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References

Bantle, J. A., and Hahn, W. E. (1976), *Cell 8,* 139-150.
Birnie, G. D., MacPhail, E., Young, B. D., Getz, M. J., and Paul, J. (1974), *Cell Differ. 3,* 221-232.
Borun, T. W., Scharff, M. D., and Robbins, E. (1967), *Biochim. Biophys. Acta 149,* 302-304.
Darnell, J. E., Jelinek, W. R., and Molloy, G. R. (1973), *Science 181,* 1215-1221.
Galau, G. A., Britten, R. J., and Davidson, E. H. (1974), *Cell 2,* 9-20.
Gelderman, A. H., Rake, A. V., and Britten, R. J. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 172-176.
Getz, M. J., Birnie, G. D., Young, B. D., MacPhail, E., and Paul, J. (1975), *Cell 4,* 121-129.
Gilmour, R. S., Harrison, P. R., Windass, J., Affara, N. A., and Paul, J. (1974), *Cell Differ. 3,* 9-22.
Gilmour, R. S., Windass, J. D., Affara, N. A., and Paul, J. (1975), *J. Cell. Physiol. 85,* 449-458.
Harrison, P. R., Birnie, G. D., Hell, A., Humphries, S., Young, B. D., and Paul, J. (1974), *J. Mol. Biol. 84,* 539-554.
Hell, A., Birnie, G. D., Slimming, T. K., and Paul, J. (1972), *Anal. Biochem. 48,* 369-377.
Herman, R. C., Williams, J. G., and Penman, S. (1976), *Cell 7,* 429-437.
Hough, B. R., Smith, M. J., Britten, R. J., and Davidson, E. H. (1975), *Cell 5,* 291-299.
Humphries, S., Windass, J., and Williamson, R. (1976), *Cell 7,* 267-277.
Perry, R. P., Kelley, D. E., and LaTorre, J. (1974), *J. Mol. Biol. 82,* 315-332.
Ryffel, G. U. (1976), *Eur. J. Biochem. 62,* 417-423.
Ryffel, G. U., and McCarthy, B. J. (1975), *Biochemistry 14,* 1379-1385.
Scherrer, K. (1974), in Control of Gene Expression, Kohn, A., Shatkay, A., Ed., New York, N.Y., Plenum Publishing Co., pp 169-219.
Scherrer, K., and Marcaud, L. (1968), *J. Cell. Physiol. Suppl 1, 72,* 181-212.
Smith, M. J., Hough, B. R., Chamberlin, M. E., and Davidson, E. H. (1974), *J. Mol. Biol. 85,* 103-126.
Wetmur, J. G., and Davidson, N. (1968), *J. Mol. Biol. 31,* 349-370.
Williamson, R., Morrison, M., Lanyon, G., Eason, R., and Paul, J. (1971), *Biochemistry 10,* 3014-3021.
Young, B. D., Birnie, G. D., and Paul, J. (1976), *Biochemistry 15,* 2823-2828.
Young, B. D., and Paul, J. (1973), *Biochem. J. 135,* 573-576.

Characterization of Dog Small Intestinal Fucolipids with Human Blood Group A Activity. Differences in Dog and Human A-Active Fucolipids†

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ABSTRACT: Glycolipids containing fucose (fucolipids) which carried human blood group A activity were isolated from a number of dog small intestines and analyzed. On the basis of sugar analysis, methylation, periodate oxidation, enzyme degradation, mass spectrometry, and immunologic studies, a structure is proposed for these substances. The ceramides of the dog fucolipids contained only hydroxylated fatty acids with 85% saturated and 15% monoenoic acids ranging from 16 to 25 carbon atoms. Sphingosine and phytosphingosine comprised 48% each of the long chain bases. An A-active fraction isolated from human small intestine was shown to have two components, one of which was immunologically distinct and the other identical with the dog intestinal fucolipids. The human fraction differed from the dog fucolipids in migration on thin-layer chromatography and contained two types of amino sugar substitution. It is proposed that the human fraction was composed of two fucolipids with incomplete structures.

The possibility that the A antigen of the human ABO (H) blood group system exists on erythrocytes as a number of variants has long been recognized (Race and Sanger, 1962; Hakomori and Strycharz, 1968; Koscielak et al., 1970; Hakomori et al., 1972). The human A antigens are also distributed on erythrocytes and/or other tissues of infrahuman species, although there is some evidence for antigenic differences in these antigens among the species (Sorensen et al., 1974; Joysey, 1959; Furuhata, 1962; Borel, 1954; Schroffel et al., 1971; Slomiany and Horowitz, 1972, 1973; Thiele and Koch, 1973; Hiramoto et al., 1973; Zweibaum et al., 1974b; Slomiany et al., 1973, 1974; Slomiany and Slomiany, 1975; Hakomori and Watanabe, 1976; Slomiany et al., 1976a, 1976b). The variations in A antigenicity which are due to structural differences and those which are due to organizational differences on the

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membrane cannot be stated without more information on both.

In this paper, the complete structure of a dog intestinal fucolipid with human blood group A activity is proposed. Additionally, it is suggested that at least one structural difference between the human and dog intestinal A-active fucolipids thus far isolated is the type of amino sugar substitution: that the human intestinal A-active fucolipid fraction is a mixture of two ceramide hexaglycoside compounds, one with the type 1 stem (β-gal 1 → 3 glcNAc) and another with the type 2 stem (β-gal 1 → 4 glcNAc), while the A-active ceramide hexaglycosides from dog intestines have only the type 2 stem.

Experimental Procedures

Materials

Ceramide mono-, di-, and trihexosides and Forssman hapten were isolated from dog and human small intestine (Smith and McKibbin, 1972; Vance et al., 1966; McKibbin, 1969). Gangliosides G_{M1} and G_{D1a} were isolated from human brain (Svennerholm, 1972). Jack bean β-*N*-acetylhexosaminidase, jack bean β-galactosidase, and fig α-galactosidase were prepared according to a procedure reported previously (Li and Li, 1972). α-*N*-Acetylgalactosaminidase and α-L-fucosidase were gifts from Dr. F. Egami of Mitsubishi-Kasei Institute of Life Sciences, Tokyo, Japan. Sodium taurocholate was purchased from Nutritional Biochemicals Co., Cleveland, Ohio. *lacto*-*N*-Fucopentaose I was a gift from Dr. Victor Ginsburg (National Institutes of Health). Melibiose, lichenin, and laminarin were purchased from K & K Laboratories, Inc., Plainview, N.Y.

Methods

A. Isolation of the Fucolipids. The procedure for the isolation of the fucolipids in native form from human and dog intestine is based on silicic acid and Florisil column chromatography, solvent fractionation, and silica gel thin-layer chromatography and has been described in detail (Vance et al., 1966; Smith and McKibbin, 1972; McKibbin, 1976). The single human intestine was from an adult male of blood group A_1.

B. Characterization of the Fucolipids. 1. Analysis of the Ceramides. Determination of the Fatty Acids. Dog fucolipid 26F-1 (100 nmol; see Table I) was hydrolyzed in 1 M H_2SO_4 in dry methanol (Svennerholm and Vanier, 1973) and the resulting fatty acid methyl esters were separated into normal and hydroxylated fractions by TLC[1] (Svennerholm and Ställberg-Stenhagen, 1968). The hydroxylated esters were converted to the trimethyl silyl derivatives (Carter and Gaver, 1967) and analyzed in a Perkin-Elmer gas chromatograph Model 900 using a 15% DEGS column at 190 °C. Fatty acid analyses of dog and human A active fucolipids by mass spectrometry have been reported elsewhere (Smith et al., 1975b).

Determination of Long Chain Bases. Fucolipid 26F-1 (100 nmol) was hydrolyzed in 1 M HCl in methanol–water 82:18 (Gaver and Sweeley, 1965). The long chain bases were analyzed as aldehydes in the gas chromatograph using a 15% DEGS column at 170 °C (Vanier et al., 1973). Long chain base analyses of dog and human A-active fucolipids by mass spectrometry have been reported elsewhere (Smith et al., 1975b).

2. Analysis of the Oligosaccharide. Determination of Constituent Sugars. The sugars released from intact or periodate oxidized fucolipid by acid hydrolysis were converted into the alditol acetate derivatives and the latter determined by gas–liquid chromatography (Smith et al., 1975a).

Methylation and Identification of Methylated Sugars. The procedure for methylation of the intact fucolipids has been described (Smith et al., 1975d). Hydrolysis of the methylated product was carried out by the method of Stellner (Stellner et al., 1973) and reduction and acetylation of the methylated sugars by the same procedure used for the determination of unmethylated sugars. The derivatives of the methylated neutral and amino sugars were identified by gas–liquid chromatography (Smith et al., 1975d). Amino sugar derivatives from three of the fucolipids were identified by Dr. S. I. Hakomori of the University of Washington using both gas–liquid chromatography and mass spectrometry.

Enzyme Degradation. One milligram of fucolipid 32F-1 and Hu-3F-1 (Table I) was incubated at 37 °C in 2 mL of a 0.05 M sodium citrate buffer (pH 4.0) containing 4 mg of sodium taurocholate with 1–2 mg (dried weight) each of α-L-fucosidase and α-*N*-acetylgalactosaminidase. *p*-Nitrophenyl β-galactoside (1.2 mg) was added to inhibit any contaminant β-galactosidase activity. The reaction was stopped after 24 h with 8 mL of chloroform–methanol 2:1 and the upper phase discarded. The lower was dried and taken up in 1 mL each of chloroform and water, shaken, and the upper water layer discarded. The chloroform layer was again dried and taken up in the 0.05 M sodium citrate buffer, pH 4.0 (containing 1 mg of taurocholate per mL), and aliquots were incubated with appropriate combinations of β-galactosidase and β-*N*-acetylhexosaminidase. The glycolipids remaining in the digests were then extracted and examined by thin-layer chromatography (Svennerholm et al., 1973).

Acid Hydrolysis of the Human A-Active Fucolipid. Since α-L-fucosidase and α-*N*-acetylgalactosaminidase produced an insufficient amount of ceramide tetrasaccharide from the human fucolipid Hu 3F-1 for further enzyme degradation work, it was refluxed in 0.3 N HCl in 2:1 chloroform–methanol for 2 to 3 h in 60 °C water bath (Vance et al., 1966). The hydrolysate was taken to dryness and partitioned between chloroform and water. The chloroform layer contained glycolipids which were separated on silica gel G thin-layer plates in the system chloroform–methanol–water–glacial acetic acid, 65:30:3:3. These substances migrated with ceramide mono-, di-, and trihexosides and several other slower moving glycolipids.

Immunologic Techniques. The preparation of rabbit antisera against a dog intestinal fucolipid (12F-1) with human blood group A activity has been described (Hiramoto et al., 1973). This antiserum had an antiimmunofluorescence titer of $^1/_{512}$ tested on canine colonic sections (Zweibaum et al., 1974a). For the Ouchterlony precipitin tests, the wells were filled three times with undiluted antiserum and 1 mg/mL solutions of the glycolipids in saline. Auxiliary lipids were not used since they did not significantly affect the activity of the simple series I A-active fucolipids (Smith et al., 1973; Slomiany et al., 1976b).

Results

Chromatographic Mobility of the Fucolipids. Figure 1 shows the mobilities of several human and dog intestine glycolipids on thin-layer plates in the solvent system chloroform–methanol–concentrated NH_4OH, 40:80:25. Both A-

[1] Abbreviation used: TLC, thin layer chromatography.

TABLE I: Composition and Properties of the A-Active Fucolipids Isolated from Dog Intestine.[a]

Species, Specimen No. and Fucolipid Designation		Sugar Molar Ratio (glc:gal:glcNAc:galNAc:fuc) Before Periodate	After Periodate	Human Blood Group A Act.	Ref	Sequence from Mass Spectrometry	Ref	Methylation of Sugars Neutral	Amino
Dog	12F-1	1:2:1:1:1	0:2:1:0:0	+	I, II			ND	ND
	12F-2	+:+:+:+:+[b]	ND	+				ND	ND
	15F-1	1:2:1:1:1	0:2:1:0:0	+	I, II	A-like	III	ND	ND
	20F-1	1:2:1:1:1	0:2:1:0:0	ND		A-like	III	A-like	Type 2
	26F-1	1:2:1:1:1	ND	ND		A-like	III	A-like	Type 2
	29F-1	ND	ND	ND		A-like	III	A-like	Type 2
	32F-1	1:2:1:1:1	0:2:1:0:0	ND		A-like	III	A-like	Type 2
Human	Hu 3F-1	1:2:1:1:1	0:2:1:0:0	+	II	A-like	III	A-like	Type 1, type 2

[a] ND, not determined. A-like, hexosamine, deoxyhexose hexose → hexosamine structure at nonreducing end. Reference I, Smith et al. (1973); II, Hiramoto et al. (1973); III, Smith et al. (1975b). [b] +, present in paper chromatograms of hydrolysates.

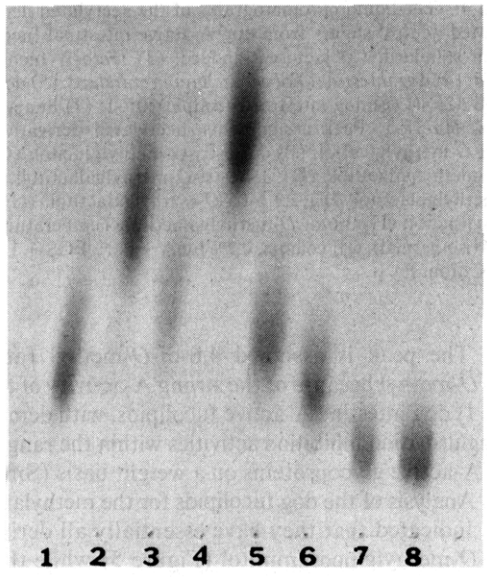

FIGURE 1: Mobility of small intestinal glycolipids with Forssman or human blood group activities on silica gel G TLC. Solvent system: chloroform–methanol–concentrated NH_4OH (40:80:25). Chromatogram sprayed with 0.2% naphthoresorcinol in absolute ethanol, and 20% aqueous H_2SO_4 (1:1) and then heated at 110 °C for 10 min. Channel 1, dog 20F-1 (A-active); 2, human Hu-3F-1 (A-active); 3, human Hu-2F-1 (Le^a-like); 4, dog Forssman hapten; 5, dog 17F-1 (H-active); 6, dog 23F-1 (Le^c-like); 7, dog 16F-2 (Le^b-like); 8, dog 23F-2 (Le^b-like).

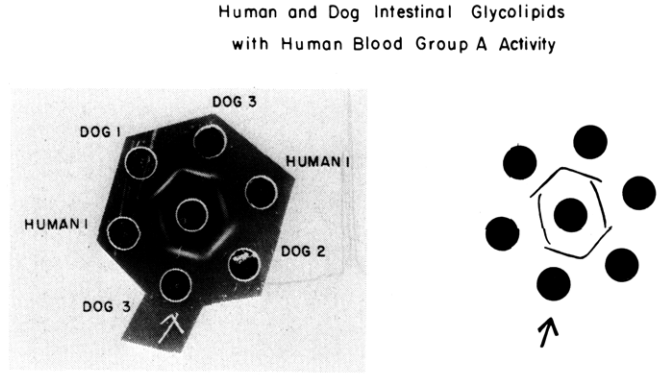

FIGURE 2: Reaction on Ouchterlony plate of three different dog and one human A-active fucolipids with rabbit anti-12F-1 (dog intestinal A-active fucolipid). Dog 1, 12F-1, 1 mg per mL saline; dog 2, 20F-1, 1 mg per mL saline; dog 3, 32F-1, 1 mg per mL saline. Human 1: Hu-3F-1, 1 mg per mL saline. Center well: Rabbit anti-12F-1 undiluted. The human compound showed two bands; a weak band which showed identity with the dog compounds is not well seen in this figure.

active fucolipids had greater mobility than the Le^b-like fucolipids which have the same number of sugar residues, and the human A-fucolipid had significantly higher mobility than the canine. In order to assess possible differences in mobility among different A-active fucolipids, several other canine samples were compared with the Forssman glycolipid as a standard. They all had relative R_f's in the range of 0.4–0.6 to that of the Forssman, and well below that of the single human sample (0.8) we have isolated thus far. The human fucolipid could not be further resolved in any of our thin-layer chromatography systems, although it apparently consists of two components.

Precipitin Tests with the Fucolipids. Rabbit antiserum prepared against an A-active fucolipid, dog 12F-1 (Hiramoto et al., 1973), reacted on Ouchterlony plates with both human and dog A-active fucolipids (Figure 2). The dog A-active fucolipids showed identity, but the human Hu-3F-1 fraction gave two bands, the stronger of which was discontinuous with the dog compounds and a very faint band which showed identity with the dog fucolipids.

Analysis of the Oligosaccharide. Results of sugar analysis of the several dog and the human A^+ intestinal fucolipids both before and after periodate oxidation are given in Table I and typical sugar patterns in gas–liquid chromatography are seen in Figure 3. All A^+ fucolipids were hexaglycosides with glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and fucose in a molar ratio of 1:2:1:1:1, respectively. Only glucosamine and galactose remained after periodate oxidation and in a molar ratio of 1:2, respectively. Paper chromatography of fucolipids after periodate treatment showed that glucose is decomposed, although some remaining glucose is detected when the more sensitive gas–liquid chromatography methods are utilized. The findings are consistent with the common structural feature of A-positive saccharide chains in which galactosamine and fucose are terminal and unsubstituted, one galactose is substituted by fucose and galactosamine, and the other galactose is substituted at the 3 position. The fact that glucose was almost completely decomposed by periodate indicated that it was for the most part substituted at its number 4 or 6 carbon. The small amount of glucose found after per-

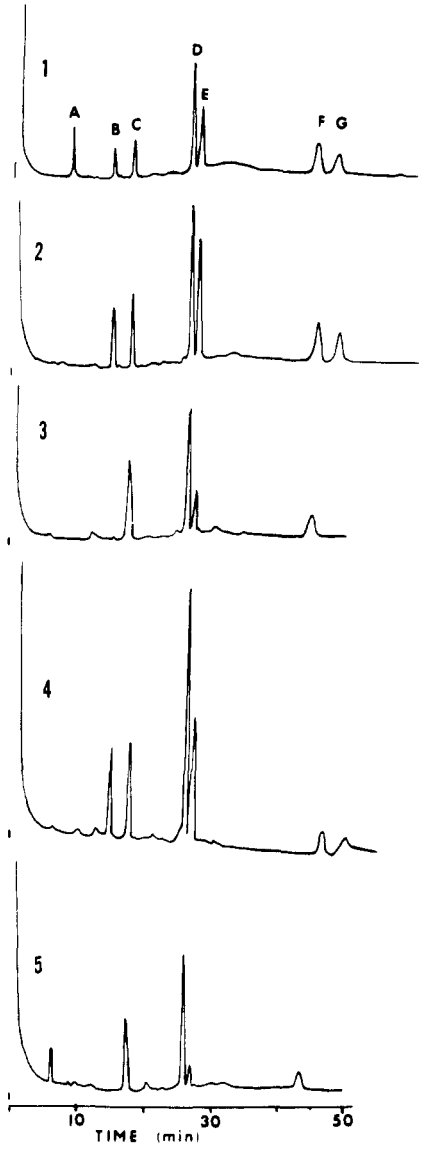

FIGURE 3: Gas–liquid chromatograms of the alditol acetates of sugars from dog and human intestinal A active fucolipids before and after periodate treatment. (1) Sugar standards: (A) erythritol; (B) fucose; (C) arabinose (internal standard); (D) galactose; (E) glucose; (F) glucosamine; (G) galactosamine. (2) Dog fucolipid 20F-1. (3) Dog fucolipid 20F-1 after periodate treatment. (4) Human fucolipid Hu 3F-1. (5) Human fucolipid Hu 3F-1 after periodate treatment.

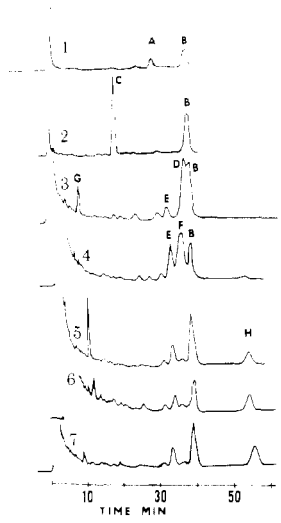

FIGURE 4: Gas–liquid chromatograms of the acetylated derivatives of methylated neutral sugars from dog A-active intestinal fucolipids. (1) Lichenin standard; (2) lactose standard; (3) *lacto-N*-fucopentaose I standard; (4) dog intestinal Forssman hapten standard; (5) dog intestine fucolipid 32F-1; (6) dog intestine fucolipid 20F-1; (7) human intestine fucolipid Hu-3F-1. Peak assignments: acetylated derivatives of (A) 2,4,6-tri-*O*-methylglucitol; (B) 2,3,6-tri-*O*-methylglucitol; (C) 2,3,4,6-tetra-*O*-methylgalactitol; (D) 3,4,6-tri-*O*-methylgalactitol; (E) 2,4,6-tri-*O*-methylgalactitol; (F) 2,3,6-tri-*O*-methylgalactitol; (G) 2,3,4-tri-*O*-methylfucitol; (H) 3,6-di-*O*-methylgalactitol. Temperature program was 160 (isothermal); 6-ft column, 0.2% EGA + 0.2% EGS + 1.4% XE-60 on Gas Chrom P.

iodate oxidation is consistent with that found after oxidation of several other glycolipids having the glycosyl- (1 → 4) glucosylceramide structure.

Methylation Studies. The methylated and acetylated alditol derivatives of the neutral sugars of the dog and human fucolipids showed identical gas–liquid chromatography patterns (Figure 4). Comparison of these patterns with methylated standards demonstrated the presence of major peaks of the acetylated derivatives of 2,3,6-tri-*O*-methylglucitol, 2,4,6-tri-*O*-methylgalactitol, 2,3,4-tri-*O*-methylfucitol, and minor peaks which coincide with 2,3,6-tri-*O*-methylglucitol and 2,3,6-tri-*O*-methylgalactitol. In Figure 4 the peak eluting at approximately 56 min is either a 2,6- or 4,6-di-*O*-methylgalactitol derivative based on comparison of methylated dog A-active fucolipids with methylated ganglioside G_{M1} and G_{D1a} standards on a 3% ECNSS-M column. The 2,6- and 4,6-di-*O*-methyl derivatives have identical elution times on this column.[2] The peak is assigned 4,6-di-*O*-methyl rather than 2,6-di-*O*-methyl because of the strong A-activity of the simple (series I) dog intestine A-active fucolipids, with demonstrated hemagglutination inhibition activities within the range of those of the A-active glycoproteins on a weight basis (Smith et al., 1973). Analysis of the dog fucolipids for the methylated amino sugars indicated that they have essentially all derivatives of 3,6-di-*O*-methylglucosaminitol (Figure 5) while the human fucolipid fraction gave derivatives of 3,6- and 4,6-di-*O*-methylglucosaminitol.

Anomeric Configuration of Glycosidic Linkages. Results from the treatment of the intact dog and human A-active fucolipids with α- and β-galactosidase and β-hexosaminidase indicated that these compounds were resistant to the action of these enzymes. Predigestion of the intact dog fucolipid (32F-1) with α-fucosidase and galactosaminidase together produced in low yield a ceramide tetrasaccharide. The latter incubated with both β-galactosidase and β-hexosaminidase gave a ceramide monohexoside, whereas incubation with β-galactosidase, then heating, then incubation with β-hexosaminidase gave a ceramide dihexoside. Treatment with β-galactosidase alone gave a ceramide trihexoside. These results indicate that the ceramide tetrasaccharide had the following sequence and anomeric configuration: gal β glcNAc β gal β glc-ceramide. This sequence is identical with that determined by mass spectrometry (Smith et al., 1975b).

Acid hydrolysis of the human A-active fucolipid fraction yielded multiple ceramide glycosides, but only a ceramide dihexoside was successfully isolated and treated with enzymes. This ceramide dihexoside was converted to a ceramide monohexoside by β-galactosidase, indicating a gal β → glc-ceramide structure.

[2]Månsson, J. E., unpublished experiments.

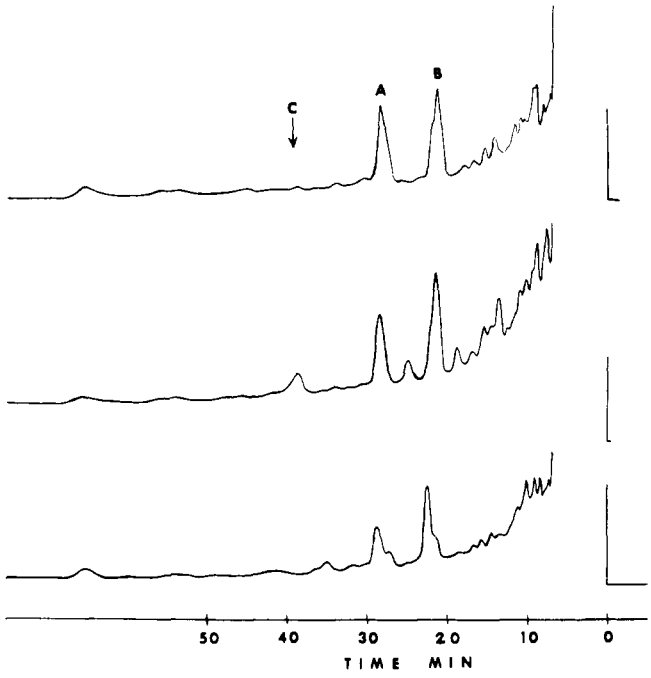

FIGURE 5: Gas-liquid chromatograms of acetylated derivatives of methylated amino sugars from dog intestinal A-active fucolipids. (Upper) Fucolipid 29F-l; (middle) fucolipid 26F-l; (lower) fucolipid 20F-l. Peak assignments (from GLC and mass spectrometry of fucolipid 20F-l): acetylated derivatives of (A) 3,6-di-*O*-methyl-2-deoxy-2-*N*-methylacetamidoglucitol; (B) 3,4,6-tri-*O*-methyl-2-deoxy-2-*N*-methylacetamidogalactitol; (C) 4,6-di-*O*-methyl-2-deoxy-2-*N*-methylacetamidoglucitol.

TABLE II: Distribution of the Hydroxy Fatty Acids[a] and the Long Chain Bases in the A^+ Fucolipid of Dog Intestine (26F-1).

	Percent
Fatty acid	
16:0	16
18:0	11
20:0	14
22:0	17
22:1	4
23:0	4
23:1	2
24:0	22
24:1	9
25:0	1
25:1	0
26:0	0
Long chain base	
t18:0	48
d18:0	1
d18:1	48
t20:0	3

[a] No nonhydroxylated fatty acids were found.

Ceramide Analysis. The fatty acid and long chain base distribution in dog 26F-1 A^+ fucolipid is given in Table II. Only hydroxy fatty acids are found with the saturated even numbered fatty acids 16 through 24 predominating. Equal amounts of sphingosine bases t18:0 and d18:1 account for essentially all of the long chain base. These results are in complete agreement with the analysis of the ceramides of dog and human A-active fucolipids by mass spectrometry (Smith et al., 1975b).

A summary of the composition and properties of 7 of the 15 A-active dog intestine fucolipids which have been isolated and of one human fucolipid is given in Table I.

With the data presented above, it is proposed that fucolipids with human blood group A activity which were isolated from dog whole small intestine have the structure *N*-acetylgalactosaminyl (α1 → 3) [fucosyl (α1 → 2)] galactosyl (β1 → 4) *N*-acetylglucosaminyl (β1 → 3) galactosyl (β1 → 4) glucosyl (1 → 1)ceramide (type 2). The dog A active fucolipids also give traces of methylated derivatives, indicating the presence of small amounts of glucose substituted 1 → 3 and galactose substituted 1 → 4. It is suggested that the human A active fucolipid fraction is composed of a mixture of two ceramide hexaglycosides with the following incomplete structures: *N*-acetylgalactosaminyl (1 → 3) [fucosyl (1 → 2)] galactosyl (1 → 3) *N*-acetylglucosaminyl (1 → 3) galactosyl (β1 → 4) glucosyl 1-1-ceramide (type 1) and *N*-acetylgalactosaminyl (1 → 3) [fucosyl (1 → 2)] galactosyl (1 → 4) *N*-acetylglucosaminyl (1 → 3) galactosyl (β1 → 4) glycosyl 1-1-ceramide (type 2). Mass spectra of the methylated human fucolipid (Smith et al., 1975b) indicate a predominance of type 1 chain since very little *m/e* 182 ion, a characteristic of type 2 chain, was found. The Ouchterlony precipitin results also indicate a disproportionate mixture of fucolipids with only a minor component giving a continuous band with the canine fucolipid (type 2).

Discussion

That fucolipids with A activity exist in multiple structural forms on erythrocytes (Hakomori et al., 1972; Koscielak et al., 1970) and gastrointestinal tissue (Slomiany et al., 1973, 1974, 1975, 1976a; Slomiany and Slomiany, 1975; Smith et al., 1975a,b) has now been documented and could account for variations in immunologic activities and migration on thin-layer chromatography. Interesting species difference among human, hog, and dog gastrointestinal A-active fucolipids have been found. Types 1 and 2 A-active fucolipids have been found in both hog (Slomiany et al., 1974, 1975) and human gastrointestinal tissue. The hog compounds occur in two series, one with a sugar sequence identical with the human, and the other with an additional galactose residue between glucosamine and glucose. Thus far, only type 2 A-active fucolipids have been found in dog intestinal tissue. Additionally, two other fucolipids with somewhat lower blood type A activity have been isolated from hog gastric mucosa. These appear to be identical in structure with those of the two series cited above but lack glucosamine (Slomiany et al., 1973, 1975). An A-Le^b hybrid has also been reported in hog gastric mucosa (Slomiany and Slomiany, 1975) and dog and human small intestine and human pancreas (Smith et al., 1975c).

The fact that the human A-active fraction could not be resolved into two components and migrated so differently from the dog A-active fucolipids remains unexplained, but is worth noting. Types 1 and 2 A active fucolipids in hog gastric mucosa had different ceramide moieties and could be separated on thin-layer chromatography, but this was not the case with the human A-active compounds.

While less is known about the composition of the ceramides of the A-active fucolipids from different sources, significant differences are found. In hog gastric mucosa, 73 to 94% of the total base composition was sphingenine and there was essentially no phytosphingosine (Slomiany et al., 1973, 1974). One of the dog intestine fucolipids contained 48% sphingenine and 48% phytosphingosine and none of the human erythrocyte

blood group substances contain more than trace amounts of phytosphingosine (Hakomori and Strycharz, 1968; Yang and Hakomori, 1971). The hog gastric mucosa fucolipids contained less α-hydroxy fatty acids, from 4.5 to 35.4% of the total, whereas the dog intestine fucolipid fatty acids were all hydroxylated. There were differences in chain length of the acids as well, those of the hog gastric mucosa ranging from 41 to 77% below C_{20}, while the dog intestine lipid contained only 27% below C_{20}.

Although material actually isolated is at best a crude indicator of the amount present in a tissue, it is clear that the secretory tissues contain much more A-active fucolipids than do erythrocytes. The average yield of nine individual dog intestines was 46 mg/kg wet tissue (317 mg/kg dry lipid-free tissue); the single human intestine yielded 159 mg/kg wet tissue (1041 mg/kg dry lipid free tissue). Pooled hexane washed hog stomach mucosa yielded 229 mg/kg dry tissue (Slomiany and Horowitz, 1973; Slomiany et al., 1973) while human erythrocytes yielded 0.15 mg/L wet tissue (Hakomori and Strycharz, 1968). It should be noted that the dog intestine A-active fucolipid described here has been identified with the A antigen of the Canine Secretory Antigen (CSA) system described by Zweibaum and associates (Zweibaum et al., 1967, 1974a,b; Zweibaum and Steudler, 1969). By use of the immunofluorescent technique, these investigators found the A antigen in 45.2% of a population of 250 mongrel dogs (Zweibaum et al., 1974b). We have isolated A-like fucolipids from intestines of 15 of a total of 28 dogs, a comparable distribution which indicated that the immunofluorescent technique was detecting fucolipids alone or with A active glycoprotein, but probably not glycoprotein alone.

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References

Borel, J. F. (1954), in Blood Groups of Animals, Matousek, J., Ed., The Netherlands, the Hague, Dr. W. Junk, p 471.

Carter, H. E., and Gaver, R. C. (1967), *J. Lipid Res. 8,* 391.

Furuhata, T. (1962), *Proc. Congr. Int. Soc. Blood Transfus., 8th, 1960,* 45.

Gaver, R. C., and Sweeley, C. C. (1965), *J. Am. Oil Chem. Soc. 42,* 294.

Hakomori, S. I., Stellner, K., and Watanabe, K. (1972), *Biochem. Biophys. Res. Commun. 49,* 1061.

Hakomori, S., and Strycharz, G. D. (1968), *Biochemistry 7,* 1279.

Hakomori, S. I., and Watanabe, K. (1976), in Glycolipid Methodolgy, Witting, L. A., Ed., Champaign, Ill., American Oil Chemists' Society Press, p 13.

Hiramoto, R. N., Smith, E. L., Ghanta, V. K., Shaw, J. F., and McKibbin, J. M. (1973), *J. Immunol. 110,* 1037.

Joysey, V. C. (1959), *Br. Med. Bull. 15,* 158.

Koscielak, J., Piasek, A., and Gorniak, H. (1970), in Blood and Tissue Antigens, Aminoff, D., Ed., New York, N.Y., Academic Press, p 163.

Li, Y.-T., and Li, S.-C. (1972), *Methods Enzymol. 28,* 702.

McKibbin, J. M. (1969), *Biochemistry 8,* 679.

McKibbin, J. M. (1976), in Glycolipid Methodology, Witting, L. A., Ed., Champaign, Ill., American Oil Chemists' Society Press, p 77.

Race, R. R., and Sanger, R. (1962), in Blood Groups in Man, Oxford, Blackwell Science Publishers, p 28.

Schroffel, J., Radas, A., Thiele, O. W., and Koch, J. (1971), *Eur. J. Biochem. 22,* 396.

Slomiany, A., and Horowitz, M. I. (1973), *J. Biol. Chem. 248,* 6232.

Slomiany, A., and Slomiany, B. L. (1975), *Biochim. Biophys. Acta 388,* 135.

Slomiany, A., Slomiany, B. L., and Horowitz, M. I. (1974), *J. Biol. Chem. 249,* 1225.

Slomiany, A., Slomiany, B. L., and Horowitz, M. I. (1976b), in Glycolipid Methodology, Witting, L. A., Ed., Champaign, Ill., American Oil Chemists' Society Press, p 49.

Slomiany, B. L., and Horowitz, M. I. (1972), *Immunochemistry 9,* 1067.

Slomiany, B. L., Slomiany, A., and Horowitz, M. I. (1973), *Biochim. Biophys. Acta 326,* 224.

Slomiany, B. L., Slomiany, A., and Horowitz, M. I. (1975), *Eur. J. Biochem. 56,* 353.

Slomiany, B. L., Slomiany, A., and Horowitz, M. I. (1976a), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 1443.

Smith, E. L., Bowdler, A. J., Bull, R. W., and McKibbin, J. M. (1973), *Immunology 25,* 621.

Smith, E. L., and McKibbin, J. M. (1972), *Anal. Biochem. 45,* 608.

Smith, E. L., McKibbin, J. M., Karlsson, K.-A., Pascher, I., and Samuelsson, B. E. (1975a), *J. Biol. Chem. 250,* 6059.

Smith, E. L., McKibbin, J. M., Karlsson, K.-A., Pascher, I., and Samuelsson, B. E. (1975b), *Biochemistry 14,* 2120.

Smith, E. L., McKibbin, J. M., Karlsson, K.-A., Pascher, I., and Samuelsson, B. E. (1975c), *Biochim. Biophys. Acta 388,* 171.

Smith, E. L., McKibbin, J. M., Karlsson, K.-A., Pascher, I., Samuelsson, B. E., and Li, S.-C. (1975d), *Biochemistry 14,* 3370.

Sorensen, A. N., Rendel, J., and Stone, W. H. (1974), *J. Immunol. 73,* 407.

Stellner, K., Saito, H., and Hakomori, S. (1973), *Arch. Biochem. Biophys. 155,* 464.

Svennerholm, L. (1972), *Methods Carbohydr. Chem. 6,* 464.

Svennerholm, L., Månsson, J.-E., and Li, Y.-T. (1973), *J. Biol. Chem. 248,* 740.

Svennerholm, L., and Ställberg-Stenhagen, S. (1968), *J. Lipid Res. 9,* 215.

Svennerholm, L., and Vanier, M.-T. (1973), *Brain Res. 55,* 413.

Thiele, O. W., and Koch, J. (1973), *Vox. Sang. 25,* 317.

Vance, W. R., Shook, C. P., III, and McKibbin, J. M. (1966), *Biochemistry 5,* 435.

Vanier, M.-T., Holm, M., Månsson, J.-T., and Svennerholm, L. (1973), *J. Neurochem. 21,* 1375.

Yang, H. J., and Hakomori, S. I. (1971), *J. Biol. Chem. 246,* 1192.

Zweibaum, A., Dussaulx, E., Smith, E. L., and McKibbin, J. M. (1974a), *Proc. Soc. Exp. Biol. Med. 147,* 883.

Zweibaum, A., Halpern, B., Veyre, C., and Oriol Palou, P. (1967), *Pathol.-Biol. 15,* 359.

Zweibaum, A., Oriol, R., Feingold, N., Dussaulx, E., Rousset, M., and Chevalier, G. (1974b), *Tissue Antigens 4,* 115.

Zweibaum, A., and Steudler, V. (1969), *Nature (London) 223,* 84.