A monosialosyl ganglioside, sialosylpentaglycosylceramide, has been isolated from chicken pectoral muscle. This ganglioside is the first to be found which contains the glycosphingolipid structure of the globo series. It was extracted from tissue with a mixture of tetrahydrofuran and aqueous KCl and purified by ion exchange and silicic acid chromatography. Enzymic hydrolysis utilizing specific glycosidases and methylation technique were employed to establish the structure which is proposed to be: NeuAca2 \( \alpha \) Gal\( \beta \)1. The long chain base was predominantly sphingosine and the fatty acids present were mainly stearate and oleate.

Although approximately 40 ganglioside structures have been defined in vertebrate tissues none has been found containing the core oligosaccharide structure of the globo series (1). In our studies of the glycosphingolipids of chicken skeletal muscle (2-4), a resorcinol-positive compound was resolved whose Rf on TLC in three different solvent systems differed from the other gangliosides found which were of the lactosylceramide and ganglio series. Here we report the detailed structure of a monosialosylpentaglycosylceramide purified from chicken skeletal muscle which contains a novel neutral core oligosaccharide chain incorporating the globo sequence (globo series).

**EXPERIMENTAL PROCEDURES**

**Materials**—Pectoral muscles from adult leghorn chickens were obtained from a local supermarket or dissected from an inbred strain of chickens after death. Extraneous tissues were separated by gross dissection and the muscle ground and stored at \(-60^\circ\) C. DEAE-Sephadex A-50, N-acetyl- and N-glycolylineuraminic acids, and \(\alpha\)-neuraminidase type IV were obtained from Sigma. Sphingosine, dihydroxyphenosine, and fatty acid methyl esters were products of Supelco, Inc., as were GC packings of 10% DEGS-PS, 3% SP-2340, 3% OV-17, and 3% SE-30 (all on Supelcoport support). Sephadex LH-20 was purchased from Pharmacia and precoated silica gel plates (Silica Gel 60) were from Brinkmann. Bio-Sil A was obtained from Bio-Rad. Ganglioside standards from human brain and neutral gangliosides were prepared in our laboratory, \(\beta\)-Galactosidase and \(\alpha\)-hexosaminidase were purified from jack bean meal (5, 6) and \(\alpha\)-galactosidase from fig powder (7).

**Glycolipid Extraction**—One kilogram of ground muscle was homogenized in 10 volumes of tetrahydrofuran:0.01 M KCl (4:1, v/v) using a Waring blender. The mixture was stirred for 3 h, then filtered and the residue extracted twice with 5 volumes of tetrahydrofuran:0.01 M KCl (8:1, v/v). The combined filtrates were concentrated in a rotary evaporator to a syrup; then 1 liter of chloroform:methanol (2:1, v/v) was added followed by 200 ml of H\(\text{2}O\) (8) in order to separate the gangliosides by partition. The lower layer was reextracted twice with theoretic upper phase containing 0.027% KCl. The combined upper layers were concentrated to dryness, dissolved in water, and dialyzed at 4 °C against four changes of distilled water.

**Ion Exchange Chromatography**—DEAE-Sephadex A-50 (C form) was converted to the acetate as previously described (9). The crude ganglioside fraction obtained after dialysis was taken to dryness in a rotary evaporator and dissolved in a small volume of chloroform:methanol (2:1, v/v) for application to the column. The neutral lipids including neutral glycolipids were eluted with methanol and the gangliosides were eluted with 5 volumes of methanol containing in succession 0.01, 0.02, and 0.2 M sodium acetate. The 0.01 M sodium acetate effluent contained only monosialogangliosides including the unusual galactosamino-containing ganglioside. Each of the fractions was concentrated to dryness and salt removed by dialysis.

**Silice Acid Chromatography**—Bio-SiA was activated overnight at 110 °C, allowed to cool, suspended in chloroform, and packed into a glass column (\(1.5 \times 45 \) cm) using a vibrator at full speed. The ganglioside fraction eluted by 0.01 M sodium acetate was dissolved in a small volume of chloroform:methanol (2:1, v/v) and applied to the column. Gangliosides were eluted first with 400 ml of solvent A (chloroform:methanol:H\(\text{2}O\), 130:70:12, v/v/v) and then with 500 ml of solvent B (chloroform:methanol:H\(\text{2}O\), 120:70:14, v/v/v). Collection volumes were 6 ml of which 50-ml aliquots from alternate tubes were taken to identify the gangliosides by TLC.

**Enzymic Sequence Analysis of the Oligosaccharide Chain**—The saccharide chain sequence of glycosyl residues and the anomeric configurations were determined by incubation of the purified ganglioside with several specific glycosidases as previously described (9). Sialic acid elimination was effected with 40 milliunits of \(\alpha\)-neuraminidase from Clostridium perfringens (1 unit of enzyme will release 1 pmol of NeuAc from bovine submaxillary mucin/min at 37 °C and pH 5.0) incubated overnight at 37 °C in a reaction mixture containing 30 \(\mu\)g of ganglioside in 200 \(\mu\)l of 0.05 M sodium acetate buffer at pH 5.0. The reaction was stopped by addition of 1 ml of chloroform:methanol (2:1), and the asialoglycolipid was recovered from the lower layer. The hydrolysis of neutral glycosphingolipids was done with \(\beta\)-galactosidase, \(\beta\)-hexosaminidase, and \(\alpha\)-galactosidase according to the procedures of Chien et al. (9). The purified glycolipid (500 \(\mu\)g) was methylated according to the procedure of Yang and Hakomori (10) and further purified on a column of Sephadex LH-20 before hydrolysis with 0.6 M H\(\text{2}SO\) in 80% aqueous CH\(\text{3}COOH\) and acetylated according to the method of Bjornrad et al. (11). The partially methylated alditol acetate derivatives were analyzed on a Finnigan Model 3300 gas chromatograph-mass spectrometer. The partially methylated glucitol and galactitol acetates were separated on 3% SP 2340 at 180 °C and the amino sugar derivative on 3% OV 17 with programmed temperature rise from 180-200 °C (2 °C/min) (12-14).

**Other Methods**—Fatty acid methyl esters were extracted with hexane after methanolysis (1.5 M anhydrous methanolic H\(\text{2}O\), 80 °C, 24 h) and analyzed on a 10% DEGS column at 190 °C. Long chain bases were determined as trimethylsilyl derivatives on a 3% SE-30 column (15) after hydrolysis (16). Sialic acids were quantitated according to the method of Svennerholm (17) as modified by Miettinen and Takki-Luukainen (18). N-Acetyl- and N-glycolylineuraminic acids (19) and neutral and amino sugars (11) were determined by gas chromatography.
RESULTS

Thin Layer Chromatography of the Purified Ganglioside—
The novel ganglioside had an Rf close to GD3 (Fig. 1A) in a neutral solvent system containing calcium (chloroform:methanol:0.25% CaCl2, 60:40:9) but a lesser Rf in alkaline conditions (chloroform:methanol:0.25 M NH4OH, 60:40:9) where it migrated slightly behind GD1a (Fig. 1B) from human brain. The best resolution of this ganglioside was achieved with tetrahydrofuran:0.1% KCl (7:3:15). In this solvent system (Fig. 1C) the novel ganglioside could be clearly separated from GD3 and GD1a and had the highest Rf of the three solvents.

Chemical Composition—The acyl groups of the ganglioside were mainly palmitic, stearic, and oleic acids (Table I). Sphingosine was the major long chain base and less than 15% was dihydrodihydrosphingosine. The ganglioside contained N-acetylgalactosamine, galactose, galactosamine, glucose, and sphingosine in a molar ratio of 1.01:3.12:0.95:1.00:0.98.

Hydrolysis of Saccharide Sequence by Specific Glycosidases—The purified ganglioside was not degraded by β-hexosaminidase, β-hexosaminidase, or α-galactosidase. When incubated with α-neuraminidase in the absence of detergent, it was hydrolyzed to a neutral glycosphingolipid with a Rf close to that of the pentaglycosylceramide purified from bovine erythrocytes (Lane 3, Fig. 2). Subsequent incubation with β-galactosidase converted it to a compound with the migration of globoside (Lane 4, Fig. 2). Further treatment of the glycosphingolipid with β-hexosaminidase, α-galactosidase, and β-galactosidase yielded tri-, di-, and monohexosylceramides (Fig. 2).

Linkages between Saccharide Units—The sites of the linkages between the saccharide units have been established by identification of the partially methylated alditol acetates by gas chromatograph-mass spectrometer. Three peaks (Fig. 3a) were obtained upon analysis of derivatives from this ganglioside and their mass spectra were those of 2,4,6-tri-O-methylgalactitol-1,3,5-triacetate (Peak 1, Fig. 3a), 2,3,6-tri-O-methylgalactitol-1,4,5-triacetate (Peak 2, Fig. 3a), and 2,3,6-tri-O-methylglucitol-1,4,5-triacetate (Peak 3, Fig. 3a). The peaks and mass spectra (Fig. 3b) are identical with those derived from the Forssman hapten glycolipid (Fig. 3c). When the triglycosyceramide derived from the chicken muscle ganglioside was analyzed, Peak 1 disappeared and a new peak (Peak 0) appeared (Fig. 4) which was identified as 2,3,4,6-tetra-O-methylgalactitol-1,5-diacetate, while Peaks 2 and 3 remained unchanged. This indicates substitution of the galactose residue of lactosylceramide at the C-4 position and the other two galactose residues at the C-3 position. The amino sugar derivative was identified as 4,6-di-O-methyl-2-deoxy-2-N-methylacetamidogalactitol-1,3,5-triacetate and was identical with that obtained from the penultimate N-acetylgalactosamide residue of Forssman glycolipid (Fig. 5).

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**TABLE I**

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<th>Fatty acids and long chain bases of sialosylpentaglycosyceramide</th>
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**DISCUSSION**

The structure of the novel ganglioside is NeuAcα2→3Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4GlcCer and should be abbreviated as V3NeuAcIV3GalGbOse4Cer according to the short symbol designation of the IUPAC-IUB Commission on Lipid Nomenclature (1976). It constitutes 9 mol % of the total gangliosides of chicken muscle. The other species of gangliosides in this muscle are GM3 (38%) and GD3 (14%) the two gangliosides containing neolactotetraose, IV3NeuAcLeOse4Cer (11%), V13NeuAcLeOse4Cer (14%), GD1a, and GT1 in smaller amounts. Hence, chicken skeletal muscle gangliosides have oligosaccharides of all four sequences known to occur in glycolipids: lactose, gangliotetraose, neolactotetraose, and globotetraose.
Although gangliosides have been studied in muscle of pig, rat (20), and rabbit (21), a similar detail of structural analysis has been reported only in human muscle where the major gangliosides in both skeletal (22) and cardiac muscle (23) are GM3, GM2, GD3, GD1a, and IV°NeuAcLeOse,Cer although their distribution in the two types of muscle differs. Recently, Iwamori and Nagai (24) characterized the monosialogangliosides of rabbit skeletal muscle and found gangliosides of lacto series containing oligosaccharides as large as octosaccharide length, but no ganglio or globo series. We have not observed the globo series ganglioside described here in our studies of human skeletal muscle (2) and suspect that there is species difference in muscle gangliosides.

REFERENCES
Novel pentahexosyl ganglioside of the globo series purified from chicken muscle.
J L Chien and E L Hogan


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