Lewis Blood Group Fucolipids and Their Isomers from Human and Canine Intestine*

John M. McKibbin†, William A. Spencer, and Edwin L. Smith
From the Department of Biochemistry, the University of Alabama in Birmingham, Birmingham, Alabama 35294
Jan-Eric Mansson
From the Department of Psychiatry and Neurochemistry, Psychiatric Research Center, University of Göteborg, Göteborg, Sweden
Karl-Anders Karlsson§ and Bo E. Samuelsson
From the Department of Medical Biochemistry, University of Göteborg, Göteborg, Sweden
Yu-Teh Li† and Su-Chen Li
From the Department of Biochemistry, Tulane University, New Orleans, Louisiana 70112

Glycolipids containing fucose linked to N-acetylglucosamine were isolated and characterized from 14 individual human and 13 individual dog intestines. From 8 of the dog intestines, Lewis a isomer fucolipids were isolated, all identical and having the structure Gal(3→4)Fucα1→3GlcNAc(3→3)Gal(β1→4)Glc-ceramide. Lewis b fucolipids were isolated from 12 of the intestines, all identical and having the structure Fucα1→2Gal(β1→4)Fucα1→3GlcNAc(β1→3)Gal(β1→4)Glc-ceramide. Lewis a fucolipids were isolated as the sole major fucolipid in 6 of the human intestines and differed from the canine isomer only in the position of the linkage of galactose to N-acetylglucosamine, having the β1→3 (type 1) rather than the β1→4 (type 2) linkage. Lewis a-fucolipid isomers commonly co-existed in canine intestine as major fucolipids whereas Lewis a and b glycolipids did not co-exist in human intestine. In all of the fucolipids, only hydroxylated fatty acids were present and phytosphingosine and sphingosine were the predominant long chain bases. These findings are of interest in the biosynthesis of these substances and in their genetic expression.

1. Isolation of the Fucolipids. Human and small intestines were taken immediately at sacrifice after experimental surgery. The small human intestines were all grossly normal and taken at autopsy, usually within 4 or 5 hours post mortem. The tissues were extracted with ethanol/water (1:1) as described previously (17) except that 10 ml of solvents mixture were used per gram of tissue. Following removal of the ethanol/water, the tissues were extracted with chloroform:methanol:water (65:35:8) over night at room temperature and subjected to preparative thin layer chromatography with the system chloroform:methanol:water (65:35:8) over night. The fraction containing the Gal(3→4)Fucα1→3GlcNAc(3→3)Gal(β1→4)Glc-ceramide was collected. The fractions were analyzed by gas chromatography as described previously (17). The fractions for the isolation of the intact lipids with sodium periodate and for the determination of the sugars present in the fucolipid were analyzed by the chromatographic system described previously (17).

2. Characterization of the Fucolipids. A. Derivatization of the Constituents. The fucolipids were hydrolyzed at 105° for 12 hours and the isolated sugars reduced to the alditoles and acetylated by the methods of Hutt and Backman (18) and Smith et al. (17). The acetylated alditols were then analyzed by gas chromatography as described previously (17). The procedure for the isolation of the intact lipids with sodium periodate and for the determination of the sugars present after oxidation were described (9). B. Selective Hydrolysis of the Fucolipids. Selective cleavage of the fucose residues for enzymatic degradation and analysis was carried out as follows. Fucose-2,3,4,6-tetraol of the fucolipids was cleaved with 10 ml of 0.1 M aqueous hydrochloric acid in a 150 ml screw cap tube and heated 2 hours at 95°. The hydrolysate was extracted with 3 ml chloroform:methanol 2:1 (v/v) and the lower layer rechromatographed with the system chloroform:methanol:water (65:35:8) over night. The lipids were detected with iodine vapor and the lacto-fettones, ceramide band removed and eluted with chloroform:methanol:water (50:50:1) over night.

3. Portions of this paper (including "Experimental Procedures" and Figs. 1-4) are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 81M-669, cite authors, and include a check or money order for $3.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Lewis Blood Group Fucolipids and Their Isomers

Table I

| Fucolipid type blood group | Number of isolates as sole major fucolipid in the intestine | Number of isolates with other major fucolipids in the intestine |
|----------------------------|-------------------------------------------------------------|---------------------------------------------------------------|
| Dog A                      | 17                                                          | 17                                                            |
| Difucosyl A                | 0                                                          | 6                                                             |
| Lewis b isomer             | 1                                                          | 0                                                             |
| Lewis a isomer             | 0                                                          | 1                                                             |
| Lewis b isomer             | 0                                                          | 1                                                             |
| Lewis b isomer             | 0                                                          | 4                                                             |
| Lewis a isomer             | 0                                                          | 4                                                             |
| Lewis b isomer and difucosyl A | 0                                                          | 3                                                             |
| Lewis b isomer and Lewis a isomer | 0                                                          | 3                                                             |
| Total                      | 51                                                         | 27                                                            |

Human

| Blood group | Number of isolates as sole major fucolipid in the intestine | Number of isolates with other major fucolipids in the intestine |
|-------------|-------------------------------------------------------------|---------------------------------------------------------------|
| A           | 1                                                          | 1                                                             |
| H           | 1                                                          | 1                                                             |
| Lewis a isomer | 6                                                          | 6                                                             |
| Lewis b isomer | 8                                                          | 5                                                             |
| Lewis b isomer and Difucosyl A | 2                                                          | 0                                                             |
| Complex fucolipid | 1                                                          | 0                                                             |
| Total       | 19                                                         | 13                                                            |

RESULTS

Dog Intestinal Lewis a Isomer Fucolipid—This substance was isolated from 8 of 37 dog intestines as a major fucolipid. In 1 intestine, it was the sole major fucolipid; in the other 7, the Leb isomer fucolipid was also present and in 4 of the latter, the difucosyl A glycolipid was present as well (Table I). Results of sugar analysis of the Leb isomer fucolipids indicated that they were pentaglucosyl ceramides containing glucose, galactose, N-acetylgalactosamine, and fucose in a molar ratio of 1:2:1:1, respectively. Of these, only galactose, glucosamine, and small amounts of glucose remained after oxidation of the intact lipids with periodate. The lipids are isomers of both the dog blood group H and human blood group Leb fucolipids but do not have these immunological activities (5).

Five of the 8 isolates have been permethylated and 3 of these have also been permethylated after selective removal of the N-acetylglucosamine. These have also been permethylated after selective removal of the fucose. Typical gas-liquid chromatograms of the partially permethylated Leb isomer fucolipid was also present and in the intact lipids with periodate. The lipids are isomers of both the dog intestine Lewis a isomer and Lewis b isomer fucolipids that were isolated from dog blood group 1:2:1:1, 1:2:1:1, and 1:2:1:4.

Analysis of the ceramide fatty acids and long chain bases of the Leb isomer fucolipids indicated that they were tetraacylceramides. The composition of the ceramides of the Lewis b isomer fucolipids has also been determined by mass spectrometry (11.12). The composition of the Lewis a and b fucolipid ceramides determined by this method is given below.

Phytosphingosine was the predominant long chain base. The analysis of the ceramide fatty acids and long chain bases of the Leb isomer fucolipids indicated that they were tetraacylceramides. The composition of the ceramides of the Lewis b isomer fucolipids has also been determined by mass spectrometry (11.12). The composition of the Lewis a and b fucolipid ceramides determined by this method is given below.
Lewis Blood Group Fucolipids and Their Isomers

Table I
Distribution of the hydroxy fatty acids and long chain bases in dog intestinal fucolipids

| Hydroxy fatty acids | Dog 31 F-1 (Lewis a isomer) | Dog 31 F-2 (Lewis b isomer) |
|---------------------|-----------------------------|-----------------------------|
|                     | %                           | %                           |
| 16:0                | 14                          | 15                          |
| 18:0                | 9                           | 10                          |
| 20:0                | 12                          | 14                          |
| 22:0                | 13                          | 16                          |
| 22:1                | 21                          | 3                           |
| 23:0                | 4                           | 5                           |
| 23:1                | 1                           | 2                           |
| 24:0                | 18                          | 26                          |
| 24:1                | 7                           | 8                           |
| 25:0                | <1                          | <1                          |
| 25:1                | <1                          | 0                           |
| 26:0                | <1                          | <1                          |

Long chain bases

|                      | %                           | %                           |
|----------------------|-----------------------------|-----------------------------|
| t18:0                | 78                          | 38                          |
| d18:0                | 3                           | 2                           |
| d18:1                | 17                          | 58                          |
| t20:0                | 2                           | 2                           |
Lewis Blood Group Fucolipids and Their Isomers

Mass spectrum and simplified formula of the permethylated derlva-
tive of a Lewis a isomer of dog small intestine (Fig 3B). The conditions
of analysis were: electron energy, 50 eV; trap current, 500 μA; ion source-
acceleration voltage, 6 kV; ion source temperature, 340°C; and probe temperature,
215°C. Peaks below m/e 80 were not reproduced.

5.80 ppm (J1.2 = 2.7 Hz). Before reduction, the α-fucose signal
was found at 4.79 ppm (J1.2 = 3.9 Hz). This great change in
chemical shift upon reduction is probably due to a deshielding
effect of nitrogen and is found for anomeric protons of sugars in
position 3 of the glucosamine (17, 20, 21). Therefore, the
down-field α-signal (small coupling constant) should be due
to fucose in position 3 of glucosamine. The resonance shown
close to the glucosamine signal was probably caused by a
nonanomeric proton, possibly H-5 of fucose.

The above data are consistent with the following structure
for the Lewis a isomer dog intestine fucolipid: Gal(β1 → 4)
[Fucα1 → 3]GlcNAc(β1 → 3)Gal(β1 → 4)Glc(β1 → 1)-cer-
amide.

This is a ceramide of lacto-N-fucopentaose III and identical
with that isolated by Yang and Hakomori (6) from human
adenocarcinoma tissue and which they have termed "human
tumor glycolipid."

Dog Intestinal Lewis b Isomer Fucolipid—This glycolipid
is separated from the blood groups H, A, and Lewis a isomer
fucolipids by its lower mobility in preparative thin layer
chromatography with silica gel. It has been isolated from 12
of the 37 dog intestines as a major fucolipid; in 2 intestines as
the sole major fucolipid, in 3 with the Lewis a isomer fucolipid, in 1
with the difucosyl A lipid (13), and in 4 with both Lewis a isomer
and difucosyl A lipids (Table I).

Results of the sugar analysis of the Lewis b isomer fucolipids
clearly show that they are hexaglycosylceramides containing
glucose, galactose, N-acetylglucosamine, and fucose in a molar
ratio of 1:2:1:2, respectively. Treatment of the fucolipids of 3
of the dogs (numbers 13, 16, and 23) with periodate resulted
uniformly in decomposition of all sugars except 1 mol each of
glucosamine and galactose. They are isomers of the human
blood group Lewis b fucolipids and share at least some of the
latter's immunological activity (5).

Several of the isolates have been permethylated including
some with small amounts of the difucosyl A glycolipid contam-
nant. Gas-liquid chromatograms of the partially methylated
alditol acetates resulting from methylation and methylation
after defucosylation are given in Fig. 1B for the dog 48 F-2
glycolipid. A peak corresponding to the 3,6 di-O-methyl-N-
acetylgalactosamine derivative has replaced the 6-O-methyl-N-
acetylgalactosamine derivative of the parent fucolipid. It is clear
that fucose and galactose are linked at the 3 and 4 positions,
respectively, of N-acetylgalactosamine.
Enzyme degradation of the defucosylated glycolipid of dog 16 F2 is shown in Fig. 5. Jack bean β-galactosidase converted the lipid to a product migrating with a standard trihexosyl ceramide; the β-galactosidase and β-N-acetylhexosaminidase together converted it to an apparent monohexosyl ceramide. Incubation with β-galactosidase, then heating to inactive, and incubation of the product with the β-N-acetylhexosaminidase gave a product which migrated with a dihexosyl ceramide. The defucosylated glycolipid was not hydrolyzed by β-galactosidase. These results indicate a Galβ → GlcNAcβ → Glc → ceramide structure.

![Fig. 5. Enzymatic hydrolysis of dog intestine Lewis b isomer fucolipid 16 F2. The fucolipid was pretreated with 0.10 M trichloroacetic acid to remove fucose. The ceramides remaining were divided and treated as follows. A, standards (STD, untreated), upper, ceramide galactoside (Gal-cer); middle, ceramide lactoside (Lac-cer); lower, defucosylated fucolipid; B, incubated with β-galactosidase, then incubated with β-N-acetylhexosaminidase, D, incubated with β-galactosidase, E, untreated, F, fucolipid before trichloroacetic acid treatment. The ceramides remaining in the digests and standards were analyzed in Silica Gel G thin-layer chromatography using the solvent system chloroform/methanol/water, 90:32:7. dF, defucosylated.](image)

Analysis of the ceramide fatty acids and the long chain bases from the fucolipid of dog 31 is given in Table II. All of the fatty acids were hydroxylated and only 25% were below 20 carbons in chain length. Sphingosine (58%) and phytosphingosine was a major long chain base.

Mass spectra of the Leh isomer fucolipid of dog intestine have been published (14) and clearly demonstrate the structure: fucose-hexose-[fucose]-hexosamine-hexose-hexose-ceramide. These spectra also confirm the composition of the ceramide fatty acids and long chain bases given in Table I of the Leh isomer fucolipid of dog 31. Only 2-hydroxy fatty acids were found and phytosphingosine was a major long chain base.

Rabbit antisera directed against dog 23 Lewis b isomer fucolipid reacted on Ouchterlony plates with solutions of this fucolipid and with Lewis b isomer fucolipids from dogs 9, 13, 16, and 31 giving a continuous band around the center well (Fig. 6). This is evidence for the identity of these 5 fucolipids.

NMR spectra were recorded for both permethylated derivative (not reproduced) and permethylated-reduced derivative of the Lewis b isomer fucolipid prepared from dogs 38 and 44 and put together (Fig. 4, spectrum 2). In agreement with the enzyme degradation, 2 β-galactoses and 1 β-glucosamine were concluded (19–21). In addition, a β-glucose signal was found for both derivatives. Two α-resonances typical for the 2 expected fucoses are shown. The fucose in position 2 did change its chemical shift only slightly upon reduction. However, the fucose in position 3 of the glucosamine shown at 5.76 ppm (J1,2 less than 2 Hz) for the reduced derivative (Fig. 4, spectrum 2) had changed from a split signal at 4.77 ppm (J1,2 = 3.2 Hz) and 4.83 ppm (J1,2 = 3.0 Hz) before reduction (not reproduced). This is conclusive for this position (21).

The above data are consistent with the following structure for the Lewis b isomer fucolipid of dog intestine: Fuc(1 → 2)Gal(β1 → 3)[Fuc(1 → 3)]GlcNAc(β1 → 3)Gal(β1 → 4)Glc(β1 → 1)-ceramide which is therefore a ceramide of lacto-N-neofucohexaoside 1. This differs from the human intestinal Lewis b fucolipid only in the linkage positions of galactose and fucose on the N-acetylgalcosamine.

**Human Intestinal Lewis a Fucolipid**—The structure of this fucolipid has been determined (13) on the basis of all of the above criteria except for methylation studies on the defucosylated lipid and NMR spectra. The fucolipid has been isolated from 6 of 16 human small intestines as the only major fucolipid present (Table I). The structure proposed (13), Gal(β1 → 3)[Fuc(1 → 4)]GlcNAc(β1 → 3)Gal(β1 → 4)Glc-ceramide, has been confirmed by methylation studies on these fucolipids (Fig. 1C) and by the NMR spectrum of the permethylated-reduced derivative (Fig. 4, spectrum 3). Compared with the nonreduced derivative (spectrum not shown), a β-resonance has moved down-field which is additional evidence for a Gal(β1 → 3)GlcNAc linkage (20, 21). The β-glucose signal had the typical high-field position (19–21).

**Human Intestinal Lewis b Fucolipid**—This glycolipid was separated from the blood groups H, A, and Lewis a fucolipids by its lower mobility in preparative thin layer silica gel plates, as was the case with the dog intestinal Lewis b isomer fucolipid. It has been isolated from 8 of 19 human intestines as a major fucolipid: in 5 of these as the only major fucolipid, in 2 along with the difucosyl A glycolipid, and in 1 with an uncharacterized high molecular weight fucolipid. All of the lipids of this group were hexaglycosylceramides containing glucose, galactose, N-acetylgalcosamine, and fucose in a molar ratio of 1:2:1:2 and by these criteria were identical with the dog intestinal Lewis b isomer fucolipid. Mass and NMR spectra of the permethylated and permethylated-reduced derivatives,
enzyme degradation studies, and permethylation studies before and after defucosylation all gave data entirely consistent with that of a ceramide of lacto-N-difucohexaose I as proposed in a preliminary note by Hakomori and Andrews (4) for the Lewis b-active glycolipid they isolated from human adenocarcinoma tissue.

**DISCUSSION**

Methylation analyses of 10 dog intestinal mono- and difucosyl glycolipids having fucose linked to N-acetylgalactosamine, 6 fucolipids with blood group H, and 9 with blood group A specificities have shown that galactose is linked exclusively to position 4 of N-acetylgalactosamine. In contrast, analyses of 10 human Lewis intestinal fucolipids, 4 difucosyl A glycolipids, and 1 with blood group H specificity have shown the galactose linked only to position 3 of N-acetylgalactosamine. The NMR spectra of reduced derivatives show characteristic and great differences in chemical shifts for α-fucose and β-galactose, whether in position 3 or position 4 of glucosamine (Fig. 4).

The distribution of the several types of fucolipids isolated from 37 individual dog intestines and 16 individual human intestines is given in Table I. These were all major fucolipids in the sense that 6 to 40 mg were recovered from the dog and 8 to 80 mg from the human small intestine. Most of the intestines contained other fucolipids in very small quantities, usually in unresolved mixtures comprising 2–5 mg of material. There were 2 major unresolved mixtures and 1 complex major fucolipid that has not been characterized. Under these circumstances there are limitations in interpretation of these data in genetic or immunologic terms.

In dog intestines, the most common fucolipids were the blood group A and Lewis b isomer present in about a half and a third of the samples, respectively, whereas the Lewis a and Lewis b predominant in 14 of the 16 human intestines. The latter did not occur together in these adult human intestines as has been reported in human cecal tumors (4). Blood groups A and H fucolipids appear to be less common in human than in canine intestines.

The findings in both dog and human intestine show marked differences in genetic expression between small intestine and red blood cells. In the dog, the ABO and Lewis isomer fucolipids are prominent in intestine but are not present in blood and are not related to canine blood typing. In man, the most common intestinal fucolipids, Le⁺ and Le⁻, do not appear in red blood cells until transferred there from the plasma proteins (22). The most common human erythrocyte fucolipids, A and H, are less common in intestine and are expressed with the type 1 chain, whereas these are nearly all of the type 2 chain in human erythrocytes.

Although no Lewis isomer fucolipids were found in human intestine, their precursor, neolactotetraosyl ceramide, is available to bone marrow, and in *in vitro* experiments, a fucosyl transferase in human blood plasma converted it to Lewis a isomer fucolipid (23). However, these isomers were not identified in a systematic isolation and resolution of human plasma glycolipids in which Lewis a and Lewis b fucolipids were isolated and identified (24, 25). Lewis a isomer has been found in human adenocarcinoma tissue (6) and in normal and Krabbe diseased human brain (26, 27).

**Acknowledgments**—We are indebted to Irmin Pascher for derivatization for mass spectrometry and NMR spectroscopy, to Karl-Erik Falk for NMR analyses, to Deanna F. Lyerly and Walter Johnson for valuable technical assistance, and to the faculty of the Departments of Pathology, Medicine, and Surgery.

**REFERENCES**

1. McKibbin, J. M. (1978) *J. Lipid Res.* 19, 131–147
2. Hakomori, S-I., and Kobata, A. (1974) in *The Antigens* (Sela, M., ed) Vol I, pp 79–140, Academic Press, New York
3. Hakomori, S-I. (1975) *Progr. Biochem. Pharmacol.* 10, 167–196
4. Hakomori, S-I., and Andrews, H. D. (1970) *Biochim. Biophys. Acta* 202, 225–228
5. Hiramoto, R. N., Smith, E. L., Ghanta, V. K., Shaw, J. F., and McKibbin, J. M. (1973) *J. Immunol.* 110, 1037–1043
6. Yang, H-J., and Hakomori, S-I. (1971) *J. Biol. Chem.* 246, 1192–1200
7. Vanier, R. W., Shook, C. P., III, and McKibbin, J. M. (1966) *Biochemistry* 5, 435–445
8. McKibbin, J. M. (1976) in *Glycolipid Methodology* (Witting, L. A., ed) pp 77–95, American Oil Chemists’ Society Press, Champaign, IL
9. Smith, E. L., McKibbin, J. M., Karlsson, K.A., Pascher, I., Samuelsson, B. E., and Li, S-C. (1975) *Biochemistry* 14, 3370–3376
10. Hakomori, S-I. (1964) *J. Biochem. (Tokyo)* 55, 205–208
11. Nakagawa, H., Yamada, T., Chien, J-L., Garidas, A., Kitamikado, M., Li, S-C., and Li, Y-T. (1986) *J. Biol. Chem.* 255, 8595–8599
12. Manson, J. E., Vanier, M. T., and Svennerholm, L. (1978) *J. Neurochem.* 30, 273–275
13. Smith, E. L., McKibbin, J. M., Karlsson, K-A., Pascher, I., Samuelsson, B. E., Li, Y-T., and Li, S-C. (1975) *J. Biol. Chem.* 250, 6059–6064
14. Smith, E. L., McKibbin, J. M., Karlsson, K-A., Pascher, I., and Samuelsson, B. E., (1975) *Biochim. Biophys. Acta* 388, 171–179
15. Li, Y-T., Manson, J-B., Vanier, M-T., and Svennerholm, L. (1973) *J. Biol. Chem.* 248, 2634–2636
16. Karlsson, K. A. (1976) in *Glycolipid Methodology* (Witting, L. A., ed) pp 97–122, American Oil Chemists’ Society Press, Champaign, IL
17. Karlsson, K. A. (1975) *Progr. Chem. Fats Other Lipids* 16, 207–230
18. Karlsson, K. A. (1974) *Biochemistry* 13, 3643–3647
19. Falk, K. E., Karlsson, K. A., and Samuelsson, B. E. (1979) *Arch. Biochem. Biophys.* 192, 164–176
20. Falk, K. E., Karlsson, K. A., and Samuelsson, B. E. (1979) *Arch. Biochem. Biophys.* 192, 177–190
21. Falk, K. E., Karlsson, K. A., and Samuelsson, B. E. (1979) *Arch. Biochem. Biophys.* 192, 191–202
22. Marcus, D. M. and Cass, L. E. (1969) *Science* 164, 553–555
23. Paczkusa, T., and Koscielak, J. (1979) *Eur. J. Biochem.* 64, 498–506
24. Hanfland, F. (1978) *Eur. J. Biochem.* 87, 161–170
25. Hanfland, F., Kladetzky, R. G., and Egli, H. (1978) *Chem. Phys. Lipids* 22, 141–145
26. Vanier, M. T., Manson, J. E., and Svennerholm, L. (1980) *FEBS Lett.* 112, 70–72
27. Svennerholm, L., Vanier, M. T., and Manson, J. E. (1980) *J. Lipid Res.* 21, 53–64
Lewis blood group fucolipids and their isomers from human and canine intestine.
J M McKibbin, W A Spencer, E L Smith, J E Mansson, K A Karlsson, B E Samuelsson, Y T Li and S C Li

J. Biol. Chem. 1982, 257:755-760.

Access the most updated version of this article at http://www.jbc.org/content/257/2/755

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/257/2/755.full.html#ref-list-1