Structural Analysis of Nine Oligosaccharides Isolated from the Urine of a Blood Group O, Nonsecretor, Woman during Pregnancy and Lactation*

(Published for re-election, August 23, 1976)

PETER HALLGREN AND ARNE LUNDBLAD

From the Department of Clinical Chemistry, University Hospital, S-221 85 Lund, Sweden

Nine oligosaccharides have been isolated from urine collected from an O(H), Le(a + b −), nonsecretor, woman during her pregnancy and subsequent lactation. One of them is a new hexasaccharide denoted lacto-N-neo-difucohexaose II: β-D-Gal-(1→4)-(α-L-Fuc-(1→3))-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-(α-L-Fuc-(1→3))-β-D-Glc. Seven of the remainder are milk oligosaccharides lacto-N-difucohexaose II and III, lacto-N-tetraose, 6'-galactosyllactose, 3-fucosyllactose, and lactose. The ninth component is a glucose-containing tetrasaccharide, α-D-Glc-(1→6)-(α-D-Glc-(1→4))-α-D-Glc-(1→4)-β-D-Glc, previously found in normal human urine.

The oligosaccharides were isolated by gel chromatography, preparative zone electrophoresis, and paper chromatography. The structures were determined by sugar and methylation analyses. Sequence analysis of tri- and tetrasaccharides was carried out by gas-liquid chromatography-mass spectrometry of reduced and permethylated oligosaccharides and mass spectrometry of reduced and permethylated oligosaccharides was carried out by gas-liquid chromatography. The structures were determined by sugar and methylation analyses. Sequence analysis of tri- and tetrasaccharides was deduced from gas chromatography-mass spectrometric studies of di- and trisaccharides and hexasaccharides.

EXPERIMENTAL PROCEDURES

Urine—Four-hour morning samples of urine were collected after 12 h of fasting from a healthy, 24-year-old female, O(H), nonsecretor, Le(a+b−). The samples were collected on 3 consecutive days in the 13th, 16th, 22nd, 26th, 35th, and 38th week of pregnancy. Samples were also taken on the 1st, 2nd, and 3rd morning after delivery and on 3 consecutive days in the 5th week of lactation. Finally, a control sample was obtained 8 months after lactation was ended.

Phenyl mercuric nitrate (30 ml of saturated solution/liter of urine) was added to prevent bacterial growth.

Oligosaccharides—Authentic samples of lacto-N-tetraose and lacto-N-fucopentosae II were a generous gift from Dr. A. Gauhe, Max Planck Institute, Heidelberg, West Germany. Lacto-N-difucohexaose II, lacto-N-neotetraose, and 3-fucosyllactose were purified from human milk as described by Kobata (3). β-D-Gal-(1→3)-D-GlcNAc, β-D-Gal-(1→3)-D-GlcNAc, β-D-Gal-(1→3)-D-GlcNAc, and β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-Glc were isolated after partial acid hydrolysis of lacto-N-tetraose. β-D-Gal-(1→4)-D-GlcNAc and β-D-Gal-(1→4)-D-Glc were isolated after partial acid hydrolysis of lacto-N-neotetraose. 6'-Galactosyllactose was purified from human milk as described by Yamashita and Kobata (10). A glucose-containing tetrasaccharide, α-D-Glc-(1→6)-α-D-Glc-(1→4)-β-D-Glc-(1→4)-β-D-Glc, was isolated as previously described (11).

Analytical Methods—Ultrafiltration of filtered urine samples was performed at 4 °C using Visking 23/32-inch dialysis tubing (Union Carbide Corp., Chicago, Ill.) and a negative pressure of 660 mmHg (12). Gel chromatography was carried out using a Sephadex G-25 (fine) column and a Bio-Gel P-2 (500 to 490 mesh) column (1.5 × 85 cm). Gel chromatographic fractions were purified by preparative zone electrophoresis (13) and paper chromatography on Whatman No. 3 paper. A maximum of 1 mg of substance was applied per cm of paper. The following solvent systems were used: (A) 2 M acetic acid, (B) ethyl acetate: acetic acid:water (3:1:1, v:v), (C) ethyl acetate: pyridine: water (10:4:3, v:v), (D) ethyl acetate: pyridine: acetic acid: water (5:5:1:3, v:v), (E) ethyl acetate: pyridine: water (2:1:2, v:v, upper phase), and (F) propan-1-ol:ethyl acetate:water (6:1:3, v:v). After development, 0.5-cm strips were cut out every 20 cm and stained with a silver dip reagent (14).

The abbreviations used are: GlcNAc, 2-acetamido-2-deoxyglucose; Fuc, L-fucose; Gal, D-galactose; Glc, D-glucose; all monosaccharides described were in the pyranoside form; GLC, gas-liquid chromatography; MS, mass spectrometry; Me, methyl; Ac, acetyl; Rf, Rlactose−T, relative retention time.

* This work was supported by grants from the Swedish Medical Research Council (03X-2) and the Medical Faculty, University of Lund. This is the first of three publications in a study of urinary oligosaccharides during pregnancy and lactation. The following papers in this series are Refs. 8 and 9.
Hydrolysate was reduced with sodium borodeuteride, methylated was added and the sample was again taken to dryness. This partial times during pregnancy and lactation were concentrated lo-

min, cooled, and evaporated to dryness. One milliliter of 1

solved in 3 ml of water, 3 mg of sodium borohydride were added. After 4 h, excess borohydride was destroyed by the addition of Dowex

3, then hydrolyzed with trifluoroacetic acid at 100° for 45

NH2OH, and solvents. The results obtained from sugar

remove impurities extracted from the chromatography papers

to be performed as a final step to sufficient amounts for structural analyses. Column chroma-

Fractions IV and V from each column were concentrated and further fractionated by preparative paper electrophoresis (Solvent A). The main carbohydrate-containing material in both Fraction IV and Fraction V was stationary and was subjected to preparative paper chromatography (Solvent C, 8 days and Solvent B, 3 days, respectively). Eight major subfrac-

Further purification of the different subfractions was achieved by rechromatography as follows: IV14 and V14 were obtained as shown in Fig. 2.

Characterization of Oligosaccharides

Six major subfractions (IV14 and V14) were present in all urine samples, but distinct differences in relative amounts were observed and are described separately (9). After quantitation, each subfraction isolated during different times of pregnancy and lactation was combined to give a normal excretion pattern of total hexose and 6-deoxyhexose from the same individual is seen in Fig. 1c. Eluted material was pooled into fractions (I to VII) as indicated.

Fractions IV and V were processed by on-line computer

RESULTS AND DISCUSSION

Isolation of Oligosaccharides

Ultrafiltrates of the urine samples obtained at different times during pregnancy and lactation were concentrated 10-

fold and fractionated on a Sephadex G-25 column. Eluted fractions were analyzed for total hexose and 6-deoxyhexose (fucose). Fig. 1 shows representative patterns observed in the 18th and 38th week of pregnancy (Fig. 1a) and in the 5th week of lactation (Fig. 1c). A normal excretion pattern of total hexose and 6-deoxyhexose from the same individual is seen in Fig. 1d. Eluted material was pooled into fractions (I to VII) as indicated.

Subfractions IV14 and V14 were homogeneous in five different solvent systems (Table II). The results obtained from sugar analysis, methylation analysis, and optical rotation measure-

Colorimetric methods were used for determining total hexose (15) and 6-deoxyhexose (fucose) (16). After hydrolysis (4 M trifluoroacetic acid, 4 h at 100°), sugar analysis was performed by GLC (17) and MS (18). Optical rotation was recorded using a Perkin-Elmer 241 polarimeter.

Methods for methylation analysis and methylation of reduced oligosaccharides have been described elsewhere (19, 20). A Perkin-Elmer model 3920 gas chromatograph was used under the following conditions: (a) glass column, 2 m, packed with 3% ECNSS-M on Gas-Chrom Q (100 to 200 mesh) at a column temperature of 185-210° for sugar alditol acetates and at 160° for partially methylated alditol derivatives; (b) glass capillary column (25 m x 0.25 mm), wall-coated with SP-1000 (LKB, Stockholm, Sweden) at a column temperature of 210° for partially methylated alditol acetates; (c) glass capillary column (25 m x 0.25 mm), wall-coated with SE-30 (LKB, Stockholm, Sweden) at a column temperature of 160° for partially methylated alditol acetates and at 210-310° for permethylated alditol derivatives of di- to tetrasaccharides. For GLC-MS, the same columns as above were used on a Varian MAT 311 A combined GLC-MS instrument. Glass capillary columns were connected directly to the ion source of the instrument. The mass spectra were recorded at an ionization potential of 70 eV, an ionization current of 1 mA, and an ion source temperature of 120°. All data were processed by on-line computer system (Spectrosystem 100 Varian MAT).

Partial Acid Hydrolysis—To about 1 mg of oligosaccharide, dissolved in 3 ml of water, 3 mg of sodium borohydride were added. After 4 h, excess borohydride was destroyed by the addition of Dowex 50 (H+), which was then removed by filtration. Boric acid was removed by evaporation with methanol. The reduced oligosaccharide was then hydrolyzed with 3 ml of 1 M trifluoroacetic acid at 100° for 45 min, cooled, and evaporated to dryness. One milliliter of 1 M NH2OH was added and the sample was again taken to dryness. This partial hydrolysate was reduced with sodium borodeuteride, methylated (20), and analyzed by GLC-MS.

TABLE I

<table>
<thead>
<tr>
<th>Trivial name</th>
<th>Structure</th>
<th>Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'-fucosyllactose</td>
<td>β-D-Gal-(1-4)-D-Glc</td>
<td>V3</td>
</tr>
<tr>
<td>3'-fucosyllactose</td>
<td>α-L-Fuc</td>
<td>V3</td>
</tr>
<tr>
<td>3'-fucosyllactose</td>
<td>β-D-Gal-(1-6)-β-D-Gal-(1-4)-D-Glc</td>
<td>V2</td>
</tr>
<tr>
<td>lacto-N-tetraose</td>
<td>β-D-Gal-(1-3)-β-D-GlcNAc-(1-3)-β-D-Gal-(1-4)-D-Glc</td>
<td>IV1</td>
</tr>
<tr>
<td>lacto-N-neotetraose</td>
<td>β-D-Gal-(1-3)-β-D-GlcNAc-(1-3)-β-D-Gal-(1-4)-D-Glc</td>
<td>IV2</td>
</tr>
<tr>
<td>lacto-N-fucopentaose I</td>
<td>α-L-Fuc</td>
<td>IV2</td>
</tr>
<tr>
<td>lacto-N-fucopentaose II</td>
<td>β-D-Gal-(1-3)-β-D-GlcNAc-(1-3)-β-D-Gal-(1-4)-D-Glc</td>
<td>IV1</td>
</tr>
<tr>
<td>lacto-N-fucopentaose II</td>
<td>α-L-Fuc</td>
<td>IV1</td>
</tr>
<tr>
<td>lacto-N-fucopentaose III</td>
<td>β-D-Gal-(1-3)-β-D-GlcNAc-(1-3)-β-D-Gal-(1-4)-D-Glc</td>
<td>IV3</td>
</tr>
<tr>
<td>lacto-N-fucopentaose III</td>
<td>α-L-Fuc</td>
<td>IV3</td>
</tr>
<tr>
<td>lacto-N-difucohexaose II</td>
<td>β-D-Gal-(1-3)-β-D-GlcNAc-(1-3)-β-D-Gal-(1-4)-D-Glc</td>
<td>IV2</td>
</tr>
<tr>
<td>lacto-N-neodifucohexaose II</td>
<td>β-D-Gal-(1-3)-β-D-GlcNAc-(1-3)-β-D-Gal-(1-4)-D-Glc</td>
<td>IV3</td>
</tr>
</tbody>
</table>

Isolation of Urinary Oligosaccharides during Pregnancy and Lactation

The table shows the structures of some of the oligosaccharides mentioned in the text.

Downloaded from http://www.jbc.org/ by guest on September 6, 2017
1016 Urinary Oligosaccharides during Pregnancy and Lactation

Fig. 1 (left). Gel chromatographic profiles of 4-h urine samples collected after 12 h of fasting from a blood group O, nonsecretor, Le (a + b−), woman in the 18th (a) and 38th (b) week of pregnancy and in the 5th week of lactation (c). A 4-h control sample from the same individual collected 8 months after lactation was ended is also shown (d). Ultrafiltered urine samples were concentrated 10-fold and applied to a Sephadex G-25 (fine) column (10 × 105 cm; void volume = 2,700 ml) and eluted with distilled water. Eluted fractions (45 ml) were analyzed for total hexose (-----) and 6-deoxyhexose (fucose) (-----). Material in Fractions IV to VI were pooled as indicated.

Fig. 2 (right). Paper chromatographic distribution of uncharged material from (A) Fraction IV and (B) Fraction V after preparative zone electrophoresis at pH 1.9. Chromatogram A was developed in Solvent C for 8 days, chromatogram B in Solvent B for 3 days, and visualized with a silver-dip reagent (14).

Table II

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>IV₁</th>
<th>IV₂</th>
<th>IV₃</th>
<th>IV₄</th>
<th>V₁</th>
<th>V₂</th>
<th>V₃</th>
<th>V₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.05</td>
<td>0.11</td>
<td>0.19</td>
<td>0.25</td>
<td>0.25</td>
<td>0.40</td>
<td>0.62</td>
<td>1.00</td>
</tr>
<tr>
<td>C</td>
<td>0.21</td>
<td>0.09</td>
<td>0.23</td>
<td>0.37</td>
<td>0.37</td>
<td>0.46</td>
<td>0.63</td>
<td>1.00</td>
</tr>
<tr>
<td>D</td>
<td>0.19</td>
<td>0.20</td>
<td>0.46</td>
<td>0.59</td>
<td>0.59</td>
<td>0.85</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.16</td>
<td>0.24</td>
<td>0.40</td>
<td>0.55</td>
<td>0.55</td>
<td>0.58</td>
<td>0.71</td>
<td>1.00</td>
</tr>
<tr>
<td>F</td>
<td>0.30</td>
<td>0.40</td>
<td>0.59</td>
<td>0.59</td>
<td>0.59</td>
<td>0.85</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

Table III

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Analytical solution</th>
<th>Relative molar proportions</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₁</td>
<td>66°</td>
<td>2.74</td>
</tr>
<tr>
<td>V₂</td>
<td>28°</td>
<td>2.23</td>
</tr>
<tr>
<td>V₃</td>
<td>156°</td>
<td>1.78</td>
</tr>
<tr>
<td>V₄</td>
<td>36°</td>
<td>1.91</td>
</tr>
<tr>
<td>V₅</td>
<td>52°</td>
<td>1.32</td>
</tr>
</tbody>
</table>

Peaks were analyzed for total hexose (-----) and 6-deoxyhexose (fucose) (-----). Material in Fractions IV to VI were pooled as indicated.

Table IV

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Analaltical solution</th>
<th>Relative molar proportions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV₁</td>
<td>66°</td>
<td>2.74</td>
</tr>
<tr>
<td>IV₂</td>
<td>28°</td>
<td>2.23</td>
</tr>
<tr>
<td>IV₃</td>
<td>156°</td>
<td>1.78</td>
</tr>
<tr>
<td>IV₄</td>
<td>36°</td>
<td>1.91</td>
</tr>
<tr>
<td>IV₅</td>
<td>52°</td>
<td>1.32</td>
</tr>
</tbody>
</table>

Peak A had a retention time and mass spectrum identical to those from an authentic sample of lactose (21). By the partial acid hydrolysis procedure described, no differentiation between 3- and 4-substituted methylated hexitols can be achieved. This symmetry effect was overcome by reduction with sodium borodeuteride. Peak B was derived from β-D-GlcNAc-(1→3)-p-Gal as indicated by its mass spectrum (Fig. 4). In the A series of fragments (22-24), aA₁ (m/e 260) and aA₂ (m/e 228) were seen which indicated the presence of a 2-acetamido-2-deoxyhexose residue at the nonreducing terminal. In the J series of fragments, ald₁ (m/e 236) and ald (m/e 226) were seen, indicating a hexitol residue at the reducing terminal. The fragmentation of the aldol chain with fragments m/e 45, m/e 46, m/e 89, m/e 90, m/e 133 was expected from a 3-substituted terminal reducing hexose residue. These fragments indicate the structure: 2-acetamido-2-deoxyhexose-(1→3)-hexitol. Peak C had a retention time and mass spectrum (Fig. 5) identical to that of a derivative of β-D-Gal-(1→3)-p-GlcNAc. The A series of fragments were aA₁ (m/e 219), aA₂, aA₃, and aA₄.
**Table IV**

<table>
<thead>
<tr>
<th>Methyl ethers</th>
<th>T values*</th>
<th>Relative molar proportions*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ECNSS-M</td>
<td>SE-30</td>
</tr>
<tr>
<td>1,2,3,5,6-Penta-O-Me-Glc-ld</td>
<td>0.38</td>
<td>0.62</td>
</tr>
<tr>
<td>1,2,5,6-Tetra-O-Me-Glc-ld</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td>2,3,4-Tri-O-Me-Fuc</td>
<td>0.65</td>
<td>0.67</td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-Me-Glc</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-Me-Gal</td>
<td>1.25</td>
<td>1.07</td>
</tr>
<tr>
<td>2,4,6-Tri-O-Me-Gal</td>
<td>2.28</td>
<td>1.50</td>
</tr>
<tr>
<td>2,3,6-Tri-O-Me-Glc</td>
<td>2.50</td>
<td>1.50</td>
</tr>
<tr>
<td>2,3,4-Tri-O-Me-Glc</td>
<td>2.49</td>
<td>1.62</td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-Me-Gal</td>
<td>3.41</td>
<td>1.81</td>
</tr>
<tr>
<td>4,6-Di-O-Me-GlcN(Me)Ac</td>
<td>-</td>
<td>4.90</td>
</tr>
<tr>
<td>6-O-Me-GlcN(Me)Ac</td>
<td>-</td>
<td>5.60</td>
</tr>
</tbody>
</table>

* Retention times of the corresponding alditol acetates relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol.

* The relative molar proportions of the partially methylated hexosaminitols could not be determined as their response factors are not known. Substantial amounts of the penta-O-methyl ethers are lost in the methylation procedure because of their high volatility.

* 4,6-Di-O-Me-GlcN(Me)Ac = (N-methyl)-2-acetamido-2-deoxy-4,6-di-O-methyl-D-glucose. About 10% of the corresponding non-N-methylated products were also identified.

---

Figure 3. GLC analysis of reduced and permethylated products obtained after partial acid hydrolysis of Fraction IV,.

The separation was performed on a glass capillary column (25 m x 0.25 mm) wall-coated with SE-30, using a linear temperature gradient of 2'°C/min between 210-310°. See the text for the identification of peaks A to I. Peaks without designation were shown to contain noncarbohydrate material.

In the trisaccharide region three peaks, E, F, and G, gave GLC-MS data identical to derivatives of P-D-Gal-(1-4)-P-D-GlcNAc-(1-3)-P-D-Gal, and P-D-GlcNAc-(1-3)-P-D-Gal-(1-4)-P-D-Gl, respectively. The mass spectra and some primary fragments for these compounds are shown in Figs. 6 to 8. The mass spectra for peaks E and F were very similar. In the A series of fragments, aA, (m/e 219), aA, (m/e 157), baA, (m/e 464), and baA, (m/e 464), and baA, (m/e 432), which suggested the structural unit 2-acetamido-2-deoxyhexose→hexitol. From the fragmentation of the alditol residue it can be concluded that it is substituted in position C-3. The mass spectrum for peak E differed from that of peak F in that no fragment baldA (m/e 296) was observed, which suggested that peak E was the derivative of a hexose-(1-3)-2-acetamido-2-deoxyhexose residue at the reducing terminal.

Further, fragments a1 (m/e 325) and b1 (m/e 319) indicated the sequence hexose→hexitol. The above data suggest that peak G is 2-acetamido-2-deoxyhexose-(1-3)-hexose→hexitol. Two further peaks H and I (relative proportions 4:1) were observed which co-chromatographed with authentic derivatives of lacto-N-tetraose and lacto-N-neotetraose, respectively. No mass spectra were obtained for these peaks. By a combination of the above data, two core tetrasaccharides identical to lacto-N-tetraose and lacto-N-neotetraose are evident and in combination with the methylation analysis we propose that Fraction IV, is a mixture of two isomeric oligosaccharides, one of which...
is identical to lacto-N-difucohexaose II and the other is a new isomer denoted lacto-N-neodifucohexaose II (Table I). No determinations of anomeric configurations were performed but the optical rotation for the mixture was $[\alpha]_D^0 -66.3^\circ$, and is in good agreement with that of lacto-N-difucohexaose II (25) ($[\alpha]_D^0 -68.8^\circ$). The optical rotation of the new component lacto-N-neodifucohexaose II is assumed to be in the same order of magnitude.

IV$_2$ - The chromatographic behavior and sugar and methylation analyses were identical to those for lacto-N-fucopentaose II (26) and lacto-N-fucopentaose III (27) (Table I). After partial acid hydrolysis and GLC, a chromatogram almost identical to that obtained from Fraction IV$_1$ (Fig. 3) was obtained, the main difference being the ratio of lacto-N-tetraose to lacto-N-neotetraose (2:1 in Fraction IV$_1$). Identification of the different products was performed as described.
Urinary Oligosaccharides during Pregnancy and Lactation

FIG. 6. Mass spectrum and some important primary fragments of peak E as its permethylated alditol derivative.

FIG. 7. Mass spectrum and some important primary fragments of peak F as its permethylated alditol derivative.

above for Fraction IV₁. We conclude that Fraction IV₂ is a mixture of the two oligosaccharides lacto-N-fucopentaose II and lacto-N-fucopentaose III.

IV₃—The chromatographic mobility, sugar and methylation analysis, and optical rotation data were all identical to those for lacto-N-tetraose (28). The sequence of the internal sugar residues was established by introducing the permethylated alditol oligosaccharide directly into the ion source of the mass spectrometer using the solid inlet. The mass spectrum of Fraction IV₃ as its permethylated alditol derivative is shown in Fig. 9. In the A series of fragments, αA₁ (m/e 219), αA₂ (m/e 187), αA₃ (m/e 155), βA₁ (m/e 464), βA₂ (m/e 432), and cabA₁ (m/e 668) are seen, and the sequence hexose→2-acetamido-2-deoxyhexose→hexose→ is indicated. Furthermore, fragments ald (m/e 236), cald (m/e 440), and bcald (m/e 685) suggested the sequence →2-acetamido-2-deoxyhexose→hexose→hexitol. These data together with the absence of fragments bcaldJ₁ (m/e 500) and caldJ₁ (m/e 296) indicates the sequence hexose→(1→3)2-acetamido-2-deoxyhexose→hexitol. Furthermore, the fragmentation of the hexitol shows that it is substituted at C-4 (Fig. 9). The above evidence strongly suggests that Fraction IV₃ is identical to lacto-N-tetraose.

IV₁ and V₁—These two fractions were found by paper
chromatography, sugar and methylation analyses, and optical rotation data to be identical and were identified as a glucose-containing tetrasaccharide previously isolated from human urine (11). GLC-MS could not differentiate between these two fractions or an authentic sample of the glucose-containing tetrasaccharide (29).

$V_3$—Sugar and methylation analyses of this fraction indicated the sequence Gal-(1→6)-Gal-(1→4)-Glc. Further support for this structure was obtained by GLC-MS studies of reduced and permethylated Fraction $V_3$. These data together with the optical rotation $[\alpha]_D^{20} +36.9^\circ$ suggest that the fraction is identical to 6'-galactosyllactose ($[\alpha]_D^{20} +34^\circ$) (30). The same component has been found previously in human milk (10) and has been investigated by GLC-MS as its permethylated alditol derivative (31).

$V_3$—The chromatographic mobility and sugar and methylation analyses were identical to those of an authentic sample of 3-fucosyllactose (Table I). The mass spectrum of the permethylated alditol derivative of Fraction $V_3$ is shown in Fig. 10. In the A series, fragments $aA_1$ (m/e 219), $aA_2$ (m/e 187), $aA_3$ (m/e 155), $bA_1$ (m/e 189), and $bA_2$ (m/e 157) show the presence of both a nonreducing terminal hexose and 6-deoxyhexose residue. In the J series, $bJ_1$ (m/e 500), $aJ_1$ (m/e 440), and $aJ_2$ (m/e 410) were seen. These fragments show that both nonreducing and terminal sugars are linked to a common hexitol residue. The primary fragments of the alditol chain suggest that it is substituted at positions C-3 and C-4 although the exact substitution positions cannot be determined unambiguously from the mass spectrum. By partial acid hydrolysis of borodeuteride-reduced and permethylated Fraction $V_3$ only one product, identified by GLC-MS as a derivative of lactose, was observed. From the data presented above together with the optical rotation data it is evident that Fraction $V_3$ is 3-fucosyllactose.

$V_3$—This fraction was studied by paper chromatography, sugar and methylation analyses, and by GLC-MS as its
permethylated alditol derivative. The results from the above analyses were identical to those obtained using a sample of lactose. The appearance of lactose in the gel chromatographic Fraction V is due to an overlap from Fraction VI, where lactose is by far the most abundant disaccharide.

**Conclusions**

It has been shown previously that nonsecretors of different ABO blood groups have a significantly lower normal excretion of fucose-containing material than secretors, and no blood group-specific oligosaccharides have been isolated from urine of nonsecretors (32). The origin of the oligosaccharides isolated from urine during pregnancy and lactation is not known. It is surprising that the excretion of milk-type oligosaccharides starts so early in pregnancy. The biosynthetic activity in the mammary gland, uterus, placenta, and fetus is high during this time and the urinary components may reflect superfluous activity of different glycosyltransferases in these organs. It is reasonable to assume that the oligosaccharides isolated during lactation are derived from the milk. Nothing is known, however, about their release from the mammary gland and transfer to the circulation. The physiological function of all these components is also unknown.

The new oligosaccharide lacto-N-neodifucohexaose II is clearly related to the known milk oligosaccharides and cannot be separated from its isomer lacto N difucohexaose II by paper chromatography. It is surprising that this compound has not as yet been found in milk. It might be expected that the best source for isolating this new isomer would be milk from Le (a− b−) nonsecretor women. The fucopentaose with a fucose residue linked α(1→3) to the glucose residue in lacto-N-tetraose has only recently been found (33).

Lacto-N-tetraose and lacto-N-fucopentaose II are present in the urine of pregnant and lactating women (6, 7), but these oligosaccharides were isolated from pooled urine and the ABO, Lewis, and secretor status of the different individuals were not considered. The glucose-containing oligosaccharide is present in normal urine but has not been detected in milk.

Pronounced differences in excretion rate for these oligosaccharides during pregnancy and lactation were observed and are reported in another paper (9).

**Acknowledgments** – We are grateful to Mr. Anders Eriks-son, Mrs. Christina Gellerstig, Mr. Lennart Holmqvist, and Mrs. Gulli Lindfors for excellent technical assistance.

**REFERENCES**

Structural analysis of nine oligosaccharides isolated from the urine of a blood group O, nonsecretor, woman during pregnancy and lactation.

P Hallgren and A Lundblad


Access the most updated version of this article at http://www.jbc.org/content/252/3/1014

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/252/3/1014.full.html#ref-list-1