactosidase from Jack bean and α-galactosidase from fig ficin were prepared according to the procedure reported previously (Li and Li, 1972).

Isolation and Purification of Fucolipids. The procedure used to isolate the fucolipids of dog whole small intestine has been described previously (Vance et al., 1966; Smith and McKibbin, 1972). All isolated fucolipids were found to be homogeneous when tested on thin-layer plates coated with silica gel G or H in three solvent systems: a neutral (system A, chloroform–methanol–H_2O; 65:35:8), a basic (system B, chloroform–methanol–concentrated NH_4OH; 40:80:25), and an acidic system (system C, chloroform–methanol–H_2O–glacial acetic acid; 65:35:4:4 or system D, 55:45:5:5) (Smith and McKibbin, 1972). These systems have been shown to separate intestinal fucolipid mixtures into various components. Additional evidence of purity was obtained by analysis of the intact methylated and intact methylated and reduced fucolipids using thin-layer chromatography and mass spectrometry.

Determination of Sugars. Fucolipids (250 μg) were analyzed for sugar after hydrolysis with 0.5 ml of 2.5 *N* HCl for 6 hr at 100–105° in an air oven; 0.25 μmol of arabinose was added to samples and standards as an internal standard. The hydrolysates were extracted with 1 ml of $CHCl_3$; the aqueous phases were lyophilized and reduced with 1.0 ml of 2% sodium borohydride. The borohydride was decomposed with 0.2 ml of 6 *M* acetic acid and the sample lyophilized. One-milliter portions of 2% HCl in methanol were added to the residue and dried with filtered air after each portion. The residue was then acetylated with 1:1 pyridine–acetic anhydride in a boiling water bath for 3 hr. The alditol acetates of the samples and sugar standards were analyzed in a Beckman GC-65 gas chromatograph using a 6-ft column (column A) of 0.2% ethylene glycol succinate + 0.2% ethylene glycol acetate + 1.4% XE-60 on Gas Chrom P (100–200 mesh, Applied Science Labs). The temperature program was 150° for 1.5 min with 2.5°/min increase until a final temperature of 220°, which was held for 25 min; helium gas flow was 60 cm^3/min.

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Periodate Oxidation. Intact fucolipid (250 μg) was taken to dryness and dissolved in 0.5 ml of 0.6 *M* sodium periodate and left in the dark for 100–115 hr at room temperature. The reaction was stopped with 6 drops of ethylene glycol and the mixture transferred to dialysis tubing with 1.5 ml of water, dialyzed exhaustively, and lyophilized. After hydrolysis with 2.5 *N* HCl, monosaccharides resistant to periodate oxidation were identified by paper chromatography (Smith and McKibbin, 1972).

Methylation. The following glycolipids and oligosaccharides were used as standards for methylation studies: dog small intestinal Forssman hapten (GalNAcα(1→3)GalNAcβ(1→3)Galα(1→4)Galβ(1→4)Glc-ceramide); lacto-*N*-fucopentaose I (Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4)Glc), melibiose (Galα(1→6)Glc); lactose (Galβ(1→4)Glc); maltose (Glcα(1→4)Glc); and lichenin (β(1→3)Glcβ(1→4)Glc polymer); 250 μg of intact fucolipids or standards was methylated according to the procedure of Hakomori (Hakomori, 1964). After the methylation, 10 ml of H_2O was added to the reaction mixture, and the mixture was shaken and then extracted three times with 3 ml of $CHCl_3$. The chloroform was then extracted two times with 4 ml of H_2O, once with 4 ml of 15% Na_2SO_3, and three times with 4 ml of H_2O, and finally evaporated to dryness with filtered air. Hydrolysis of methylated neutral sugars was accomplished with 1 ml of 90% formic acid for 4 hr at 100° in an air oven. After drying, the residue was further hydrolyzed in 0.33 *N* H_2SO_4 for 12 hr at 100° in an air oven. The hydrolysate was passed through 0.5 g of Dowex 1-X8 (acetate form) in a small pipet and washed with 2 ml of H_2O. The hydrolysate and washing were taken to dryness and reduced and acetylated by the procedure used for sugar analysis. The partially methylated alditol acetates were analyzed using column A and the temperature program as described for the fully acetylated alditols except the initial temperature was held for 3.5 min. For analysis of both the amino sugars and neutral sugars the hydrolysis conditions and neutralization were carried out according to Stellner et al. (Stellner et al., 1973b), and reduction and acetylation by the procedure used for carbohydrate analysis described above. For identification of the methylated amino sugar, a 6-ft column packed with 3% cyanoethylsilicone–ethylene glycol succinate on Gas Chrom Q (Applied Science Labs.) at 190° and with a gas flow rate of 30 cm^3/min was used (column B). Amino sugar standards were from dog and human intestinal fucolipids and were identified by gas chromatography and mass spectrometry by Dr. S. I. Hakomori of the University of Washington.

Enzyme Degradation. Jack bean β-*N*-acetylhexosaminidase, Jack bean β-galactosidase, and fig α-galactosidase were used for enzymatic degradation of fucolipid; 30–50 μg of the intact fucolipid or defucosylated lipid (derived from intact fucolipid by mild acid hydrolysis in 0.1 *M* trichloroacetic acid for 1–2 hr at 80°) was dissolved in 0.1 ml of 0.05 *M* sodium citrate (pH 4.0) containing 100 μg of sodium taurocholate and incubated with 0.5–2 units of appropriate enzymes at 37° for 16–24 hr (Svennerholm et al., 1973). After the incubation, the reaction mixtures were shaken with 4 volumes of chloroform–methanol, 2:1, and centrifuged. The lower layer was evaporated to dryness and analyzed by thin-layer chromatography on silica gel G plates, using chloroform–methanol–water, 60:32:7, as solvent. The glycolipids on the plates were revealed by heating the plates at 120° after spraying with 1% orcinol in 3% H_2SO_4.

Mass Spectrometry. The glycolipid was methylated according to the procedure of Hakomori (Hakomori, 1964) using the reagent proportions as described elsewhere (Karlsson, 1974). The methylated glycolipid was reduced with $LiAlH_4$ (Karlsson, 1973, 1974). Mass spectrometry was performed on an MS 902 instrument (AEI Ltd., Manchester, England) equipped with a separate probe heater. The conditions of analysis are given in the legends for the reproduced spectra. To avoid an unrepresentative fatty acid profile due to possible molecular distillation effects during the probe heating, the reproduced spectra were recorded at the maximum intensity of the total ion current. This has been shown satisfactory for samples with a fatty acid composition known by gas chromatography. The mass numbers were obtained by counting by hand and are nominal mass numbers and not exact masses. The interpretation of spectra was based on earlier data of complex glycolipids (Karlsson, 1973; Karlsson, et al., 1974a–c), and on unpublished spectra of different fucolipids from human and dog small intestine.[1]

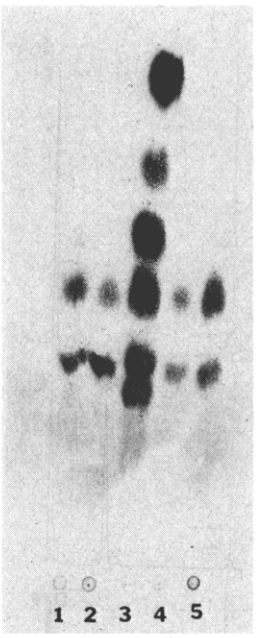

FIGURE 1: Paper chromatography of the hydrolysates from periodate treated intact fucolipids. (1) Dog 16 intestine F-1; (2) dog 16 intestine F-2 (Le^b active); (3) standard sugars, order from origin: galactosamine, glucosamine, galactose, glucose, mannose, fucose; (4) dog 17 intestine F-1 (H active); (5) human 2 intestine F-1 (Le^a active). Developing solvent system: 1-butanol–pyridine–water 5:3:2.

Immunologic Techniques. The preparation of rabbit anti-sera against dog intestinal fucolipids with various human blood types has been described (Hiramoto et al., 1973).

Results

From the small intestines of 21 individual dogs four compounds (9F-1, 17F-1, 24F-1, 28F-1) were isolated and found by either immunologic or methylation studies to have an H structure. 9F-1 and 17F-1 were immunologically H-active (Hiramoto et al., 1973) and 24F-1 and 28F-1 gave methylation patterns consistent with the H-structure (terminal Fuc(1→2) (Gal-). Two of the dog intestinal H-active fucolipids were accompanied by an Le^b-like fucolipid (9F-1,

[1] K.-A. Karlsson, J. M. McKibbin, I. Pascher, B. E. Samuelsson, and E. L. Smith, in preparation.

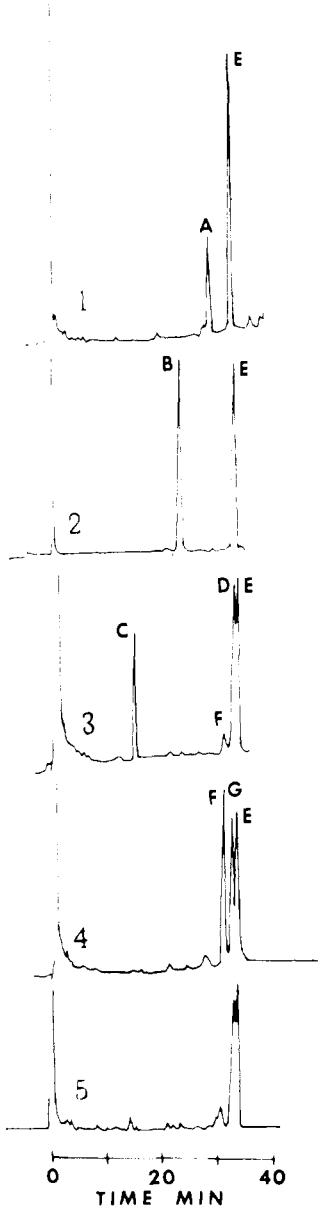

FIGURE 2: Gas-liquid chromatograms of the acetylated derivatives of methylated neutral sugars from the blood group H-active fucolipid 28F-1. (1) Lichenin standard; (2) lactose standard; (3) lacto-*N*-fucopentaose I standard (peak C amplified for clarity); (4) dog intestine Forssman hapten standard; (5) H-active dog intestinal fucolipid 28F-1. Peak assignments: acetylated derivatives of A, 2,4,6-tri-*O*-methylglucitol; B, 2,3,4,6-tetra-*O*-methylgalactitol; C, 2,3,4-tri-*O*-methylfucitol; D, 3,4,6-tri-*O*-methylgalactitol; E, 2,3,6-tri-*O*-methylglucitol; F, 2,4,6-tri-*O*-methylgalactitol; G, 2,3,6-tri-*O*-methylgalactitol. Temperature program was 150° for 3.5 min with a 2.5°/min increase until 220°. Column was 0.2% ethylene glycol acetate + 0.2% ethylene glycol succinate + 1.4% XE-60 (6 ft).

17F-1), and two isolated without evidence of any other fucolipid (24F-1, 28F-1).

Analysis of the Oligosaccharide. Results of sugar analysis on the H-active fucolipids indicated that these compounds were pentaglycosides containing glucose, galactose, glucosamine, and fucose in a molar ratio of 1:2:1:1. These results were supported by mass spectra data. After periodate oxidation, only 1 mol of galactose and glucosamine remained (Table I, Figure 1).

Methylation Studies. The partially methylated alditol acetates of both an H-active oligosaccharide of known structure and the H-active fucolipid gave identical elution

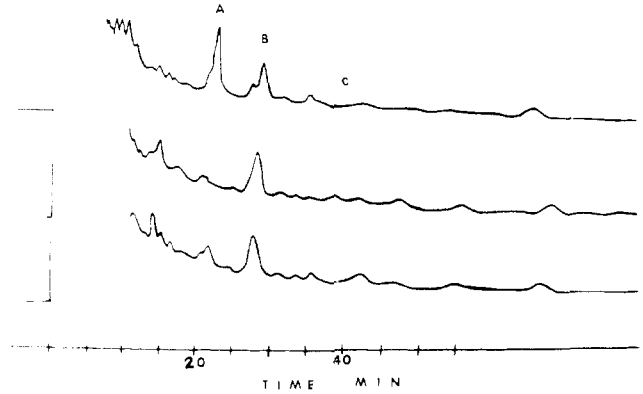

FIGURE 3: Gas-liquid chromatograms of acetylated derivatives of partially methylated hexosaminitols from dog intestinal blood type H fucolipids: upper, fucolipid 20F-1 (blood type A); middle, fucolipid 28F-1 (blood type H); lower, fucolipid 24F-1 (blood type H). Peak assignments (from GLC and mass spectrometry of fucolipid 20F-1): acetylated derivatives of A, 3,4,6-tri-*O*-methyl-2-deoxy-2-*N*-methylacetamidogalactitol; B, 3,6-di-*O*-methyl-2-deoxy-2-*N*-methylacetamidoglucitol; C, 4,6-di-*O*-methyl-2-deoxy-2-*N*-methylacetamidoglucitol. (expected location from other standards).

patterns in gas chromatography (Figure 2). Examination of the peaks of the H-active fucolipid indicated derivatives of 2,3,4-tri-*O*-methylfucitol, 2,4,6- and 3,4,6-tri-*O*-methylgalactitol, and 2,3,6-tri-*O*-methylglucitol. It could not be determined whether or not the derivative of 2,3,4-tri-*O*-methylglucitol was present, since it migrates with those of 2,3,6-tri-*O*-methylglucitol or 3,4,6-tri-*O*-methylgalactitol depending on whether column A or B was used. Amino sugar analysis revealed only the acetylated derivative of 3,6-di-*O*-methylglucosaminitol (Figure 3).

Anomeric Configuration of Glycosidic Linkages. Results of the treatment of intact and defucosylated H-active 17F-1 with degradative enzymes indicated that while the intact fucolipid was resistant to the action of α- and β-galactosidase and β-hexosaminidase, the defucosylated compound could be degraded. Jack bean β-galactosidase converted the defucosylated H-active 17F-1 to a glycolipid with mobility very close to trihexosylceramide (Galα(1→4)Galβ-(1→4)Glc ceramide) isolated from the kidney of a Fabry's patient. The newly formed trihexosylceramide was, in turn, converted to lactosylceramide by Jack bean β-*N*-acetylhexosaminidase. The combined action of β-galactosidase and β-*N*-acetylhexosaminidase converted the defucosylated H-active 17F-1 into glucosylceramide. The sequence and the anomeric configuration of the defucosylated compound was therefore concluded to be Galβ→GlcNAcβ→Galβ→Glc-ceramide. This sequence was supported by mass spectra data of the intact fucolipid.

Mass Spectrometry. As shown before (Karlsson, 1973, 1974; Karlsson et al., 1974a–c) sugar composition and sequence as well as ceramide structure may be derived from methylated and reduced methylated derivatives both concerning neutral glycolipids and gangliosides. In the present case mass spectra were recorded for the two derivatives of samples 17F-1, 24F-1, and 28F-1. The fragmentation patterns were very similar for the three glycolipids and only 17F-1 will be discussed in detail.

The spectrum of the methylated glycolipid is reproduced in Figure 4, and a simplified formula for the interpretation is shown in Figure 5. The only terminal sugar indicated is fucose (deoxyhexose) at *m/e* 189 and 157 (189 minus methanol). No evidence for hexose (*m/e* 219) or hexosamine

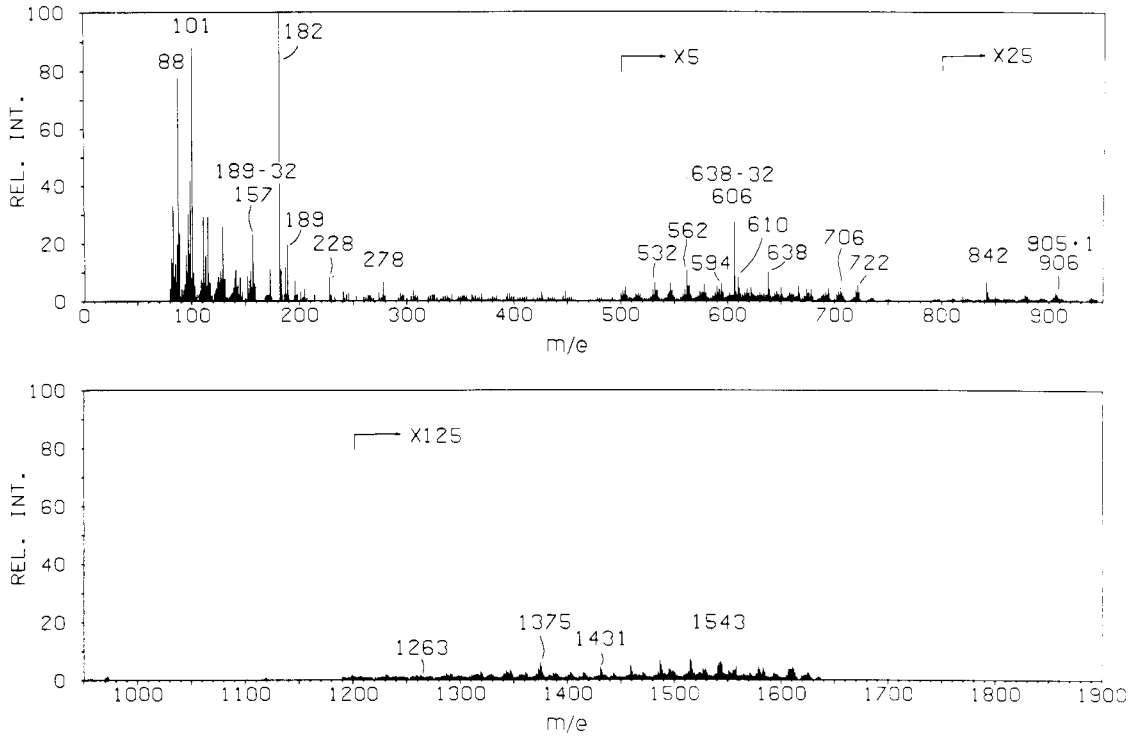

FIGURE 4: Mass spectrum of the methylated derivative of a H active glycolipid (17F-1) of dog small intestine. The conditions of analysis were: electron energy 45 eV, acceleration voltage 5.7 kV, filament current 500 μA, ion source temperature 310°, and probe temperature 330°.

Table I: A Summary of the Dog Intestinal Fucolipids with H Activity.

Dog F-1 Compound	Blood Group Activity	Methylated Sugar Pattern	Mass Spectra Structure	mg Isolated	mg Isolated per g of Dry Lipid-Free Tissue	Molar Ratio of Glc:Gal:GlcNAc:Fuc		Sequence and Anomeric Configuration of Sugars by Specific Glycosidases
						Before Periodate	After Periodate	
9F-1	H-like[d]	N.D.[a]	N.D.	11.7	0.259	1:2:1:1	0:1:1:0	N.D.
17F-1	H-like	N.D.	H-like[c]	15.6	0.197	1:2:1:1	0:1:1:0	Galβ → GlcNAcβ → Gal β → Glc-Cer
24F-1	N.D.	H-like[c]	H-like	6.9	0.083	N.D.	0:+:+:0Ψ[b]	N.D.
28F-1	N.D.	H-like	H-like	17.5	0.201	N.D.	0:+:+:0Ψ	N.D.

[a] N.D. = not determined. [b] Ψ, qualitative paper chromatography. [c] H-like. Consistent with the terminus of blood group H active oligosaccharide. Chains: fucosyl(1→2)galactosyl(1→4)*N*-acetylglucosaminyl(1→3)galactose. [d] Confers agglutination activity on rabbit erythrocytes tested against *Ulex* lectin (Hiramoto et al., 1973).

(*m/e* 260) as terminal sugars was found. The peaks at *m/e* 638 and 606 are due to a trisaccharide as shown in Figure 5 and *m/e* 842 corresponds to a tetrasaccharide with an additional hexose. The heaviest ceramide ions are indicated at *m/e* 722 and 706 (722 − 16) and is due to the molecular species phytosphingosine-hydroxytetracosanoic acid (see Figure 5). The analogous species with hydroxyhexadecanoic acid is found at *m/e* 610 and 594. Evidence for sphingosine-hydroxyhexadecanoic acid is shown at *m/e* 562. The heaviest ion interpreted is at *m/e* 1543 and probably originates from cleavage between the two methoxy groups of phytosphingosine. As this ion contains the fatty acid a series of ions due to the fatty acid spectrum is seen down to *m/e* 1431 (hydroxyhexadecanoic acid). The second series at *m/e* 1375 − 1263 has been indicated in Figure 5 and is probably due to a loss of the two terminal sugars (fucose-hexosamine). A rearrangement ion of low abundance at *m/e* 906 (compare Figure 5) is evidence for two hexoses next to ceramide.

The mass spectrum of the reduced derivative is reproduced in Figure 6 with an accompanying formula in Figure 7. The amide groups of ceramide and the hexosamine were reduced to the corresponding amines with a loss of 2 times 14 mass units. In this case molecular weight ions were recorded at *m/e* 1755 (hydroxytetracosanoic acid) and 1643 (hydroxyhexadecanoic acid). Intense peaks at *m/e* 1471 − 1359 are due to loss of the phytosphingosine chain (see Figure 7) and give semiquantitatively the fatty acid composition (*m/e* 1471 for hydroxytetracosanoic acid and 1359 for hydroxyhexadecanoic acid). Only smaller amounts, if any, of normal fatty acids may be present according to the spectrum. The series of peaks (1471 − 1359) is also conclusive for the number of sugars present (three hexoses, one hexosamine, and one fucose). The ion at *m/e* 1403 is due to a loss of the fatty acid (see Figure 7) and is also evidence for five sugars as well as phytosphingosine as the dominating long-chain base. As for the methylated derivative (Figure 4) only fucose was found as the terminal sugar (*m/e* 189 and 157). A successively increasing number of sugars are represented by the ions at *m/e* 624 and 640 (three sugars), 828 (four

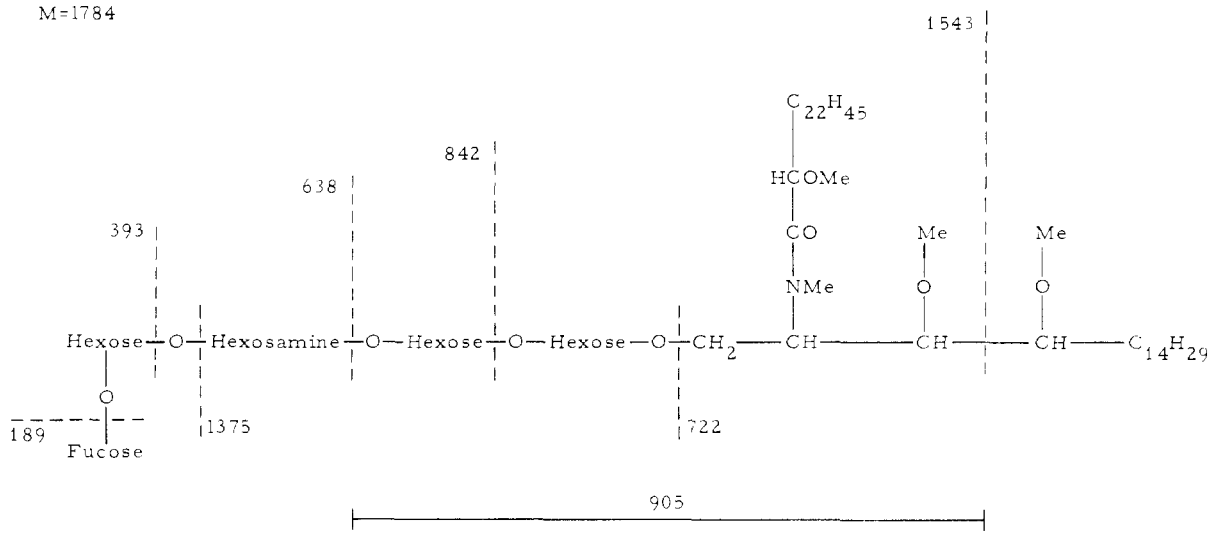

FIGURE 5: Simplified formula for the interpretation of the mass spectrum of Figure 4. The heaviest ceramide species found with phytosphingosine and hydroxytetracosanoic acid is reproduced.

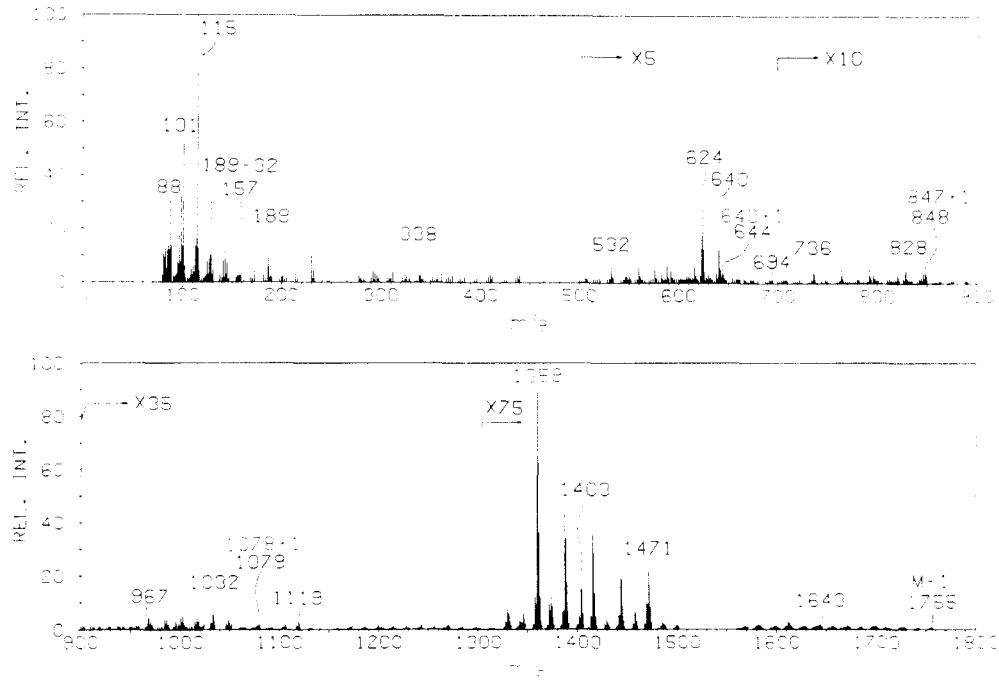

FIGURE 6: Mass spectrum of the reduced methylated derivative of a H active glycolipid (17F-1) of dog small intestine. The conditions of analysis were: electron energy 30 eV, acceleration voltage 4.4 kV, filament current 500 μA, ion source temperature 310°, and probe temperature 340°.

sugars), and 1032 (five sugars). Further sequence information is obtained by the rearrangement ions at *m/e* 644, 848, and 1079 (explained below the formula of Figure 7; peaks at 532, 736, and 967 are due to the molecular species with hydroxyhexadecanoic acid). These data and the sugar peaks of the spectrum of the methylated derivative (Figure 4), conclusively give the sequence fucose-hexose-hexosamine-hexose-hexose-ceramide.

The base peak of the methylated derivative (Figure 4) is at *m/e* 182. This abundant ion which originates[2] from the hexosamine ($C_9H_{12}O_3N$) is only found for "type 2" carbohydrate chain (Galβ(1→4)GlcNAc) and not for glycolipids with "type 1" chain (Galβ(1→3)GlcNAc).

Immunologic Activity. At least two fucolipids containing glucose, galactose, glucosamine, and fucose in a molar ratio of 1:2:1:1 have been isolated from dog intestine: the H-active lipid and an isomer. The former conferred agglutination activity on rabbit erythrocytes tested against *Ulex* lectin whereas the latter did not (Hiramoto et al., 1973). In contrast; antisera raised against one of these isomers, fucolipid 23F-1, gave strong and continuous precipitin bands with both types of fucolipid on Ouchterlony plates.

The data presented above is consistent with the following structure for the dog intestine H-active fucolipid: fucosyl-α(1→2)galactosylβ(1→4)*N*-acetylglucosaminylβ(1→3)galactosylβ(1→4)glucosyl-ceramide. The fatty acids were C_{16}-C_{24} hydroxy fatty acids and the major long chain base phytosphingosine, although sphingosine was also present.

Discussion

Blood group H-active substances have been reported in

[2] E. L. Smith, J. M. McKibbin, K.-A. Karlsson, I. Pascher, and B. E. Samuelsson, submitted.

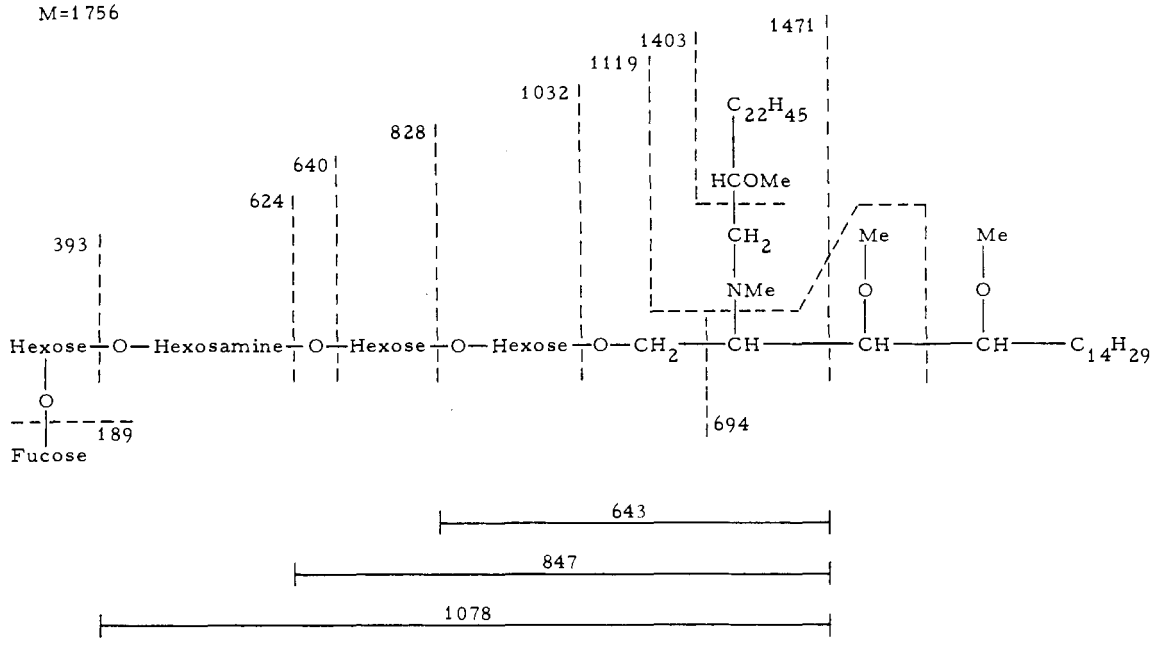

FIGURE 7: Simplified formula for the interpretation of the mass spectrum of Figure 6. Compare Figure 5.

Table II: Structures of H-Active Fucolipids from Several Animal Sources.[a]

Source	Proposed Structure	Predominant Fatty Acids % of Total	Predominant Long Chain Base	Ref
Pig stomach	Fucα(1→2)Galα(1→3)Galβ(1→4)Glc-ceramide	C16:1....15 C18:0....17 C24:1....9	Sphingosine (93%)	Slomiany et al., 1974
Canine small intestine	R-β(1→3)Galβ(1→4)Glc-ceramide	Only hydroxy fatty acids	Phytosphingosine	This paper
Human erythrocytes	R-β(1→3)Galβ(1→4)Glc-ceramide*	C24:0....44 C22:0....14 C18:0....11	Sphingosine (90%)	Stellner et al., 1973a
Human erythrocytes	R-β(1→3)Galβ(1→4)Glc-ceramide*	C24:1....20 C24:0....31 C22:0....18	N.D.	Koscielak et al., 1973
Human erythrocytes	R-β(1→ 3)Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4)-Glc-ceramide*	C24:0....32 C22:0....20 C16:0....11	N.D.	Koscielak et al., 1973
Human erythrocytes	R-β(1→6)→ R-β(1→3)→ Galβ→GlcNAc→Galβ(1→4)Glc-ceramide	N.D.	N.D.	Watanabe et al., 1974

[a] * denotes no hydroxy fatty acids. R = Fucα(1→2)Galβ(1→4)GlcNAc.

dog tissue (Horowitz et al., 1961; Hiramoto et al., 1973) but have not been characterized. Structures for the H-active fucolipids isolated from all sources are listed in Table II. The oligosaccharide terminus of all of these is identical with that of the blood group H-active human glycoproteins (Watkins, 1967) except for the one from hog gastric mucosa (Slomiany et al., 1974) which lacks glucosamine. The oligosaccharide portion of the dog intestine fucolipid is identical with that isolated from human erythrocytes (Koscielak et al., 1973; Stellner et al., 1973a), both containing the "type 2" (Galβ(1→4)GlcNAc) linkage. Although the "type 1" linkage (Galβ(1→3)GlcNAc) is found in blood group H-active glycoproteins (Watkins, 1967), it has not been found in significant amounts in any dog intestine fucolipids thus far. Since the type 1 linkage appears to be necessary for formation of Lewis^{a,b}-active fucolipids (which contain the fucosylα(1→4)GlcNAc linkage) its absence correlates with our failure to find an Le^a active fucolipid in dog intestine. Although the difucosyl Le^b-like[2] lipids isolated along with the H-active lipids of dogs 9 and 17 did react with human anti-Le^b agglutinins (Hiramoto et al., 1973) it seems more probable that it has Le^d structure (Gunson and Latham, 1972; Oriol et al., 1975). Although all of the lipids listed in Table II are type 2, a type 1 fucolipid containing sialic acid but otherwise identical with the dog intestine and human erythrocyte fucolipids was isolated from bovine liver (Wiegandt, 1973). The pentasaccharide remaining after removal of sialic acid and ceramide from this lipid had blood group H activity when tested with *Ulex* lectin.

The isomer of the H-active fucolipid, also present in dog intestine, has an oligosaccharide structure identical with the latter except that fucose is linked to glucosamine instead of galactose,[3] and thus may have Le^c type structure (Gunson

[3] K.-A. Karlsson, I. Pascher, B. E. Samuelsson, E. L. Smith, and J. M. McKibbin, unpublished.

and Latham, 1972). The immunologic difference between these substances has been cited above.

The ceramides of the dog intestine fucolipids are more polar than all of the others listed in Table II since all fatty acids are hydroxylated and phytosphingosine is a major long chain base. No hydroxy fatty acids were reported for any of the other H-active fucolipids.

The amount of the H-active fucolipid isolated ranged from 0.083 to 0.259 mg/g of dry lipid-free tissue (0.0121–0.0378 mg/g of fresh tissue) or about three- to sixfold greater than from human erythrocyte membranes (Stellner et al., 1973a). Canine intestine is obviously a good source of this hapten even though the latter is apparently not a factor in canine blood typing (Smith et al., 1973) and is probably absent from erythrocyte membranes. This situation differs from that in man where blood group active fucolipids are found in both tissues.

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