*Carbohydrate Research*, 151 (1986) 213–223
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

# MOUSE LIVER GANGLIOSIDES*,†

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(Received September 6th, 1985; accepted for publication in revised form, November 20th, 1985)

## ABSTRACT

The major gangliosides from mouse liver were purified and characterized by t.l.c., g.l.c., sialidase treatment, and a methylation study. GM3(NeuAc), GM3(NeuGc), GM2(NeuGc), GM1(NeuGc), and GDla(NeuGc, NeuGc) were identified. The structural identification of three of the gangliosides, GM2(NeuGc), GM1(NeuGc), and GDla(NeuGc, NeuGc), was supported by the results of $^1$H-n.m.r. analysis, and the structures of GM3(NeuGc), GM2(NeuGc), and GM1(NeuGc) were further confirmed by negative-ion fast-atom bombardment mass spectrometry. Ganglioside mapping showed that there was polymorphic variation of gangliosides in the liver of inbred strains of mice and that the major gangliosides were GM3(NeuGc) in WHT/Ht, GM2(NeuGc) in BALB/c and C3H/He, and GM2(NeuGc), GM1(NeuGc), and GDla(NeuGc, NeuGc) in ICR mice. Gangliosides containing *N*-acetylneuraminic acid, except for GM3(NeuAc), were not detected as major gangliosides in the strains of mice we analyzed.

## INTRODUCTION

The polymorphic variation of glycosphingolipids as blood group active substances on human erythrocytes has been extensively reported[2–4], and the variation is considered to be controlled genetically through the regulation of the levels of glycosyltransferase activities[5]. In the course of glycolipid analysis of various mammalian organs, we found similar polymorphic variation of gangliosides in mouse liver[6–9] as well as in erythrocytes of dog[10], horse[11], cattle[11], cat[12], and mouse[13,14].

We have shown that there are at least three polymorphic types of liver gangliosides in mice[7]. The first type has GM3(NeuGc), but not gangliosides longer than GM3 as the major component. The second has GM2(NeuGc), but not GM1(NeuGc) and GDla(NeuGc, NeuGc). The third has GM2(NeuGc),

*Dedicated to Roger W. Jeanloz.

†Abbreviations: The nomenclature for the gangliosides is based on the system of Svennerholm[1]. Sialic acid species of gangliosides are indicated in parentheses. GDla(NeuGc, NeuGc) denotes IV$^3$NeuGc-, II$^3$NeuGc-GgOse$_4$Cer, and GDla(NeuGc, NeuAc), IV$^3$NeuGc-, II$^3$NeuAc-GgOse$_4$-Cer.

0008-6215/86/$ 03.50 

GM1(NeuGc), and GDla(NeuGc, NeuGc). By a mating experiment between strains of the first and second types, we proved that GM2(NeuGc) expression is controlled as a dominant trait by an autosomal single gene that regulates the level of activity of *N*-acetylgalactosaminyltransferase to synthesize GM2(NeuGc) from GM3(NeuGc)[15]. We also proved[7] that GM1(NeuGc) expression is controlled by the single gene located at the centromeric side just outside the H-2 complex on mouse chromosome 17. We then confirmed that GM1(NeuGc) expression is regulated by the level of activity of galactosyltransferase that synthesizes GM1(NeuGc) from GM2(NeuGc)[16]. These studies were based on the structures we tentatively proposed for the major liver gangliosides[7].

Ghidoni *et al.*[17] independently reported strain differences in liver gangliosides among inbred strains of mice, as well as individual differences in a closed colony line. Recently, Ghidoni *et al.*[18,19] reported the results of a study of ganglioside metabolism using an experimental system involving mouse liver and radiolabelled GM1(NeuAc). In these studies, the gangliosides they identified were GM2(NeuAc), GM1(NeuAc), and GDla(NeuGc, NeuAc) in addition to GM3(NeuAc), GM3(NeuGc), and GM2(NeuGc). In contrast to our findings, GM2(NeuAc), GM1(NeuAc), and GDla(NeuGc, NeuAc) were reported as the major gangliosides.

With this background, we report here the precise identification of six major gangliosides purified from mouse liver and discuss the discrepancy between the results obtained by Ghidoni's group[17] and ours.

## MATERIALS AND METHODS

*Mice.* — The WHT/Ht strain was maintained in our institute, the DBA/2NCrj, BALB/cAnCrj, C3H/HeNCrj, and Crj:CD-1(ICR) strains were purchased from Charles River Japan Co. Ltd., and the C57BL/10Snslc strain was from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka. Mice aged 7–12 weeks were used for the analysis.

*Purification of the major liver gangliosides.* — ICR liver (1100 g) was used for the purification of gangliosides A, B, C, and D, WHT/Ht liver (60 g) for that of ganglioside A′, and DBA/2 liver (141 g) for that of ganglioside B′. The livers were successively extracted with 20 vol. each of chloroform–methanol mixtures (2:1, 1:1, and 1:2, v/v). The purification procedures for the six gangliosides were essentially the same, consisting of acetone precipitation, Folch's partitioning, alkaline hydrolysis, dialysis, and column chromatography on DEAE-Sephadex and Iatrobeads as described previously[14,20]. The yields of purified gangliosides A, A′, B, B′, C, and D were 24, 13, 118, 50, 105, and 28 mg, respectively. These purified gangliosides were used for the component analysis. For fast-atom bombardment mass spectrometry (f.a.b.–m.s.), gangliosides A, B, and C were further purified by h.p.l.c. on an ODS column (3 mm i.d. × 30 cm) with methanol–water mixtures as elution solvents according to the method of Handa and Kushi[21]. Gangliosides B, C, and D, thus purified, were also subjected to $^1$H-n.m.r. analysis.

*Methods for structural analysis.* — After methanolysis with 0.8M HCl–methanol for 18 h at 80°, the carbohydrate composition was determined by g.l.c. of the trimethylsilylated methyl glycosides[22]. Sialic acid species were analyzed by the g.l.c. method of Yu and Ledeen[23]. Fatty acid methyl esters were recovered with hexane after the methanolysis for carbohydrate analysis and analyzed by g.l.c.[20]. Sphingosine bases were analyzed by the method of Ando and Yu[24].

*Permethylation studies.* — The purified gangliosides A, A′, B, B′, C, and D (~1 mg each) were permethylated by the method of Hakomori[25] as slightly modified by Kundu *et al.*[26], and then hydrolyzed[20] in 80% acetic acid containing 0.7M HCl for 18 h at 70°. After reduction and acetylation[25], the partially *O*-methylated alditol acetates were analyzed by g.l.c. on a capillary column of crosslinked 5% phenylmethylsilicone (0.31 mm i.d. × 25 m).

*Sialidase treatment.* — The purified gangliosides (~20 μg as sialic acid) were incubated in 50mM acetate buffer at pH 5.5 with 0.05 units of sialidase (type VI, Sigma Chemical Co., St. Louis) for 24 h. After the incubation, the products were desalted on a C18-Sep Pak cartridge (Waters Associates, Inc., Milford)[27] and then analyzed by t.l.c. with 55:45:4:6 (v/v) chloroform–methanol–5M ammonium hydroxide–0.4% aqueous $CaCl_2$ for development.

*Mass spectrometry.* — The partially methylated alditol acetates were separated by g.l.c. as already described and the peaks were confirmed by the mass spectra obtained with a Shimadzu GCMS-QP1000 mass spectrometer. Negative-ion fast-atom bombardment mass spectrometry (f.a.b.–m.s.) of intact gangliosides A, B, and C was performed with a JEOL JMS-DX303 mass spectrometer equipped with a JMA-DA5000 computer system with triethanolamine as the matrix. The accelerating voltage was 2.1 kV and the primary beam for the bombardment was 3.0 kV $Xe^0$.

*Proton nuclear magnetic resonance spectroscopy.* — The purified gangliosides B, C, and D were analyzed in 0.4 mL of dimethyl sulfoxide-$d_6$ containing 2% $D_2O$ and 1% tetramethylsilane with a JEOL 400MHz JNM-GX400 n.m.r. spectrometer at 24°.

*Ganglioside mapping.* — A mapping procedure that is a slight modification of the method of Iwamori and Nagai[28] was developed by introducing h.p.l.c. on a DEAE-silica column, as reported previously[20]. The injected samples were lipids obtained by lyophilization of the dialyzate after alkaline hydrolysis performed as described for the purification. The concentration of ammonium acetate in methanol was programmed for gradient elution as follows; zero for the first 20 min and then linearly increased to 0.02M at 70 min, to 0.04M at 80 min, to 0.08M at 190 min, and finally to 0.12M at 210 min. The flow rate was 1.5 mL/min and 3-mL fractions were collected. The gangliosides in an aliquot of each fraction were applied to a t.l.c. plate that was developed with 55:50:4:6 (v/v) chloroform–methanol–5M ammonium hydroxide–0.4% aqueous $CaCl_2$.

*Thin-layer chromatography.* — The homogeneity of the purified gangliosides was determined by on t.l.c. plates developed with either 55:45:10 (v/v) chloroform–

methanol–0.2% aqueous $CaCl_2$ or 60:40:4:6 (v/v) chloroform–methanol–5M ammonium hydroxide–0.4% aqueous $CaCl_2$. The gangliosides were detected by spraying with the resorcinol reagent and heating the plates at 95°. Neutral glycolipids were detected by spraying orcinol–sulfuric acid reagent and heating the plates at 110°. The following gangliosides were used as references; a mixture of bovine brain gangliosides, GM3(NeuAc) purified from dog erythrocytes[29], GM3(NeuGc) from horse erythrocytes[29], GM2(NeuAc) from the brain of a case of Tay–Sach's disease[30], GM2(NeuGc) from C3H/He mouse erythrocytes[11], lactosylceramide[2] and sialylneolactotetraosylceramide(NeuAc)[2] from human erythrocytes, GDla(NeuAc, NeuAc) from bovine brain, and GDla(NeuAc, NeuGc) and GDla(NeuGc, NeuGc) from bovine adrenal gland[22].

## RESULTS

*T.l.c. of the purified gangliosides.* — As shown in Fig. 1, the purified gangliosides A, A′, B, B′, C, and D were homogenous on t.l.c. developed with either the neutral solvent system or the solvent system containing ammonia. The results obtained with both solvent systems confirmed that gangliosides A and A′ comigrated with the reference GM3(NeuGc), B and B′ with the reference GM2(NeuGc), and D with GDla(NeuGc, NeuGc). Ganglioside C comigrated with the reference GM1(NeuAc) on t.l.c. developed with the neutral solvent system but migrated much slower than GM1(NeuAc) on t.l.c. developed with the solvent system containing ammonia. This behavior is characteristic of a ganglioside containing *N*-glycolylneuraminic acid. As shown in Fig. 1(B), the molecular species of gangliosides with different sialic acid components are clearly separated from each other with the solvent system containing ammonia, and the results indicate that gangliosides A, A′, B, B′, C, and D do not contain detectable amounts of the corresponding molecular species of gangliosides containing *N*-acetylneuraminic acid. As to GDla, the reference GDlas were clearly separated into molecular species according to the different sialic acid components. This indicates that the liver GDla contained 2 mol of *N*-glycolylneuraminic acid and not one mol each of *N*-acetyl- and *N*-glycolyl-neuraminic acid.

*Carbohydrate structures of gangliosides A, A′, B, B′, C, and D.* — The carbohydrate compositions of the six gangliosides are listed in Table I, and the results of the methylation study are presented in Table II.

Gangliosides A from ICR and A′ from WHT/Ht contained galactose, glucose, and *N*-glycolylneuraminic acid in the molar ratio of 1:1:1, and sialidase treatment produced a neutral glycolipid that comigrated with lactosylceramide. These results, together with the results of the methylation study, indicate that gangliosides A and A′ are GM3(NeuGc).

Gangliosides B from ICR and B′ from DBA/2 contained galactose, *N*-acetylgalactosamine, glucose and *N*-glycolylneuraminic acid in the molar ratio of 1:1:1:1, and the sialic acid of these gangliosides was resistant to sialidase. These results and

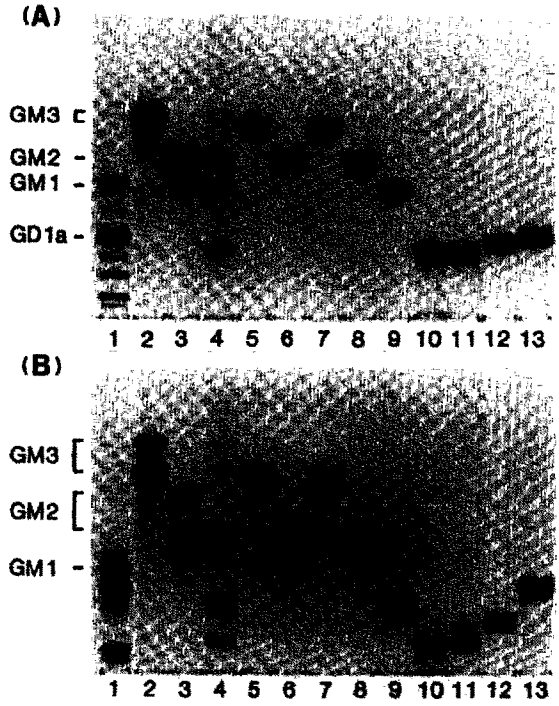


Fig. 1. Thin-layer chromatograms of the purified gangliosides from mouse liver. Lane 5, ganglioside A′ from WHT/Ht liver; lane 6, ganglioside B′ from DBA/2 liver; lanes 7, 8, 9, and 10, gangliosides A, B, C, and D from ICR liver, respectively. The other lanes contained reference gangliosides: lane 1, a mixture of bovine brain gangliosides; lane 2, GM3(NeuAc) and GM3(NeuGc) from top to bottom; lane 3, GM2(NeuAc) and GM2(NeuGc) with double bands, from top to bottom; lane 4, total gangliosides from ICR liver; lane 11, GD1a(NeuGc, NeuGc); lane 12, GD1a(NeuAc, NeuGc); and lane 13, GD1a(NeuAc, NeuAc). Solvent systems: (*A*) chloroform–methanol–0.2% aqueous $CaCl_2$ (55:45:10, v/v), and (*B*) chloroform–methanol–5M ammonium hydroxide–0.4% aqueous $CaCl_2$ (60:40:4:6, v/v). The bands were detected with resorcinol reagent.

TABLE I

CARBOHYDRATE COMPOSITIONS [a] OF THE LIVER GANGLIOSIDES

| *Component sugars* | *Ganglioside* | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *A′* | *B* | *B′* | *C* | *D* |
| Galactose | 0.98 | 1.00 | 1.00 | 1.05 | 2.00 | 1.98 |
| Glucose | 1 | 1 | 1 | 1 | 1 | 1 |
| *N*-Acetylgalactosamine | – | – | 1.16 | 1.14 | 1.03 | 1.16 |
| *N*-Glycolylneuraminic acid | 1.15 | 0.98 | 1.11 | 1.05 | 1.16 | 2.42 |
| *N*-Acetylneuraminic acid | – | – | – | – | – | – |

[a]Expressed as molar ratio relative to glucose as 1; –: not detectable.

TABLE II

COMPOSITIONS OF PARTIALLY METHYLATED ALDITOL ACETATES[a]

| *Methylated alditol acetate* | *Ganglioside* | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *A'* | *B* | *B'* | *C* | *D* |
| 1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol | – | – | – | – | 0.98 | — |
| 1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol | 0.84 | 0.96 | – | – | – | 1.01 |
| 1,3,4,5-Tetra-*O*-acetyl-2,6-di-*O*-methylgalactitol | – | – | 1.03 | 1.08 | 0.99 | 1.00 |
| 1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol | 1 | 1 | 1 | 1 | 1 | 1 |
| 1,5-Di-*O*-acetyl-3,4,6-tri-*O*-methyl-2-deoxy-2-*N*-(methylacetamido)galactitol | – | – | + | + | – | – |
| 1,3,5-Tri-*O*-acetyl-4,6-di-*O*-methyl-2-deoxy-2-*N*-(methylacetamido)galactitol | – | – | – | – | + | + |

[a]Expressed as molar ratios relative to 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol as 1; +: detected at the molar ratio of 0.1–0.3; –: not detectable.

the results of the methylation study indicate that gangliosides B and B' are GM2(NeuGc).

Ganglioside C contained galactose, *N*-acetylgalactosamine, glucose, and *N*-glycolylneuraminic acid in the molar ratio of 2:1:1:1, and the ganglioside was resistant to sialidase. These results, together with the results of the methylation study, indicate that ganglioside C is GM1(NeuGc).

Ganglioside D contained galactose, *N*-acetylgalactosamine, glucose, and *N*-glycolylneuraminic acid in the molar ratio of 2:1:1:2 and it was converted into a ganglioside that comigrated with ganglioside C on t.l.c. following treatment with neuraminidase. These results, together with the results of the methylation study, indicate that ganglioside D is GDla(NeuGc, NeuGc).

*Compositions of the hydrophobic portions of gangliosides A, A', B, B', C, and D.* — The fatty acid compositions and sphingosine bases are presented in Table III. The sphingosine base is predominantly C18 sphingenine in all of the six gangliosides. The major fatty acids are C22:0, C23:0, C24:0, and C24:1 in all of the gangliosides and ganglioside D also contained C18:0. These results indicate that the hydrophobic portions of the six gangliosides are quite similar and that gangliosides A, B, C, and D might have a precursor and product relationship.

*Negative-ion f.a.b.–m.s. of the gangliosides.* — The molecular weights of gangliosides A, B, and C determined from the spectra were quite consistent with the values calculated from the proposed structures in this study. The major molecular species of ganglioside A in the form of $[M - H]^-$ were 1251, 1265, 1277, and 1279, which indicate GM3(NeuGc) containing C22:0 fatty acid–C18 spingenine, C23:0 fatty acid–C18 sphingenine, C24:1 fatty acid–C18 sphingenine, and C24:0 fatty acid–C18 sphingenine, respectively. Those of ganglioside B were 1454, 1468, 1480 and 1482, indicating GM2(NeuGc) with C22:0–C18, C23:0–C18, C24:1–C18, and C24:0–C18, respectively. Those of ganglioside C were 1616, 1630,

TABLE III

COMPOSITIONS[a] OF THE HYDROPHOBIC PORTIONS

| *Fatty acids* | *Ganglioside* | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *A'* | *B* | *B'* | *C* | *D* |
| 16:0 | 8 | 2 | 2 | 4 | 2 | 6 |
| 18:0 | 10 | 3 | 3 | 11 | 2 | 21 |
| 20:0 | 5 | 3 | 3 | 2 | 4 | 3 |
| 22:0 | 27 | 39 | 29 | 23 | 28 | 29 |
| 23:0 | 12 | 16 | 15 | 14 | 15 | 11 |
| 24:0 | 19 | 27 | 22 | 19 | 27 | 17 |
| 24:1 | 19 | 10 | 26 | 27 | 22 | 13 |
| Sphingosines | | | | | | |
| 16 sphingenine | tr | tr | tr | tr | tr | tr |
| 18 sphingenine | 100 | 100 | 100 | 100 | 100 | 100 |
| 18 sphinganine | tr | tr | tr | tr | tr | tr |

[a]Expressed as percentages; tr: trace amount.

1642, and 1644, indicating GM1(NeuGc) with C22:0–C18, C23:0–C18, C24:1–C18, and C24:0–C18, respectively.

*$^1$H-N.m.r. analysis of the gangliosides.* — The resonance regions for anomeric protons in the n.m.r. spectra of gangliosides B, C, and D are shown in Fig. 2. In these spectra, the anomeric-proton (H-1) signals of *N*-acetylgalactosamine of gangliosides B (4.56 p.p.m.), C (4.63 p.p.m.), and D (4.76 p.p.m.) were shifted upfield, compared with those of the corresponding gangliosides containing *N*-acetylneuraminic acid, that is, GM2(NeuAc) 4.79 p.p.m., GM1(NeuAc) 4.86 p.p.m., and GDla(NeuAc, NeuAc) 4.85 p.p.m.[31,32]. This phenomenon might be explained by the possibility that the anomeric proton of *N*-acetylgalactosamine is affected differently by the *N*-glycolyl group of the neuraminic acid. Other anomeric protons were assigned as follows: glucose at 4.15 p.p.m. and galactose at 4.30 for ganglioside B, glucose at 4.15 p.p.m., the internal galactose at 4.29 p.p.m., and the terminal galactose at 4.19 p.p.m. for ganglioside C, and glucose at 4.14 p.p.m., the internal galactose at 4.26 p.p.m., and the terminal galactose at 4.24 p.p.m. for ganglioside D. These values are quite comparable to those for GM2(NeuAc), GM1(NeuAc), and GDla(NeuAc, NeuAc) reported by Gasa *et al.*[31] and Koerner *et al.*[32]. All of these anomeric protons show coupling constants of 7–9 Hz, indicating that the sugars have the $\beta$ configuration. These n.m.r. results support the structural identification of gangliosides B, C, and D described here.

*Ganglioside mapping of mouse liver.* — The mappings are presented in Fig. 3 to confirm the ganglioside compositions in view of the complete identification of the ganglioside structures already described. WHT/Ht liver contained GM3(NeuGc) as a major ganglioside with a small amount of GM3(NeuAc), and also very minor monosialogangliosides longer than GM3(NeuGc). BALB/c liver

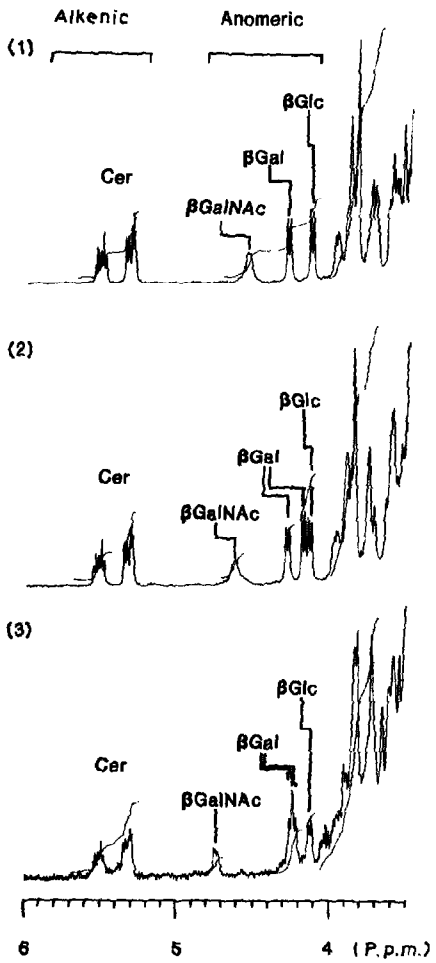


Fig. 2. $^1$H-N.m.r. spectra of the purified gangliosides. (1) ganglioside B, (2) ganglioside C, and (3) ganglioside D.

contained GM2(NeuGc) as the major ganglioside with detectable amounts of GM3(NeuAc), GM3(NeuGc) and GM1(NeuGc), and an unknown component that was eluted just before the disialogangliosides. However, no detectable amounts of GM2(NeuAc) and GM1(NeuAc) were found. C3H/He liver contained GM2(NeuGc) as the major ganglioside and small amounts of GM3(NeuAc), GM3(NeuGc), and GM1(NeuGc) and certainly no detectable amounts of GM2(NeuAc) and GM1(NeuAc). ICR liver contained GM2(NeuGc), GM1(NeuGc), and GD1a(NeuGc, NeuGc) as the major gangliosides with small amounts of GM3(NeuAc) and GM3(NeuGc). In this case also, GM2(NeuAc), GM1(NeuAc), and GD1a(NeuGc, NeuAc) or (NeuAc, NeuGc) were not detectable.

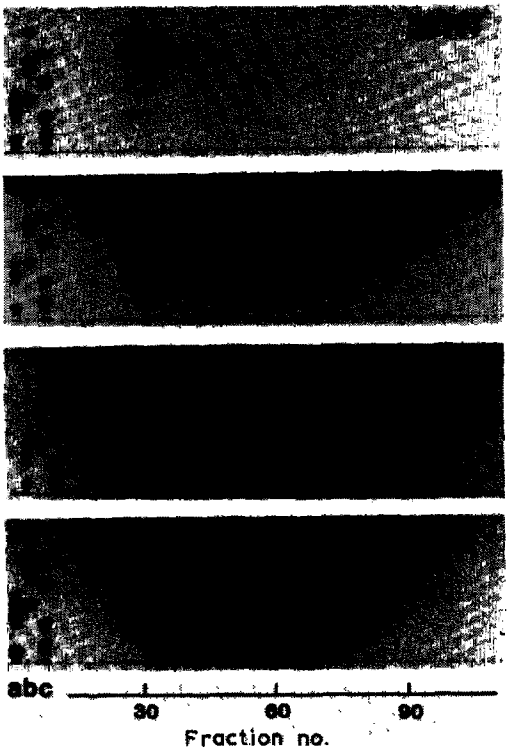


Fig. 3. Ganglioside mapping of WHT/Ht, BALB/c, C3H/He and ICR liver. The reference gangliosides in lanes a, b, and c were as follows: a, a mixture of bovine brain gangliosides; b, GM3(NeuAc), GM2(NeuAc), sialylneolactotetraosylceramide(NeuAc), and GM1(NeuAc), from top to bottom; and c, GM3(NeuGc), GM2(NeuGc), GM1(NeuGc), and GD1a(NeuGc, NeuGc), from top to bottom. The solvent system was chloroform–methanol–5M ammonium hydroxide–0.4% aqueous $CaCl_2$ (55:50:4:6, v/v). The bands were detected with resorcinol reagent. Monosialogangliosides are eluted around fraction number 30 and disialogangliosides around fraction number 60. The other conditions for h.p.l.c. are given in the text.

## DISCUSSION

We present this paper for the following two reasons. One is that our previous studies were based on the chemical structures we tentatively proposed, and correct characterization is absolutely necessary to confirm our previous results[6–9,13–16]. The other reason is that Ghidoni *et al.*[17] reported different results for the structures of GM2, GM1, and GDla. They reported that the sialic acid species of GM2 purified from Balb/cAnNCrlBR mice was a mixture of *N*-acetyl- and *N*-glycolyl-neuraminic acid in a ratio of 2:3. GM1 purified from C3H/HeNCrlBR mice was found to carry *N*-acetylneuraminic acid exclusively, and also GDla purified from DBA/2NCrlBR mice was found to carry *N*-glycolylneuraminic acid at the terminal galactose and *N*-acetylneuraminic acid at the internal galactose.

In this study, we demonstrated that the gangliosides purified from ICR liver were GM3(NeuAc), GM3(NeuGc), GM2(NeuGc), GM1(NeuGc), and GDla(NeuGc, NeuGc), and those from WHT/Ht and DBA/2 liver were GM3(NeuGc) and GM2(NeuGc), respectively. Gangliosides containing *N*-acetylneuraminic acid, such as GM2(NeuAc), GM1(NeuAc), and GDla(NeuGc, NeuAc), were not detected as major gangliosides.

There are at least two possible explanations for the discrepancy between the results obtained by Ghidoni *et al.*[17] and by us. First, if both sets of results are correct, we must consider the strain differences, e.g., between Balb/cAnNCrlBR and BALB/cAnCrj, C3H/HeNCrlBr and C3H/HeNCrj, DBA/2NCrlBR and DBA/2NCrj, and Crl:CD$^{R}$-l(CR)BR and Crj:CD-l(ICR) mice; this reasoning gives us a working hypothesis that the differences in the sialic acid species of GM2, GM1, and GDla between these inbred strains provide a good model system, which can be used to prove the mode of genetic control of the expression of *N*-glycolylneuraminic acid in these gangliosides. The second possibility is that the identification of the sialic acid species by Ghidoni's group[17] may not be correct, because they did not use a solvent system that can separate gangliosides with different molecular species of sialic acid from each other. The procedure used for the identification of sialic acid species is not clearly described in their paper, but seems to employ g.l.c. analysis of free sialic acids released on mild acid hydrolysis or sialidase treatment. At present we think that the g.l.c. method of Yu and Ledeen[23] is the simplest and most reproducible means of determining whether the sialic acid is *N*-acetyl- or *N*-glycolyl-neuraminic acid.

Ghidoni's group studied the metabolism of gangliosides by injecting radiolabelled GM1(NeuAc) into mice and analyzing the radioactive metabolites in the ganglioside fraction obtained from the liver[18,19]. We have postulated that the main biosynthetic pathway for mouse liver gangliosides is as follows: GM3(NeuAc) → GM3(NeuGc) → GM2(NeuGc) → GM1(NeuGc) → GDla(NeuGc, NeuGc). However, experiments are needed to determine the substrate specificities of monoxygenases, sialyltransferases, and other glycosyltransferases involved in ganglioside synthesis in the liver. The *in vivo* experiments performed by Ghidoni *et al.* are a good approach for this purpose.

The correct understanding of the chemical structures of the gangliosides is necessary for the further investigation of ganglioside metabolism and the control mechanisms for ganglioside expression.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan, and Special Coordination Funds for Promoting Science and Technology from the Science and Technology Agency of Japan.

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