

# Glycosyltransferases in Human Blood

## I. GALACTOSYLTRANSFERASE IN HUMAN SERUM AND ERYTHROCYTE MEMBRANES

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**ABSTRACT** Human serum and hemoglobin-free erythrocyte membranes were found to contain a galactosyltransferase which catalyzes the transfer of galactose from UDP-galactose to specific large and small molecular weight acceptors. The requirements for enzyme activity were found to be similar for the enzymes from both sources. However, the membrane-bound enzyme depended on a detergent for maximal activity.  $Mn^{++}$  was an absolute requirement for transfer and uridine nucleoside phosphates were inhibitors. The most effective acceptor for galactose was a glycoprotein containing *N*-acetylglucosamine residues in the terminal position of its oligosaccharide side chains. *N*-acetylglucosamine was also an acceptor. While the presence of  $\alpha$ -lactalbumin in the incubation medium resulted in a significant decrease in the transfer of galactose to *N*-acetylglucosamine, glucose, which was not an acceptor for galactose in the absence of  $\alpha$ -lactalbumin, became an excellent acceptor. The serum enzyme catalyzed the transfer of 54 nmoles of galactose per milliliter of serum per hour and its apparent  $K_m$  for UDP-galactose was  $7.5 \times 10^{-6}M$ . The membrane enzyme had a similar apparent  $K_m$ . Using a quantitative assay system the enzyme was found to be present in all individuals studied, regardless of their blood type, secretor status, or sex.

### INTRODUCTION

Glycoproteins and glycolipids have been shown to be present in the plasma membranes of many mammalian cells including erythrocytes (1-9). Although numerous biological roles have been ascribed to these substances such as ABO and MN blood group antigenicity (3-8), ability to inhibit influenza virus hemagglutination reac-

tions (9), tumor antigenicity (10-11), ion and macromolecular transport (12), and control of cellular adhesion (13, 14), little is known about their chemistry and biosynthesis. In the synthesis of glycoproteins and glycolipids glycosylation is achieved by a family of related enzymes, i.e., a multiglycosyltransferase system (15). In general, they are membrane-bound in the tissues and each such enzyme transfers a specific sugar from a nucleotide-sugar substrate to an acceptor of discrete structure.

We have recently reported the presence of two glycosyltransferases, an *N*-acetylgalactosaminyltransferase (16) and a sialyltransferase (17) in erythrocyte membranes and in the serum of man. The *N*-acetylgalactosaminyltransferase was detected on the erythrocyte membranes and in the sera only of individuals of blood type A and AB but not in those of types B or O. In contrast, the sialyltransferase was present in all individuals studied regardless of their blood groups. Unfortunately, due to the lack of available substrates with low specific radioactivity, a comparative study of the kinetics of these enzymes from the two sources could not be performed.

Galactosyltransferases, which catalyze the transfer of galactose from UDP-galactose<sup>1</sup> to low and high molecular weight acceptors, have been described in various tissues and secretions (18-25). The present investigations were carried out in order to develop a quantitative assay system for the galactosyltransferase in blood. The catalytic properties and acceptor specificity of this glycosyl-

<sup>1</sup> *Abbreviations used in this paper:* AMP, ADP, ATP, mono, di, and triphosphoadenosine; Fuc, fucose; Gal, galactose; GalN, galactosamine; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcN, glucosamine; GlcNAc, *N*-acetylglucosamine; GMP, GDP, GTP, mono, di, and triphosphoguanosine; Man, mannose; OSM, ovine submaxillary mucin; UDP-Gal and UDP-galactose, uridine diphosphate galactose; UPM, UDP, UTP, mono, di, and triphosphouridine.

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transferase were examined in human serum and in erythrocyte membranes. The relationships of this enzyme to the ABO and MN blood group activities and to the secretor status of the individual were also studied.

## METHODS

**Preparation of samples.** Hemoglobin-free erythrocyte membranes were prepared from fresh blood obtained from volunteers according to a previously published modification (17) of the method of Dodge et al. (26). Serum was obtained from fresh plasma by allowing it to clot at 4°C as described (17).

**Preparation of glycosyltransferase acceptors.** On the basis of previous work (27, 28), which allowed definition of their molecular structure, and in particular of the end groups involved in glycosyltransferase reactions, two glycoprotein acceptors were chosen: firstly fetuin, whose carbohydrate moiety ends in the sequence GlcNAc-Gal-sialic acid and secondly, ovine submaxillary mucin (OSM), possessing the sequence of GalNAc-sialic acid. Fetuin was isolated from fetal calf serum by the method of Spiro (29). The fetuin acceptor was prepared by removing the sialic acid and the galactose moieties of fetuin by mild acid hydrolysis, followed by the Smith degradation procedure as described by Spiro (27). The ovine submaxillary mucin acceptor was prepared by treatment of the mucin with neuraminidase (*Vibrio cholerae*), employing essentially the method used by Payza, Rizvi, and Pigman (30) for the preparation of sialic acid free porcine submaxillary mucin.

**Enzyme assay.** Unless stated otherwise, incubations of the reaction mixtures were carried out at 30°C for 2 hr in a shaking water bath and were terminated by the addition of 5 ml cold 1% phosphotungstic acid in 0.5 N HCl. Bovine serum albumin (0.8 mg) was added as a carrier protein and the precipitate was washed three times with cold 1% phosphotungstic acid, dissolved in 0.5 ml of NCS solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.) at 50°C and counted in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) with toluene-phosphor. When monosaccharides were used as the acceptor, the reaction was stopped by heating the tubes at 100°C for 30 sec. Tubes were then centrifuged and the supernatant fluid was passed through a column (9 × 20 mm) packed with a mixed bed resin (Dowex-1 X 8 formate form and Dowex-50W X 8 hydrogen form). The precipitate was washed twice with distilled water and washings were also passed through the column. The combined effluent and washings were dried at 100°C in a counting vial and counted as described above. Values were corrected for quenching or self-absorption by the use of an external standard. Control incubations were performed in all instances in the absence of either exogenous acceptor or enzyme and in calculating enzyme-mediated incorporation into the acceptors the sum of the radioactivity incorporated into both controls was subtracted from the total radioactivity.

The incubation mixture for the assay of galactosyltransferase contained the following: 122,000 dpm of UDP-D-galactose-<sup>14</sup>C (6 mCi/mmmole) prepared by mixing UDP-D-galactose-<sup>14</sup>C (298 mCi/mmmole, New England Nuclear Corp., Boston, Mass.) with unlabeled UDP-D-galactose (Sigma Chemical Co., St. Louis, Mo.), enzyme (20 μl of serum or 0.5 mg protein of erythrocyte membrane), 1.5 mg of the fetuin acceptor, 13 μmoles of MnCl<sub>2</sub>, 0.33 mg of Triton X-100 (only in erythrocyte membrane assays), and 66 μmoles of

cacodylateacetate buffer, pH 7.5, in a final volume of 0.33 ml. When low molecular weight acceptors were employed, 2 μmoles of acceptor were added to a similar incubation mixture except that the serum was dialyzed before use as a source of the enzyme to remove potential small molecular weight acceptors. The protein contents of the samples were determined by the method of Lowry, Rosebrough, Farr, and Randall (31).

**α-Lactalbumin preparation.** Bovine α-lactalbumin was obtained from Schwarz-Mann Laboratories, Orangeburg, N. Y. The material was dissolved in water (15 mg/ml). To eliminate contaminating galactosyltransferase activity, the sample was heated for 20 min at 80°C.

**Identification of the labeled reaction product.** For the identification of the labeled sugar incorporated into glycoprotein acceptors, the reaction product was precipitated and hydrolyzed in 3 N HCl under nitrogen at 100°C for 4 hr. The hydrolysate was filtered through glass wool and the HCl evaporated at 50°C. The residue was dissolved in water and chromatographed on Whatman 3 MM paper in ethyl acetate-pyridine-water (12:5:4 v:v) for 15 hr at 25°C. A 2 cm wide strip was cut and the strip was divided into 2.0-cm long sections. Each section was cut into small squares and counted in a Packard Tri-Carb liquid scintillation spectrometer with toluene-phosphor. The standard monosaccharides used for chromatography were D-galactosamine, D-glucosamine, D-galactose, D-glucose, D-mannose, and L-fucose, obtained from Mann Research Labs, Inc., New York.

TABLE I  
Requirements of Human Serum and Erythrocyte Membrane Galactosyltransferases

Incubation mixture	Enzyme activity dpm/2 hr	Per cent activity %
Serum enzyme		
Complete assay mixture*	19,250	100
Minus enzyme	11	0.1
Minus acceptor	16	0.1
Minus MnCl <sub>2</sub>	35	0.2
Minus MnCl <sub>2</sub> plus 25 mM EDTA	33	0.2
Minus MnCl <sub>2</sub> plus 21 mM MgCl <sub>2</sub>	20	0.2
Minus MnCl <sub>2</sub> plus 21 mM CaCl <sub>2</sub>	19	0.1
Plus 0.33 mg Triton X-100	19,996	103.8
Erythrocyte membrane enzyme		
Complete assay mixture*	2,400	100
Minus enzyme	11	0.5
Minus acceptor	11	0.5
Minus MnCl <sub>2</sub>	12	0.5
Minus MnCl <sub>2</sub> plus 25 mM EDTA	15	0.6
Minus MnCl <sub>2</sub> plus 21 mM MgCl <sub>2</sub>	20	0.8
Minus MnCl <sub>2</sub> plus 21 mM CaCl <sub>2</sub>	18	0.7
Minus 0.33 mg Triton X-100	195	8.1

\* The complete incubation mixture contained the following: 122,000 dpm of UDP-D-galactose-<sup>14</sup>C (6 mCi/mmmole), 20 μl of serum or 0.5 mg protein erythrocyte membrane, 1.5 mg of fetuin acceptor, 13 μmoles of MnCl<sub>2</sub>, 0.33 mg of Triton X-100 (for membrane assay only) and 66 μmole of cacodylate-acetate buffer pH 7.5 in a final volume of 0.33 ml.

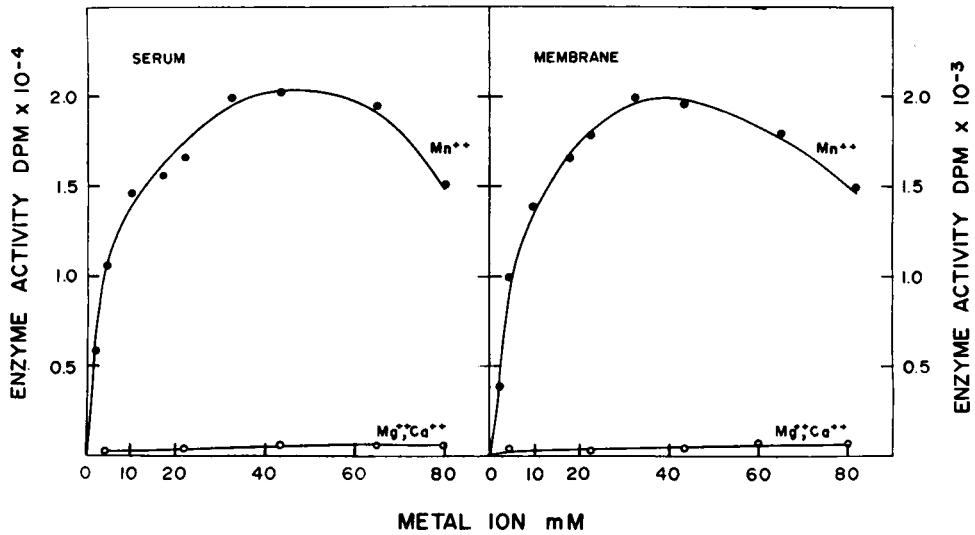


FIGURE 1 The effect of  $Mn^{++}$  concentration on the galactosyltransferase activity of serum and erythrocyte membrane. Incubations were carried out as described in Methods for the standard assay procedure.

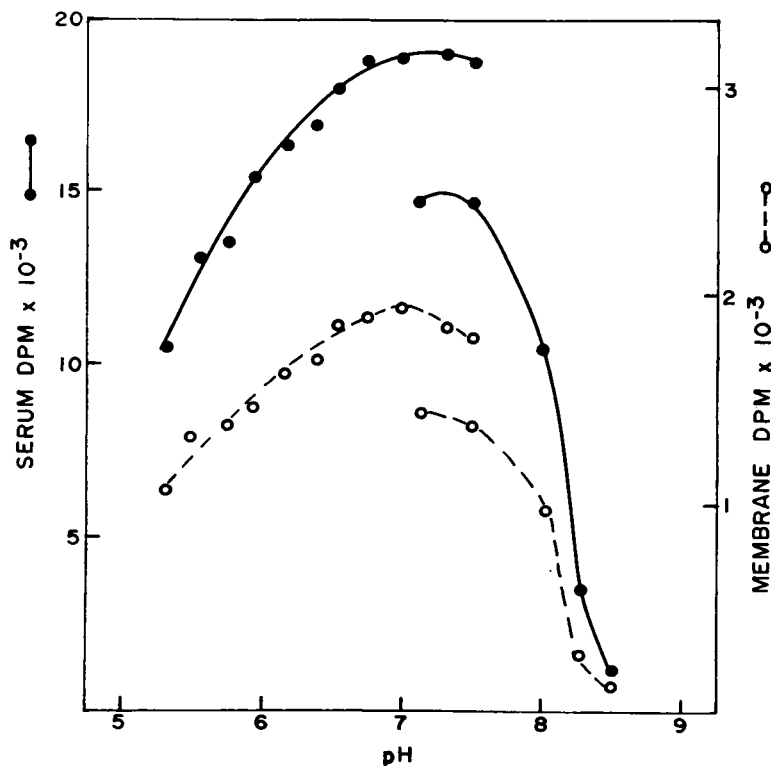


FIGURE 2 The effect of pH on the transfer of galactose by serum and erythrocyte membranes from UDP-Gal to fetuin acceptor. ●—●, serum; ○—○, erythrocyte membranes; from pH 5.3 to 7.5 cacodylate-acetate buffer; from 7.2 to 8.5 Tris-acetate buffer. Incubation conditions are those described in Methods.

## RESULTS

*Serum and erythrocyte membrane galactosyltransferase.* The conditions for optimal enzyme activity are shown in Table I. Virtually no incorporation of galactose occurred when the acceptor was deleted. Manganous ion was an absolute requirement for the incorporation of galactose by the enzyme from serum or erythrocyte membrane. Neither  $\text{Ca}^{++}$  nor  $\text{Mg}^{++}$  was able to substitute for  $\text{Mn}^{++}$ . The optimal  $\text{Mn}^{++}$  concentration was 43 mM for either enzyme (Fig. 1). Of 13 detergents tested, Triton X-100 proved most effective but was only required for the erythrocyte membrane enzyme. Sodium deoxycholate gave results comparable to those obtained with Triton X-100. The enzyme from both sources had a pH optimum of 7.0 (Fig. 2) and maximal incorporation occurred at 30°C (Fig. 3). The incorporation of galactose into the acceptor under the standard incubation conditions was linear with regard to enzyme concentration up to 75  $\mu\text{l}$  of serum and 0.5 mg protein of erythrocyte membrane (Fig. 4) and the reaction was proportional to the length of incubation for up to 6 hr (Fig. 5). The serum and erythrocyte membrane enzymes retained full activity on storage for 5 wk at  $-20^\circ\text{C}$ . The enzyme from both sources was completely inhibited by 1.25 mM *p*-chloro-mercuribenzoate. The apparent  $K_m$  values of the serum and erythrocyte membrane enzymes for UDP-galactose were  $7.5 \times 10^{-6}$  M and  $5.0 \times 10^{-6}$  M, respectively (Fig. 6). Fig. 7 shows the effect of varying the concentration of the fetuin acceptor on galactose incorporation with the serum and erythrocyte membrane enzymes. Acceptor saturation under the standard assay conditions was achieved by the addition of 1.0 mg of the fetuin acceptor to the serum assay and 0.5 mg to the erythrocyte membrane enzyme incubation mixture.

*Effect of nucleoside phosphates on galactosyltransferase activity.* Table II shows the effects of various

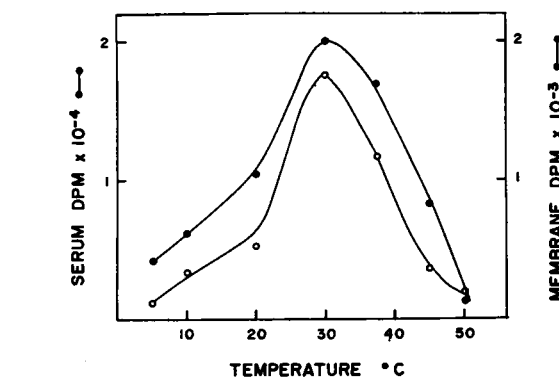


FIGURE 3 The effect of temperature on galactosyltransferase activity of serum and erythrocyte membranes. Incubation conditions are those described in Methods.

nucleoside phosphates on the incorporation of galactose into the fetuin acceptor by the serum enzyme. Additions of adenosine or guanosine phosphates had little influence on the transfer of galactose at concentrations of either 1 mM or 10 mM. In contrast, the incorporation of galactose was markedly inhibited by UDP, UTP, and UMP even at concentrations of 0.1 mM; UDP showed the most significant inhibition.

*Characterization of incorporated sugar.* The radioactive sugar incorporated into fetuin by the serum and erythrocyte membrane galactosyltransferase with UDP-galactose as the substrate was examined. Galactose was the only radioactive compound detected as shown in Fig. 8, indicating that no conversion to other sugar nucleotides occurred during the reaction.

*The effect of  $\alpha$ -lactalbumin on the galactosyltransferase acceptor specificity.* Table III shows the acceptor specificity of the galactosyltransferase from serum and erythrocyte membranes. In the absence of  $\alpha$ -lactalbumin, the enzyme from either source did not

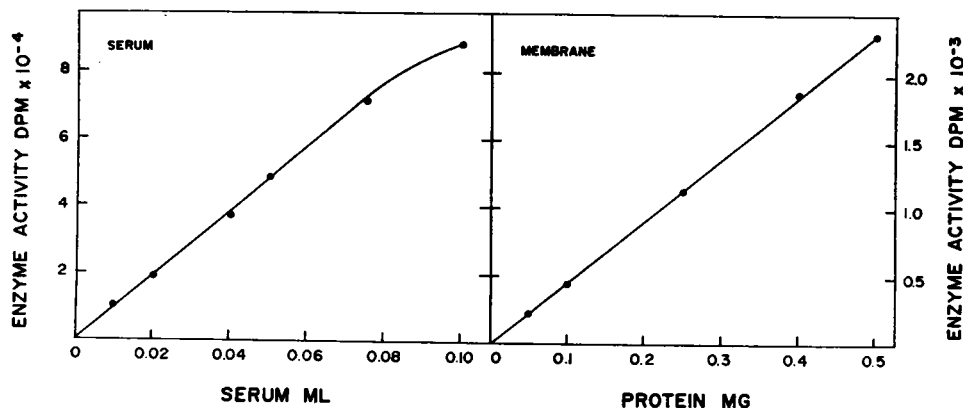


FIGURE 4 Effect of varying enzyme concentration on incorporation of galactose into fetuin acceptor. Incubation conditions are those described in Methods.

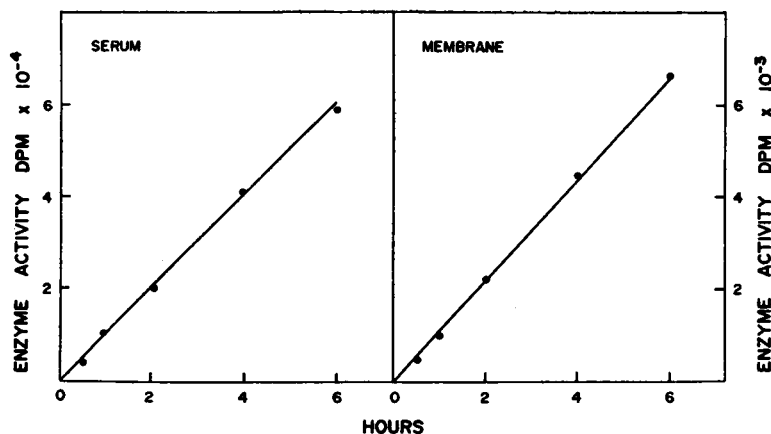


FIGURE 5 Effect of incubation time on the degree of incorporation of galactose into fetuin acceptor. Conditions for assay are those described in Methods.

utilize glucose as an acceptor. When  $\alpha$ -lactalbumin was added, the transfer of galactose to glucose to form lactose occurred. When  $\alpha$ -lactalbumin was absent from the incubation mixture, *N*-acetylglucosamine and the fetuin acceptor with terminal *N*-acetylglucosamine residues were the most effective acceptors for the enzyme from either source. By contrast the presence of  $\alpha$ -lactalbumin markedly diminished the transfer of galactose to *N*-acetylglucosamine by either enzyme. However,  $\alpha$ -lactalbumin had little effect on the transfer of this sugar to the fetuin acceptor.

The transfer of galactose to *N*-acetylgalactosamine was detected only when the serum enzyme was used. When an OSM with terminal *N*-acetylgalactosamine residues was the acceptor, moderate incorporation of galactose was catalyzed by the serum enzyme but a low level of transfer occurred with the erythrocyte mem-

brane preparation. Using these acceptors no effect on glycosylation was observed when  $\alpha$ -lactalbumin was included in the reaction mixture.

The level of the galactosyltransferase in serum and on erythrocyte membranes was examined in individuals differing in blood group and secretor status (Table IV). These results indicate no significant difference in the galactosyltransferase level among the groups studied.

## DISCUSSION

The enzyme studied herein catalyzes the transfer of galactose from UDP-galactose to specific large and small molecular weight acceptors (Fig. 9). A galactosyltransferase with similar acceptor specificities to the enzyme examined here is required for the biosynthesis of serum glycoproteins (32) and for intermediate stages in the biosynthesis of ABO blood group substances

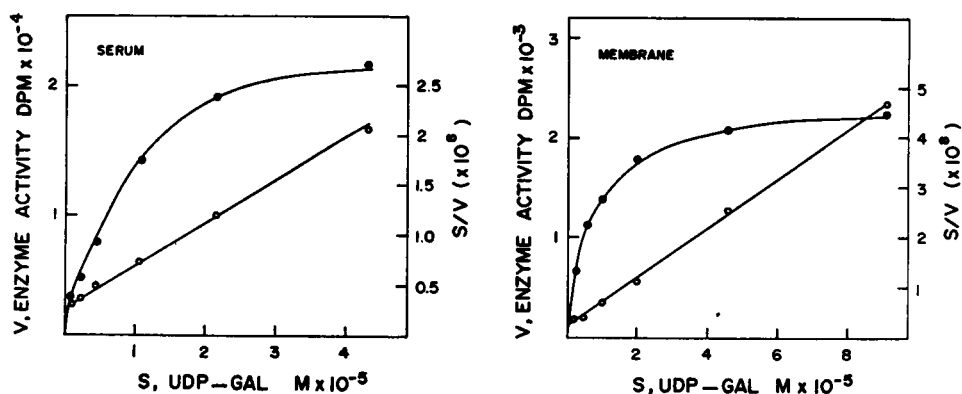


FIGURE 6 Effect of UDP-galactose concentration on the incorporation of galactose into fetuin acceptor. Incubation conditions are those described in Methods. The apparent  $K_m$  values for UDP-galactose were  $7.5 \times 10^{-6}$  M and  $5.0 \times 10^{-6}$  M for the serum and erythrocyte membrane enzymes, respectively. V, ●—● and S/V, ○—○.

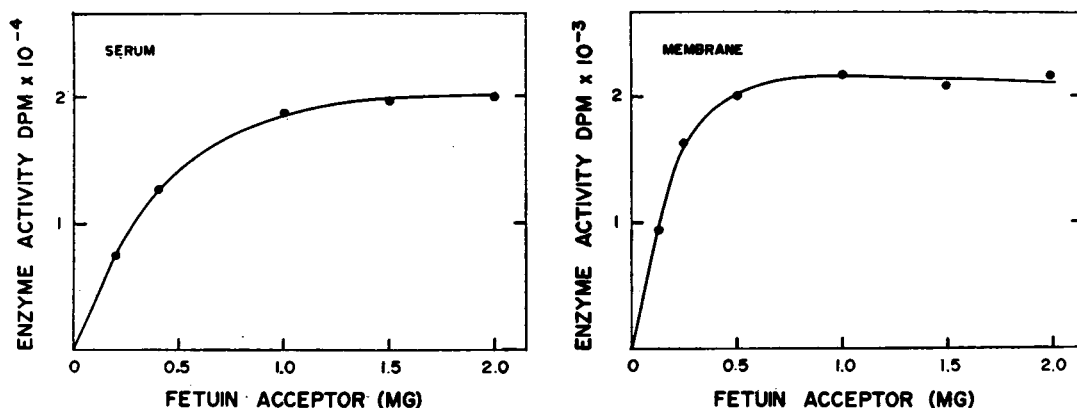


FIGURE 7 Effect of acceptor concentration on incorporation of galactose. Incubation conditions used were those described in the Methods.

TABLE II  
Effect of Nucleoside Phosphates on Galactosyltransferase Activity

Nucleoside phosphate added	Serum		Erythrocyte membrane	
	Enzyme activity	Per cent change	Enzyme activity	Per cent change
	dpm/2 hr	%	dpm/2 hr	%
None added	28,750		2,096	
UTP 0.1 mM	23,715	-17.5	1,380	-34.1
1.0 mM	11,460	-60.1	833	-60.3
10.0 mM	1,880	-93.5	128	-93.9
UDP 0.1 mM	12,930	-55.1	1,144	-45.4
1.0 mM	2,370	-91.8	260	-87.6
10.0 mM	250	-99.1	33	-98.4
UMP 0.1 mM	23,700	-17.6	1,885	-10.1
1.0 mM	8,380	-70.9	891	-57.5
10.0 mM	1,240	-97.2	139	-93.4
ATP 1.0 mM	25,580	-11.1	2,048	-2.3
10.0 mM	23,020	-20.0	1,557	-25.7
ADP 1.0 mM	27,240	-5.3	1,968	-6.1
10.0 mM	23,200	-19.3	1,705	-18.7
AMP 1.0 mM	27,320	-5.0	1,961	-6.4
10.0 mM	26,960	-6.2	1,810	-13.7
GTP 1.0 mM	28,780	+0.1	ND	
10.0 mM	22,400	-22.1	ND	
GDP 1.0 mM	27,530	-4.2	ND	
10.0 mM	24,640	-14.3	ND	
GMP 1.0 mM	27,780	-3.4	ND	
10.0 mM	27,530	-4.2	ND	

The assays were performed as described in Methods except that a 25  $\mu$ l portion of serum was used. The concentration of nucleoside phosphates indicated are the final concentrations in the incubation mixture. Per cent change represents the difference in activities between the incorporation in the presence and absence of nucleoside phosphate. ND, not determined.

(33) as well as for the formation of lactose in lactating mammary glands (34).

In the present studies such an enzyme has been found to be located in the serum and on erythrocyte membranes of all individuals studied, regardless of their blood type or secretor status. The galactosyltransferase from both sources had very similar catalytic properties. Both had an absolute requirement for  $Mn^{++}$  and had similar temperature, metal ion concentration, and pH optima and had the same acceptor specificities. They differed, however, in their requirement for a detergent. While a detergent was required for the expression of the erythrocyte membrane enzyme, its

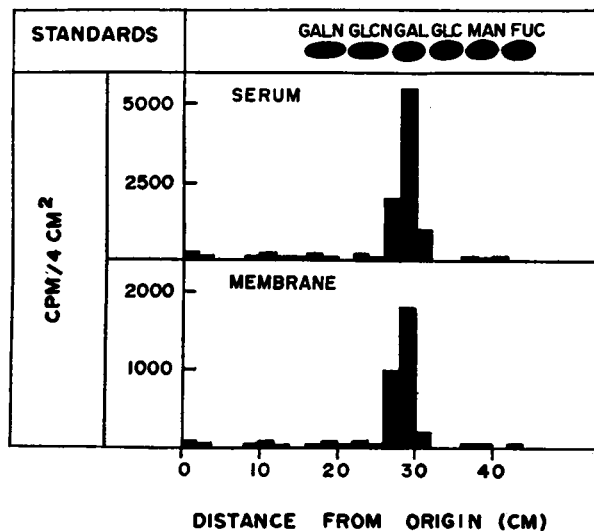


FIGURE 8 Chromatographic identification of incorporated sugar. Fetuin was used as the acceptor. The reaction mixture was scaled up three-fold for the erythrocyte membrane incubation. The conditions used were the same as in the standard assay procedure. Hydrolysis and paper chromatography were performed as described in the Methods.



fer of galactose to *N*-acetylglucosamine was markedly reduced by the presence of  $\alpha$ -lactalbumin but the addition to *N*-acetylgalactosamine was only marginally affected, indicating there may be two different enzymes involved. Schachter, McGuire, and Roseman (20) have recently reported that there appears to be at least two galactosyltransferases present in porcine submaxillary gland, one adding galactose to *N*-acetylglucosamine, another to a *N*-acetylgalactosamine-terminating acceptor.

Clearly the most immediate questions which arise concern the origin and biological functions of these enzymes. The serum glycosyltransferases may well be of diverse origin and may arise not only from blood cells but also from other sources, e.g., liver, brain, mammary gland, etc. Equally, the demonstration of these enzymes in the red cell membrane does not prove that they originate or play a vital role in that tissue, that is, their presence may be a result of adsorption from serum. However, the effect of detergents suggests that they are bound to structural components. Furthermore, since glycoproteins may be involved in various cell surface phenomena it is tempting to postulate a role for the soluble glycosyltransferases in the synthesis or maintenance of surface components, be they concerned with blood group determination, transport processes, cellular adhesion, tumor antigenicity, or perhaps with the clearance of specific components from the circulation. In light of recent findings (38-40) that desialization of glycoproteins with consequent exposure of galactose as a terminal sugar leads to their rapid removal by the liver from the circulation, the need to preserve intact the carbohydrate moiety of serum glycoprotein is evident. Purification of the serum glycosyltransferases, together with data on the turnover rates of the enzymes in various organs and in different phases of organ function or disease are clearly needed. The fact that the serum, erythrocyte membrane and other (19, 23) galactosyltransferases share with the A protein of lactose synthetase a susceptibility to modification by  $\alpha$ -lactalbumin is also of considerable interest. It is reasonable to suppose that other glycosyltransferases may interact with modifiers, altering the substrate or acceptor specificities of these enzymes, thus providing a general mechanism in the regulation of glycoprotein biosynthesis.

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