Uridine Diphosphate-\(\alpha\)-N-acetyl-\(\beta\)-galactosamine: \(\alpha\)-Galactose \(\alpha\)-3-\(\alpha\)-N-Acetyl-\(\beta\)-galactosaminyltransferase, a Product of the Gene That Determines Blood Type A in Man

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SUMMARY

An \(N\)-acetyl-\(\beta\)-galactosaminyltransferase which occurs in human milk from donors of blood type A or AB but not in milk from donors of blood type B or O has been purified 55-fold, and its properties have been studied. The enzyme catalyzes the following reaction which results in the formation of structural determinants of blood type A.

\[
\text{UDP-\(\alpha\)-GalNAc } + \text{ O-\(\alpha\)-d-Gal} \xrightarrow{\text{Mn}^{++}} \text{O-\(\alpha\)-L-Fuc-(1 \rightarrow 2)O-\(\alpha\)-D-Gal} \xrightarrow{\text{UDP}} \text{O-\(\alpha\)-L-Fuc-(1 \rightarrow 2)}
\]

This study, the enzyme has been purified and its properties studied. In addition, the structure of the reaction product has been elucidated.

EXPERIMENTAL PROCEDURE

Materials—UDP-\(N\)-acetyl-\(\beta\)-galactosamine was prepared from \(N\)-acetyl-\(\beta\)-galactosamine 1-phosphate and UMP morpholidate by the method of Roseman et al. (4). \(N\)-Acetyl-\(\beta\)-galactosamine 1-phosphate was synthesized by the method of Kim and Davidson (5), and UMP-morpholidate was obtained from the Sigma Chemical Company. UDP-\(N\)-acetyl-\(\beta\)-galactosamine labeled with 14C in the acetyl group (91 \(\mu\)Ci per \(\mu\)mole) was obtained from the International Chemical and Nuclear Corporation (Irvine, California).

2'-Fucosyllactose, \(3\)-fucosyllactose, lacto-\(N\)-tetraose, lacto-\(N\)-fucopentaose I, lacto-\(N\)-fucopentaose II, lacto-\(N\)-fucopentaose III, and \(\Lambda\)-tetrasaccharide C were isolated from human milk by methods previously reported (6, 7). \(\Lambda\)-fucopentaose was prepared by mild acid hydrolysis of \(\Lambda\)-fucotetraose C by the method of Kuhn and Gauhe (8). 2'-O-\(\alpha\)-L-Fucopyranosyl-\(\beta\)-galactosyl and 2-O-\(\alpha\)-L-Fucopyranosyl-N-talosyl were prepared from 2'-fucosyllactose by alkaline hydrolysis as described by Kuhn, Baur, and Gauhe (9).

Lacto-difucotetraose, lacto-\(N\)-difucohexaose I, and lacto-\(N\)-difucohexaose II were kindly donated by Dr. A. Gauhe. 2-O-\(\alpha\)-L-Fucopyranosyl-\(\beta\)-galactosyl, 4-O-\(\alpha\)-L-Fucopyranosyl-\(\beta\)-galactopyranoside, and 6-O-\(\alpha\)-L-Fucopyranosyl-\(\beta\)-galactopyranoside were the generous gifts of Dr. O. P. Bahl. 3-O-\(\alpha\)-N-Acetyl-\(\beta\)-galactosaminyl-\(\alpha\)-galactosyl and 3-O-\(\alpha\)-N-Acetyl-\(\beta\)-galactosaminyl-\(\beta\)-galactosyl were kindly provided by Drs. Z. Yoshizawa and T. Yamakawa, respectively.

Purified \(\alpha\)-N-acetyl-\(\beta\)-galactosaminidase from bovine liver was kindly provided by Dr. B. Weissman. \(\Lambda\)-Labeled oligosaccharides were tested as substrates for the enzyme as follows: 1 \(\mu\)mole of oligosaccharide was incubated with 12 pg of enzyme in 20 \(\mu\)l of 0.1 M sodium citrate buffer, pH 4.5, and 15 \(\mu\)l of bovine

(1.) The distribution of the enzyme, in addition to its acceptor specificity, made it likely that the transferase formed the following structure known to be a determinant of A specificity (2, 3).

\[
O-\alpha\text{-GalNAc}-(1 \rightarrow 3)-O-\beta\text{-d-Gal} \ldots O-\alpha\text{-L-Fuc}-(1 \rightarrow 2)
\]
serum albumin for 24 hours at 37°C. The reaction mixture was applied on Whatman No. 3MM filter paper and chromatographed for 14 hours with Solvent 1, to be described below. The chromatogram was then scanned for 14C activity for detection of the formation of free N-acetyl-d-galactosamine-14C. Under the above conditions of incubation, the enzyme liberates 0.06 pmole of N-acetyl-d-galactosamine per hour per mg of protein from blood group substance A isolated from hog stomach. 2

Samples of milk were obtained from members of the Metropolitan Washington Chapter of the La Leche Society and stored at −20°C until used. Upon thawing, the milk was centrifuged at 2°C for 20 min at 1000 × g. The solidified lipid was removed by filtering the milk through glass wool at 2°C, and the defatted milk obtained in this way was used as a starting point for the enzyme purification. The blood type and secretor status of the donors was determined on samples of blood and saliva by Mrs. Mary McGinniss at the blood bank of the National Institutes of Health.

Soluble blood group substance A purified from human ovarian cyst mucin was kindly provided by Dr. Donald M. Marcus of the Albert Einstein College of Medicine.

**Paper Chromatography**—Descending paper chromatography was performed with the following solvent systems: Solvent 1, upper layer of ethyl acetate-pyridine-H2O (2:1:2); Solvent 2, ethyl acetate-pyridine-acetic acid-H2O (5:5:1:3); and Solvent 3, 1-butanol-ethanol-H2O (4:1:1). For the separation of N-acetyl-β-glucosamine from N-acetyl-d-galactosamine, Solvent 1 was used with borate-impregnated paper (10). Oligosaccharides were located with AgNO3 reagent (11) and radioactivity with a Vanguard automatic chromatogram scanner.

**Analytical Procedures**—Hexosamine was measured by the method of Rimington (12), fucose was measured by the method of Dieche and Shelttes (13), glucose was measured with Glucostat reagent (Worthington Biochemical Corporation), and galactose was measured (14) by galactose oxidase (Worthington Biochemical Corporation).

Because N-galactosamine also reacts with galactose oxidase and, under the conditions of assay, gives 75% of the color of N-galactose, assays for galactose in oligosaccharides that contained N-galactosamine were corrected for the contribution of N-galactosamine. d-Galactosamine was determined by its 14C activity.

**Enzyme Assay**—The standard assay mixture for transferase activity contained 0.01 pmole of UDP-N-acetyl-d-galactosamine-14C (68,000 cpm); 0.2 pmole of 2'-fucosyllactose; 0.5 pmole of MnCl2; 2.5 μmoles of Tris buffer, pH 7.5; and enzyme, in a total volume of 50 μl. The mixture was incubated at 37°C for 24 hours, and the reaction was stopped by heating in a boiling water bath for 2 min. The solution was transferred to Whatman No. 3MM paper as a 2-cm band and subjected to electrophoresis in pyridine-acetic acid buffer, pH 5.4 (15), for 60 min at 50 volts per cm in a high voltage electrophorator (Gilson Medical Electronics, Middleton, Wisconsin). The paper was cut 10 cm from the anodal end to remove the residual UDP-N-acetyl-d-galactosamine-14C which migrates there, and the remaining paper was chromatographed for 16 hours with Solvent 2 to separate the reaction product from the free N-acetyl-d-galactosamine which is formed during incubation. Both of these sugars remain near the origin during electrophoresis. The section of the chromatogram containing the product was assayed for 14C activity in 5 ml of Dray's solution (16) in a scintillation counter. Background 14C activity was determined from the corresponding area of another chromatogram derived from an incubation mixture in which 2'-fucosyllactose was omitted.

A unit of enzyme is defined as the amount of enzyme that will catalyze the formation of 1 μmole of product per hour under the standard assay conditions. Specific activity is expressed as units of enzyme activity per mg of protein as measured by the method of Lowry et al. (17) with bovine serum albumin as a standard.

**Results**

**Enzyme Purification**

**Distribution of Enzyme**—The enzyme is found only in milk from donors with A or AB blood type, and its level in this group varies considerably, as shown in Table I. The fractionation scheme was worked out with milk from Donor E. K., who had the highest level of enzyme activity. E. K. belongs to the subgroup AX but, as yet, no difference has been found in the properties of this enzyme obtained from milk donors belonging to the subgroup AX. Both enzymes fractionate in a similar way, and the other properties described in this paper are indistinguishable. All of the following operations were performed at 4°C unless otherwise specified.

**Ammonium Sulfate Fractionation**—Ammonium sulfate was slowly added with stirring to 1 liter of defatted milk until 36% saturation was reached. The turbid solution was centrifuged for 20 min at 10,000 × g, and the resulting precipitate, which contained less than 10% of the transferase activity, was discarded. Ammonium sulfate was then added to the supernatant solution to 42% saturation. After centrifugation, the precipi-

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1. B. Weissman, unpublished results.

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**Table I**

| Donor | Blood type/ ABO Lewis | Secretor status | N-Acetyl-d-galactosaminyltransferase-14C transferred to 2'-fucosyllactose |
|-------|-----------------------|-----------------|---------------------------------------------------------------|
| J. M. | A1 (b+)               | Secretor        | 580                                                          |
| M. K. | A1 (b+)               | Secretor        | 250                                                          |
| D. C. | A1 (b+)               | Secretor        | 310                                                          |
| J. B. | A2 (b+)               | Secretor        | 100                                                          |
| E. K. | A2 (a--b--)           | Secretor        | 1400                                                         |
| J. C. | A1B (b+)              | Secretor        | 820                                                          |
| L. M. | A2 (a+)               | Nonsecretor     | 330                                                          |
| B. B. | A1 (a+)               | Nonsecretor     | 140                                                          |
| G. H. | B (b+)                | Secretor        | 0                                                            |
| R. S. | B (b+)                | Secretor        | 0                                                            |
| N. C. | B (b+)                | Secretor        | 0                                                            |
| L. J. | O (b+)                | Secretor        | 0                                                            |
| L. N. | O (b+)                | Secretor        | 0                                                            |
| C. D. | O (b+)                | Secretor        | 0                                                            |
| J. S. | O (a+)                | Nonsecretor     | 0                                                            |
| D. R. | O (a+)                | Nonsecretor     | 0                                                            |
N-Acetyl-α-galactosaminyltransferase and Blood Type A

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FIG. 1. Sephadex G-200 chromatography of the enzyme. —— absorbance at 280 μν; --- "hydrolase" activity that liberates N-acetyl-α-galactosamine from UDP-N-acetyl-D-galactosamine; hatched area, N-acetyl-α-galactosaminyltransferase activity.

TABLE II

Purification of an N-acetyl-α-galactosaminyltransferase from human milk

| Purification step | Protein | Total activity | Specific activity | Yield | Puriﬁcation |
|------------------|---------|----------------|------------------|-------|------------|
| Defatted, dialyzed milk | 10,500 | 210,000 | 20 | 100 | 1 |
| (NH₄)₂SO₄ fractionation | 1,639 | 164,000 | 100 | 78 | 5 |
| Sephadex G-200 chromatography | 194 | 163,000 | 531 | 49 | 27 |
| MnCl₂ fractionation | 90 | 98,700 | 1,085 | 47 | 55 |

tate, which contained about 80% of the transferase activity,⁴ was dissolved in 50 ml of 0.05 m Tris buffer, pH 7.5, and dialyzed overnight against the same buffer. The dialyzed solution was then centrifuged for 15 min at 10,000 × g, and the small precipitate was discarded.

Sephadex G-200 Chromatography—Aliquots, 5 ml each, of the enzyme preparation were fractionated by passage through a column of Sephadex G-200 (2 × 90 cm) that had been washed overnight with 0.05 m Tris buffer, pH 7.5. The column was eluted with the same buffer, and the eluate was collected in 5-ml fractions. Each fraction was assayed for protein by absorbance measurements at 280 μν and for enzymatic activity. As shown in Fig. 1, this fractionation resulted in a substantial purification of the transferase and a separation from it of a hydrolase which liberated free N-acetyl-α-galactosamine from the labeled substrate (probably the combined action of a nucleotide pyrophosphatase and a phosphatase). The latter activity was eluted in the void volume along with most of the protein. Fractions 16 to 24 from all of the runs were pooled and concentrated to 4 ml by filtration through a collodion bag (Carl Schleicher and Schuell, Keene, New Hampshire).

Manganese Chloride Fractionation—To 4 ml of the concentrated enzyme solution at 0° was added 1 ml of 0.1 m MnCl₂ and, after mixing, the solution was warmed to 37° for 2 min. A precipitate of inactive protein formed which was removed by centrifugation at 1000 × g for 5 min at room temperature. The supernatant solution was dialyzed overnight against 1 liter of 0.05 m Tris buffer, pH 7.5, concentrated to 4 ml by filtration through a collodion bag, and stored at −20°. The enzyme at this stage was used for most of the studies described in this paper. It is quite stable, and no loss of activity was observed after 4 months of storage.

A summary of the purification scheme is given in Table II.

Properties of the Enzyme

Kinetics—The reaction rate is proportional to protein concentration and is linear for at least 20 hours as shown in Fig. 2.

Effect of pH—The activity of the enzyme as a function of pH is shown in Fig. 3. The optimum pH is approximately 7.5.
Metal Requirement—The requirement for Mn++ is shown in Fig. 4. The optimal concentration of Mn++ is 0.01 mM and at higher concentrations a slight inhibition occurred. Magnesium ion had no effect at any concentration tested. At 0.0125 mM concentration a slight inhibition occurred. Magnesium under the above conditions included n-galactose, n-glucose, n-mannose, α- and β-methyl-β-D-galactopyranosides, N-acetyl-n-glucosamine, 4-O-α-L-fucopyranosylmethyl-β-D-galactopyranoside, 6-O-α-L-fucopyranosylmethyl-β-D-galactopyranoside, lactose, 2-O-α-L-fucopyranosyl-n-talose, lacto-difucotetraose, lacto-N-tetraose, lacto-N-neotetraose, lacto-N-tetraose, lacto-N-difucohexaose I and lacto-N-difucohexaose II.

Acceptor Specificity—Various sugars were tested for their ability to act as acceptors for N-acetyl-D-galactosamine by replacing 2'-fucosyllactose in the "standard assay" by an equal amount (0.2 μmole) of the sugar to be tested. In addition to 2'-fucosyllactose and lacto-N-fucopentaose I (1), only 2-O-α-L-fucopyranosylmethyl-β-D-galactopyranoside and 2-O-α-L-fucopyranosyl-n-galactose were active acceptors. Inactive acceptors under the above conditions included n-galactose, n-glucose, n-mannose, α- and β-methyl-β-D-galactopyranosides, N-acetyl-n-glucosamine, 4-O-α-L-fucopyranosylmethyl-β-D-galactopyranoside, 6-O-α-L-fucopyranosylmethyl-β-D-galactopyranoside, lactose, 2-O-α-L-fucopyranosyl-n-talose, fucosyllactose, lacto-difucotetraose, lacto-N-tetraose, lacto-N-neotetraose, lacto-N-fucopentaose II, lacto-N-fucopentaose III, lacto-N-difucohexaose I, and lacto-N-difucohexaose II.

Effect of Substrate Concentration—The effect of UDP-N-acetyl-D-galactosamine concentration on the reaction rate with 2'-fucosyllactose as an acceptor is shown in Fig. 5. From the reciprocal plot shown in the inset, the \( K_m \) value for the nucleotide can be calculated to be 2.5 \( \times 10^{-4} M \).

The effect of varying the concentrations of the four active acceptors is shown in Fig. 6A. From the reciprocal plots shown in Fig. 6B, the \( K_m \) values for the acceptors can be calculated to be 7.4 \( \times 10^{-4} M \) for 2-O-α-L-fucopyranosyl-d-galactose, 5.0 \( \times 10^{-4} M \) for 2-O-α-L-fucopyranosylmethyl-β-D-galactopyranoside, 4.0 \( \times 10^{-4} M \) for 2'-fucosyllactose, and 3.5 \( \times 10^{-4} M \) for lacto-N-fucopentaose I. The highest \( V_{max} \) was obtained with 2-O-α-L-fucopyranosylmethyl-β-D-galactopyranoside, twice as high as with the other acceptors.

CHARACTERIZATION OF THE REACTION PRODUCTS

Isolation—For characterization of the reaction products, relatively large amounts were prepared with 2'-fucosyllactose and lacto-N-fucopentaose I as acceptors. The following procedure was used. A reaction mixture containing 37 μmoles of 2'-fucosyllactose (or lacto-N-fucopentaose I); 3.2 μmoles of UDP-N-acetyl-d-galactosamine-14C (6 \( \times 10^6 \) cpm); 100 μmoles of MnCl₂; 500 μmoles of Tris-HCl buffer, pH 7.5; and 73 units of purified enzyme in a final volume of 10 ml was incubated for 96 hours at 37°C. A drop of toluene was present to inhibit bacterial growth. The reaction mixture was heated at 100°C for 2 min, and coagulated protein was removed by centrifugation. The supernatant solution was passed through a mixed bed resin column (1 x 3 cm) containing AG 50 (H⁺) and AG 3 (OH⁻), and eluate and washings (3 bed volumes of H₂O) were combined and concentrated to 0.5 ml under vacuum. The solution was transferred to Whatman No. 3MM paper as a 15-cm band and chromatographed for 2 days when 2'-fucosyllactose was used as an acceptor and for 5 days when lacto-N-fucopentaose I was used as an acceptor. The oligosaccharides were located by their UV activity, eluted with H₂O, and rechromatographed under the same conditions. With 2'-fucosyllactose as an acceptor, 1.9 μmoles of product (P₁₁) were obtained, whereas 1.1 μmole of product (P₁₁) was isolated with lacto-N-fucopentaose I as an acceptor.

Composition—Aliquots of P₁ and P₁₁ were hydrolyzed in 1 N HCl for 1 hour at 100°C, and the monosaccharides in the hydrolysates were analyzed by paper chromatography with Solvent I. Glucose, galactose, fucose, and galactosamine were found in the hydrolysate of P₁₁ whereas glucose, galactose, fucose, glucosamine, and galactosamine were found in the hydrolysate of P₁. The ratios of these monosaccharides (Table III) show that P₁ and P₁₁ are formed by the addition of 1 N-acetyl-D-galactosaminyl residue to 2'-fucosyllactose and to lacto-N-fucopentaose I, respectively.

Partial Hydrolysis—Partial hydrolysis of P₁ and P₁₁ resulted in the formation of the oligosaccharides shown in Fig. 7. Free N-acetyl-D-galactosamine which could be detected by shorter chro-
TABLE III

Composition of the reaction products

Colorimetric analysis of fucose was carried out on the intact oligosaccharides, whereas the colorimetric analyses of the other sugars were done after hydrolysis in 1 N HCl for 2 hours at 100°C.

| Oligosaccharide                      | Molar ratio | Glucose | Galactosamine | Fucose | Hexosamine | N-Acetyl-D-galactosamine |
|--------------------------------------|-------------|---------|---------------|--------|------------|-------------------------|
| 2'-Fucosyllactose                   | 1.00        | 1.02    | 0.96          | 0.01   |
| Product derived from 2'-fucosyllactose (P1) | 1.00        | 1.10    | 0.95          | 0.95   |
| Lacto-N-fucopentaose I              | 1.00        | 2.03    | 0.95          | 0.93   |
| Product derived from lacto-N-fucopentaose I (PII) | 1.00        | 1.98    | 0.91          | 1.93   |

Fig. 7. Chromatographic analysis of the oligosaccharides resulting from acid hydrolysis of the purified products derived from 2'-fucosyllactose (P1) and lacto-N-fucopentaose I (PII). The conditions of hydrolysis were 0.1 N HCl for 30 min at 100°C. Chromatography was carried out on Whatman No. 3 MM paper for 30 hours with Solvent 1. A, PI before hydrolysis; B, PI after hydrolysis; C, PII before hydrolysis; D, PII after hydrolysis. The smaller peaks shown in D were obtained by scanning the chromatogram at one-third sensitivity. The standard sugars at the bottom of the figure are: 1, lacto-N-difucohexaose I; 2, lacto-N-fucopentaose I; 3, lacto-N-fucopentaose II; 4, lacto-N-tetraose; 5, 2'-fucosyllactose; 6, lactose; and 7, 3-O-acetyl-l-D-galactosaminyl-l-D-galactose.

The chromatographic runs were formed in both cases and was identified by rechromatography on borate-impregnated paper with Solvent 1. Oligosaccharides P1-2 and PII-4 had the same chromatographic mobility as 3-O-acetyl-l-D-galactosaminyl-l-D-galactose and on hydrolysis in 1 N HCl for 1 hour at 100°C, both liberated only galactosamine and galactose. This result indicated that the N-acetyl-l-D-galactosamine was being transferred to galactose in both cases, and, as fucosyl residues are quite acid labile, that P1-2 is GalNAc + Gal + Glc, PII-2 is GalNAc + Gal + GlcNAc, PII-3 is GalNAc + Gal + GlcNAc + Gal, and PII-4 is GalNAc + Gal + GlcNAc + Gal + Glc. Starting with 600 mpmoles of P1, 80 mpmoles of P1-2 and 312 mpmoles of P1-3 were obtained. Starting with 600 mpmoles of PII, 39 mpmoles of PII-3, 35 mpmoles of PII-4, and 190 mpmoles of PII-5 were obtained. Results of the periodate oxidation (Fig. 2) of P1-2 and PII-4 (the...
The anomeric configuration of the N-acetyl-n-galactosamine can be assigned an α configuration from three independent lines of evidence: (a) the disaccharides \( \text{Pr}_{1-2} \) and \( \text{Pr}_{11-2} \) in Fig. 7 had the same chromatographic mobility as 3-0-α-D-N acetylgalactosaminyl-β-D-galactose in Solvent 3, which clearly separates 3-0-α-D-N acetylgalactosaminyl-β-D-galactose \( (R_{\text{lactose}} = 1.62) \) from 3-0-β-D-N acetylgalactosaminyl-β-D-galactose \( (R_{\text{lactose}} = 1.44) \);

(b) all of the N-acetyl-β-D-galactosamine is liberated from \( \text{Pr}_1 \) and \( \text{Pr}_2 \) by purified bovine liver α-α-N-acetylgalactosaminidase, which does not hydrolyze β-linked N-acetyl-β-D-galactosamine; and

(c) both \( \text{Pr}_1 \) and \( \text{Pr}_{11} \) are very potent haptenic inhibitors of the precipitation of soluble blood group substance \( \text{A}_1 \) by anti-\( \text{A}_1 \) serum as shown in Fig. 9. Both oligosaccharides inhibited the precipitation reaction by 50% at concentrations of approximately 10 mmoles in the 0.35-ml reaction mixture. Inhibition of anti-A sera is quite specific for α-linked N-acetyl-β-D-galactosamine (19), and, under the conditions of incubation given in Fig. 9, no inhibition was observed with 300 mmoles of the two acceptors, 2'-fucosyllactose and lacto-N-fucopentaose I.

The above results establish the structures of \( \text{Pr}_1 \) and \( \text{Pr}_{11} \) to be those shown in Fig. 10. It is possible that these oligosaccharides occur naturally as oligosaccharides with their chromatographic mobility and, with \( \text{A} \)-haptene activity, occur in milk from “secretors” with blood type A (1). Whether they are identical with \( \text{Pr}_1 \) and \( \text{Pr}_{11} \) is not known. The levels of these naturally occurring haptens in milk ranged from 0.2 to 13 mmoles per ml (Table IV) in “secretors” with blood type A, assuming similar inhibitory powers to those of \( \text{Pr}_1 \) and \( \text{Pr}_{11} \). The oligosaccharides were not found in milk from “secretors” with blood type B or O or in milk from a “nonsecretor” with blood type A.

### Table IV

| Donor | Blood type | Secretor status | Haptenic oligosaccharide |
|-------|------------|-----------------|--------------------------|
|       |            |                 | \( \text{F}_1 \) | \( \text{F}_{11} \) |
| B. J. | A\(_1\)    | Secretor        | 13                       | 4                       |
| E. K. | A\(_2\)    | Secretor        | 3                        | 2                       |
| E. W. | A\(_1\)    | Secretor        | 0.4                      | 0.2                     |
| M. C. | A\(_1\)    | Secretor        | 8                        | 4                       |
| M. R. | A\(_1\)    | Secretor        | 0.2                      | 0.3                     |
| K. G. | A\(_1\)    | Secretor        | 6                        | 1                       |
| B. B. | A\(_1\)    | Nonsecretor     | <0.1                     | <0.1                    |
| H. S. | O          | Secretor        | <0.1                     | <0.1                    |
| M. S. | B          | Secretor        | <0.1                     | <0.1                    |

**DISCUSSION**

The transferase described in this paper is the product of the \( \text{A} \) gene that determines blood type (cf. Reference 19) and produces the \( O-\alpha-\text{D-GalNAc}(1 \rightarrow 3)-\text{O}-\beta-\text{D-Gal} \) structures that occur in red cell glycolipids responsible for blood type A (20-22) in glycoproteins of mucous secretions (23) as well as in oligosaccharides of urine (24) and milk (Table IV) of persons of blood type A. The same enzyme is involved in the synthesis of all of these classes of compounds, as the structure is not found in similar material from persons with blood type B or O, and family studies show that the inheritance of blood type A is controlled by one gene (cf. Reference 25).

The enzyme transfers \( \text{N-acetyl}-\text{D-galactosamine to galactosyl residues that are substituted with L-fucosyl residues on the C-2 position of the } \text{galactosyl residue of 2'}-\text{fucosyllactose and the second galactosyl residue of lacto-N-fucopentaose I. As shown in Fig. 8, the galactosyl residues of both } \text{Pr}_1 \) and \( \text{Pr}_{11} \) are completely resistant to periodate oxidation which can occur only if the \( \text{N-acetyl}-\text{D-galactosamine is attached to the C-3 position of these galactosyl residues} \) (see Fig. 10). As expected, all of the galactosyl residues of lactose and 2'-fucosyllactose and half of the galactosyl residues of lacto-N-tetraose are destroyed by periodate (Fig. 8).

### Fig. 10. Structure of the oligosaccharides \( \text{Pr}_1 \) and \( \text{Pr}_{11} \) formed by the addition of N-acetyl-D-galactosamine to 2'-fucosyllactose and lacto-N-fucopentaose I, respectively.

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**Milk, 40 ml of each type, was fractionated by Sephadex chromatography as described previously (6).** Appropriate fractions were pooled (e.g. Fractions 10 to 38 in Fig. 1 of Reference 6) and chromatographed with Solvent 1 for 4 days. The areas of the chromatogram corresponding to \( \text{Pr}_1 \) and \( \text{Pr}_{11} \) were eluted with \( \text{H}_2\text{O} \), and the haptenic activity of the eluate was tested in the system described in the legend of Fig. 9. The concentration of inhibitor was estimated from the curves of Fig. 9.
then, N-acetyl-d-galactosamine is added to the same d-galactosyl residue; and, finally, a second d-fucose is added to the N-acetyl-d-glucosaminyl residue (28).

The antigens on the erythrocyte surface responsible for blood group A are thought to be glycolipids, but their structures are not established as yet (29). According to the recent work of Wiegandt (30), about one-half of the gangliosides found in the erythrocyte have either lacto-N-tetraose or lacto-N-neotetraose in their oligosaccharide chains. This finding suggests that oligosaccharide P1 may be a structural determinant for blood group A in the glycolipids of the erythrocyte. Eto et al. (31) recently isolated a glycolipid, Gal-(1 \→ 3)-Gal-(1 \→ 3)-GlcNAc-(1 \→ 3)-Gal-(1 \→ 4)-Glc-ceramide, from rabbit erythrocyte which shows weak blood group B haptenic activity.

N-Acetyl-d-galactosaminyltransferases with properties similar to the one described in this paper have been observed in preparations from glands of humans (32) and pigs (33–35).

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