The carbohydrate fraction of human milk includes a number of chemically defined oligosaccharides which contain the 6-deoxy-

The Journal of Biological Chemistry
Vol. 240, No. 3, March 1965
Printed in U.S.A.

Biosynthesis of Fucosyllactose and Other Oligosaccharides
Found in Milk*

ARTHUR P. GROLLMAN,† CLARA W. HALL, AND VICTOR GINSBURG

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health,
United States Public Health Service, Bethesda, Maryland 20014

(Received for publication, April 22, 1964)

The carbohydrate fraction of human milk includes a number of chemically defined oligosaccharides which contain the 6-deoxy-

The present report describes a particular enzyme from lactating canine mammary tissue which catalyzes the synthesis of fucosyllactose, the simplest member of this group, by transfer of L-fucose from guanosine diphosphate to lactose.

If fucosyllactose were formed by the preceding reaction in vivo, the lactose moiety of the trisaccharide should arise from free lactose and, as a consequence, resemble it in 14C distribution after administration of a labeled precursor. This metabolic relationship was investigated in lactating dogs by modifying the technique used by Wood, Siu, and Schambrey (5) in their extensive investigations of lactose synthesis by the bovine mammary gland. Glycerol-14C was injected into the artery supplying the mammary gland, and the amount and distribution of radioactivity were determined in the oligosaccharides subsequently isolated from the milk. In addition to lactose and fucosyllactose, sialyllactose and a higher oligosaccharide fraction were isolated, and the specific activities of their component sugars were determined. The results indicate that D-glucose is the initial acceptor to which other sugars are sequentially transferred in the biosynthesis of fucosyllactose as well as other oligosaccharides found in milk.

EXPERIMENTAL PROCEDURE

Materials

UTP, GTP; UDP-N-acetyl-D-glucosamine, UDP-D-glucose, and GDP-D-mannose were purchased from the Sigma Chemical Company. Lactose-1-14C, glycerol-14C, and hexokinase were obtained from the California Corporation for Biochemical Research, and oxytocin from the Parke, Davis and Company. TPNH was a gift from Dr. J. Strominger, D-mannose-1-phosphate-14C was donated by Dr. G. Ashwell, UDP-D-galactose-14C was supplied by Dr. E. Maxwell, and D-galactose oxidase was provided by Dr. B. L. Horecker. O-(N-Acetylneuraminyl)-2-(2→3)-O-β-D-galactopyranosyl-(1 → 4)-D-glucose (3′-sialyllactose), O-(N-acetylneuraminyl)-2-(2→3)-O-β-D-galactopyranosyl-(1 → 4)-D-glucose (3′-sialyllactose), O-(N-acetylneuraminyl)-2-(2→3)-O-β-D-galactopyranosyl-(1 → 4)-D-glucose (6′-sialyllactose), and 2′-fucosyllactose were kindly provided by Dr. Adeline Gauhe and Dr. Richard Kuhn. 2-Fucosyllactose was prepared by alkaline hydrolysis of 2′-fucosyllactose (4). Crystalline β-galactosidase from Escherichia coli was a gift of Dr. M. Nirenberg, and nucleotide pyrophosphatase, purified from snake venom, was obtained from Dr. R. J. Hilman.

GDP-L-fucose was prepared enzymatically from GDP-D-mannose with an extract of Aerobacter aerogenes (6). Freshly harvested bacteria, 25 g, suspended in 40 ml of 0.05 M potassium phosphate buffer, pH 7.4, were disrupted sonically for 15 minutes in a Raytheon 10-ke sonic disintegrator. Under conditions of the standard assay (6), 1 ml of the supernatant solution obtained after centrifugation for 30 minutes at 18,000 × g converted 2D-mannose to GDP-D-mannose per hour to GDP-L-fucose. In a typical large scale preparation, 40 ml of the enzyme solution were incubated for 3 hours at 23° with 100 μmoles of GDP-D-mannose, 20 μmoles of TPN, 600 μmoles of glucose 6-phosphate, 10 mmole of MgCl2, and 1 mmole of EDTA in 960 ml of 0.05 M Tris buffer, pH 8.0. Endogenous glucose 6-phosphate dehydrogenase present in the crude sonic extract served to generate TPNH in this system. After cooling to 0°, 100 ml of 2.5 N perchloric acid were added, the precipitated protein was removed by centrifugation, and 0 g of acid-washed Norit was added to absorb the nucleotide fraction containing the GDP-L-fucose. The charcoal was washed twice with water and then eluted with three 400-ml portions of 50% ethanol containing 0.1% NH4OH. Nucleotides in the combined eluates were concentrated under reduced pressure and subjected to chromatography in Solvent III. Ultraviolet-absorbing material with the same chromatographic mobility as authentic GDP-L-fucose was eluted and purified by chromatography in Solvent IV. The nucleotide thus obtained showed the absorption spectra of a guanosine nucleotide and migrated as a single compound in three chromatographic solvents and on paper electrophoresis at pH 3.6. Hydrolysis with 0.01 M HCl for 10 minutes at 100° released a single neutral sugar, which was characterized as fucose by paper chromatography and by its absorption spectra in the cysteine-sulfuric
acid reaction (7). In this preparation, 28 pmoles of GDP-L-fucose were obtained for an overall yield of 28%.

GDP-n-mannose-14C was synthesized chemically by condensing n-mannose 1-phosphate-14C with guanosine 5'-phosphoromorpholidate as described by Roseman et al. (8). GDP-L-fucose-14C (specific activity, 70,700 c.p.m. per pmole) was prepared by the enzymatic reduction of GDP-n-mannose-14C according to the procedures described in the preceding section for the purification of nonradioactive GDP-L-fucose. GDP-L-fucose-3H (specific activity, 25,000 c.p.m. per pmole), presumably tritiated in the C-4 position of L-fucose, was prepared in an analogous manner from GDP-n-mannose and TPNH (specific activity, 6.3 \times 10^4 c.p.m. per pmole). L-Fucose 1-phosphate-3H was prepared from GDP-L-fucose-3H by treatment with nucleotide pyrophosphatase, while L-fucose-3H was prepared from L-fucose 1-phosphate by enzymatic dephosphorylation.

Methods

Quantitative Determinations—L-Fucose was measured by the cysteine-sulfuric acid reaction (7), n-galactose by its reducing value (9) or by a specific n-galactose oxidase described by Avigad et al. (10), and n-glucose by coupling the hexokinase and n-glucose 6-phosphate dehydrogenase reactions and measuring the formation of TPNH at 340 mμ. Sialic acid was determined by the thiobarbituric acid assay (11), sialylactose by measuring sialic acid after treatment with neuraminidase, lactose by the increase in n-glucose after hydrolysis with β-galactosidase, and fusocyllactose by its reducing value (9).

Chromatography—Paper chromatography by the descending technique was employed with the following solvent systems: I, pyridine-ethyl acetate-water (1.0:3.6:1.15); II, 1-propanol-ethyl acetate-water (1.0:3.6:1.15); III, ethanol-1 m ammonium acetate (7:3); and IV, isobutyl alcohol-0.5 m NH₄OH (5:3). Radioactivity on chromatograms was detected with a Vanguard strip scanner. Nucleotides were located by ultraviolet light, and unlabelled sugars with the AgNO₃ reagent (12).

Radioactivity Determinations—14C- and 3H-labeled compounds were determined in a Packard Tri-Carb liquid scintillation spectrometer in a system consisting of 0.02 ml of water, 1.0 ml of ethanol, and 10 ml of toluene containing 400 mg of 2,5-diphenyloxazole and 5 mg of p-bis-2'-(5'-phenyloxazolyl)benzene per 100 ml (13).

Preparation of Particulate Enzyme—Mammary tissue was obtained by mastectomy of mongrel dogs during the early weeks of lactation. The tissue was minced, rendered free of connective tissue and fat, suspended in 8 volumes of 0.25 m sucrose, and homogenized for two 1-minute periods in a Waring Blender. This and all subsequent operations were carried out at 0–4°. The crude homogenate was filtered through two layers of cheesecloth to remove extraneous tissue, and through glass wool to remove congealed lipid. The supernatant solution obtained after centrifugation for 10 minutes at 2,000 \times g, and for 30 minutes at 95,000 \times g, was recentrifuged for 1 hour at 100,000 \times g. Sedimented particles were suspended in 0.25 m sucrose and resedimented by centrifugation for 1 hour at 100,000 \times g. The washed particles were then suspended in a buffer composed of 0.1 m Tris, pH 7.5, and 0.01 m mercaptoethanol (0.5 ml for every gram of tissue), and used for the experiments described in this paper.

Injection of Glycerol-14C into Pudendal Artery—The pudendal artery in the dog is adjacent to the pudendal vein, and the two vessels are easily exposed by a 2-inch semicircular incision near the lateral edge of the caudal mammary gland (14). The two vessels were divided by blunt dissection, and indwelling catheters were inserted with purse string sutures. Glycerol-14C, diluted in 0.9% NaCl, was injected over 5 seconds through the arterial cannula while at the same time the pudendal vein was occluded proximal to the venous cannula and the venous return was withdrawn into a syringe.

Isolation and Identification of Radioactive Oligosaccharides of Canine Milk

In a typical experiment, 5.1 ml of milk were diluted to 20 ml with water and centrifuged for 10 minutes at 3,000 \times g at 0°; the solidified layer of lipid was removed with a spatula, and trichloroacetic acid was added to a final concentration of 10%. After centrifugation, the supernatant solution was extracted seven times with equal volumes of ether to remove trichloroacetic acid and lipid material and was brought to pH 8.0 with NH₄OH; the deproteinized, defatted solution was used for isolation of oligosaccharides. It contained 5.3 pmoles of thiobarbituric acid-reacting material, measured as sialic acid after treatment with neuraminidase, and 990 pmoles of reducing sugars, measured as lactose.

Sialyllactose—Sialyllactose, 4.8 pmole, was isolated from the extract as described by Mayron and Tokes (15) by chromatography on Dowex 1 and charcoal-Cellite columns. It was then further purified by paper electrophoresis in 0.05 m ammonium formate buffer, pH 4.0, followed by paper chromatography in pyridine-ethyl acetate-acetic acid-water (5:5:1:4), which separates 3'-sialyllactose (Rlactose 0.68) from 6'-sialyllactose (Rlactose 0.54). After the final purification, 2.4 pmoles of sialyllactose with the chromatographic mobility of the 3' isomer and 2.1 pmoles with the mobility of the 6' isomer were recovered. After treatment with neuraminidase, lactose and N-acetylgalactosamine were separated by paper electrophoresis and eluted. The lactose was then hydrolyzed with 1 N H₂SO₄ for 1 hour at 100°, or with β-galactosidase; both procedures yielded equal quantities of glucose and galactose after separation of the hydrolysis product by chromatography.

Lactose—Compounds which failed to adsorb to Dowex 1 were passed through a mixed bed ion exchange resin and then adsorbed on charcoal-Cellite, 1:1 (16). The column was eluted successively with water and with 8%, 15%, and 60% ethanol. The 8% eluate, which contained 90% of the lactose, was reduced in volume and the lactose was crystallized. The yield of lactose was 350 pmoles; this amount was recrystallized twice from ethanol-water (melting point and mixed melting point, 201–202°, uncorrected). Treatment of the crystals with β-galactosidase released equal quantities of glucose and galactose after separation of the hydrolysis product by chromatography.

Fucosyllactose—Fucosyllactose was eluted from the charcoal-Cellite with 15% ethanol accompanied by some lactose and unidentified higher oligosaccharides. The volume of this fraction was reduced, and the sugars were separated by paper chromatography in Solvent I. The area corresponding to standard fucosyllactose was eluted, resulting in the isolation of 15 pmoles of trisaccharide. Hydrolysis of this compound in 1 N H₂SO₄ for 2 hours at 100° yielded equimolar quantities of fucose, glucose,

1 Four experiments were carried out with essentially similar results. The details from one of these are described in the text, and the data are represented in Table I.
and galactose. Milder hydrolysis with 1 N acetic acid for 2 hours at 100° followed by paper chromatography in Solvent I produced two reducing compounds with Rf values corresponding to fucose and lactose. Treatment of the latter compound with β-galactosidase yielded only glucose and galactose. Alkaline hydrolysis of the trisaccharide in 0.025 M Na₂CO₃ for 5 minutes at 100° formed a disaccharide with the same Rf as 2'-fucosylgalactose, which yielded fucose and galactose after hydrolysis in 1 N H₂SO₄ for 2 hours at 100°.

Further identification was provided by adding 20 μmoles of authentic 2'-fucosyllactose to an aliquot of the radioactive trisaccharide containing 3040 c.p.m. The crystalline tosylhydrazone derivative was prepared (4), and the specific activity was determined as 151 c.p.m. per μmole. After two recrystallizations from propenol-water, the specific activity was 152 c.p.m. per μmole (theoretical, 152 c.p.m. per μmole).

Higher Oligosaccharides—A number of neutral oligosaccharides were eluted from the charcoal-Celite column by 60% ethanol. These oligosaccharides were not characterized and were combined as the “higher oligosaccharides” of canine milk.

**EXPERIMENTS AND RESULTS**

**Studies In Vitro**

**Formation and Identification of Fucosyllactose**—When GDP-L-fucose-3H and lactose were incubated with the enzyme (Reaction A, Fig. 1), two radioactive products were formed, one with the chromatographic mobility of fucose, and a second, more slowly migrating compound (Reaction Product I) with the mobility of 2'-fucosyllactose. When unlabeled GDP-L-fucose and lactose-1-14C were incubated with enzyme (Reaction A, Fig. 2) the only radioactive product (Reaction Product II) showed the chromatographic properties of authentic 2'-fucosyllactose. As shown in Reaction B, Fig. 2, this compound was not formed in the absence of GDP-L-fucose.

Reaction Products I and II were eluted and subjected to certain chemical procedures described by Kuhn et al. (4) for the characterization of 2'-fucosyllactose (Table I). Both products had the same chromatographic mobility as authentic 2'-fucosyllactose in three solvent systems and gave the expected radioactive products on chemical degradation. Following strong acid hydrolysis, radioactive fucose was formed from Product I and radioactive glucose from Product II; both sugars were identified chromatographically. Mild acid hydrolysis again released fucose from Product I and radioactive glucose from Product II; both sugars were identified chromatographically. Mild acid hydrolysis again released fucose from Product I and a labeled compound with the mobility of lactose from Product II. Alkaline hydrolysis resulted in the formation of a labeled sugar from Product I which had the same chromatographic properties as 0-fucosyl-(1→2)-galactose and which yielded free fucose on strong acid hydrolysis. The same radioactive sugar was not observed after similar treatment of Product II. Alkaline hydrolysis distinguishes 2'-fucosyllactose from an isomer occurring in milk in which the fucosyl group is glycosidically linked to the 3-position of glucose (17).

![Fig. 1. Formation of fucosyllactose-3H.](http://www.jbc.org/)

![Fig. 2. Formation of fucosyllactose-14C.](http://www.jbc.org/)
in the crystalline tosylhydrazones. The specific activity of these derivatives was not lowered by recrystallization.

**Assay for Formation of Fucosyllactose**—The formation of fucosyllactose was followed by determining incorporation of radioactivity from labeled GDP-L-fucose into a compound migrating with 2'-fucosyllactose in Solvent II. The standard reaction mixture is described in the legend of Fig. 1. Following incubation with enzyme, 0.1 μmole of carrier 2'-fucosyllactose was added; the reaction mixture was heated for 3 minutes in a boiling water bath, precipitated protein was discarded, and the supernatant solution was deionized with a mixed bed ion exchange resin (Resyn, Fisher Scientific Company). The deionized solution was evaporated to a small volume, applied as a band on Whatman No. 3MM paper, and chromatographed with Solvent II for 36 hours. Radioactivity incorporated into fucosyllactose and other neutral compounds was measured by scanning the chromatogram or by cutting out appropriate areas and determining radioactivity directly with a liquid scintillation spectrometer. As the specific activity of the starting GDP-L-fucose is known, the yield of trisaccharide can be calculated.

**Properties of GDP-L-fucose:Lactose Fucosyltransferase**—Formation of fucosyllactose by the particulate enzyme requires both GDP-L-fucose and lactose, as shown in Fig. 1. The trisaccharide was not formed in the absence of lactose (B) or when GDP-L-fucose was replaced by L-fucose 1-phosphate (C) or L-fucose (D).

As shown in Table III, trisaccharide formation requires Mg++ and is stimulated by GTP. The latter effect is not specific for GTP, since other nucleotides such as UTP, UDP-N-acetyl-D-glucosamine, UDP-D-glucose, or GDP-D-mannose stimulate the reaction to the same extent. This unspecific stimulation is probably related to a protection of GDP-L-fucose against breakdown. The enzyme preparation contains both nucleotide pyrophosphatase and phosphomonoesterase activity as evidenced by the formation of free L-fucose from GDP-L-fucose and L-fucose 1-phosphate (Fig. 1), but, under conditions of the assay, the rate

---

**Table I**

Chromatographic properties of 3H- and 14C-labeled reaction products

<table>
<thead>
<tr>
<th>Reaction product</th>
<th>Hydrolysis conditions</th>
<th>Standard</th>
<th>R_&lt;sub&gt;G&lt;/sub&gt; glucose of radioactivity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Solvent I</td>
</tr>
<tr>
<td>I</td>
<td>Strong acid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fucosyllactose</td>
<td>0.15 (0.15)</td>
</tr>
<tr>
<td>I</td>
<td>Mild acid&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Fucose</td>
<td>1.7 (1.7)</td>
</tr>
<tr>
<td>I</td>
<td>Mild alkali&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Fucosylgalactose</td>
<td>0.46 (0.45)</td>
</tr>
<tr>
<td>II</td>
<td>Strong acid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fucosyllactose</td>
<td>0.15 (0.10)</td>
</tr>
<tr>
<td>II</td>
<td>Mild acid&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Glucose</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td>II</td>
<td>Mild alkali&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Lactose</td>
<td>0.30 (0.30)</td>
</tr>
</tbody>
</table>

<sup>a</sup> R_<sub>G</sub> glucose of corresponding standard is shown in parentheses.

<sup>b</sup> Hydrolyzed in 1.0 N H<sub>2</sub>SO<sub>4</sub> for 1 hour at 100°.

<sup>c</sup> Hydrolyzed in 1.0 N acetic acid for 2 hours at 100°.

<sup>d</sup> Hydrolyzed in 0.025 N Na<sub>2</sub>SO<sub>4</sub> for 5 minutes at 100°.

---

**Table II**

Tosylhydrazone derivatives of fucosyllactose-<sup>3H</sup> and fucosyllactose-<sup>14C</sup>

Carrier 2'-fucosyllactose (20 μmoles) was added to Reaction Product I, which contained 960 c.p.m. of <sup>3H</sup>, and to Reaction Product II, which contained 8580 c.p.m. of <sup>14C</sup>. Tosylhydrazone derivatives of the two samples were then prepared by the method of Kuhn et al. (4) as follows. The samples were evaporated to dryness at 40°, and the residue was taken up in 0.2 ml of methanol. Acetonitrile, 0.2 ml, containing 4.0 mg of p-tolylsulfonylhydrazine was added, and the mixture was heated for 40 minutes at 100° in a sealed ampoule and allowed to crystallize at room temperature for 3 days. The mother liquors were decanted, and the crystals were washed with three portions of cold methanol-acetonitrile (1:1). The derivatives were then recrystallized from hot 80% 1-propanol. Part of the colorless needles thus obtained were sealed ampoule and allowed to crystallize at room temperature in the indicated solvents. Standard compounds were added to each before chromatography.

A number of unidentified radioactive compounds were obtained. There was no radioactivity in the area corresponding to fucosylgalactose.
of formation of fucosyllactose is linear for at least 2 hours, as shown in Fig. 3. The yield of trisaccharide, based on GDP-fucose added, ranged between 1 and 12% with various enzyme preparations. Sodium fluoride (0.025 M) or sodium phosphate (0.1 M) did not decrease the rate of breakdown of GDP-L-fucose or increase the amount of product formed. In Fig. 4 the formation of fucosyllactose is illustrated as a function of enzyme concentration and is linear over the indicated range. In Fig. 5 the effect of increasing lactose concentration on the synthesis of fucosyllactose is shown. From the reciprocal plot shown in the inset, the $K_m$ for lactose was calculated to be $7 \times 10^{-3}$ M.

The enzyme lost half of its original activity after 36 hours at 4°C. Active preparations could be obtained from frozen mammary tissue stored for several weeks at -20°C. In addition to the 25,000 to 100,000 × g particulate fraction used in the experiments described above, enzyme activity was found in the fraction sedimenting between 2,000 and 25,000 × g. This heavier fraction, however, has significantly higher nucleotide pyrophosphatase activity than the lighter fraction, which results in a lower yield of trisaccharide. The supernatant solution obtained after centrifugation at 100,000 × g does not catalyze the synthesis of fucosyllactose.

**Table III**

*Formation of Fucosyllactose*

The complete system contained 0.1 μmole of GDP-L-fucose-3H, 1.7 μmoles of lactose, 0.2 μmole of MgCl₂, 25 μmoles of Tris buffer, pH 8.0, and 0.1 ml of enzyme in a final volume of 0.25 ml. After incubation for 2 hours at 37°C, the reaction mixtures were treated as described in the text under “Assay for Formation of Fucosyllactose.”

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Fucosyllactose formed (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>0.62</td>
</tr>
<tr>
<td>Complete system minus MgCl₂</td>
<td>0.03</td>
</tr>
<tr>
<td>Complete system plus 5 μmoles of GTP</td>
<td>2.14</td>
</tr>
<tr>
<td>Complete system with heated enzyme (3 minutes at 100°C)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Fig. 3.** Rate of synthesis of fucosyllactose. The reaction mixtures were the same as in Reaction A of Fig. 1. At the indicated times the samples were treated as described in the text under “Assay for Formation of Fucosyllactose.”

**Fig. 4.** Effect of enzyme concentration on the rate of formation of fucosyllactose. Reaction mixtures were the same as in Reaction A of Fig. 1 except that 5 μmoles of lactose were used instead of 1.7 μmoles and the amount of enzyme was varied as indicated. After 2 hours of incubation at 37°C the mixtures were treated as described in the text under “Assay for Formation of Fucosyllactose.”

**Fig. 5.** Effect of lactose concentration on rate of formation of fucosyllactose. Reaction mixtures were the same as in Reaction A of Fig. 1 except that the concentration of lactose was varied. After incubation for 2 hours at 37°C, the mixtures were treated as described in the text under “Assay for Formation of Fucosyllactose.”

*Synthesis of Lactose*—The particulate preparation used in this paper is similar to the fraction from the mammary gland of the lactating guinea pig used by Watkins and Hassid (18) in the enzymatic synthesis of lactose. The preparation from canine tissue also synthesized lactose by transfer of β-galactose from UDP-β-galactose to β-glucose when assayed by the method employed by these authors.

*Studies in Vivo*

Distribution of Radioactivity in Canine Milk Oligosaccharides after Intra-arterial Injection of GlyceroP₄C—Components of the purified oligosaccharides were obtained by partial or total hydrolysis as described above, and their specific activities were determined (Table IV). In every case the specific activity of
injected intravenously and milk was expressed manually from the mammary glands. Glycerol-\textsuperscript{14}C, 150 μc (5 μc per μmole), was then determined in a liquid scintillation spectrometer with a system consisting of 0.02 ml of water, 1.0 ml of ethanol, and 10 ml of toluene containing 400 mg of 2,5-diphenyloxazole and 5 mg of p-bis-(5'-phenyloxazolyl)benzene per 100 ml (13).

The part,iculate enzyme preparation described in the present paper catalyzes both this reaction and the further synthesis of the trisaccharide, fucosyllactose, by addition of L-fucose to lactose. Canine mammary tissue thus possesses the requisite specific enzymes for the synthesis of fucosyllactose from UDP-L-fucose, UDP-D-galactose, and GDP-L-fucose, each of which has been found in mammary tissue or milk (20). Demonstration of the total synthesis in vivo of fucosyllactose from UDP-D-galactose and D-glucose.

the sugar nucleotide precursors is not practical because of the high Km for lactose of the L-fucosyltransferase (Fig. 5). This consideration would have less significance in vivo, since the concentration of lactose in the mammary gland is very high.

After injection of glycerol-\textsuperscript{14}C, the distribution of radioactivity in milk oligosaccharides following injection of glycerol-\textsuperscript{14}C into artery supply mammary gland

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>Specific activity of oligosaccharide (c.p.m./μmole)</th>
<th>Specific activity of hydrolysis products (c.p.m./μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>2100</td>
<td>2000</td>
</tr>
<tr>
<td>Fucosyllactose</td>
<td>3340</td>
<td>&lt;100</td>
</tr>
<tr>
<td>3'-Sialyllactose</td>
<td>3490</td>
<td>1590</td>
</tr>
<tr>
<td>6'-Sialyllactose</td>
<td>3480</td>
<td>1590</td>
</tr>
<tr>
<td>Higher oligosaccharides</td>
<td></td>
<td>1870</td>
</tr>
</tbody>
</table>

* Although these compounds had the same chromatographic mobility as the indicated isomers, the possibility that either may be the 2' or 4' isomer has not been excluded.

**DISCUSSION**

Two isomers of fucosyllactose, 2'-fucosyllactose and 3-fucosyllactose, are known to occur in human milk (17), and no other position isomers have been reported. The identity of the enzymatically synthesized trisaccharide is based on the characterization of two reaction products, one labeled in the fucose moiety derived from GDP-L-fucose-\textsuperscript{3H}, and the other in the glucose moiety derived from lactose-D-\textsuperscript{14}C. Each of these products proved to be identical with authentic 2'-fucosyllactose by paper chromatography, partial and total acid hydrolysis, alkaline degradation, and carrier crystallization as the tosylhydrazone. Formation of fucosylgalactose by alkaline hydrolysis, in addition to the chromatographic properties, distinguishes the 2' isomer from 3-fucosyllactose. The stability of 1,2-glycosidic bonds to alkal is well known and has been reported by Kuhn et al. specifically for the case of fucosyllactose (19). Nevertheless, methylation studies would be required to establish rigorously that the enzymatic product is 2'-fucosyllactose.

Watkins and Hassid (18) showed that lactose can be enzymatically synthesized from UDP-D-galactose and D-glucose. The particulate enzyme preparation described in the present paper catalyzes both this reaction and the further synthesis of the trisaccharide, fucosyllactose, by addition of L-fucose to lactose. Canine mammary tissue thus possesses the requisite specific enzymes for the synthesis of fucosyllactose from UDP-D-galactose, UDP-D-galactose, and GDP-L-fucose, each of which has been found in mammary tissue or milk (20). Demonstration of the total synthesis in vitro of fucosyllactose from UDP-D-galactose and D-glucose.
lactose was found to be distributed equally between L-fucose and D-galactose, while the D-glucose moiety remained relatively unlabeled. The low specific activity of D-glucose would be anticipated if this hexose arose from the large endogenous D-glucose pool. Since we have shown that canine mammary tissue possesses the requisite enzymes for the synthesis of fucosyllactose, a pathway consisting of known reactions can be constructed illustrating the biosynthesis of this trisaccharide and its metabolic relationship to glycerol (Fig. 6).

The interpretation of the distribution of radioactivity in the two sialylactose isomers is analogous to that described for fucosyllactose. Both sialic acid and D-galactose are equally labeled, indicating their origin from hexose phosphate, while glucose is relatively unlabeled. These results are in accord with the findings of Jourdian, Carlson, and Roseman (28), who demonstrated an enzyme in goat mammary gland that catalyzes the synthesis of sialylactose from CMP-sialic acid and lactose.

D-Glucose isolated from higher oligosaccharides contained negligible radioactivity. Although the exact composition of this fraction was not determined, glucose is at the reducing end of the principal oligosaccharides found in milk (2). These facts suggest that the higher heterosaccharides of milk arise by sequential glycosyl transfer from sugar nucleotides catalyzed by specific transferases, glucose serving as the initial acceptor molecule. Nucleotide-linked oligosaccharides occur in milk (29-31), but their role, if any, in the synthesis of free oligosaccharides is not known.

**SUMMARY**

A particulate enzyme from mammary tissue of lactating dogs catalyzes the synthesis of fucosyllactose by transfer of D-fucose from guanosine diphosphate L-fucose to lactose. The trisaccharide was characterized by its chromatographic properties, by identification of hydrolysis products, and by carrier dilution of its tosylhydrazone derivative. Since the same enzyme preparation catalyzes the synthesis of lactose from uridine diphosphate N-galactose and guanosine sugar nucleotides to the initial acceptor, free glucose.

The synthesis in vivo of fucosyllactose and other oligosaccharides found in milk was studied in dogs by injecting glycerol-14C into the artery supplying the mammary gland. Lactose, fucosyllactose, sialylactose, and certain higher oligosaccharides have been subsequently isolated from milk, and the specific activity of their constituent sugars has been determined. Lactose obtained by partial hydrolysis of fucosyllactose and from two isomers of sialylactose had approximately the same specific activity as free lactose, while the specific activities of D-galactose, L-fucose, and xylose acid were approximately equal. The glucose moiety contributed less than 5% to the total specific activity of the lactose moiety in these trisaccharides.

These observations indicate that D-glucose of these oligosaccharides arises directly from free D-glucose rather than through phosphorylated intermediates. Taken together with its known terminal position in these oligosaccharides, the results suggest that D-glucose is the initial acceptor in their biosynthesis.

**Acknowledgments**—We are indebted to Dr. Gerald Austen and Dr. Sara Jane Uhrich for performing the surgical operations necessary to this study.

**REFERENCES**

Biosynthesis of Fucosyllactose and Other Oligosaccharides Found in Milk
Arthur P. Grollman, Clara W. Hall and Victor Ginsburg


Access the most updated version of this article at http://www.jbc.org/content/240/3/975.citation

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/240/3/975.citation.full.html#ref-list-1