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ENZYMES INVOLVED IN THE BIOSYNTHESIS OF GLYCOCONJUGATES. A UDP-2-ACETAMIDO-2-DEOXY-D-GLUCOSE: β-D-GALACTOPYRANOSYL-(1→4)-SACCHARIDE (1→3)-2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYLTRANSFERASE IN HUMAN SERUM*

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ABSTRACT

A 2-acetamido-2-deoxy-β-D-glucopyranosyltransferase (*N*-acetylglucosaminyltransferase) that catalyses the transfer of 2-acetamido-2-deoxy-D-glucose from UDP-2-acetamido-2-deoxy-D-glucose to terminal nonreducing β-D-galactosyl residues in disaccharides, oligosaccharides, glycoproteins, and glycolipids has been detected in human serum. The preferred acceptors are those with β-D-galactosyl residues linked (1→4) to the subterminal sugar residue. Activity is greatest when the second sugar residue is 2-acetamido-2-deoxy-D-glucose but β-D-Gal*p*-(1→4)-D-Glc and β-D-Gal*p*-(1→4)-D-Man are also good acceptors. Compounds with β-D-galactosyl groups linked (1→3) to 2-acetamido-2-deoxy-D-glucose are relatively poor acceptors and β-D-Gal*p*-(1→6)-D-GlcNAc is inactive. Oligosaccharides substituted with an L-fucose unit on the β-D-galactosyl unit or on the adjacent sugar residue failed to accept 2-acetamido-2-deoxy-D-glucose. Similarly, glycoproteins with L-fucose or sialic acid substituents are less effective acceptors before removal of these sugars to expose more β-D-galactosyl end-groups. The transferred 2-acetamido-2-deoxy-β-D-glucopyranosyl residue is cleaved from the enzymic reaction products by the *N*-acetyl-β-D-glucosaminidase from Jack beans. Methylation analysis of the products of 2-acetamido-2-deoxy-D-glucosyl transfer to *N*-acetyllactosamine [β-D-Gal*p*-(1→4)-D-GlcNAc] and lactose [β-D-Gal*p*-(1→4)-D-Glc] revealed that the terminal, nonreducing D-galactosyl group in both these acceptors had been 3-*O*-substituted with 2-acetamido-2-deoxy-D-glucose. Hence, the enzyme in human serum catalyses, in the presence of Mn^{2+}, the reaction UDP-GlcNAc + β-D-Gal*p*-(1→4)-R → β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-R + UDP and is a UDP-2-acetamido-2-deoxy-D-glucose: β-D-galactopyranosyl-(1→4)-saccharide (1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyltransferase.

*Dedicated to Professor Elvin A. Kabat.

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INTRODUCTION

Mammalian tissues and body fluids contain glycoproteins, glycosphingolipids, and free oligosaccharides in which a 2-acetamido-2-deoxy-D-glucose unit is joined in β-(1→3) glycosidic linkage to a D-galactose unit. Despite the widespread occurrence of this disaccharide unit, comparatively little is known about the *N*-acetylglucosaminyltransferase that catalyses its formation. Membrane-bound enzymes that transfer 2-acetamido-2-deoxy-D-[^{14}C]glucose from UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose to terminal, nonreducing β-D-galactosyl groups were reported[1] in preparations from human, baboon, and rabbit stomachs but the products of 2-acetamido-2-deoxy-D-glucose transfer were not further characterised. Basu *et al.*[2] described an enzyme in rabbit bone marrow which catalysed the transfer of 2-acetamido-2-deoxy-D-[^{14}C]glucose to lactosylceramide. The labelled trisaccharide, when cleaved from the ceramide moiety, cochromatographed with *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose and the enzyme was therefore inferred to be the (1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyltransferase involved in the synthesis of blood-group active glycosphingolipids.

The present paper describes a (1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyltransferase in human serum that catalyses the addition of 2-acetamido-2-deoxy-D-glucose to unsubstituted terminal nonreducing β-D-galactopyranosyl groups in both low-molecular-weight acceptors and in macromolecules.

EXPERIMENTAL

Materials. — UDP-2-acetamido-2-deoxy-D-[U-^{14}C]glucose (300 Ci/mol) was purchased from Amersham, U.K. Unlabelled UDP-2-acetamido-2-deoxy-D-glucose, lactose [β-D-Gal*p*-(1→4)-D-Glc], melibiose [α-D-Gal*p*-(1→6)-D-Glc], D-galactose, 2-acetamido-2-deoxy-D-glucose, phenyl β-D-galactopyranoside, *p*-nitrophenyl lactoside, β-D-Gal*p*-(1→3)-D-Ara*f*, lactulose [β-D-Gal*p*-(1→4)-D-Fru*f*], β-D-Gal*p*-(1→4)-D-Man, and *N*,*N*′-diacetylchitobiose [β-D-Glc*p*NAc-(1→4)-D-GlcNAc] were purchased from Sigma Chemical Co. Ltd., Dorset, U.K.

Synthetic *N*-acetyllactosamine [β-D-Gal*p*-(1→4)-D-GlcNAc] and lacto-*N*-biose I [β-D-Gal*p*-(1→3)-D-GlcNAc] were generously supplied by Professor R. U. Lemieux, University of Edmonton, Alberta, Canada. Also supplied by Professor Lemieux were four oligosaccharides glycosidically linked to an aliphatic spacer arm[3]: β-D-Gal*p*-(1→4)-D-Glc*p*NAc-O-$(CH_2)_8CO_2CH_3$, β-D-Gal*p*-(1→3)-D-Glc*p*NAc-O-$(CH_2)_8CO_2CH_3$, β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-D-Gal*p*-O-$(CH_2)_8CO_2CH_3$, and β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*-O-$(CH_2)_8CO_2CH_3$.

Methyl α- and β-D-galactopyranoside were purchased from Koch–Light Laboratories Ltd., Colnbrook, Bucks, U.K. 2′-Fucosyllactose [α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-D-Glc], 3-fucosyllactose {β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-

Glc}, lacto-*N*-tetraose [β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc], and lacto-*N*-*neo*tetraose [β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc] were isolated from human milk as described by Anderson and Donald[4]. Lacto-*N*-fucopentaose I [α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc], lacto-*N*-fucopentaose II {β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc}, lacto-difucotetraose {α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc}, lacto-*N*-difucohexaose I {α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc}, lacto-*N*-difucohexaose II {β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-Glc} and β-D-Gal*p*-(1→6)-D-GlcNAc were gifts of Dr. A. Gauhe and the late Professor R. Kuhn, Max-Planck-Institut für Medizinische Forschung, Heidelberg, G.F.R. α-D-Gal*p*-(1→4)-D-Gal was isolated from Okra pods by the method of Whistler and Conrad[5], and β-D-Gal*p*-(1→3)-D-Glc was synthesised as described by Kuhn and Baer[6]. α-D-Gal*p*-(1→6)-D-GlcNAc and α-D-Gal*p*-(1→6)-D-GalNAc were prepared by enzymic transgalactosylation[7]. The chemically synthesised, branched pentasaccharide[8], β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-[β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)]-D-Gal was kindly supplied by Dr. C. Augé, Université de Paris-Sud, Orsay, France.

Lactosylceramide [β-D-Gal*p*-(1→4)-D-Glc*p*-Cer], globoside [β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Glc*p*-Cer], and lacto-*N*-*neo*tetraosylceramide [β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*-Cer] were gifts of Dr A. Gardas, Warsaw, Poland.

Blood-group specific glycoproteins were isolated from ovarian cyst fluids as described by Morgan[9]. Fl. 14 (5.6% sialic acid) and No. 484 (5.8% sialic acid) were precursor glycoproteins obtained from cyst fluids of individuals who were non-secretors[10] of blood group A, B, H, Le^a, and Le^b. No. 350 (19% sialic acid)[11] and No. 445 (3.7% sialic acid) were blood-group Le^a-active preparations isolated from cyst fluids of patients who were ABH non-secretors. No. 277 and MH were blood-group HLe^b-active preparations isolated from cyst fluids from blood-group O secretors, MA was a blood-group A-active glycoprotein isolated from a blood-group A-secretor cyst-fluid, and No. 413 a blood-group B-active glycoprotein isolated from a blood-group B-secretor cyst-fluid.

α_1-Acid glycoprotein was a gift from Dr. K. Schmid, Boston University Medical Centre, Boston, Mass., U.S.A. Fetuin was purchased from Sigma Chemical Co., Dorset, U.K., and Type XIV pneumococcal polysaccharide was a gift from Dr. Michael Heidelberger, New York University, N.Y., U.S.A. Tamm–Horsfall glycoproteins from blood group Sd(a +) and Sd(a −) individuals[12] were isolated from urine by the method of Tamm and Horsfall[13], and freed from lipid as described by Soh *et al.*[12].

Jack bean *N*-acetyl-β-D-hexosaminidase (E.C. 3.2.1.30), tunicamycin, and bis-(*p*-nitrophenyl) phosphate were purchased from Sigma Chemical Co. Ion-ex-

change resins, AG 1-X4 (200–400 mesh) and AG 50 W-X8 (200–400 mesh) were obtained from Bio-Rad Laboratories Ltd., Watford, U.K.

Assay of 2-acetamido-2-deoxy-D-glucopyranosyltransferase activity. — The reaction mixtures used for assays of 2-acetamido-2-deoxy-D-glucopyranosyltransferase activity are given in the Tables and Figures. At the end of the incubation period, the products were separated by chromatography on Whatman DE-81 paper in 10:4:3 (v/v) (solvent *a*) or 2:1:1 (v/v) (solvent *b*) ethyl acetate–pyridine–water, or in 5:1:1:3 (v/v) propan-1-ol–ethyl acetate–pyridine–water (solvent *c*). The chromatograms were scanned on a Packard Radiochromatogram scanner and the radioactive areas were cut out and counted in a Nuclear Chicago Scintillation Counter.

Larger-scale preparation of products of 2-acetamido-2-deoxy-β-D-glucopyranosyl transfer. — In order to establish the positional linkage of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue transferred to *N*-acetyllactosamine and lactose, the products of synthesis were prepared in milligram amounts. Fractionation experiments with ammonium sulphate (data not shown) established that the major part of the transferase activity in serum was concentrated in the fraction precipitating between 25 and 50% saturation. This fraction was prepared from 20 mL of blood-group O serum, the redissolved precipitate was dialysed against 0.15M sodium chloride until free from ammonium sulphate, and used as the enzyme source to prepare the product with *N*-acetyllactosamine. The dialysed solution (total volume 4 mL) was added to the following mixture: manganese chloride, 80 μmol; ATP, 4 μmol; sodium azide, 65 μmol; *N*-acetyllactosamine, 80 μmol; UDP-2-acetamido-2-deoxy-D-[U-^{14}C]glucose, 12 μmol (2.2×10^6 c.p.m.), 2-acetamido-2-deoxy-D-glucose, 100 μmol; and Tris · HCl buffer, pH 8.5, 350 μmol. The mixture (total volume 8 mL) was incubated for 90 h at 37° and, at the end of this period, absolute ethanol (8 mL) was added. The precipitated protein was removed by centrifugation for 10 min at 16 000 r.p.m., the precipitate was washed with 50% ethanol (v/v, 10 mL), and after centrifugation the supernatant was pooled with the supernatant from the first spin. The combined solutions were reduced to a volume of ~4 mL by rotary evaporation and passed through columns (0.6×5 cm) of AG1-X4 (AcO^-) and AG50W-X8 (H^+) ion-exchange resins. The final eluate was reduced to a small volume and the trisaccharide product separated from other reaction products by chromatography in solvent *a* on Whatman DE-81 paper for 40 h. The trisaccharide product was eluted and purified further by repeated chromatography in solvents *a* and *b* on Whatman No. 40 paper. The yield of trisaccharide (Product N) was 3.76 μmol.

The compound synthesised by the transfer of 2-acetamido-2-deoxy-D-glucose to lactose was prepared by essentially the same method except that 30 μmol of UDP-2-acetamido-2-deoxy-D-[U-^{14}C]glucose were used and all the other components of the reaction mixture were increased proportionally. The trisaccharide product was purified as described earlier, except that the first chromatography step was carried out on Whatman DE-81 paper in solvent *c*. The yield of trisaccharide (Product L) was 7.5 μmol.

Sugar analysis. — 2-Amino-2-deoxy-hexoses and -hexitols were determined with an amino acid analyser[14]. Neutral sugars were analysed by gas chromatography as trimethylsilyl derivatives[15] following resin hydrolysis (AG50, H^+) of the oligosaccharides[16]. An open, tubular capillary column (38 m) coated with SE-30, and a temperature programme from 140–240° at 4°/min, were used for the separation.

Methylation. — Methylation of oligosaccharides was performed with methyl iodide in *N*,*N*-dimethylformamide. The catalyst was barium oxide–barium hydroxide, and the methylated oligosaccharides were hydrolysed with 0.25M sulfuric acid in 90% (v/v) acetic acid[17]. The hydrolysates were separated into neutral and basic fractions by passage through ion-exchange resins[18]. The neutral sugar fractions were converted into alditol acetates[17] and examined by g.l.c. on (a) a 38-m SE-30 capillary column and (b) a 39-m SP-2250 capillary column, both temperature-programmed from 160–220° at 1°/min. The basic amino sugar methyl ether fraction was examined with an amino acid analyser[18]. Methylated sugars were identified by their retention times in comparison with standard compounds.

Removal of sialic acid from glycoproteins. — The glycoproteins (20 mg) were dissolved in 50mM sulfuric acid (3 mL). The solutions were heated for 1 h at 80°, and then dialysed against distilled water, and the indiffusible material was dried from the frozen state.

Treatment of ^{14}C-labelled products with N-acetyl-β-D-hexosaminidase. — Jack bean *N*-acetyl-β-D-hexosaminidase was dialysed against 10mM phosphate buffer (pH 6.0) and 200 μL (1 unit) was added to 50-μL aliquots of the products of 2-acetamido-2-deoxy-D-[U-^{14}C]glucose transfer (5–15 000 c.p.m.; 1–3 nmol). The mixtures were incubated for 16 h at 37°, and the reaction products were separated by chromatography on Whatman No. 40 paper in solvent *a*.

RESULTS

Human sera from fifteen normal blood donors, unselected for ABO blood groups, were tested for their capacity to transfer 2-acetamido-2-deoxy-D-[^{14}C]glucose from UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose to the acceptor substrate lactose under the conditions given for the complete mixture in Table I. Chromatography of the reaction products on Whatman DE-81 paper in solvent *a* revealed, with all the sera tested, a radioactive product in the trisaccharide area ($R_{lactose}$ 0.62) which was not present in the control mixtures from which the lactose acceptor had been omitted. The same product was observed when the enzyme sources were (a) serum from an individual with the rare blood-group Bombay O_h phenotype[19], (b) eight serum samples separated from human-cord blood, and (c) serum from an adult with the blood-group i phenotype[19]. When *N*-acetyllactosamine was used as the acceptor substrate in place of lactose, a radioactive trisaccharide product was formed with $R_{lactose}$ 0.81 in solvent *a*. All the samples of human serum examined thus contained an *N*-acetylglucosaminyltransferase which

TABLE I

REQUIREMENTS FOR *N*-ACETYLGLUCOSAMINYLTRANSFERASE ACTIVITY

Component	*Incorporation of [^{14}C]GlcNAc into*			
	N-Acetyllactosamine		*Lactose*	
	C.p.m.	*nmol/h/mL of serum*	*C.p.m.*	*nmol/h/mL of serum*
Complete mixture[a]	12 268	6.97	5667	3.22
Minus acceptor	387	0.22	180	0.10
Minus serum	255	0.14	180	0.10
Minus ATP	9471	5.38	4385	2.49
Minus Mn^{2+}	319	0.18	302	0.17
Minus Mn^{2+}, plus Co^{2+}	4959	2.81	2912	1.65
Minus Mn^{2+}, plus Fe^{2+}	1684	0.96	982	0.56
Minus Mn^{2+}, plus Mg^{2+}	683	0.39	559	0.32
Complete mixture[a]				
Plus Co^{2+}	7856	4.46	3446	1.97
Plus 25mM EDTA	320	0.18	328	0.19
Plus 30mM 2-acetamido-2-deoxy-D-glucose			7950	4.54
Plus 0.5 μg tunicamycin	12 253	6.96	5526	3.14

[a]The complete mixture contained in a total volume of 100 μL: UDP-[^{14}C]GlcNAc, 20 nmol (110 000 c.p.m.); $MnCl_2$ 1.0 μmol; ATP, 0.05 μmol, NaN_3, 0.8 μmol, acceptor (*N*-acetyllactosamine or lactose) 0.25 μmol, sodium cacodylate buffer, pH 7.0, 5 μmol; human group O serum, 20 μl. When components were omitted from the reaction mixture the volume was made up to 100 μL with water. The mixtures were incubated for 16 h at 37°, and at the end of this period the reaction products were separated by chromatography on DE 81 paper in solvent *a*. The c.p.m. given in the Table represent the counts in the area of the chromatogram where the trisaccharide product was detected when the complete mixture was used.

catalysed the addition of 2-acetamido-2-deoxy-D-glucose to two low-molecular-weight acceptors having terminal nonreducing β-D-galactopyranosyl groups.

Requirements for activity of the N-*acetylglucosaminyltransferase.* — The capacity of the *N*-acetylglucosaminyltransferase in a group O serum sample to transfer 2-acetamido-2-deoxy-D-[^{14}C]glucose to lactose and *N*-acetyllactosamine was tested over a range of pH values (Fig. 1). Both disaccharides were acceptors of 2-acetamido-2-deoxy-D-glucose over a broad pH range with apparent optima extending from pH 6 to 8.5. Most of the experiments to be described subsequently were carried out at pH 7.0.

Divalent cations were required for the transfer of 2-acetamido-2-deoxy-D-glucose to either lactose or *N*-acetyllactosamine, and all activity disappeared on addition of 25mM EDTA (Table I). At a 10mM concentration, the order of effectiveness was $Mn^{2+} > Co^{2+} > Fe^{2+} > Mg^{2+}$. At this same concentration, Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Ca^{2+} ions failed to give detectable activation. The effects of varying Mn^{2+} and Co^{2+} concentrations are shown in Fig. 2; with either acceptor substrate, optimal activity was observed at a 10mM concentration of Mn^{2+} ions and at a 5mM concentration of Co^{2+} ions.

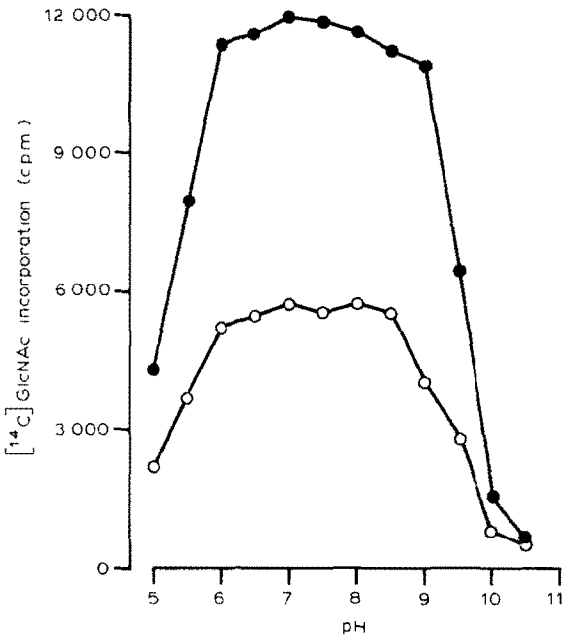

Fig. 1. Effect of pH on *N*-acetylglucosaminyltransferase activity. The reaction mixtures were the same as the "complete" mixture in Table I, except that sodium cacodylate buffer was used for the pH range 5.0–7.0, Tris · HCl buffer for the pH range 7.5–8.5, and glycine–sodium hydroxide buffer for the pH range 9.0–10.5: (●—●) Acceptor substrate, *N*-acetyllactosamine; (○—○) acceptor substrate, lactose

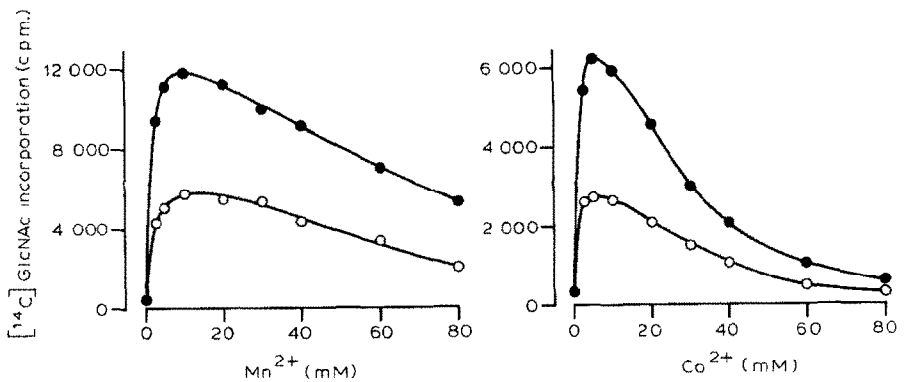

Fig. 2. Metal ion requirements of *N*-acetylglucosaminyltransferase. The reaction mixtures were the same as the "complete" mixture in Table I, except that the concentrations of Mn^{2+} and Co^{2+} ions were varied: (●—●) Acceptor substrate, *N*-acetyllactosamine; (○—○) acceptor substrate, lactose.

When UDP-2-acetamido-2-deoxy-D-glucose is incubated with human serum, it is subjected to the combined degradative action of nucleotide phosphohydrolases and an *N*-acetyl-α-D-glucosaminidase[20]. The amount of 2-acetamido-2-deoxy-D-[^{14}C]glucose incorporated into an acceptor substrate is, therefore, to an extent dependent on the degradative effect of these enzymes on the nucleotide sugar donor,

as well as on the *N*-acetylglucosaminyltransferase activity. Under the conditions used for the complete reaction mixture described in Table I, some 40–50% of the radioactivity was found as free sugar at the end of the incubation period. Omission of ATP, which inhibits the phosphohydrolases, resulted in increased breakdown of UDP-2-acetamido-2-deoxy-D-glucose and lower incorporation of 2-acetamido-2-deoxy-D-[^{14}C]glucose (Table I). An increased ATP concentration, however, also inhibited the transferase activity. Addition of 2-acetamido-2-deoxy-D-glucose to inhibit the *N*-acetyl-α-D-glucosaminidase reduced the amount of radioactivity released as free sugar to ~25% and resulted in an increase of the incorporation into acceptor (Table I). Although 2-acetamido-2-deoxy-D-glucose was not routinely added, it was included in reaction mixtures used for the large-scale preparation of the products of 2-acetamido-2-deoxy-D-glucose transfer to lactose and to *N*-acetyllactosamine.

The antibiotic tunicamycin selectively blocks the biosynthesis of some glycoproteins by inhibition of 2-acetamido-2-deoxy-D-glucose transfer reactions; at a concentration of 5 μg/mL, the antibiotic completely inhibits the enzyme catalysing the transfer of 2-acetamido-2-deoxy-D-glucose to lipid intermediates[21]. At this same concentration, tunicamycin was without inhibitory action on the *N*-acetylglucosaminyltransferase that utilises *N*-acetyllactosamine or lactose as acceptor substrates (Table I).

*The effect of incubation time and enzyme concentration on the incorporation of 2-acetamido-2-deoxy-*D*-[^{14}C]glucose.* — Incorporation of 2-acetamido-2-deoxy-D-[^{14}C]glucose into either lactose or *N*-acetyllactosamine continued to increase when serum was incubated with the appropriate additives for periods up to 24 h (Fig. 3), although the reaction ceased to be linear after ~12 h. Similarly, incorporation of radioactivity into the acceptors increased with the volume of serum added

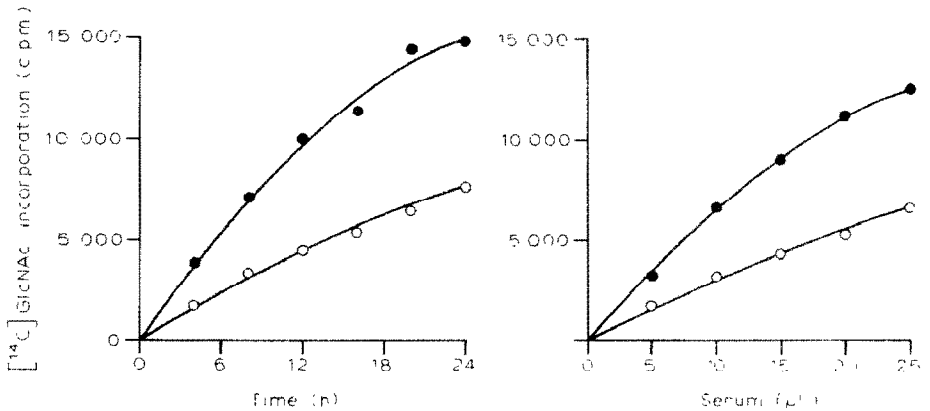

Fig. 3. Effect of incubation time and enzyme concentration on *N*-acetylglucosaminyltransferase activity. The reaction conditions were the same as for the "complete" mixture in Table I, except that (a) the mixtures were incubated for various times and (b) various amounts of serum were added (●—●) Acceptor substrate, *N*-acetyllactosamine; (○—○) acceptor substrate, lactose

TABLE II

TRANSFER OF 2-ACETAMIDO-2-DEOXY-D-[^{14}C]GLUCOSE TO VARIOUS LOW-MOLECULAR-WEIGHT ACCEPTORS BY THE *N*-ACETYL-D-GLUCOSAMINYLTRANSFERASE IN HUMAN SERUM[a]

Acceptor	*Incorporation of [^{14}C]GlcNAc*		*Relative mobility of product*[b]		
	C.p.m.	*nmol/h/mL*	R_{LNT}	R_{Lac}	R_{GlcNAc}
Phenyl β-D-Gal*p*	529	0.30			1.57
β-D-Gal*p*-(1→4)-D-Glc	6323	3.59		0.62	
p-Nitrophenyl β-D-Gal*p*-(1→4)-β-D-Glc	6556	3.72			1.35
β-D-Gal*p*-(1→4)-D-GlcNAc	13 291	7.55		0.81	
β-D-Gal*p*-(1→4)-D-Glc*p*NAc-O-$(CH_2)_8CO_2CH_3$	13 716	7.79			1.35
β-D-Gal*p*-(1→4)-D-Man	6197	3.52		0.78	
β-D-Gal*p*-(1→4)-D-Fru	208	0.12		0 78	
β-D-Gal*p*-(1→3)-D-GlcNAc	404	0 23		0.74	
β-D-Gal*p*-(1→3)-D-Glc	533	0.30		0.61	
β-D-Gal*p*-(1→3)-D-Glc*p*NAc-O-$(CH_2)_8CO_2CH_3$	184	0.10			1.38
β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-O-$(CH_2)_8CO_2CH_3$	10 851	6.17			1.15
β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc	11 797	6.70	0.76	0.30	
β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc	708	0.40	0.67	0 26	
β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc	296	0.17	0.48	0.19	
β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-D-Gal	14 285	8.12	0.25	0.09	

[a]The acceptor substrates were all tested at a concentration of 2.5mM. The reaction mixtures were the same as in Table I, except for those containing the di- and tri-saccharides glycosidically linked to $(CH_2)_8CO_2CH_3$. An additional component, bis-(*p*-nitrophenyl) phosphate (0.15 μmol) was added to these reaction mixtures to inhibit decomposition by esterases in the serum. The reaction mixtures were incubated for 16 h at 37°. At the end of this period the mixtures containing the di- and tri-saccharides linked to $(CH_2)_8CO_2CH_3$ were separated by paper chromatography on DE-81 paper in solvent *b*. The remaining reaction mixtures were separated by chromatography on DE-81 paper in solvent *a*. [b]Mobility relative to lacto-*N*-tetraose, R_{LNT}; relative to lactose, R_{Lac}; and relative to 2-acetamido-2-deoxy-D-glucose, R_{GlcNAc}.

to the reaction mixtures up to a volume of 25 μL (Fig. 3), although the relationship ceased to be linear when volumes greater than 10 μL were used.

Acceptor specificity of the N*-acetylglucosaminyltransferase with low-molecular-weight acceptors.* — A variety of oligosaccharides were tested as possible acceptors for the 2-acetamido-2-deoxy-D-[^{14}C]glucose transfer catalysed by the serum *N*-acetylglucosaminyltransferase. Details of the reaction mixtures and methods of separation are given in Table II. When these same conditions were used, the following compounds did not function as acceptors (<100 c.p.m. were detected in the region of the chromatogram where the product would be expected to be located): D-galactose, 2-acetamido-2-deoxy-D-glucose, methyl α- and β-D-galactopyranoside, α-D-Gal*p*-(1→4)-D-Gal, α-D-Gal*p*-(1→6)-D-GlcNAc, α-D-Gal*p*-

(1→6)-D-GalNAc, α-D-Gal*p*-(1→6)-D-Glc, β-D-Gal*p*-(1→3)-D-Ara, β-D-Gal*p*-(1→6)-D-GlcNAc, *N*,*N*′-diacetylchitobiose, α-L-Fuc*p*-(1→2)-D-Gal, 2′-fucosyllactose, 3-fucosyllactose, lactodifucotetraose, lacto-*N*-fucopentaose I, lacto-*N*-difucohexaose I, lacto-*N*-difucohexaose II, and β-D-GlcNAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*-O-$(CH_2)_8CO_2CH_3$.

All the compounds that functioned as acceptors of 2-acetamido-2-deoxy-D-[^{14}C]glucose contained unsubstituted, terminal nonreducing β-D-galactopyranosyl residues (Table II). An L-fucose substituent on either the terminal β-D-galactosyl group or on the subterminal sugar residue prevented the addition of 2-acetamido-2-deoxy-D-glucose. Lacto-*N*-fucopentaose II appeared to be a very weak acceptor (Table II), but this incorporation probably resulted from a minor contaminant in the oligosaccharide preparation. The best acceptors were those compounds containing β-D-galactopyranosyl groups linked (1→4) to 2-acetamido-2-deoxy-D-glucose units, and the next most effective were those in which the β-D-galactopyranosyl group was joined in the same positional linkage to D-glucose or D-mannose units. Compounds with β-D-galactopyranosyl groups linked (1→3) to 2-acetamido-2-deoxy-D-glucose or D-glucose were by comparison very weak acceptors (Table II), and *O*-β-D-galactopyranosyl-(1→6)-2-acetamido-2-deoxy-D-glucose failed to give detectable incorporation. The presence of the hydrophobic spacer arm $(CH_2)_8CO_2Me$ attached to *N*-acetyllactosamine, or the aromatic group in *p*-nitrophenyl β-D-lactoside, did not affect the capacity of the terminal, nonreducing β-D-galactopyranosyl group in these compounds to function as acceptors of 2-acetamido-2-deoxy-D-[^{14}C]glucose (Table II). The branched pentasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-[β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)]-D-Gal gave a level of incorporation comparable with that given with *N*-acetyllactosamine and lacto-*N*-*neo*tetraose, suggesting that the 2-acetamido-2-deoxy-D-glucose unit was most probably being transferred to the branch terminating with the β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc structure.

Anomeric linkage of the transferred 2-acetamido-2-deoxy-D-[^{14}C]glucose unit. — The labelled products synthesised by the transfer of 2-acetamido-2-deoxy-D-[^{14}C]glucose to lactose, *N*-acetyllactosamine, β-D-Gal*p*-(1→4)-D-Man, lacto-*N*-*neo*tetraose, and the branched pentasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-[β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)]-D-Gal were treated with Jack bean *N*-acetyl-β-D-hexosaminidase. Paper chromatography of the reaction products in solvent *a* revealed in each instance one radioactive spot which co-chromatographed with 2-acetamido-2-deoxy-D-glucose, showing that the transferred sugar was completely cleaved by the *N*-acetyl-β-D-hexosaminidase. Treatment of nitrophenyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside with the hexosaminidase resulted in hydrolysis of the β-D- and not of the α-D-linked compound. The *N*-acetylglucosaminyltransferase in serum was therefore transferring 2-acetamido-2-deoxy-D-glucose in the β form.

Apparent K_m and V_{max} values for low-molecular-weight acceptor substrates. — Several of the low-molecular-weight substrates which functioned as acceptors

TABLE III

KINETIC CONSTANTS FOR THE *N*-ACETYLGLUCOSAMINYLTRANSFERASE WITH LOW-MOLECULAR-WEIGHT ACCEPTOR SUBSTRATES[a]

Acceptor substrate	*Apparent* K_m *(mM)*	V_{max} *(nmol/h/mL)*	*Efficiency* (V_{max}/K_m)
(1) β-D-Gal*p*-(1→4)-D-GlcNAc	2.4	16	6.6
(2) β-D-Gal*p*-(1→4)-D-Glc	2.6	7	2.7
(3) β-D-Gal*p*-(1→4)-D-Man	2.5	13	4.3
(4) β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc	3.8	22	5.8
(5) β-D-Gal*p*-(1→3)-D-GlcNAc	10	1	0.1
(6) β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc	19	7	0.4
(7) β-D-Gal*p*-(1→3)-D-Glc	22	6	0.3
(8) Phenyl β-D-Gal*p*	100	11	0.1

[a]The reaction mixtures were the same as the "complete mixture" given in Table I, except that acceptor substrates Nos 1–4 were tested at a range of concentrations from 0.25 to 2.5mM, and acceptor substrates Nos 5–8 at a range of concentrations from 2 5 to 25mM.

for 2-acetamido-2-deoxy-D-glucose were tested at a range of concentrations. Lineweaver–Burk plots of $1/v$ versus $1/[S]$ showed straight-line relationships and enabled apparent K_m and V_{max} values to be calculated (Table III). All the compounds with terminal, nonreducing β-D-galactopyranosyl groups joined in (1→4) linkage to the subterminal sugar residues gave apparent K_m values between 2.4 and 3.8 mM and V_{max} values between 7 and 22 nmol/h/mL of serum. The compounds containing β-D-galactopyranosyl groups joined in (1→3) linkage to the subterminal sugar residue had higher K_m values, in the range of 10 to 22mM, and lower V_{max} values, in the range 1–7 nmol/h/mL of serum. Of all the acceptors tested, the least efficient was phenyl β-D-galactopyranoside. The results, therefore, confirm the preference of the *N*-acetylglucosaminyltransferase for β-D-galactopyranosyl residues substituting at O-4 of the subterminal sugar residue.

Characterisation of products synthesised with N-*acetyllactosamine and lactose as acceptor substrates.* — The products formed by transfer of 2-acetamido-2-deoxy-D-glucose to *N*-acetyllactosamine and lactose were synthesised on a milligram scale for structural investigations. Sugar analysis of the compound (Product N) synthesised with *N*-acetyllactosamine as acceptor substrate revealed the presence of D-galactose and 2-acetamido-2-deoxy-D-glucose in the molar ratio of 1:2.07, confirming that one 2-acetamido-2-deoxy-D-glucose unit had been added to form a trisaccharide. On methylation, followed by hydrolysis, the trisaccharide yielded, on g.l.c. of the neutral sugar methyl ethers, one peak that had a retention time corresponding to the derivative of 2,4,6-tri-*O*-methylgalactose on both SE-30 and SP-2250 columns. The standard 3,4,6-tri-*O*-methyl-D-galactose derivative had an identical retention time on the SE-30 column, but was clearly distinguishable from the 2,4,6-tri-*O*-methyl derivative on SP-2250. When added to a standard mixture of

methyl ethers, the methylation product of the trisaccharide was observed to increase the height of the 2,4,6-tri-*O*-methylgalactose peak. The other possible tri-*O*-methylgalactose derivatives (2,3,6-, 3,4,6- and 2,3,4-) were not detectable in the methylation products of the trisaccharide. The 2-acetamido-2-deoxy-D-glucose unit had therefore been transferred to O-3 of the β-D-galactopyranosyl group in *N*-acetyllactosamine. The hexosamine methyl ether fraction gave two peaks; one had a retention time corresponding to the methyl ether of the standard 2-amino-2-deoxy-3,6-di-*O*-methylglucose, and the second a retention time corresponding to standard 2-amino-2-deoxy-3,4,6-tri-*O*-methylglucose. Hence, these results confirmed that the trisaccharide had the structure β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-GlcNAc.

Product L, enzymically synthesised by the transfer of 2-acetamido-2-deoxy-D-glucose to lactose, was found on sugar analysis to contain galactose, glucose, and 2-acetamido-2-deoxyglucose in the molar ratios of 1:1.03:0.94, confirming that one 2-acetamido-2-deoxyglucose unit had been added to form a trisaccharide. Following methylation and hydrolysis, the neutral sugar methyl ethers gave two peaks on gas chromatography; one had a retention time corresponding to the derivative of standard 2,4,6-tri-*O*-methyl-D-galactose on both SE-30 and SP-2250 columns, and the second peak was coincident with authentic 2,3,6-tri-*O*-methyl-D-glucose on both columns. No evidence was obtained for the presence of any other tri-*O*-methylgalactose derivative. The hexosamine methyl ether fraction gave one peak on the amino acid analyser with a retention time corresponding to the methyl ether of 2-amino-2-deoxy-3,4,6-tri-*O*-methylglucose. The methylation evidence therefore demonstrates that the 2-acetamido-2-deoxy-D-glucose unit had been transferred to O-3 of the β-D-galactopyranosyl group in lactose to give a trisaccharide with the structure β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc.

Acceptor specificity with macromolecular acceptors. — The serum *N*-acetylglucosaminyltransferase was tested for its action on macromolecular substances known to contain β-D-galactopyranosyl end-groups (Table IV). Of the blood-group specific glycoproteins isolated from ovarian cysts, the precursor glycoprotein Fl. 14 was the most efficient acceptor. This substance was isolated from an ovarian cyst of an individual of the rare Le(a-b-) nonsecretor[10] phenotype and, hence, lacked the L-fucosyl substituents that are added by the blood group *H* and *Le* gene-specified glycosyltransferases[23]. Treatment of Fl. 14 with weak acid to remove sialic acid resulted in enhanced incorporation of 2-acetamido-2-deoxy-D-[^{14}C]glucose (Table IV) and this effect was even more marked with a second precursor glycoprotein, No. 484, also obtained from an Le(a-b-) nonsecretor individual. One Le^a-active glycoprotein, No. 350, with a very high sialic acid content[11] failed to accept 2-acetamido-2-deoxy-D-[^{14}C]glucose, whereas a second Le^a-active preparation, No. 445, with a much lower content of sialic acid, was a relatively good acceptor (Table IV). In blood-group Le^a glycoproteins, the Type 2 [β-D-Gal*p*-(1→4)-D-GlcNAc] chain endings lack the fucose additions catalysed by the *H* gene-specified glycosyltransferase[23]. Hence, more unsubstituted β-D-galac-

TABLE IV

TRANSFER OF 2-ACETAMIDO-2-DEOXY-D-[^{14}C]GLUCOSE TO MACROMOLECULAR ACCEPTORS BY THE *N*-ACETYL-D-GLUCOSAMINYLTRANSFERASE IN HUMAN SERUM[a]

Acceptor	*Incorporation of [^{14}C]GlcNAc*	
	C.p.m	*nmol/h/mL of serum*
Blood-group glycoproteins from ovarian cysts:		
Precursor Fl. 14	13 305	1.89
Asialo precursor Fl. 14	20 812	2.96
Precursor 484	442	0.06
Asialo precursor 484	16 065	2.28
Le^a 350	0	0
Le^a 445	6182	0.88
H MH	0	0
H 277	296	0.04
A MA	0	0
B 413	631	0.09
Serum glycoproteins:		
Fetuin	0	0
Asialo fetuin	10 695	1.52
α_1-Acid glycoprotein	14 855	2.11
Asialo α_1-acid glycoprotein	63 110	8.96
Tamm-Horsfall urinary glycoproteins:		
Sd(a +) W.M.	5708	0.81
Asialo Sd(a +) W.M.	34 524	4.49
Sd(a −) A.S.	34 234	4.86
Asialo Sd(a −) A.S.	39 390	5.59
Pneumococcus Type XIV polysaccharide:	12 436	1.77
Glycosphingolipids:		
Lactosylceramide	608	0.09
Globoside	0	0
Lacto-*N*-*neo*tetraosylceramide	600	0.09

[a]The reaction mixtures were the same as in Table I, except that 500 μg of the macromolecular acceptors were used in place of the low-molecular-weight substrates and Triton X-100 (final concentration 0.1%) was added to the mixtures containing glycosphingolipids. The total volume was 150 μL. The mixtures were incubated for 64 h at 37°, and the reaction products in those containing exogenous glycoproteins and polysaccharides were separated by chromatography on Whatman 3MM paper in solvent *c*. The exogenous acceptors remained at the origin on the chromatograms. In the absence of exogenous acceptors, incorporation of [^{14}C]GlcNAc occurred into endogenous acceptors (19 000 c.p.m.). This figure has been subtracted from the c.p.m. given in the Table. The reaction mixtures containing exogenous glycosphingolipids were extracted twice with 2:1 (v/v) chloroform–methanol, and the combined extracts chromatographed[22] on Whatman 3MM paper in 1% sodium tetraborate, pH 9.1.

topyranosyl end-groups are to be expected in an Le^a preparation than in an H-active glycoprotein. In agreement with this expectation, two H-active preparations proved to be very poor acceptors of 2-acetamido-2-deoxy-D-[^{14}C]glucose (Table IV). A blood-group A and a blood-group B glycoprotein, in which the H-structures

are further substituted with 2-acetamido-2-deoxy-D-galactose and D-galactose, respectively[23], were likewise poor acceptors for the *N*-acetylglucosaminyltransferase.

The asparagine-linked oligosaccharide chains in fetuin[24] and α_1-acid glycoprotein[25] terminate at the nonreducing ends with the β-D-Gal*p*-(1→4)-D-GlcNAc structure substituted on the D-galactosyl residue with sialic acid. Untreated fetuin failed to accept 2-acetamido-2-deoxy-D-[^{14}C]glucose but was a fairly good acceptor after removal of sialic acid. α_1-Acid glycoprotein accepted some 2-acetamido-2-deoxy-D-[^{14}C]glucose in the untreated state and after removal of sialic acid was the most efficient acceptor substrate of all those tested (Table IV).

The carbohydrate chains in Tamm–Horsfall urinary glycoprotein[13] are asparagine-linked, but as yet little information is available on their detailed structure. The association of the human Sd^a blood-group character with this glycoprotein[12,26], however, has revealed that in Sd^a positive individuals some of the carbohydrate chains terminate in 2-acetamido-2-deoxy-D-galactose and others in D-galactose units, whereas in Sd^a negative individuals the 2-acetamido-2-deoxy-D-galactose units are missing. When Tamm–Horsfall glycoproteins were tested as acceptors of 2-acetamido-2-deoxy-D-[^{14}C]glucose, the Sd^a negative preparation was a better acceptor than the Sd^a positive glycoprotein (Table IV). Both variants were converted into more efficient acceptors by removal of sialic acid.

The capsular polysaccharide from *Pneumococcus* Type XIV consists of tetrasaccharide repeating-units with β-D-galactopyranosyl groups linked (1→4) to 2-acetamido-2-deoxy-D-glucose units in the main chain[27]. This polysaccharide also functioned as an acceptor for the serum *N*-acetylglucosaminyltransferase (Table IV).

In comparison with the glycoproteins, the glycosphingolipids tested were very poor acceptors of 2-acetamido-2-deoxy-D-[^{14}C]glucose. However, weak but definite incorporation was observed with the two glycolipids having terminal β-D-galactopyranosyl groups, namely lactosylceramide and lacto-*N*-*neo*tetraosylceramide, whereas globoside, which has a terminal, nonreducing 2-acetamido-2-deoxy-β-D-galactopyranosyl group (Table IV), gave no detectable incorporation.

The radioactive product formed by the transfer of 2-acetamido-2-deoxy-D-[^{14}C]glucose to the precursor glycoprotein Fl. 14 was digested with Jack bean *N*-acetyl-β-D-hexosaminidase under the conditions used for hydrolysis of the low-molecular-weight products. All the radioactivity was cleaved from the macromolecular product by this treatment and the released sugar cochromatographed in solvent *a* with 2-acetamido-2-deoxy-D-glucose.

When amounts of the precursor glycoprotein Fl. 14, ranging from 100 to 1000 μg, were included in the reaction mixture, with the other conditions as given in Table IV, incorporation of 2-acetamido-2-deoxy-D-[^{14}C]glucose was linear with amounts up to 400 μg of acceptor, and continued to increase up to 1000 μg. A Lineweaver–Burk plot of $1/v$ versus $1/[S]$ gave a straight-line relationship, and an apparent K_m of 1.5mM was calculated for the glycoprotein substrate by assuming a

M_r 1×10^6 and 300 available acceptor sites per molecule. This latter figure can only be an approximation, but it is not dissimilar from the apparent K_ms calculated for the low-molecular-weight substrates having (1→4)-linked β-D-galactopyranosyl nonreducing end-groups (Table III).

Competition experiments for acceptor substrates. — The acceptor requirements of the glycoprotein and glycolipid substrates suggest that, in the series of experiments described in this paper, the same enzyme is involved in the transfer of 2-acetamido-2-deoxy-D-[^{14}C]glucose to low-molecular-weight and macromolecular acceptor substrates. Definitive competition experiments to establish whether one or more enzymes are involved cannot be carried out with crude serum as the enzyme source, because it is not possible to separate the endogenous acceptors from the exogenous macromolecular substrates, and the amount of 2-acetamido-2-deoxy-D-[^{14}C]glucose incorporated into the endogenous material was found to vary according to the amount of exogenous low-molecular-weight substrate used (data not shown). However, in conditions judged to be enzyme-limiting from the K_m data, the addition of either lactose or *N*-acetyllactosamine to the incubation mixtures containing the asialo precursor glycoprotein Fl. 14 resulted in a decrease in the incorporation of 2-acetamido-2-deoxy-D-[^{14}C]glucose into both the disaccharide and glycoprotein acceptor molecules (Table V). These experiments, thus, support the inference that a single enzyme is involved in the transfer of 2-

TABLE V

COMPETITION EXPERIMENTS WITH DIFFERENT ACCEPTOR SUBSTRATES[a]

Acceptor			*Incorporation of [^{14}C]GlcNAc (c.p.m.) into*		
N-Acetyl-lactosamine	*Lactose*	*Asialo precursor glycoprotein Fl. 14*	*N-Acetyl-lactosamine*	*Lactose*	*Asialo precursor glycoprotein Fl. 14*[b]
+			10,030		
	+			4,270	
		+			13,793
+	+		7,360	2,145	
+		+	7,819		7,281
	+	+		3,222	7,800

[a]The reaction mixtures contained in a total volume of 200 μL: UDP-[^{14}C]GlcNAc (40 nmol, 220 000 c.p.m.); $MnCl_2$ (2.0 μmol); ATP (0.10 μmol); NaN_3 (0.8 μmol); one or two of the following components: lactose (1.0 μmol), *N*-acetyllactosamine (1.0 μmol), and asialo Fl. 14 (1 mg); sodium cacodylate buffer, pH 7.0 (15 μmol); and group O serum (20 μL). The reaction products were separated by chromatography in solvent *c* on DE-81 paper for the products synthesised with lactose or *N*-acetyllactosamine, or Whatman 3MM paper for the product synthesised with asialo Fl. 14. Assays containing both disaccharide acceptors and asialo Fl. 14 were set up in duplicate, and one mixture was separated on DE-81 paper for the detection of the labelled trisaccharide products, and the other on Whatman 3MM paper for measurement of incorporation into the glycoprotein acceptor. The plus sign indicates the presence of an acceptor in the incubation mixture. [b]Incorporation into endogenous acceptors included in this measurement.

acetamido-2-deoxy-D-[^{14}C]glucose to the macromolecular and low-molecular-weight substrates.

DISCUSSION

The only *N*-acetylglucosaminyltransferase previously described in human serum[28] utilised, as an exogenous acceptor, α_1-acid glycoprotein that had been depleted of sialic acid, D-galactose, and 2-acetamido-2-deoxy-D-glucose by the sequential action of exo-glycosidases. The D-mannosyl groups exposed by the exo-glycosidase treatment were assumed to be the acceptors for the transferred 2-acetamido-2-deoxy-D-glucose unit. This transferase has not been further characterised, but its specificity requirements resemble those of enzymes found in other sources which synthesise β-D-GlcNAc→D-Man linkages[29]. The transferase in human serum described in the present paper is a different enzyme that transfers 2-acetamido-2-deoxy-β-D-glucose units to terminal β-D-galactopyranosyl groups in oligosaccharides, glycoproteins, and glycosphingolipids. The work on this enzyme arose from an attempt, which has as yet proved unsuccessful, to find the transferase catalysing the addition of 2-acetamido-2-deoxy-D-glucose to O-6 of D-galactose to form the branching structure first isolated from ovarian cyst glycoproteins by Lloyd, Kabat, and Licerio[30], and subsequently found in blood-group active glycolipids[31] and polyglycosylceramides[32]. The blood-group I antigenic complex is associated with this branched structure[33–35], and, even before the nature of the determinant structures had been fully elucidated, Kościelak[36] had suggested that the *I* gene could be considered to be the structural gene coding for the (1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyltransferase that catalyses the formation of the branch point. This enzyme is presumably functional in haemopoietic tissue and, by analogy with the blood-group *H*, *A*, and *B* gene-specified transferases[23], might be expected to occur in human serum. However, the *N*-acetylglucosaminyltransferase detected in serum that utilised lactose and *N*-acetyllactosamine as acceptor substrates was present in the serum of an adult with the i blood-group phenotype[19] and in several samples of serum separated from cord blood. The red cells of new born infants and adult i individuals are deficient in I activity and, hence, might be expected to lack the transferase responsible for the synthesis of the branch point. Methylation analysis of the trisaccharide products synthesised with *N*-acetyllactosamine and lactose as acceptor substrates confirmed that the enzyme was not catalysing the addition of a 2-acetamido-2-deoxy-D-glucose unit to O-6 of the β-D-galactopyranosyl end-groups. The terminal disaccharide unit at the non-reducing end in both trisaccharides was β-D-Glc*p*NAc-(1→3)-D-Gal, thus demonstrating that the enzyme under investigation was a (1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyltransferase. To our knowledge, this is the first time that the properties and detailed acceptor requirements of this enzyme have been recorded, although the enzyme in particulate membrane preparations from K-652 cells that catalysed the incorporation of 2-acetamido-2-deoxy-D-glucose into erythroglycan[37] probably had the same specificity.

Examination of a range of low-molecular-weight acceptor substrates revealed that D-galactose itself was not an acceptor, but certain disaccharides with terminal nonreducing β-D-galactopyranosyl groups were very efficient acceptors (Table II). The transferase had a marked preference for compounds in which the terminal, nonreducing β-D-galactopyranosyl groups are attached by a (1→4) glycosidic linkage to the subterminal sugar residue. Hence, although the tissue of origin of the transferase is unknown, the specificity requirements for β-D-Gal*p*-(1→4)-R show that the enzyme has the potential for synthesising the β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-R structures that occur in many glycoconjugates, including the blood-group active glycolipids[31], the poly(lactosamine) chains of erythroglycan[38], and oligosaccharides in human milk[39] and urine[40]. The experiments with both low-molecular-weight and macromolecular acceptors indicated that an L-fucosyl or sialyl substituent on the terminal β-D-galactopyranosyl group inhibited the transfer of 2-acetamido-2-deoxy-D-glucose (Tables II and III). Chain elongation by this *N*-acetylglucosaminyltransferase would, therefore, be halted if an L-fucosyl- or sialyltransferase acted first on the precursor oligosaccharide chains.

With the increasing demand for oligosaccharides of known structure for specificity studies on monoclonal antibodies, endo- and exo-glycosidases, and glycosyltransferases, this *N*-acetylglucosaminyltransferase should be a useful reagent for the biosynthesis of oligosaccharides having β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-R end groups. Serum is a relatively readily available source and, as shown in this paper, even crude serum can be employed for the preparation of trisaccharides on a milligram scale. The preference of the enzyme for Type 2 [β-D-Gal*p*-(1→4)-D-GlcNAc] chain endings should also make the transferase, when purified, a valuable reagent for assessing the relative abundance of Type 1 [β-D-Gal*p*-(1→3)-D-GlcNAc] and Type 2 chain endings in glycoproteins of the mucin-type that carry a mixture of these chains[23].

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