GALACTOSYLTRANSFERASES: PHYSICAL, CHEMICAL, AND BIOLOGICAL ASPECTS

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I. INTRODUCTION

During the past decade, a number of articles have appeared on glycosyltransferases.[1-4] These enzymes are involved in the synthesis of oligosaccharides and are generally named after the sugar which they transfer from a nucleotide-sugar donor to an acceptor such as glycoprotein, glycolipid, and polysaccharide, as well as to simple sugars.[5] These enzymes are highly specific for both the type of acceptor they will use and the linkage attachment to the acceptor. Accordingly, these enzymes can be classified on the basis of acceptor and the linkage in the products formed. Thus, *N*-acetylglucosamine: (β1→4)-galactosyltransferase will catalyze the transfer of galactose moiety to *N*-acetylglucosamine (GlcNAc) in β1→4 linkage. For the enzyme asialo-, agalacto-, α1-acid glycoprotein (α1→2 Gal):fucosyltransferase, the acceptor is asialo-, agalacto-, α1-acid glycoprotein and the linkage formed with the fucose will be of the α1→2 type.[6] For the sake of convenience, they are also referred to by group names, such as, in the above case, galactosyltransferases and fucosyltransferases. The general name glycosyltransferases applies to many enzymes included in this group. Recently, galactosyltransferase, one of the enzymes in this group, has received a great deal of attention due to its possible role in malignancy[1] and cell surface phenomena.[2-4] The object of this review is to discuss physical, chemical, and biological aspects of galactosyltransferases.

II. GENERAL CONSIDERATIONS

A. Reaction

Galactosyltransferase (GT) is involved in many reactions of biological importance, where an addition of galactose (gal) to a suitable acceptor is required. A general reaction is shown below:

UDP-gal + GlcNAc-(saccharide)-protein or (acceptor)
Metal ↓ GT
Gal β1→4-GlcNAc-(saccharide)-protein
or
Gal-acceptor

Thus, the nature of the products depends on the nature of the acceptor. A number of GTs using various kinds of acceptors, biological (GM_2)[7] as well as organic (GlcNAc)[8,9] have

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been described in the literature. Therefore, the properties of these enzymes differ among themselves. The enzyme involved with one kind of acceptor from one source may have some different properties from the enzyme from a different source for the same acceptor. It is noteworthy that none of the glycosyltransferases, including GT, can utilize more than one type of sugar donor;[5,10,11] although specificities of the enzyme(s) for different acceptors may not alter, the kinetic properties do change. For example, GT can utilize GlcNAc in free form as well as when bound to a glycoprotein, but the km of the free form is much higher than the bound form.[12]

B. Source of GT and its Localization

Two types of GT activities have been reported: (1) insoluble and (2) soluble. Most cell-associated GTs have been found associated with membranes mainly on Golgi and smooth endoplasmic reticulum[13,14] and mitochondria.[15,16] Thus, the Golgi apparatus has been identified as the subcellular site for the sequential addition of at least some of the sugar moieties to glycoprotein.[14] For this reason, the galactose transfer to glycoprotein or GlcNAc[17,18] is now considered to be the best marker for the Golgi apparatus, at least in the tissues such as liver, that produce glycoprotein primarily for export. The activity of GT has been found associated with the membranes of the Golgi apparatus, not with their content.[19] Evidence for association of GT with the membrane of the Golgi apparatus has come from subcellular fractionation[13,14] and autoradiography following incubation with radiolabeled sugar residues.[20,21] The evidence presented on the basis of subcellular fractionation for the localization of GTs are indirect. A direct approach will be to use antibody directed against GT and determine the locale of the enzyme by immunochemical techniques. Recently, Roth and Berger[22] have developed monospecific antibodies against GT in rabbit and used them to localize GT in HeLa cells by immunofluorescence and protein A gold technique at electron microscope level. Specific immunofluorescence is observed in a juxtanuclear cytoplasmic region which is identified, on immunostained thin sections of low-temperature Lowicryl K4M-embedded HeLa cells, as Golgi apparatus. The protein A gold technique indicated that GT is compartmentalized in the cisternal stack in two to three cisternae of the trans side of the Golgi apparatus (Figure 1). A combination of pre-embedded thiamine pyrophosphatase (TPP) cytochemistry with postembedded immunostaining for GT revealed that the two enzymes are co-distributed. However, the acid phosphatase-positive, trans-most cisternae is negative for GT. The close topographical association of both GT and TPP (or nucleoside diphosphatase) suggests a concerted action of both enzymes in glycosylation (Figure 1).

Some of the glycosyltransferases, including GT, are also bound to the outer surface of the cell plasma membrane where the enzymes retain their catalytic activities and/or their binding properties. It has been speculated that these enzymes are all associated with Golgi at one stage, and later transferred to the cell surface, but so far there is no convincing evidence to prove this.[20,23] Shur and Roth[2,3] have published an excellent review on cell-surface glycosyltransferases. These surface enzymes are involved in cell-to-cell recognition and will be discussed later in this review.

The best example of soluble GT is from milk,[8,9,24,25] which catalyzes the synthesis of lactose by transferring galactose to glucose in the presence of α-lactalbumin. In the presence of this modifier protein the galactose is preferentially transferred to GlcNAc, synthesizing *N*-acetyllactosamine.[8,26] Colostrum GT is the same as milk GT.[27] Soluble GT activity has also been detected in human serum and erythrocyte membrane,[28,29] cerebrospinal fluid,[30] amniotic fluid,[31] vitreous humor,[32] and human effusion.[33]

C. Measurement of GT Activity

GT activity is generally measured by using radiolabeled nucleotide sugar. Some investigators have measured GT activity indirectly by measuring UDP formation and following

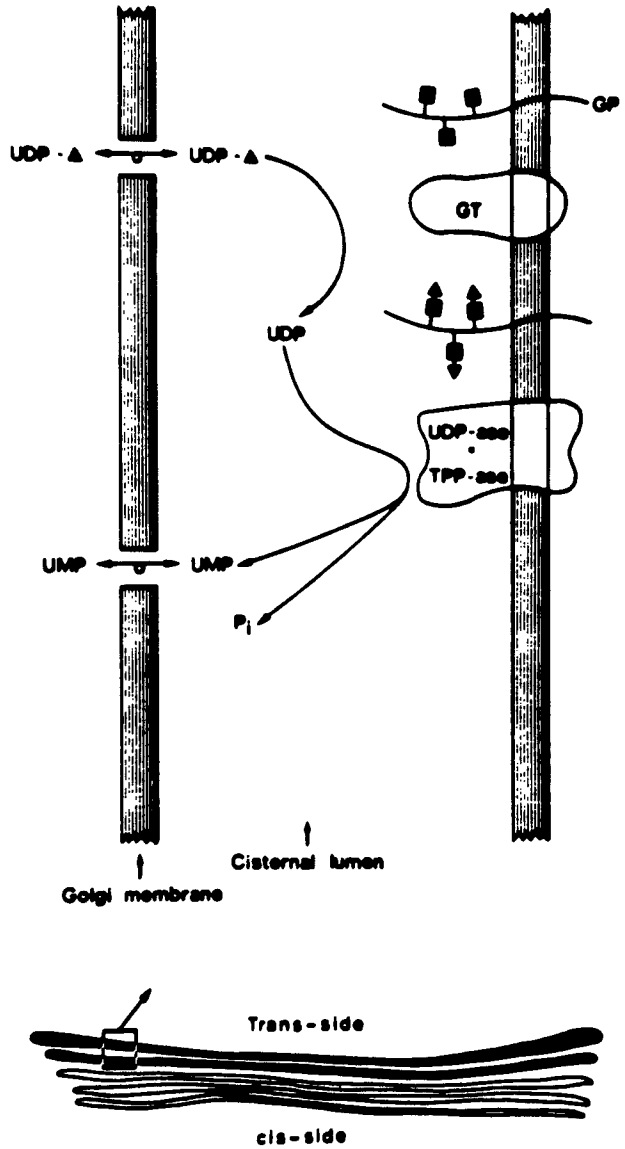


FIGURE 1. Schematic localization of both GT and TPPase (or nucleoside diphosphatase) in *trans* cisternae of the Golgi apparatus in HeLa cells. On the basis of kinetic evidence, a carrier-mediated transport mechanism for glucose, UDP-galactose, and UMP through Golgi membranes and a diffusion barrier for UDP were postulated. GT catalyzes the transfer of galactose (▲) to glycoprotein acceptors containing GlcNAc (■) at the nonreducing end of the heteroglycan. This reaction generates free UDP, which is immediately hydrolyzed by UDPase (or TPPase). This prevents the buildup of inhibiting concentrations of UDP. (From Roth, J. and Berger, E. G., *J. Cell Biol.*, 92, 223, 1982. With permission.)

the reaction in a spectrophotometer. Some of these procedures are discussed below.

1. Radioactive Procedure

A number of investigators have used the modification of the procedure published by Kim and associates.[28,29] In this assay, the GT is incubated at 37°C in a total volume of 50 μℓ

containing 100 m*M* 2-(*N*-morpholine)ethanesulfonic acid, pH 6.3, Triton® X-100 (0.1 to 0.5%), $MnCl_2$ (10 to 25 m*M*), a suitable acceptor and UDP-(^{3}H) galactose. After 1 hr, the reaction is terminated by adding 10 $\mu\ell$ of 7.5% sodium tetraborate/0.2 *M* EDTA. Samples are now subjected to high voltage electrophoresis (HVE) for 40 min at 1700 V in 1% sodium tetraborate, pH 9, as previously described[34] to separate the galactosylated products from unused UDP-galactose (UDP-gal) and degradation products, such as (^{3}H)-galactosephosphate and (^{3}H)-galactose. The origins, containing the GT products, are dried and counted in a liquid scintillation counter. The enzyme activity is calculated by nanamoles of galactose incorporated per hour per milligram protein.[35]

UDP-galactose which is used as a substrate for the GT may either have H or C labeled. The abovesaid method for GT determination has been modified at many steps involving the use of acceptors and separation of galactosylated products. Many researchers have avoided the use of HVE and have continued to search for a simpler procedure. In one method, the reaction is stopped by a mixture of 5% (w/v) trichloroacetic acid (TCA) and 1% (w/v) phosphotungstic acid (PTA). The precipitated product galactosylated acceptor glycoprotein is filtered on appropriate filter discs, washed in TCA-PTA and ethanol-ether (1:1, v/v). The incorporation of radioactivity into an acceptor glycoprotein is measured on the dried filter disc in a scintillation counter.[9,36]

The method of choice for the separation of galactosylated product from degradation products depends on the type of acceptor used. In case of macromolecule acceptors, such as a glycoprotein, the separation by filtration has been very useful, whereas, in the case of micromolecule acceptors such as GlcNAc and glucose, separation by HVE has been very useful.

At present, two principle methods exist to isolate the labeled glycoprotein. Both techniques involve precipitation of the acceptor from the assay solution. In one case, the precipitate is collected by filtration through glass-fiber discs,[37] while the second procedure involved repeated washings, centrifugation, and resuspension of the precipitate, followed by liquid scintillation counting.[38] Both of these separation procedures have been lengthy and complex. Accordingly the procedure is not useful for screening of large numbers of samples such as column fractions. Recently, Baxter and co-workers[39,40] have reported a rapid and sensitive disc assay procedure for the determination of glycoprotein glycosyltransferases. This procedure is similar to the radioactive method except that the labeled acceptor protein, after termination of the reaction, is spotted on cellulose paper discs and washed in 10% (w/v) TCA, and finally, in ethanol/ether (2:1) and ether, and dried under a heat lamp, followed by counting the discs in a xylene based scintillation medium for the measurement of GT activity.

2. Spectrophotometric Procedure

Some investigators have used the enzymatic measurement of UDP to measure lactose synthetase activity.[41-43] In yet another similar method, the formation of lactose enzymatically is measured to monitor lactose synthetase activity.[44] In spite of the simplicity, the enzymatic procedures have never been very popular because of problems associated with the crude sample and perhaps the sensitivity. Recently, an NAD coupled enzyme assay has been published by Roth and associates.[45] The UDP produced in the reaction is converted to UDP-glucuronic acid in the presence of a series of enzymes and ATP, glucose-1-phosphate and NAD^+ as follows:

$$\text{UDP-Gal} + \text{acc} \xrightarrow{\text{GT}} \text{acc-Gal} + \text{UDP}$$

$$\text{UDP} + \text{ATP} \xrightarrow{\text{NPK}} \text{UTP} + \text{ADP}$$

$$\text{UTP} + \text{Glu-l-P} \xrightarrow{\text{PPase}} \text{UDP-glu} + \text{PP}_i$$

$$\text{UDP-glu} + 2\ \text{NAD}^+ \xrightarrow{\text{DHase}} \text{UDP-glucoronic acid} + 2\ \text{NADH} + 2\ \text{H}^+$$

Thus, 2 mol of NADH are produced stoichiometrically per mole of UDP. The progress of the reaction is monitored at 340 nm in a spectrophotometer. According to the authors, activities calculated from NADH production from a partially purified bovine milk GT and dialyzed fetal calf serum GT were similar to those determined from assays that use radioactive sugar nucleotide substrates. The method has been used further to localize the GT activities on 7.5% nondenaturing polyacrylamide gels after electrophoresis by incubating the gel with an agarose indicator gel containing the coupled enzyme system. An NADH fluorescence is produced in the presence of GlcNAc, the intensity of which increases with increasing GT activity applied to the gel. Previous techniques for detecting GT in gels involved slicing of gels, eluting enzymes from the slices, and assaying enzyme activity by standard radioactivity assay.[33] The spectrophotometric assay is useful for GT that produces at least 1 to 2 nmol of UDP per hour. However, the sensitivity can be increased by NADH fluorescence at 460 nm instead of absorbance at 340 nm.

Recently, Ram and Munjal,[46] while determining the GT activities in clinical samples and a commercial GT, reported a problem with the above-described spectrophotometric procedure. The same sample at various dilutions did not give corresponding true value of GT at that dilution. To alleviate this problem, the authors modified the procedure[46] as follows: a 1-mℓ assay mixture consists of the following chemicals in ($\mu\ell$): 40 m*M* $MnCl_2$ (250), 13 mg/mℓ NAD (50), 3.05 mg/mℓ UDP-gal (20), 0.01% Triton® X-100 (10), 1000 U/mℓ NPK (10), 25 U/mℓ PPase (10), 1 U/mℓ DHase (20), dialyzed test sample (25,50, or 100), plus 100 m*M* cacodylate buffer, pH 7.5, to bring volume to 980 $\mu\ell$. The reaction is started at 37°C by adding 20 $\mu\ell$ of 1*M* GlcNAc. The sample is added in amounts so as to produce an absorbance change/min between 3×10^{-4} to 1.6×10^{-3}. If the absorbance does not fall in this range, the sample is appropriately diluted with cacodylate buffer.

In summary, the radioactive procedure, in spite of the time consumption and disposal problems of the radioactive material, is still the method of choice. Certain determinations cannot be performed using the enzymatic assay. For example, the effect of inhibitors, temperature, or pH cannot be studied by the enzymatic assay, as the multiple enzymes used in the assay mixture may also be affected. However, the enzymatic method has distinct advantages when such physical parameters are not being studied.

3. Immunoassay Procedure

A prerequisite of an immunoassay for GT is the development of an antibody against this enzyme. Antibody response towards the soluble antigens is normally poor, due to the fact that they may be readily cleared from the circulation, either by some metabolic pathway or by excretion, through routes that largely bypass lymph nodes, spleen, and other reservoirs of immunopotent cells.[47] Secondly, the production of monoclonal antibodies also requires a great deal of work. To the knowledge of the authors, to date, only one report claims to have produced monoclonal antibody against GT.[48] Recently, monospecific rabbit anti-human milk GT antibodies have also been developed by Verdon et al.[49] They developed an ELISA by conjugating the GT antibody with alkaline phosphatase, which would react with the substrate *p*-nitrophenyl phosphate in microtiter plate. The absorbance at 405 nm will quan-

Table 1
ACTIVITY[a] OF GT WITH LOW- AND HIGH-MOLECULAR-WEIGHT ACCEPTORS[b]

Acceptor	(^{14}C) Galactose incorporated (nmol/min)	
	No addition	α-Lactalbumin added
$GlcNH_2$	0	0
GlcNAc	67.4	36.1
GlcNAc-GlcNAc	35.8	42.8
GlcNAc-GlcNAc-$(Asp)_n$	13.4	19.6
Gal-GlcNAc	0	0
Fuc-Gal-Glc	0	0
ManNAc	0	0
GalNAc	0	0
GalNAc-$(Thr\text{-}Ala\text{-}Ala)_n$	0	0
Glc	0.3	11.0
Maltose	0.8	1.1
Cellobiose	0.2	0.3
Fetuin (minus sialic acid)	1.2	0.1
Fetuin (minus sialic acid and gal)	89.0	78.0
α_1-Acid glycoprotein (minus sialic acid)	0	0
α_1-Acid glycoprotein (minus sialic acid and gal)	77.4	69.4
Sheep submaxillary mucin (minus sialic acid)	0.8	1.2
Ovalbumin	28.4	27.2
Ichthyocol	0	0

[a] Activity of GT was determined by radioactive method using UDP-(^{14}C) gal.[50]
[b] Concentrations used: low-molecular-weight acceptors, 10 m*M*; high-molecular-weight acceptors, 15 m*M*.

titate GT in a sample. The assay is based on competition with GT-phosphate conjugate. The detection limit of the standard curve is 30 ng/mℓ. Currently, this assay is suitable only for quantitation of GT in body fluids.

D. Acceptor (Substrate) Requirements for GT

A number of compounds, natural and synthetic, have been used as acceptors for GT. There are a number of distinct GT enzymes which may use free GlcNAc, exposed GlcNAc residues from Asn type glycoproteins, exposed GalNAc residues from Ser Thr type glycoproteins, glycolipids, or collagen as acceptors. The extent of reaction with an acceptor depends on the type of acceptor used. Small-molecular-weight compounds such as GlcNAc and glucose are as good acceptors as larger-molecular-weight compounds such as glycoproteins and glycolipids.

One criterion for a good substrate is that the terminal sugar of a glycoprotein or a glycolipid should be GlcNAc[50] or, in some cases, GalNAc.[51,52] The galactose is added on the nonreducing end of the terminal sugar. Some of the most commonly used acceptors for plasma GT have been listed in Table 1. The table includes aminosugars, monosaccharides, oligosaccharides, and glycoproteins. It is clear from the table that only compounds containing terminal GlcNAc were acceptors. The reactivity of the plasma GT with a glycoprotein acceptor follows the same pattern. Galactose is transferred only to those glycoproteins which have GlcNAc as a terminal residue in the table. Glucose can become the acceptor when α-lactalbumin is present in the system. It is now believed that α-lactalbumin modulates the action of GT.[8] It is also known that at least GTs from bovine milk and mammary gland[41,53,54]

Table 2
GLYCOLIPIDS AS ACCEPTORS OF GT[a] IN RAT BONE MARROW CELLS

Acceptor glycolipid	(^{14}C) Galactose incorporated (nmol/mg protein/30 min)
GA_2	0.72
GM_2	0.65
CMH	0.13
CDH	0.25
GM_3	ND[b]
GM_{1a}	ND
GA_1	ND
Fuco-GA_1	ND
Fuco-GM_{1a}	ND
Paragloboside	0.92

[a] The activity of GT was determined by radioactive method.[58]
[b] ND, not detected.

are composed of two protein fractions, designated as A and B, which are required for catalytic activity. The B protein has been identified as α-lactalbumin, the common milk whey-protein. The A protein catalyzes the reaction with GlcNAc, which is inhibited by α-lactalbumin. In the presence of B protein, glucose is the preferred acceptor. This kind of phenomenon has also been observed with GTs derived from sources other than milk or mammary gland.[50] This indicates that such type of GTs are similar to lactose synthetase, at least in their catalytic properties.

Fetuin, a mucoprotein of a molecular weight of 48,400 daltons, generally isolated from fetal calf serum[55] has been the most popular glycoprotein acceptor used by most of the investigators in their studies. However, for fetuin to act as an acceptor, its sialic acid is removed by mild acid hydrolysis and the penultimate galactose removed by periodate treatment.[56] This treatment leaves the carbohydrate chains with exposed terminal GlcNAc residues which can accept incoming galactose from UDP-gal.[56] Mucin from the submaxillary gland, with sialic acid removed by acid hydrolysis or by treatment with neuraminidase,[57] also acts as an acceptor for many GTs.[29,50] Recently, Cheng and Bona[52] have reported the acceptor specificities of human tracheal epithelium GT in the presence and absence of α-lactalbumin using the following compounds as the acceptors: asialo OSM (ovine submaxillary mucin), GalNAc, acid-treated porcine submaxillary mucin, and human tracheobronchial mucin, both untreated and neuraminidase treated. The activity of the enzyme is not affected by α-lactalbumin, which shows that their GT is different from GlcNAc or glucose reactive GTs. Apparently, their enzyme transfers galactose to the GalNAc Gal β1→3 residue at nonreducing termini in mucin. The glycolipid acceptors are presented in Table 2. The use of these acceptors has been reviewed by Taki et al.[58] The data indicates that GM_2, GA_2, and paragloboside are found to be the good acceptors in bone marrow cells. Also, it has been reported that in the chick neural retinal cells and gangliosides, synthesis of GM_2, and use of GM_2 as an acceptor proceeds simultaneously.[7,59]

Some investigators, during the measurement of GT, have consistently observed some GT activity in their samples even when there is no exogenous acceptor.[60] We have also encountered this problem during the measurement of GT by spectrophotometric procedure.[46] This necessitated the dialysis of the sample, which removed most of the endogenous activity. Recently, Podolsky and Weiser[61] reported purification of an endogenous acceptor from sera and effusions of patients with extensive malignant disease. Although the acceptor acted as

substrate for both normal and cancer-associated human GT isoenzymes, it had a higher affinity for the cancer-associated GT isoenzyme than for the normal isoenzyme. This endogenous substrate was characterized and found to be asparagine-glucosamine type of glycoprotein with an approximate molecular weight of 3600 daltons. The acceptor also inhibited the growth of transformed cells and tumors but had little or no effect on normal cells and tissues.[62,63] These authors postulated that this cancer-associated glycopeptide acceptor (CAGA) was probably shed or secreted from the tumor cell.

E. Purification of GT

GT was first described by Watkins and Hassid[53] as a particulate enzyme in rat and guinea pig mammary gland. Later, a similar enzyme was found as a soluble enzyme in bovine milk[54] and was partially purified.[24] There was no significant improvement in purification of GT until the advent of the affinity chromatography columns. Andrews[8] became the first to utilize the α-lactalbumin-Sepharose column for the purification of GT. Earlier, the role of α-lactalbumin in modulation of the activity of the A protein of lactose synthetase had been indicated.[8,26] The B protein of lactose synthetase, which is characterized as α-lactalbumin remains bound to the A protein in milk.[41,63] In the presence of GlcNAc or glucose, the A protein binds to the α-lactalbumin. Later, GT can be eluted from the column by withdrawing GlcNAc or glucose from the buffer. Thus, α-lactalbumin linked to some insoluble support is being used very often as one of the steps for purification of GT. Using the α-lactalbumin affinity column, milk GT has been purified in a number of laboratories.[8,64,65] Before loading the crude material on the α-lactalbumin affinity column, most of the extraneous protein is removed either by ammonium sulfate precipitation or by passing the crude material through gel filtration or a series of ion-exchange columns. Andrews[8] used two gel filtration steps before loading his sample on the affinity column and is of the opinion that partially purified GT sample binds better to the α-lactalbumin affinity column. He also reported that passage of the A protein through the α-lactalbumin-Sepharose column is retarded much more in the presence of GlcNAc than in the presence of glucose, as used by Trayer et al.[66] in their purification procedure.

A number of specific ligands structurally related to portions of the substrates of the GT can be immobilized to cyanogen bromide-activated agarose.[67] The UDP-hexanolamine-agarose, GlcNAc-agarose, and galactosyl pyrophosphate-agarose adsorbents have been evaluated with regards to their binding affinities to GT.[68] Binding of GT on UDP-hexanolamine-agarose is increased by Mn^{2+} ions and decreased by EDTA, urea, borate, or magnesium ion. The affinity of the enzyme for GlcNAc-agarose is enhanced considerably by UDP or UMP and is decreased by borate, urea, or GlcNAc. The galactosyl-pyrophosphate-agarose has a low capacity and specificity of binding for GT. By manipulating the column conditions, GT can be isolated using the above adsorbents.[68] Of all the three above-mentioned immobilized compounds, the GlcNAc-agarose has become the most popular adsorbent.

Recent methods of GT purification have usually included one α-lactalbumin-agarose and one GlcNAc-agarose column.[9,33,69] Podolsky and Weiser[33] have purified GT I and GT II isoenzymes from effusion using nor-leucine-Sepharose column initially, followed by separation of the two isoenzymes activities on DEAE-cellulose column. The two activities then were purified separately on α-lactalbumin-agarose columns, and finally, on GlcNAc-Sepharose column and the end product gave one band on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. The GT enzyme can also be purified by passing the crude material repeatedly through α-lactalbumin-Sepharose column until it becomes pure.[70-72] However, preventive measures should be taken during each stage to avoid the loss in GT activity.[36]

Due to the possible role of GT isoenzymes in malignancy, most of the recent efforts have been directed towards the purification of the tumor-associated isoenzyme[73,74] to homogeneity

so that a test can be developed to measure only tumor-associated isoenzyme and not the normal isoenzyme. Podolsky and Weiser[33] isolated and purified GT isoenzymes from the effusions of cancer patients. The cancer GT was found to elute together with immunoglobulins at all steps during the purification.[33] Recently, Wilson et al.[69] have claimed to have obtained purified GT without contamination of immunoglobulins by passing the enzyme through anti-human serum and anti-human IgG immunoadsorbent columns. We have included protein A-Sepharose column as a step in our purification scheme to remove immunoglobulins from GT preparations. Protein A of *Staphylococcus aureus* interacts mostly with the Fc part of IgG molecules from many species[75,76] and to a lesser extent with IgA[77] and IgM.[78]

Most of the information on purification and characterization of GT has come from milk, serum, blood, effusions, and tumor tissues. The homogeneity of the GT enzyme has remained of major concern. Because of the diversity of the nature of GT isolated from a particular source and the type of linkage it catalyzes, the purification schemes have differed widely from one laboratory to another. For example, the purification scheme of hydroxylysyl GT is very different from the usual purification procedure of many GTs from milk, blood, and serum. To purify hydroxylysyl GT, separation on collagen-agarose affinity column was undertaken.[79] To prepare affinity column, citrate-soluble collagen is first prepared from rat skin and coupled to agarose by cyanogen-bromide activation.[79] The column is washed with enzyme buffer (0.12 *M* NaCl, 2 m*M* $MnCl_2$, 50 μ*M* dithiothreitol, 1% (v/v) glycerol, and 50 m*M* Tris-HCl buffer, pH 7.4) until absorbance of the eluate at 225 nm is less than 0.05, and the enzyme is then eluted either with 80 mℓ 50% (v/v) ethylene glycol in enzyme buffer[80,81] or with 2 g of dialyzable collagen peptides[82] in 2 mℓ of enzyme buffer, followed by enzyme buffer. In the elution with ethylene glycol, the fractions are pooled on the basis of enzyme activity; whereas, when the peptides are used they are pooled beginning with the first to show any increase in absorbance and continuing until the last one after the peptide peak that still has an absorbance above 2.0 at 225 nm. The pool, after concentration, is further purified on Sephadex® G-150 and the fraction containing most of the enzyme activity constitutes the final enzyme pool. The binding of hydroxylysyl GT to collagen-agarose is highly variable. This suggests that minor differences in coupling are critical for the binding of this enzyme to collagen-agarose.[82]

Recently, a β1,3 GT that transfers galactose from UDP-galactose to *N*-acetylgalactosaminide:mucin to yield galactosyl β1,3-*N*-acetylgalactosaminide mucin has been purified from swine trachea membrane homogenate.[83] The enzyme, which is present in the microsome fraction, is solubilized and purified to homogeneity by a procedure which includes affinity chromatography on Cowper's gland mucin. This enzyme shows a high specificity for *N*-acetylgalactosaminide residues linked to Ser and Thr, and the most active glycosyl acceptors are macromolecular mucin glycoproteins containing free *N*-acetylgalactosaminyl residues linked to a polypeptide chain.

F. Homogeneity of Various GTs

Some investigators have claimed to have isolated GT in pure form as judged by SDS polyacrylamide gel electrophoresis (SDS-PAGE). One of the main problems during the purification of GT to homogeneity has been the substantial loss in activity, especially when the enzyme is considerably pure. On the other hand, there is no conformity in the specific activities of GT, even when purified from similar sources. Andrews[8] purified milk GT 1150-fold with specific activity of 5.74, while Trayer and Hill[64] achieved 11,750-fold purification with specific activity of 14,100 essentially using the same affinity procedure, and reported GT to be homogeneous by SDS-PAGE, ultracentrifugal analysis, and gel filtration criteria. Many investigators have obtained homogeneous preparations of GTs as shown by SDS-PAGE from plasma,[50,84] rat liver microsomal fractions and serum,[70,71] calf serum,[85] and pleural effusions.[33] Recently, substantial progress has been made in procurement of GT

enzyme to a very high degree of purity. In our laboratory, we have purified an immunoglobulin-free GT from malignant pleural effusion to about 41,000-fold with a specific activity of 551.[72]

G. Stabilization of GT Activity

Like most other enzymes, GT is very sensitive, and if proper care is not taken during purification and/or after the enzyme has been obtained in pure form, the loss of activity is rapid. In many cases, it has been reported that Triton® X-100, a nonionic detergent, stabilized GT activity. Accordingly, a number of investigators[70,72] have incorporated Triton® X-100 into their buffers for purification of GT enzyme. Triton® X-100 not only stabilizes GT activity, but it also causes stimulation of membrane-bound (particulate) enzyme. The membrane bound enzyme is normally extracted with 1% Triton® X-100 to release most of the GT activity.[71] Cheng and Bona[52] used 2% Triton® X-100 to obtain maximum α-*N*-acetylgalactosaminide β1→3 GT from human tracheal epithelium.

Fraser et al.[36] have studied in detail the effects of different additives (glycerol, *p*-methylbenzenesulfonyl fluoride [PMSF], albumin, ammonium sulfate, and EDTA) on the stability of serum and membrane GT enzymes. The stability of the enzyme was studied by storage in assay vials at 0 to 5°C and at −20°C for a period of up to 35 days for the membrane enzyme, and for up to 60 days for the serum enzyme. In all cases albumin (2%) had the greatest stabilizing effect on the enzyme activity. Crude serum enzyme retained almost full activity at both temperatures up to 60 days. For the purified serum enzyme, the stabilizing effect of ammonium sulfate (<1%) was next to albumin, and glycerol (10%) was third in retaining GT activity at 0 to 5°C, while the enzyme virtually lost its activity when stored with agents like EDTA (2 m*M*), PMSF (0.1%), and water. Glycerol was the second best stabilizer for the membrane enzyme at either of the two temperatures. Plasma GT from blood group B subjects which converts blood group O-red blood cells to B-cells had a full retention of activity over a period of 10 days at 4°C in the presence of EDTA (1 m*M*), NaCl (0.2 *M*), and glycerol (5%). Sodium chloride or EDTA alone prevented 50% loss in activity and glycerol 25% loss in activity over the same period of time at the above temperatures.[84]

Lysolecithin has been reported to activate membrane-bound GT.[36] The extent of activation is related to the degree of binding of lysolecithin to the membrane. The lysolecithin-extracted GT from microsomes has been purified in its presence throughout the purification procedure. Rat liver phenylalanine hydroxylase is also stimulated by lysolecithin, which exposes a sulfhydryl group of the enzyme, thereby altering its conformation.[86,87] In view of this, lysolecithin may indirectly act upon the enzyme to modulate its activity. The mechanism of activation of GT by lysolecithin and Triton® X-100 are very similar, as both the reagents are surfactants. Purified GT has been reported to be lipophilic in nature and rapidly loses activity and aggregates, if not supplemented with either Triton® X-100 or serum albumin.[88]

We have conducted storage stability studies at 4°C and −20°C on GT isolated from effusion of a patient with liver cirrhosis in the presence of Triton® X-100, Nonidet P40, bovine serum albumin (BSA), lysolecithin, choline chloride, glycerol, and chloroform.[72] The presence of 0.05% Triton® X-100 in the enzyme appears to be the best in preserving partial GT activity. Over a period of 2 months, a loss of 50% GT activity was observed at 4°C. However, at −20°C, a complete loss in activity occurred in 15 days unless 5 m*M* GlcNAc was also present with Triton® X-100. Nonidet P40 also follows the similar pattern. BSA (5 mg/mℓ) maintains partial GT activity only at −20°C. Glycerol (5%) helps in maintaining GT activity both at 4°C and −20°C but the loss in activity is faster at 4°C than at −20°C. Lysolecithin, choline chloride, and chloroform appear to be of limited value in preserving GT activity over an extended period of time.

Table 3
REQUIREMENT OF MANGANESE IONS FOR GT MEASUREMENT

Source and type of GT	Mn^{2+} (m*M*)	Ref.
Normal and diseased liver serum, erythrocyte membrane	43	28
Human serum	40	94
α-*N*-acetylgalactosaminide β3 GT (asialo OSM GT)	20—25	52
Plasma blood group B	15—30	84
Rat liver and serum	12.5	70
Golgi fraction from cultured neoplastic	>10	101
Tumor	7.5—10	90
Brain glycoprotein GT	10	258
Effusion GT I and GT II isoenzymes	10	37
Human serum (normal and cancer)	7.5—10	95
Microsomal, pig thyroid	5	88
Rabbit erythrocyte cell surface GT	5	37
Hydroxylsyl GT	2	80

III. PHYSICAL ASPECTS OF GT

A. pH

Most of the GTs are active in the pH range of 6 to 8.[28,33,37,70,88-90] The optimum pH depends on the type of GT and the source from which it is isolated. Rat liver microsomal GT has maximum activity at pH 6.5. A complete loss in activity occurs below pH 5.0 and above pH 8.5.[71] Kim et al.[28] observed maximal human serum GT activity at pH 7.0. In contrast, Podolsky and Weiser[37] found a bimodal relationship with optima at pH 6.3 and pH 7.2 for human sera GT. Enzyme activity was consistently depressed at a pH of approximately 6.8. GT of a Golgi fraction from cultured neoplastic mast cells had a pH optimum of 6.5 with approximately 50% of the activity remaining at pH 5.5 and 8.0. In our laboratory, GTs isolated from malignant and nonmalignant pleural effusions were fairly active between pH 5 and 8 with maximum activity occurring at pH 7.[72] The pH optima for hydroxylsyl GT[91-93] and rabbit erythrocyte cell surface GT[37] are in the range of 7 to 7.4 and 7.4 to 7.7, respectively.

B. Temperature

Various investigators have measured GT activity between 30 to 37°C, which is considered to be an optimal temperature range for GT[28,72] and for many other enzymes. We have observed maximum GT activity for pleural effusion between 37 and 40°C, and a considerable loss in GT activity occurred below 30 and above 50°C.[72]

C. Co-Factors

Manganese is a well known co-factor for GT. Its presence is essential for the optimal activity of the enzyme. The optimum concentrations of Mn^{2+} required for various kinds of GTs from many sources have been summarized in Table 3. The concentration ranges from a low of 2 m*M* to a high of 43 m*M*. There is no agreement on the required concentration of Mn^{2+}, even for the GTs isolated from the same source.[94,95] Other divalent cations such as Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Cu^{2+}, and Cd^{2+} have been tried but none of these could replace except for Cd^{2+}, which yielded 3 to 5% of the activity observed with Mn^{2+}, of GT purified from two different solid lines of tumors produced in hamsters.[90] Babad and Hassid[24]

have reported that Mg^{2+} was 25% as effective as Mn^{2+}. According to one report,[52] Cd^{2+} could substitute for Mn^{2+} at equivalent concentrations, giving about 90% of the activity obtained in the presence of Mn^{2+} Other cations in this case, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, and Fe^{2+} were only partially effective. Polycationic compounds, e.g., spermine, spermidine, putracine, cadavarine, and poly-L-lysine at a low (2.5 m*M*) $MnCl_2$ concentration caused 70 to 130% increase in original GT activity. However, at a high concentration (25 m*M*) of $MnCl_2$, the polycationic compounds did not affect the enzyme activity. In canine tracheal tissue, it has been shown that spermine and other cationic compounds stimulate the activity of asialo OSM GT but not asialo OSM neuraminyltransferase and GlcNAc GT activities.[96] Spermine enhancement of the activity of human tracheal α-*N*-acetylgalactosaminide β1→3 GT at low $MnCl_2$ concentration but not α-*N*-acetylgalactosaminide α2→6 neuraminyltransferase activity,[52] favors the synthesis of longer carbohydrate chains. Recent reports indicate that polyamine levels and their metabolism are altered in individuals with cystic fibrosis.[97-99] This factor may contribute to the synthesis of mucins with elongated oligosaccharide chains by airways epithelium in patients suffering from this disease.[100]

The concentration of Mn^{2+} required for optimum activity of GT also depends on the concentration of acceptor. In a recent study[101] at optimal OSM (substrate) concentration, Mn^{2+} was found to be inhibitory at a concentration greater than 10 m*M*. In contrast, when an excess of OSM (inhibitory levels, 10 mg/mℓ) was used, higher concentration of Mn^{2+} became necessary for optimal GT activity.

D. Studies on Interaction of α-Lactalbumin, Mn^{2+}, Substrate, and UDP Galactose

A number of studies have shown that various chemical reagents inactivate GT.[102-105] Modification of GT by sulfhydryl reagents, such as *p*-chloromercuribenzoate,[102] *N*-ethyl maleimide,[102] trypsin,[102] lactoperoxidase,[103] iodine monochloride,[104] and UV light[105] results in loss of enzymatic activity. However, the presence of certain substrates protects GT enzyme against such inactivation, suggesting that a conformational change occurs upon binding of substrate to GT. Circular dichroism studies[106] have also shed some light on substrate-induced conformational changes of GT, possibly involving tryptophan and tyrosine residues. The interaction of GT with small ligands and with α-lactalbumin is discussed below.

1. Interactions of Mn^{2+} and UDP-gal to GT

Earlier reports have indicated that Mn^{2+} is required for catalytic activity and must be bound to GT prior to the addition of substrate.[107-109] Binding studies of Mn^{2+}, α-lactalbumin, and UDP-gal with bovine milk GT can be followed, to a certain extent, utilizing difference spectroscopy.[110] The difference spectra of GT obtained with small ligands Mn^{2+}, UDP-gal, and UDP in various combinations are shown in Figure 2.[110] The spectra, which is small ($\Delta\epsilon$ <500 m^{-1} cm^{-1}) and featureless, applies to Mn^{2+} (Figure 2a). There are no correlations between peak intensities, $MnCl_2$, and enzyme concentrations. The interaction of UDP or UDP-gal with GT produces different spectra with a large negative trough at 254 nm. This kind of spectra is characteristic of nucleotide binding to a number of enzymes.[111-113] The difference spectra obtained in the presence of UDP-gal and 2 m*M* $MnCl_2$ (Figure 2b) has a major positive peak at 284 nm, a small positive peak at 298 nm, and a large negative peak at 254 nm. Similar spectra are obtained with UDP and 2 m*M* $MnCl_2$ (Figure 2c). The difference spectra in the presence of 100 m*M* GlcNAc and 2 m*M* $MnCl_2$ has a small positive peak at 288 nm and a second positive peak (not shown) at 280 nm. When GlcNAc, UDP, and 2 m*M* $MnCl_2$ are present, the peak at 284 nm is increased significantly and the peak at 298 nm becomes a shoulder (Figure 2d). Glucose does not alter the shape of the difference spectra in the presence of UDP and 2 m*M* $MnCl_2$ (Figure 2e). The difference in intensities of spectra is also noted in various cases (Figure 2).

The negative trough at 254 or 257 nm represents the absorption change of UDP or UDP-gal upon interaction with the enzyme since UDP-gal can generate similar spectra.[110] The

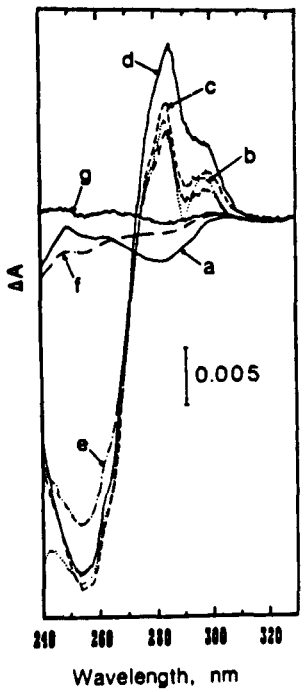


FIGURE 2. Difference spectra of GT produced by: (a) 2 m*M* $MnCl_2$; (b) 99.4 μ*M* UDP-gal + 2 m*M* $MnCl_2$; (c) 84.7 μ*M* UDP + 2 m*M* $MnCl_2$; (d) 79.1 μ*M* UDP + 100 m*M* GlcNAc + 2 m*M* $MnCl_2$; (e) 79.0 μ*M* UDP + 100 m*M* glucose + 2 m*M* $MnCl_2$; (f) 97.0 μ*M* UDP-gal + 20 μ*M* $MnCl_2$; and (g) 96.0 μ*M* UDP-gal. (g) overlaps base-line, which is either for water in R_1, R_2, S_1, and S_2, or for enzyme of identical concentration (4.47 μ*M*, A_{280} = 0.36) in R_1 and S_1 and water in R_2 and S_2. The enzyme (specific activity, 13 to 19) was (a) 8.48 μ*M*; (b) 7.18 μ*M*; (c) 5.69 μ*M*; (d) 4.19 μ*M*; (e) 4.16 μ*M*; (f) 5.43 μ*M*; (g) 5.38 μ*M*. The components in each cell were as follows, (a) R_1, enzyme, R_2, $MnCl_2$, S_1, enzyme + $MnCl_2$; S_2, none. (b) R_1, enzyme + $MnCl_2$; R_2, $MnCl_2$ + UDP-gal; S_1, enzyme + $MnCl_2$ + UDP-gal; S_2, $MnCl_2$. (c) R_1, enzyme + $MnCl_2$; R_2, $MnCl_2$ + UDP; S_1, enzyme + $MnCl_2$ + UDP; S_2, $MnCl_2$. (d) R_1, enzyme + $MnCl_2$ + GlcNAc; R_2, $MnCl_2$ + UDP + GlcNAc; S_1, enzyme + $MnCl_2$ + UDP + GlcNAc; S_2, $MnCl_2$ + GlcNAc. (e) R_1, enzyme + $MnCl_2$ + glucose; R_2, $MnCl_2$ + UDP + glucose; S_1, enzyme + $MnCl_2$ + UDP + glucose; S_2, $MnCl_2$ + glucose. (f) R_1, enzyme + $MnCl_2$, R_2, $MnCl_2$ + UDP-gal, S_1, enzyme + $MnCl_2$ + UDP-gal; S_2, $MnCl_2$. (g) R_1, enzyme; R_2, UDP-gal; S_1, enzyme + UDP-gal; S_2, none. (From Takese, K. and Ebner, K. E., *J. Biol. Chem.*, 256, 7269, 1981. With permission.)

contribution of tryptophan, tyrosine, and phenylalanine are small at these wavelengths. This type of spectrum would be expected if UDP-gal is bound to GT by hydrogen bonding to uracil or through electrostatic interactions between the phosphate groups of UDP-gal, and charged groups in the protein. It is also possible that the electrostatic interaction involved the bound Mn^{2+} on GT. The fact that the UDP and UMP are effective inhibitors of the enzyme, while uridine is a poor inhibitor,[24,114] suggests the importance of the phosphate groups in the interaction. The peak at 284 nm may be ascribed to tyrosine residues, which

are involved in the binding of UDP-gal, and is consistent with the role of tyrosine in the activity of GT.[104] However, the small positive peak obtained for GT spectra at 298 nm may be due to the change in the electrostatic environment of tryptophan residues as previously suggested.[105,115,116] Recently, it has been postulated that tryptophan residue is also involved in the binding site for UDP-gal.[110] So far, the role of phenylalanine, if any, in these interactions is not clear.

It has been reported that bovine colostrum GT has two nonequivalent binding sites with dissociation constants for the enzyme-Mn^{2+} complex in micromolar (Site I) and the millimolar ranges (Site II).[117,118] The presence of two manganese binding sites has also been shown in bovine milk GT.[119,120] The binding of Mn^{2+} to Site I is independent of substrate(s) and must occur prior to substrate binding and prior to a second Mn^{2+} binding to Site II, which, in contrast, is affected by UDP-gal.[120]

The difference spectra (Figure 2) of 2 m*M* $MnCl_2$ does not indicate a specific interaction with tyrosine or tryptophan residues of GT. Although a small change in the far UV region has been previously reported,[121] Geren et al.[106] did not observe any changes in the far and near UV circular dichroism spectra of GT in the presence of Mn^{2+}. Also, indications are that Mn^{2+} alone can bind to GT and free UDP-gal can bind to the enzyme·Mn complex.[107-109] Recently, Takase and Ebner[110] have hypothesized that the difference spectra is caused by the interactions of Mn·UDP(gal) with the enzyme·Mn(I) complex or the free enzyme, or by the interaction of free UDP(gal) with the enzyme·Mn(I) complex or the enzyme·Mn(II) complex. In the absence of $MnCl_2$ (Figure 2), the UDP-gal and UDP plus GlcNAc spectra are unremarkable and even lack some of the features of the 2 m*M* $MnCl_2$ spectra. From the intensities at 250 to 260 nm, one is led to conclude that the extent of binding of UDP-gal is essentially zero in the absence of $MnCl_2$ and GlcNAc. Accordingly, it seems that Mn^{2+} is required for significant binding of UDP-gal or UDP to GT, and the tyrosine or tryptophan residues are involved in the binding of UDP-gal or UDP to the enzyme·Mn complex. This is supported by many earlier observations.[104,105] It is possible that tyrosine and tryptophan are involved in the binding sites for UDP-gal or the binding of UDP-gal or UDP causes a conformational change in GT where tyrosine and tryptophan residues are involved and that may or may not be immediately located in the direct binding site of UDP-gal or UDP.

The binding of Mn^{2+} and UDP-gal precedes GlcNAc reactions with the enzyme.[110] This is apparent from the spectra (Figure 2) where no change in absorbance was observed at 284 nm with the process, enzyme·Mn + GlcNAc → enzyme·Mn·GlcNAc, although an increase in absorbance is registered when UDP-gal is present. This may be due to the binding of UDP to the enzyme·Mn complex which causes a conformational change in the GlcNAc binding site, which allows some tyrosine residues to be involved in the binding of GlcNAc or that most likely by a conformation change, the binding of UDP results in transition to a state which undergoes an additional conformation change involving tyrosine residue(s) upon binding of GlcNAc to this enzyme·Mn·complex. Another possibility is that the binding of GlcNAc causes displacement of a tyrosine residue(s) near the UDP, the polarity of the environment surrounding this residue does not change but that, with bound UDP the tyrosine residue comes close to the phosphate groups of UDP, which results in positive tyrosine perturbation spectra. In contrast to GlcNAc, glucose appears to have little effect (Figure 2). The apparent ineffectiveness of glucose has been ascribed to its high Kd value[122] for GT even in the presence of Mn^{2+} and UDP; however, the possibility that the binding of glucose produces a spectral effect similar to GlcNAc cannot be ruled out.

2. *Interaction of α-Lactalbumin*

It is well known that α-lactalbumin binds GT. However, the exact mechanism of its binding to GT has not been worked out. Some of the evidence for α-lactalbumin binding to bovine milk GT has come from spectral studies.[110] The difference spectra of the interaction

between GT and α-lactalbumin have been observed in the presence of 2 m*M* $MnCl_2$ and 100 m*M* GlcNAc or glucose (Figure 3a and b). These spectra indicate the involvement of Trp residues in the interaction. The shape and magnitude of the difference spectra in the presence of GlcNAc are a little different from those in the presence of glucose. This suggests that while the difference in magnitude may be due to the difference in the extent of their interaction, the difference in shape might indicate a difference in the mode of interaction. Although the shape of the spectrum in the presence of GlcNAc is that of a typical Trp perturbation, its origin in the interaction is not clear. A similar interaction of GT and α-lactalbumin is indicated by the difference spectra in the presence of $MnCl_2$, UDP (Figure 3c), $MnCl_2$, UDP and GlcNAc (Figure 3d), and $MnCl_2$, UDP, and glucose (Figure 3e). All spectra show peak or shoulder at 290 nm, which is not present in the difference spectra for the interactions with the above small ligands. In order to compare the interactions of GT and α-lactalbumin in the presence and absence of UDP, the sum of the difference spectral intensities for the GT-small ligands interactions (Figure 2), and for the GT-α-lactalbumin interaction in 2 m*M* $MnCl_2$ and 100 m*M* GlcNAc or glucose (Figure 3a and b), is indicated by crosses at several wavelengths in Figures 3c, d, and e. Good agreements are seen in Figure 3d, where disagreements are significant for the positive peaks in Figures 3c and e. Because the presence of UDP can facilitate the interaction of GT and α-lactalbumin, and because UDP alone may not be as effective as GlcNAc or glucose in forming the GT-α-lactalbumin complex,[117,123] the disagreements may be accounted for by the difference in the extent of the interaction of the two proteins under different conditions, and in the case of Figure 3e, by the possible effect of glucose binding which becomes significant in the presence of α-lactalbumin. The agreements in Figure 3d suggest that the interaction of GT and α-lactalbumin is nearly maximum in 2 m*M* $MnCl_2$ and 100 m*M* GlcNAc. The Kd value of 1.6 μm[123] for the interaction of enzyme·Mn·GlcNAc and α-lactalbumin predicts 82% interaction under the present conditions. These data indicate that the tryptophan residues are involved in the interaction of GT and α-lactalbumin and that these do not interact with UDP bound to GT and that the interaction of the two proteins does not involve at least the uracil ring of UDP. Moreover, the additive effect seen at 284 and 290 nm in Figure 3 also suggests that the tryptophan residues involved in the interaction of GT and α-lactalbumin are not located in the region which undergoes a conformational change induced by the interactions with UDP and GlcNAc, and that the Tyr residues involved in the interactions with UDP and GlcNAc are not affected by the interaction with α-lactalbumin. Recently, the similarities in primary and three-dimensional structure of α-lactalbumin to lysozyme has been reported.[124-127] The proposed three-dimensional structure of α-lactalbumin has a cleft which corresponds to the sugar binding site of lysozyme. Whether the presence of a cleft in the α-lactalbumin molecule plays any role in the binding of glucose and GlcNAc or the galactose moiety of UDP-gal in various GT-α-lactalbumin complexes is not yet clear.[110]

In one recent[128] study, 2-hydroxy-5-nitrobenzyl bromide (HNB-Br), which reacts with the Trp residues in proteins was used to determine the role of Trp in the GT and α-lactalbumin interaction. Both GT and α-lactalbumin, either alone or in combination incorporated 1 to 2 HNB (bound mole/mole) into GT and 0.1 to 0.3 HNB into α-lactalbumin with up to a 60% parallel loss of both GlcNAc and GT activities. In the presence of 2 m*M* $MnCl_2$ and 100 m*M* GlcNAc, which leads to a significant interaction between GT and α-lactalbumin, there was about 50% reduction in the incorporation of HNB into GT and α-lactalbumin. There was only a slight decrease of incorporation of HNB into either GT or α-lactalbumin alone in the presence of 2 m*M* $MnCl_2$ and 100 m*M* GlcNAc. This indicates that conditions favoring the interaction of GT and α-lactalbumin showed a perturbation of the HNB group as well as a decrease in the positive peak at 290 nm. These observations show that some Trp residues in the GT molecule become shielded by the interaction of α-lactalbumin, while some other Trp residues though not essential for the interaction, may be involved in catalytic activity

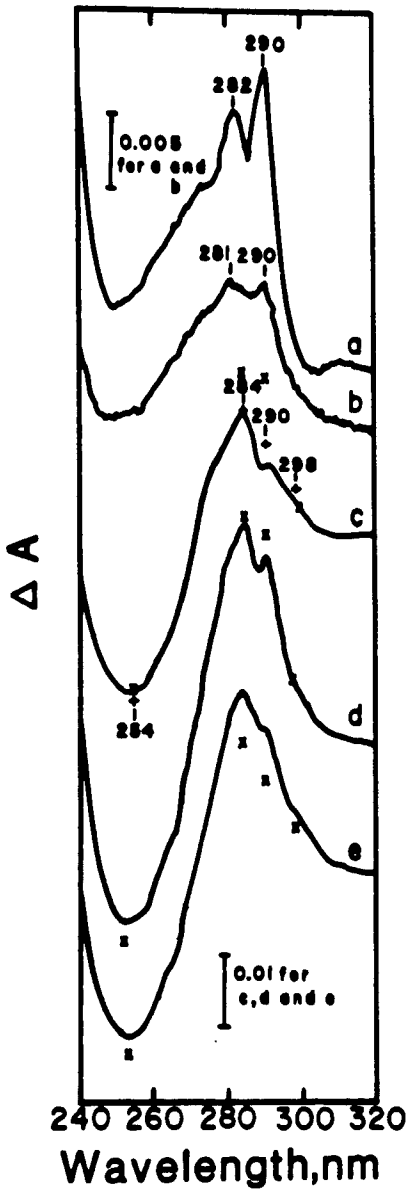


FIGURE 3. Difference spectra produced by the interactions of GT with α-lactalbumin and with small ligands. All the solutions contained 2 m*M* $MnCl_2$. (a) R_1, enzyme + GlcNAc; R_2, α-lactalbumin + GlcNAc; S_1, enzyme + α-lactalbumin + GlcNAc; S_2, GlcNAc. (b) R_1 enzyme + glucose; R_2, α-lactalbumin + glucose; S_1, enzyme + α-lactalbumin + glucose; S_2, glucose. (c) R_1, enzyme; R_2, α-lactalbumin + UDP; S_1, enzyme + α-lactalbumin + UDP; S_2, none. (d) R_1, enzyme + GlcNAc; R_2, α-lactalbumin + GlcNAc + UDP; S_1, enzyme + α-lactalbumin + GlcNAc + UDP; S_2, GlcNAc. (e) R_1, enzyme + glucose; R_2, α-lactalbumin + glucose + UDP; S_1, enzyme + α-lactalbumin + glucose + UDP; S_2, glucose. The concentration of GT was 4.19 μ*M* (specific activity, 13). The concentrations of α-lactalbumin were (a) 10.9 μ*M*; (b) 11.1 μ*M*; (c) 11.6 μ*M*; (d), 13.0 μ*M*; (e), 13.1 μ*M*. The concentrations of GlcNAc and glucose were 100 m*M*. The concentration of UDP was 79 μ*M*. The crosses are the sum of the effects of UDP ± GlcNAc or glucose on GT (spectra are shown in Figure 2) and the interaction of GT and α-lactalbumin (spectra a and b) as follows: x and + associated with spectrum c are the sum of spectrum c in Figure 2 and spectrum a here and of spectrum c in Figure 2 and spectrum b here, respectively. The concentration differences of protein and UDP were corrected in the above summation. x associated with spectrum d is the sum of spectrum d in Figure 2 and spectrum a here. x associated with spectrum e is the sum of spectrum e in Figure 2 and spectrum b here. (From Takase, K. and Ebner, K. E., *J. Biol. Chem.*, 256, 7269, 1981. With permission.)

Table 4
COMPARISON OF THE RATIO OF THE APPARENT Km AND V VALUES OF GlcNAc WITH VALUES OF GlcNAc ANALOGS WHEN USED AS ACCEPTOR SUBSTRATES FOR GT ENZYME

Analog	Km*GlcNAc/Km analog	VGlcNAc/V analog
Monofluoro *N*-acetylgucosamine	0.43	0.62
Trifluoro *N*-acetylglucosamine	1.03	1.07
β-methyl-*N*-acetylglucosamine	1.64	1.82
N-propionylglucosamine	—	—
N-benzoylglucosamine	—	—
p-azidophenyl-*N*-acetylglucosaminide	11.0	1.79
p-nitrophenyl-*N*-acetylglucosaminide	40.0	2.40
β-methylumbelliferyl-*N*-acetylglucosaminide	72.9	0.70
β-methylumbelliferyl-β-D-glucoside	—	—
Naphthol-AS-LC-*N*-acetyl-β-D-glucosaminide	370	1.31
Naphthol-AS-BI-*N*-acetyl-β-D-glucosaminide	925	1.38
N-acetyl-5-bromoindolyl-β-D-glucosaminide	2090	2.05

* All Kms and Vs were apparent values.

From Geren, C. R., Magee, S. C., and Ebner, K. E., *Arch. Biochem. Biophys.*, 72, 149, 1976. With permission.

of GT enzyme. Since HNB-Br is reported to be specific for Trp-26 and 118 in α-lactalbumin, these residues may be the ones which are protected by the interaction with GT.[128]

As indicated earlier, affinity of GT to bind α-lactalbumin[129] has been a property made use of in most of the procedures used for the purification of GT enzyme. *O*-glycosidic analogs of GlcNAc are good substrates for GT, and as the substituted groups become more hydrophobic, the affinity of binding of these analogs is increased.[43] This is responsible for a decrease in apparent Km as much as 2000-fold. A number of substituted *O*-glycosidic analogs are presented in Table 4. The *N*-acetyl group appears to be essential for activity since β-methylumbelliferyl-β-D-glucoside is not a substrate. Also, it has been reported that trifluoro-GlcNAc is as good a substrate as GlcNAc, which indicates that substitution of the hydrogen atoms in the $-CH_3$ group by fluorine atoms does not affect the galactosyl acceptor activity. The studies with the GlcNAc analogs suggest that GT may have a hydrophobic pocket near or containing the binding site for GlcNAc.[43] The binding of GT has been shown with hydrophobic compounds leucine, leucine amide, norleucine, valine, ε-amino-*n*-caprioc acid, and tyrosine immobilized to agarose in the presence of 1.25 *M* ammonium sulfate. The dissociation of GT from hydrophobic compounds takes place by the decreased molarity of ammonium sulfate. Geren et al.,[43] and more recently, other investigators[33] have used this property in purification of their GTs by using norleucine-Sepharose column as one of the steps. Inclusion of $MnCl_2$, UDP, and GlcNAc or β-methylumbelliferyl-GlcNAc does not alter the binding of GT to norleucine-Sepharose. These conditions should form a dead-end complex of enzyme-Mn^{2+}-UDP-β-methylumbelliferyl-*N*-acetylglucosaminide and mask the GlcNAc binding site. Lack of change in the binding of GT under these conditions indicates that the GT is binding to the norleucine-Sepharose at a site or sites other than GlcNAc site.

E. Kinetic Properties of GT Enzyme

Kinetic studies of GTs have provided useful information on the affinity and binding

Table 5
Km VALUES OF VARIOUS GT ENZYMES

Source or type of GT	Compounds	Km (*M*)	Ref.
Human Serum	UDP-gal	7.5×10^{-6}	28
Erythrocyte membrane	UDP-gal	5.0×10^{-6}	28
Erythrocyte cell surface	UDP-gal	7.5×10^{-6}	37
Erythrocyte cell surface	SGF-fetuin	4.0×10^{-6}	37
Rat Serum	UDP-gal	12.5×10^{-6}	70
Tumor GT-I_H and GT-II_H	SGF-fetuin	1.7×10^{-6}	90
GT I_H	UDP-gal	2.0×10^{-5}	90
GT II_H	UDP-gal	1.0×10^{-5}	90
Human plasma	Mn^{2+}	4.0×10^{-4}	50
Human plasma	UDP-gal	24×10^{-6}	50
Human plasma	α_1-acid glycoprotein	2.0×10^{-4}	50
Human plasma	GlcNAc	3.9×10^{-6}	50
Human plasma	Glucose (+ α-LA)	2.9×10^{-3}	50
Membrane GT, rat	UDP-gal	10.8×10^{-6}	71
Serum GT B	GlcNAc	2.3×10^{-3}	51
Serum GT B	GalNAc	8.0×10^{-2}	51
Collagen GT	UDP-gal	$20—30 \times 10^{-6}$	80, 82, 93
Effusion GT I and GT II	UDP-gal	1.3×10^{-5}	33
GT I and GT II	Ovalbumin	2.0×10^{-3}	33
GT I and GT II	GlcNAc	5.0×10^{-3}	33
GT I	SGF-fetuin	2.0×10^{-3}	33
GT II	SGF-fetuin	2.0×10^{-4}	33
Brain	UDP-gal	3.0×10^{-5}	258
Human malignant effusion	UDP-gal	0.015×10^{-6}	72
Human malignant effusion	GlcNAc	3.225×10^{-6}	72
Human malignant effusion	Mn^{2+}	6.250×10^{-6}	72
Human nonmalignant effusion	UDP-gal	0.021×10^{-6}	72
Human nonmalignant effusion	GlcNAc	7.142×10^{-6}	72
Human nonmalignant effusion	Mn^{2+}	2.500×10^{-6}	72

capability of UDP-gal, substrates, and the co-factors toward the enzyme. Some of the binding studies on GT have been discussed in the preceding section.

The Km values for GT for UDP-gal, a few substrates and Mn^{2+} isolated from different sources are listed in Table 5. Conflicting values for Km have been obtained by different investigators. The discrepancy may have been caused due to the differences in their experimental conditions and the purity of the GT enzymes.

Although GT II isoenzyme isolated from malignant pleural effusion differs in molecular size and in some other properties from GT I isoenzyme isolated from normal effusion,[33,73,74] both isoenzymes have identical Km values (Table 5) for GlcNAc alone. However, with DSG-fetuin where the proximate acceptor is the same as GlcNAc, the Km values are different. These investigators suggested that this difference may be due to difference in interaction of the two isoenzymes as the extended glycoprotein structure may affect the efficiency as an acceptor for sugar in DSG-fetuin.[33] Also, α-lactalbumin, a modifier protein, seems to affect these two isoenzymes differently. For GT I, addition of α-lactalbumin to the reaction mixture reduces utilization of GlcNAc in favor of glucose. Correspondingly, however, the relative decrease in glucosamine substrate activity of GT II was much less. The Ki for α-lactalbumin evaluated with GlcNAc substrate activity was found to be 10×10^{-6} *M* for GT I and 50×10^{-6} *M* for GT II isoenzymes. Similarly, the two isoenzymes (GT I_H and GT II_h) from tumors produced in outbred hamsters by subcutaneous injection of polyoma transformed BHK cells differed in their Kms for UDP-gal; GT I_H was twice that of GT II_H.[90]

Two kinds of GT activities, GT A and GT B have been reported by Berger et al.[51] in the normal human sera with A and O blood group activities. GT A had activity with asialomucin but little, if any, with the other acceptors, while GT B had very high activity with ovalbumin and asialo-agalacto-glycoprotein. Although, GlcNAc and GalNAc were also utilized by GT B, the Km's for GalNAc and GlcNAc were 850 m*M* and 2.3 m*M*, respectively. Moreover, only GT B interacted with α-lactalbumin to yield lactose when glucose was used as an acceptor.

As indicated earlier, the interaction between α-lactalbumin and GT applies only to enzymes which use GlcNAc as an acceptor. The α-lactalbumin or B protein,[63,130] in the presence of glucose, plays an important role in the lactose synthetase (UDP-galactose:D-glucose-1-galactosyltransferase, EC 2.6.1.22) system, whereby lactose is synthesized.[131] The concentration of α-lactalbumin in bovine milk is about 1 to 3 mg/mℓ,[131] while in bovine mammary tissue it is approximately 0.35 mg/g, wet weight.[130] The concentration of glucose in lactating bovine and lactating rat, on the other hand, is 0.432 mg/mℓ and between 0.072 to 0.18 mg/mℓ, respectively, indicating that α-lactalbumin is necessary to ensure maximum synthesis of lactose at the relatively low concentration of glucose found in tissue. It is possible that the amount of lactose found in the milk is influenced by the ability of the α-lactalbumin to lower the Km for glucose, in order that maximum synthesis may occur. It is, however, not clear what regulates the lactose content of milk.[132] In the bovine system, the Km for glucose is lowered from 1.4 *M* in the absence to 5×10^{-3} *M* in the presence of α-lactalbumin. Accordingly, glucose seems to be a very poor substrate for pure lactose synthetase A. The effect of α-lactalbumin in lowering Km value for glucose has been reported for many GTs.[28,33,50,70,71]

Double reciprocal plots of initial velocity at various concentrations of Mn^{2+} with varying concentrations of α_1-acid glycoprotein and fixed concentration of UDP-gal yield a series of lines that intersect to the left of the vertical axis and above the horizontal axis for the human plasma GT.[50] A secondary plot of the intercept and the slope is found to be linear. A similar double reciprocal plot is obtained with varying amounts of UDP-gal at a fixed concentration of Mn^{2+}. In contrast, the initial velocity pattern obtained for a fixed concentration of α_1-acid glycoprotein but with varying amounts of Mn^{2+} at a particular concentration of UDP-gal shows an intersect to the left of the vertical axis. In this case, the secondary plot of slopes and intercepts against the reciprocal of UDP-gal is found to be linear, but a plot of the slopes formed a line that passed through the origin. These results indicate a sequential mechanism in which all substrates must be added to the enzyme before the product(s) is released. Thus, it seems that Mn^{2+} modifies some enzyme form(s) before the addition of UDP-gal. In this case, the rate of UDP-gal in combination with the enzyme-Mn^{2+} complex is greater than the rate at which Mn^{2+} dissociates from the same complex. This mechanism is consistent with known Mn^{2+}-dependent enzymic reactions that have been reported that form enzyme-Mn^{2+} complex.[133] The same mechanism is true with bovine milk GT[107] and perhaps with the GTs of goat colostrum,[27] human milk,[8] thyroid,[134] pig serum or liver,[135] rabbit gastric mucosa,[136] and rat serum,[70] as all of them show similar properties.

IV. CHEMICAL ASPECTS OF GT

A. Molecular Weight

Isoenzymes of GT have been recognized in tumor tissues, malignant effusions, and sera of cancer patients[37,62,90,137,138] using PAGE in the presence and absence of SDS. The purified GTs from normal and cancer effusions were designated as GT I and GT II isoenzymes. Carcinoma-associated GT II isoenzyme has a lower mobility on SDS-PAGE with an ap-

Table 6
MOLECULAR WEIGHTS OF VARIOUS GT ENZYMES

Source and type of GT	Mol wt	Technique	Ref.
Milk	40,000—44,000	SDS-PAGE, gel filtration, sedimentation equilibrium sucrose density	64
Plasma	85,000	Sephadex® G-150	50
	84,000	Bio-gel® P-150	
	90,000	Bio-gel® P-200	
Rat liver microsomal	65,000—70,000	SDS-PAGE	71
Effusion GT I	54,000	SDS-PAGE	33
Effusion GT II	76,000	SDS-PAGE	33
Fetal calf serum	47,800	Diffusion and sedimentation coefficients	85
Microsomal, pig thyroid	74,000	SDS-PAGE	88
	70,000	Sephadex® G-100	
Human milk	55,000	SDS-PAGE	9
Malignant ascites	55,000	SDS-PAGE	9
Amniotic fluid	55,000	SDS-PAGE	9
Mucin: β1,3 swine trachea GT	82,000	SDS-PAGE	83
	90,000	Sephadex® G-100	
Malignant effusion	74,131	SDS-PAGE	72
Nonmalignant effusion	107,151	Gel-filtration	
Human serum	49,000	SDS-PAGE	144

proximate molecular weight of 76,000 daltons. In contrast, GT I isoenzyme is a faster moving glycoprotein, and has a molecular weight of 54,000 daltons (Table 6). Although Gerber et al.[9] initially, were able to detect, in one crude ascites sample, a slower moving GT-active peak corresponding to cancer-associated GT isoenzyme, later this peak disappeared after purification. Recently, a GT-active peak (GTℓ) was detected from the sera of cancer patients on DEAE cellulose column. The peak was far apart from normal GT peak.[139] There have been a number of speculations regarding the origin of various forms of GTs. For example, lower molecular weight forms may represent a cleavage product of higher molecular weight form or may arise due to endogenous proteolysis.[33,140-142] Since GT I and GT II isoenzymes initially were described as discrete entities on the basis of their kinetic and compositional differences (peptide-map), the abovesaid speculation did not seem to apply. However, a recent report[69] indicated that earlier preparations of GT I and GT II isoenzymes were contaminated with immunoglobulins and their amino acid compositions need reevaluation. Podolsky and Weiser[33] have also indicated that their GT I preparation seems to be similar to the other purified GTs, including GTs from human whey and rat mesenteric lymph node.[8,25,143]

Using a number of techniques, Trayer and Hill[64] have determined the molecular weight of milk GT (lactose synthetase A) ranging between 40,000 to 44,000 daltons. Since reducing conditions during SDS-PAGE or gel filtration did not alter the molecular weight of GT, it seems to indicate that it is a single polypeptide chain. Using SDS-PAGE, Fujita-Yamaguchi[144] determined the molecular weight of 49,000 daltons for human plasma GT. Gerber and associates[9] have reported a molecular weight of 55,000 daltons for each of the three GTs isolated from milk, malignant ascites, and amniotic fluid. This result is in contrast to the observations made by Podolsky and Weiser[33,95] who have reported higher molecular weight for their malignant GT preparation. Since PAGE, under nondenaturing conditions, revealed a difference in electrophoretic mobilities between the milk, ascites, and amniotic fluid GT

enzymes, it seems to indicate that these GTs have different charge. In our laboratory, we have also observed electrophoretic mobility differences in the malignant and nonmalignant GT enzymes under nondenaturing conditions. However, the SDS-PAGE gave the same molecular weight for both enzymes.[72]

The enzymes isolated by Gerber et al.[9] appear to be heterogeneous, as the milk enzyme showed 13 different forms on isoelectrofocusing in the presence and absence of urea. Comparative isoelectrofocusing of the three GTs revealed identical patterns for the amniotic and ascitic GT enzymes, while only partial identity with the milk enzyme. Since neuraminidase treatment of the ascites and milk GTs gave similar mobility patterns, this indicated that sialic acid content in the three GTs was different. The electrophoresis under nondenaturing conditions also supports the above observation. The prominent five forms from milk, ascites, and amniotic fluid focused at pH 4.9, 5.18, 5.52, 5.98, and 6.44 showed GT activities. More acidic bands are found in amniotic and ascitic GTs. Milk GT is a sialoglycoprotein. It appears that sialic acid residue(s) determine the isoelectric point but not the extent of the heterogeneity in GT preparations. However, differences in the content of Asp and Glu vs. Asn and Gln may reflect postsynthetic modification by deamidation and are likely to affect the surface charge.[145] Heterogeneity may also be due to the difference in amino acid at the amino terminal. Gerber et al.[9] have found Leu, Tyr, and Val in two different GT enzyme preparations. Heterogeneity of milk GT has also been reported by Barker et al.,[68] who detected three forms ranging in molecular weights from 43,000 to 54,000 daltons. Turco and Heath[85] found milk GT similar to fetal calf serum GT in various properties. In many cases, molecular weights of GTs determined by SDS-PAGE and gel-filtration have different values.[83,88,144] It has been reported that molecular weight determined by gel-filtration may be in error if the protein contains substantial carbohydrate.[50] Higher molecular weight obtained by gel-filtration for GlcNAc mucin:β1,3 GT may also have been due to the binding of the enzyme to Triton® X-100 present in the buffer.[83]

B. Carbohydrate Composition

GTs are glycoproteins as they stain with periodic acid-schiff reagent.[70,146] About 10 to 15% of their net weights is carbohydrate.[37,64] The information available on the exact carbohydrate composition of GT is scarce due to the nonavailability of purified GT for analysis. Compositions of rabbit erythrocyte and milk GTs are presented in Table 7. Neutral hexose constitutes a greater part of carbohydrate in GT. The membrane GT contains significant amounts of D-mannose, D-galactose, and lesser amounts of sialic acid. Presence of significant amounts of GlcNAc in both soluble and insoluble GTs indicate the asparagine-type linkage.[147] This is also supported by the presence of large amounts of asparagine (14%) in the protein structure of GT.[37]

Trayer and Hill[64] predicted that glucosamine and galactosamine, which were identified in acid hydrolysates of the A protein are probably present as their acetylated derivatives in the native enzyme. They identified the presence of galactose, mannose, and glucose qualitatively in their GT preparations. Analyses of carbohydrates in GT I and GT II isoenzymes using chemical assay and gas-liquid chromatography method showed that GT II had twice the amount of carbohydrate (9%) present in GT I (4%).[33] This difference could be due to an increase in hexosamine content in GT II relative to GT I. No significant amount of sialic acid could be detected in either GT I or GT II isoenzymes. Mannose, glucosamine, and galactose were present in small amounts.

C. Amino Acid Composition

Analyses of amino acid composition of GTs have also been hindered due to limited availability of purified enzymes. Amino acid compositions of a serum, microsomal, and milk GTs are given in Table 8.[64,71] Rat liver microsomal and rat serum GTs have similar amino acid compositions. Both contain a large number of Ser, but only a small number of Leu residues. The membrane GT has more His, Arg, and Glu residues, with much fewer

Table 7
CARBOHYDRATE CONTENT OF INSOLUBLE AND SOLUBLE GT ENZYMES

Carbohydrate	Cell-membrane GT[37] (μg/mg)	Soluble GT[64] (μg/mg)
L-Fucose	1.6	—
N-acetylneuraminic acid	22.3	—
D-galactose	71.0	11.0
GlcNAc	42.5	11.0
D-mannose	66.0	PND[a]
Neutral hexose	137.0	81.0
Hexosamine	51.0	—
Sialic acid	20.0	20.0
Glucose	—	PND[a]

[a] PND: Present but not determined quantitatively.

Table 8
AMINO ACID COMPOSITIONS OF RAT SERUM, LIVER MICROSOMAL, AND MILK GT ENZYMES

	Calculated number of residues per molecule			
Amino acid	Serum[a]	Microsomal[a]	Bovine milk[b]	Serum[c]
Lysine	51	46	18	15
Histidine	11	15	11	12
Arginine	4	8	17	25
Aspartic acid	35	36	38	43
Threonine	21	21	16	17
Serine	135	137	26	30
Glutamic acid	76	83	29	33
Proline	14	15	24	32
Glycine	106	92	25	35
Alanine	42	39	15	23
Half-cystine	ND[d]	ND	5	5
Valine	14	15	19	31
Methionine	ND	ND	8	5
Isoleucine	9	10	16	15
Leucine	13	15	27	27
Tyrosine	7	6	15	16
Phenylalanine	7	8	16	16
Tryptophan	ND	ND	5	9

[a] Based on a molecular weight of 68,000, minus 12% for carbohydrate.[71]
[b] Based on a molecular weight of 42,000, minus 12% for carbohydrate.[64]
[c] Based on a molecular weight of 49,000, minus 11% for carbohydrate.[144]
[d] ND, not determined.

Lys and Gly residues. Although milk GT has similar amounts of Asp and His; Gly, Ser, Glu, and Ala residues in milk GT are much less than in microsomal and serum GTs. Another feature of milk GT is the high Pro content. High amide content is found in A protein, which explains the observation that A protein appears to have an isoelectric point of about pH 8, as judged by its behavior on ion-exchange chromatography and electrophoretic system.[64] Minor differences in amino acid compositions of GT I and GT II isoenzymes have been reported.[33] GT II isoenzyme contained the highest concentration of Gly, followed by Lys, Ala, Leu, Val, Ser, and Phe.

V. BIOLOGICAL ROLE OF GT

A. Biosynthesis of Lactose and Other Glycoproteins

The GT enzyme plays an important role in the biosynthesis of glycoproteins and glycolipids. It adds galactose from UDP-gal to an acceptor such as GlcNAc, glycoprotein, or glycolipid having GlcNAc or GalNAc in the end, depending on the type of GT. When a glycoprotein acceptor carries GlcNAc sugar at the terminal, the linkage is β1-4. With terminal GalNAc in the glycoprotein, β1-3 linkage is the most common.[51,52,83] It is well known that GT uses GlcNAc as an acceptor. In the presence of α-lactalbumin, glucose can also serve as an acceptor. In the first case, however, the product is acetyllactosamine, while in the second case, the product is lactose. This occurs not only in the case of mammary gland GT but also with GTs from liver and other tissues.[135] Biosynthesis of oligosaccharide chains in glycoproteins proceeds via stepwise addition of monosaccharides from sugar nucleotide substrates to the growing oligosaccharide acceptor.[5] Each step is catalyzed by a glycosyltransferase which is specific for not only the glycosyl donor but also the acceptor molecule.[5,148] The sequence of addition of sugars determines both the structure and the amount of the final product, as demonstrated by the synthesis of porcine and ovine submaxillary mucins.[148-153]

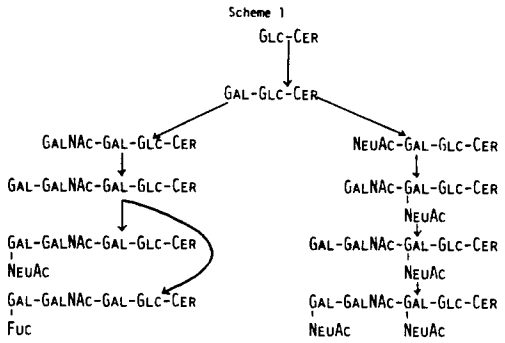
It has been suggested that synthesis of secretory glycoproteins is associated with the initial synthesis of a signal sequence peptide.[154,155] This signal sequence contains the necessary information to direct polysome attachment to the endoplasmic reticulum where vectoral discharge of the protein into lumen and subsequent glycosylation occurs. For many of the proteins studied, the signal sequence is located at the N-terminal end of the polypeptide and is cleaved off by a specific peptidase prior to completion of the protein chain. To identify the subcellular sites of glycoprotein synthesis, three methods have been proposed: (1) incorporation of radioactive monosaccharide precursors with subsequent microscopic autoradiography or subcellular fractionation to determine the distribution of incorporation into glycoproteins, (2) the isolation of membrane fractions and determination of transferase activities within each subcellular fraction, and (3) direct localization of GT (or glycosyltransferases) by the use of the electron microscope.

In the incorporation studies, generally the monosaccharide precursor becomes associated with a particular membrane fraction and later, the labeled glycoprotein becomes associated with other membrane system en route to its destination. A precursor-product relationship is thus defined. Fucose has become the monosaccharide of choice for in vitro incorporation studies since it is not readily metabolized. Subcellular localization of glycoprotein synthesis is also determined by localizing the glycosyltransferases. The localization of the enzyme in the cell provides a fair indication as to where in the cell the action of GT takes place. The presence of GT and other glycosyltransferases on endoplasmic reticulum and Golgi apparatus indicate that these are the sites of glycosylation. Subcellular immunolocalization of glycosyltransferases will be useful if antibodies can be raised against the pure GT enzyme.[22] Since the immunological method is noninvasive and does not depend upon incorporation assays, it permits better analyses of cell surface glycosyltransferases. Antibodies to GT have now been developed.[22,48,82,144,156]

The process of glycoprotein biosynthesis involves three phases: initiation, processing, and elongation. GTs activities towards asparagine-linked acceptor have been reported.[147,150,157-162] Initiation of Asn-linked oligosaccharide involves (1) synthesis of a large oligosaccharide-lipid·precursor, and (2) transfer of the oligosaccharide to a protein acceptor. The lipid compounds involved in the glycosylation of asparagine residues in proteins are called dolichol phosphates (Dol-P), which were first detected in chemical studies on the unsaponifiable fraction of biological material.[163] For example, lipid-linked galactose, the Dol-P-gal has been obtained by incubation of UDP-gal, Dol-P, and an enzyme from *Acetobacter xylinum*.[164] A number of Dol-P-P-glycoconjugates are known to be effective in the glycosylation of cellular proteins.[161,165-167] However, in general, the sequence Asn-X-Ser (Thr)- is required for Asn-linked glycosylation.[168] The next step in the glycosylation process is called processing, where large oligosaccharide undergoes the stepwise removal of all glucose and α-1,2 linked mannose residues. During this phase, the oligosaccharide structure is drastically altered in preparation for subsequent additions of other sugars. The glycosyltransferases are involved in the addition of sugar to the processed oligosaccharide. This process, termed as elongation, then determines the oligosaccharide structure of a glycoprotein. Most GTs purified to date transfer the galactose to GlcNAc linked to Asp. However, the GT enzyme isolated from tracheal epithelium transfers the galactose to 0-Ser linked GalNAc, forming the linkage Gal β1→3 GalNAc α Ser (Thr).[52,83] A membrane GT (2-acetamido-2-deoxy-D-Glucose 4β GT) from pig trachea which synthesizes galactosyl-β1→3 GlcNAc forming 1→3 linkage has also been reported.[35] The base labile nature of the product has confirmed the 1→3 type of linkage.[169,170] The β-galactosidase linkage in the β1→3 GalNAc α Ser (Thr) structure is commonly found in mucous glycoproteins,[171,172] including human tracheobronchial mucous glycoproteins.[173,174] The presence of asialo OSM GT, which is responsible for Gal β1→3 Gal NAc α Ser (Thr), has also been demonstrated in several tissues.[175-177]

Microheterogeneity of carbohydrate structure is characteristic of mucous glycoproteins.[148-151] Synthesis of oligosaccharide chains with different carbohydrate structures may be controlled by relative amounts of different enzymes[148,153] and the sequence of addition of different sugars.[152,153] The glycosyltransferases that are involved in mucin carbohydrate chain synthesis can be classified into two groups, namely those which elongate and those which terminate oligosaccharide chain synthesis. The relative activities of chain elongation vs. chain termination enzymes may be a major determinant of the length of carbohydrate chains. The role of GT as chain-elongating enzyme and neuraminyltransferase(s) as a chain-terminating enzyme has been well documented.[34,155] The relative amounts of galactosyl-to-neuraminyltransferase found in ovine (1:18) and porcine (1:1) submaxillary glands has given further support to the above hypothesis. Because of the prevalence of Gal β1→3 GalNAc α Ser (Thr) structure in mucin carbohydrate chains,[178,179] the α-GalNAc β3 GT becomes a key enzyme in the early stage of the regulation of mucin oligosaccharide synthesis. Factors which modulate the activities of the enzymes in these two classes may modify substantially the structure of mucin oligosaccharide(s).

GT enzyme also participates in the biosynthesis of collagen.[82] The posttranslational processing of collagen occurs in two stages. Intracellular modifications, together with the synthesis of the polypeptide chains, result in the formation of the triple-helical procollagen molecule. Extracellular processing converts this molecule into collagen and incorporates it into a stable, cross-linked fibril. Glycosylation of certain hydroxylysyl residues to galactohydroxylysyl and glucosylgalactosylhydroxylysyl residues occurs during intracellular modifications. The GT involved in the above process of galactosylation of collagen has been demonstrated in chick embryo extract,[79] guinea pig skin,[180] rat kidney cortex,[181] and human platelets.[182]



SCHEME 1. Glycolipid metabolic pathway in rat bone marrow cells.[58]

B. Biosynthesis of Glycolipids

Several glycosyltransferases have been identified in the biosynthesis of glycolipids. Most of the information on GTs has come from studying the metabolism of the brain cells. The gangliosides such as GM_{1a} and GM_{1b} are synthesized through GM_3. This type of ganglioside biosynthesis has been established in many tissues.[59,183,184] The presence of *N*-acetylgalactosaminyltransferase catalyzing the formation of GA_2 from CDH at the first step of the ganglioside synthetic pathway seems to control the glycolipid metabolism and composition. It follows that this affects the membrane properties of the cells. Taki et al.[58] have reported the formation of GM_{1b} through GA_2 and GA_1 in the bone marrow cells involving *N*-acetylgalactosaminyltransferase, sialyltransferase, and fucosyltransferase. They showed the formation of GA_1 on incubation of UDP-gal and GA_2 with the bone marrow cell homogenate. The abovesaid enzymes' activities and the glycolipid biosynthesis have been demonstrated to be characteristic of the free-type rat ascites hepatoma.[185] Recently, GA_1 has been proposed as a surface marker of natural killer cells by many investigators.[186-188] Since glycolipids are believed to be present on the outer surface of the cell membrane, this type of glycolipid metabolism is thought to be important in relation to the immunological reaction which takes place on the cell surface.

Handa and Burton[189] were the first to report the biosynthesis of GA_2 from CDH. Following this report, GA_1 biosynthesis was studied by Yip and Dain.[190] Yip and associates[190,191] demonstrated that GA_1 was converted to a sialidase-labile ganglioside by a sialyltransferase in rat brain. They indicated that the sialic acid residue was linked to the terminal galactosyl unit of GA_1 by means of an ultra-microscale permethylation procedure.[191] Taki et al.[58] have confirmed the existance of GM_{1b} biosynthesis through asialogangliosides, as well as biosynthetic pathway through GM_3 in rat bone marrow cells. Glycolipid metabolic pathway in rat bone marrow cells is shown in Scheme 1 as proposed by Taki et al.[58] A glycolipid (Glc-Cer) acts initially as an acceptor to which carbohydrates are added in the presence of glycosyltransferases. The major pathway for the biosynthesis of glycolipid in rat bone marrow cells is through asialogangliosides. It has also been observed that the profile of ganglioside composition of rat bone marrow cells is variable, depending on the age of the rat. This suggests that glycosyltransferases could be affected by cell differentiation or aging. [58]

Recently, Pierce[7] reported the presence of GM_1 synthetase on neural retinal cells of a 9-day-old chick embryo. This enzyme transfers galactose to the terminal GalNAc of GM_2 to synthesize GM_1.[59] The enzyme activity is present on the external surface of intact cells without any concentration gradient along the dorsoventral axis of the neural retina. The presence of ganglioside GM_2 on the surface of the retina cells and tectum is implicated in the retinotectal adhesion.[192] Thus, the presence of GM_1 synthetase and GM_2 and oligo-GM_2 on the surface of neural retina cells during neuromorphogenesis indicates its probable involvement in intercellular adhesion, by binding to its acceptors on opposing surfaces.

A number of studies have demonstrated the presence of UDP-gal·ceramide galactosyltransferase (Cer GT) in microsomes as well as in purified myelin of rat brain.[193-196] Costantino-Ceccarini[193,194] studied the Cer GT in subcellular fractions of rat forebrain during development using zonal centrifugation on linear gradients. The developmental change in distribution of Cer GT in adult animals towards myelin containing fraction could indicate that the replacement of galactoceramide in compact myelin could be carried out in close proximity to compact myelin (mesaxon, paranodal loops) rather than in the distant oligo-dendrocyte perikaryon.

Cer GT activity has also been reported in glial cell lines. Starting from the transplantable mouse glioma G26, Sundarraj et al.[197] isolated several clonal cell lines with some oligodendroglial features. Dawson[198] has shown that two of the G26 cell lines (G26-20 and G26-24) have the capacity to synthesize galactocerebrosides (CerGal) and sulfatides, which are the lipid markers of myelin and oligodendroglia in the CNS. Dawson and Kernes[199] reported the presence of an active sulfatide-synthesizing enzyme in G26-20 and G26-24 cells and showed the stimulating effect of cortisol on the sulfatide biosynthesis. However, CerGal was not detected on the cell surface of G26-24 cells by the use of anti-CerGal antiserum.[200] Rat glioma C6 cell line,[201] on the other hand, has been described as an astrocytoma, although some oligodendroglial markers were also reported to be present in this glial cell line[202] as well as in the subclone C6TK cells.[203] In view of this, it seems that C6 cells have the capacity to express some properties of both differentiated astroglial and oligodendroglial cells.[204] CerGal was not detected in C6 cell lines,[205,206] and only traces of sulfatides were reported;[207] however, increased concentrations of glucocerebroside (CerGlc), a common glycolipid in cell lines of neuronal and glial origin, was found in C6 cells.[205]

Cer GT has been purified from rat brain microsomes[208] and the properties studied.[209] However, Cer GT has not been well documented in glial cell lines. Recently, Neskovic et al.[206] have found 16 to 30 times lower Cer GT activity in the G26-20 and G26-24 oligodendroglioma clonal cell lines than in the myelinating rat brain. However, Cer GT levels in G26-20 and G26-24 cell lines were comparable to the levels found in young rat brain before the start of the myelination process. Also, the enzymatic assay for Cer GT in cultured glial cells has been complicated by a rapid conversion of UDP-gal to UDP-glucose due to an increased UDP-gal-4′-epimerase activity in certain glial cell clones.[206] It appears that the mechanisms regulating UDP-gal-4′-epimerase activity and levels of UDP sugars in glial cell lines differ from those in brain tissue.

C. Cell Surface Activity of GT

Recently, a number of investigators have reported that glycosyltransferases are also present on the outer surfaces of some cells.[2-4] The presence of glycosyltransferases on the surface of the cell can be analogized with lectins capable of interacting with surface glycosides. Thus, the role of glycosyltransferases in contact-mediated cell interaction and some related phenomena such as fertilization, morphogenesis, hemostasis, cell migration on cellular and acellular substrates have been described.[2-4] It appears that cell surface glycosyltransferases are regulated in the normal physiological process and their activities might change during pathological conditions.[2-4]

1. Surface GT Activity During Physiological Process

a. Concanavalin A Binding Site and Agglutination Reaction

Concanavalin A (Con A), a lectin isolated from Jack beans, is known to preferentially agglutinate certain cell types such as virally transformed cells,[210] mitotically active cells,[211] and various erythrocytes.[212] Although the mechanism of agglutination remains unclear, it is known that Con A binding involves interaction with the mannoside residues of the glycoproteins.[213]

Agglutination of rabbit erythrocytes has been observed with Con A.[37] Agglutination reaction with Con A appears to be proportional to the surface activity, i.e., readily agglutinated cells show higher GT activity than the unagglutinated cells. GT contains a considerable amount of mannose and the evidence has been presented that this sugar in GT binds to Con A. Moreover, treatment of GT with mannosidase, which removes the mannoside residues, results in the loss of Con A agglutinability to erythrocytes.[37,214] Thymus and spleen cells contain cell surface GT, and they agglutinate Con A to some extent depending on the activity of the enzyme.[215] A change in the cell surface will cause the cells to behave differently for agglutinability. Thus, malignant cells are agglutinated by Con A much more readily than their normal counterpart,[3] indicating the differences in their cell surface glycoproteins and the availability of the mannoside residues.

b. Hemostasis

Platelet-collagen adhesion takes place after wounding of endothelium, initiating hemostasis. Among the events taking place in this process included is glycosyltransferase induced adhesion of the cells to endothelial collagen.[15,216-218] A collagen-GT has been demonstrated in isolated platelet plasa membrane. Since the intact platelets do not show any GT activity, this indicates that GT may be buried within the intact platelets plasma membrane, or exposed only to the cytoplasmic side.[182] A parallel behavior of cell adhesion and GT activity has also been demonstrated.[219,220]

c. Intestinal Cell Differentiation

There have been reports that indicate biochemical differences between the cell surface membranes of the differentiated and undifferentiated crypt cells.[221,222] The intestinal epithelium is continually replenished by mitotically active, undifferentiated cells located in the base of the crypts. These cells migrate and mature upon villus. Golgi and surface membrane isolated from villus and crypt cell homogenates by sucrose gradient sedimentation have shown the presence of glycosyltransferase activities.[223] Increased activities of glycosyltransferase on the crypt and villus cell surfaces might be a reflection of their participation either in adhesion to the underlying basal lamina or in the synthesis of brush border glycoconjugates as indicated by Roseman.[5]

d. GT in the Immune System

In some cases, it has been proposed that the interaction of T-lymphocytes with foreign antigens may involve lectin-like interaction due to the presence of a variety of surface glycosyltransferases.[224-227] According to a recent report, the surface glycosyltransferases present on cytotoxic T-lymphocytes may participate in T-cell cytolysis of their target cells.[228] Moreover, the surface GT activity increases with the increase in binding capacity of T cells to the target cells, indicating the recognition, binding, and subsequent glycosylation of these cells.

2. Cell Surface GT During Fertilization, Development, and Embryogenesis

In the process of fertilization, a cell-to-cell contact is necessary; hence, it is not surprising that glycosyltransferases have been located on the surface of sperm[2,229,230] and alga gametes.[231,232] Lectin-like behavior towards specific egg-coat carbohydrate of egg receptor isolated from sea urchin sperm indicates an involvement of complementary proteins and carbohydrates in fertilization. The sperm may attach to eggs by virtue of the presence of GT or other glycosyltransferases on their surface to the acceptor on the egg surface. In a study where nine enzymes from sperm were assayed, only GT showed increased activity in conjunction with the increased sperm transmission.[233] It has been suggested that although these enzymes may not be directly involved in gamete binding, they do participate in the

synthesis of complex carbohydrates required for cell-to-cell interaction.[2] Most of the work on the involvement of glycosyltransferase(s) in fertilization comes from studies on algal mating[231] and mouse fertilization.[2] In the green alga *Chlamydomonas*, gamete recognition first occurs between + and − gamete flagellar membrane of the same species. When the isolated + and − flagellar membrane vesicles are mixed, a many-fold increase in transferase activity occurs. Upon mixing the sexually incompetent + and − vesicles, no such increase in enzyme activity is seen, indicating that glycosylation between gametes not only requires sexually competent cells, but also demonstrates species specificity. The surface GT enzyme may be involved in gamete adhesion.[231]

The enzymes GT, sialyltransferase, and *N*-acetylglucosaminyltransferase have been found to be present on the surface of washed mature mouse sperm.[230,233] These enzymes show increased activities when assayed against soluble exogenous acceptors such as the outer egg coats zona pellucida digest, but show very little or moderate activities against endogenous sperm acceptors. This seems to indicate that sperm have surface glycosyltransferases which are involved in egg binding.[2] Since the sperm are incapable of synthesizing carbohydrates from free sugars, and they do not possess endoplasmic reticulum or the Golgi complex where most of the intracellular transferases are located, this seems to further support the view that glycosyltransferases are localized on the sperm surface. Recently, a number of biochemical and genetic probes have been used to understand the role of GT during fertilization.

a. Surface GT on T/t Complex and its Involvement in Cellular Interaction

Involvement of glycosyltransferases, particularly GT, in cellular interaction is currently being studied using T/t complex.[2,233] While T alleles play a role in embryonic development and tail formation, the effects of t alleles are considered pleiotropic. Although t alleles (t/t) do not affect tail development, mice with T/t alleles, however, have short tails. The t alleles can interact with T alleles to form a T/t mouse with absence of tail formation.

There are methods for cloning and isolating genes adjacent to T/t complex genes by using biochemical probes. A protein with a molecular weight of 63,000 daltons with a pI of 6.9, possibly allelic to tail-interaction factor has been used as a biochemical probe to identify genes.[234] This protein is more acidic when synthesized by t-bearing cells, and is a direct transcriptional product of genes located in this segment of the T/t complex.[235] It has been shown that t mutants are metabolically different.[236] Accordingly, t/t homozygotes which accumulate excessive intracellular lipids can be distinguished from the wild type. Metabolic differences have also been reported in t-bearing sperm.[237] The metabolic differences could also contribute towards the differences observed in sperm motility,[238] thus, accounting for altered transmission frequencies.[239] The mutant genes of T/t alleles have been reported to interfere with a variety of cellular interactions during development and fertilization.[240] A number of reports[241,242] indicate that the cell surface is the site of action of T/t-complex alleles, and these genes interfere with the same process in which surface glycosyltransferases are thought to participate. According to Durr et al.,[230] t alleles dramatically affect fertilization, while sperm surface GT galactosylate egg carbohydrate. The spatial and temporal distribution of GT activity in normal chick embryo[243,244] coincides well with the distribution of pathology seen in t/t and T/T mouse embryo.[240] Thus, T/t complex mutations have been used as a probe to define further any involvement of surface GT during fertilization and development. The determination of surface GT activities on normal and t-bearing sperm have, thus, contributed towards our greater understanding of the phenomena of fertilization and development.

Among the enzymes determined on sperm from segregation-distorting +/t males vs. wild type (+/+) controls indicate that activities of GT towards GlcNAc in heterozygote (+/t) preparations was two times higher compared to the normal (+/+) littermates.[233] Endogenous acceptor activity was found to be low on washed sperm but high in the initial sperm

supernatant. Egg zona pellucida digests served as a good acceptor source. Recently, Shur and Bennett[233] reported that increased GT activity on (+/t) sperm due in part to a deficiency of some inhibitory component was normally made by (+/+) sperm. Shur[245] has also analyzed GT activity on sperm bearing various recombinant t-chromosomes in order to evaluate the role of GT in stimulation of a specific function of the T/t complex. Moreover, when the distal portions of the T/t complex, associated with embryonic lethality and increased t-sperm transmission frequency are present, only then the activity of GT in sperm is increased.

Involvement of GT in binding zona pellucida GlcNAc residues has already been discussed. According to a recent report,[246] freshly isolated epididymal sperm are coated with high levels of sperm GT acceptors, which are shed from the sperm surface prior to sperm zona pellucida binding. These sperm-bound galactosyl acceptors inhibit sperm zona pellucida binding when added back to in vitro fertilization assay. Also, sperm galactosyl acceptors present in epididymal fluids are immunoprecipitated by antiserum raised against F9 teratocarcinoma cells[247] and are sensitive to endo-β-galactosidase digestion. Anti-F9 antiserum stimulates sperm zona pellucida binding in conjugation with decreased galactosylation of sperm substrate and increased galactosylation of GlcNAc. These studies encouraged Shur[2,248] to study F9 teratocarcinoma cell surface GT. He found that GT activity towards an endogenous acceptor was very high. Based on several experiments, it was suggested that F9 antiserum recognized the endogenous substrate for F9 cell surface GT. Moverover, in the presence of anti-F9 antiserum, endogenous acceptors are displaced, increasing the availability of enzyme(s) for glycosylation of exogenous acceptors.[2]

Recently, Shur[2] reported that GT is a surface receptor for poly (*N*)-acetyllactosamine glycoconjugates on embryonal carcinoma (EC) cells. The product(s) is sparingly soluble in organic solvents, and following pronase digestion the product is still larger than conventional glycopeptides, and is highly sensitive to endo-β-galactosidase degradation. Solubilized polylactosaminyl glycoconjugates serve as competitive exogenous acceptors for the surface GT. In addition, the endogenous galactosyl acceptor(s) reacts with antiserum directed against EC cell poly (*N*)-acetyllactosamines. Anti-EC antiserum concurrently inhibits galactosylation of endogenous acceptors, stimulates galactosylation of an exogenous acceptor, and immunoprecipitates 74% of the reaction product.[2] Differentiated EC cells no longer react with anti-EC antiserum and no longer show anti-EC antiserum effects on surface GT activity. Forced galactosylation with UDP-gal releases polylactosaminyl substrates from the cell surface. In the absence of UDP-gal, release of glycoconjugate(s) is reduced significantly. An inhibitor of GT, UDP-dialdehyde[249] prevents glycoside release from the cell surface. These data indicate that one of the surface receptors for poly-(*N*)-acetyllactosamine glycoconjugate may be a GT enzyme.

b. Embryonic Surface GT

Gastrulation is a complex cellular and tissue interaction during which migration and inductive events lead to the formation of organ rudiments. Autoradiographic examination of the chick embryo after radiolabeled sugar incorporation have revealed the activities of glycosyltransferases in the notochordsomite-lateral plate mesoderm vicinity, within the optic cup-skin ectoderm junction and in the area vasculosa.[243,244,250] These studies have demonstrated the presence of galactosyl-*N*-acetylglucosaminyl-, sialyl-, and fucosyltransferases on the surface of the embryonic chick cells. [244] Each of the four glycosyltransferases shows a spatially and temporally characteristic distribution of activity. Of the four glycosyltransferases, GT is by far the most active during migration and inductive interactions of cells. It appears that these transferases might be required for the migratory and inductive behavior of these tissues by binding their glycosyl acceptors in the extracellular matrix.[243,244,251] An in vitro migration of SV-40 transformed mouse fibroblasts away from explants when cultured on certain sugar substrates have been shown and are considered to be due to the presence of surface glycosyltransferases.[252]

Surface GT activity during mouse embryogenesis has been studied.[2] Increased concentration of surface GT was detected on 8-day wild-type embryos. The activity was localized primarily on the newly formed primitive streak and the resultant mesenchymal tissues and is consistent with the GT distribution in early chick embryos.[243] By 10 days of gestation, GT level dropped considerably, with the exception of head and early limb bud mesenchyme. The galactosylated product in this case was characterized to be a large, poly (*N*)-acetyllactosamine extracellular glycoconjugate as discussed earlier. Synthesis of poly-*N*-acetyllactosamine glycoconjugates by early mouse embryos have also been reported by many other investigators.[253-255]

To explore the function of surface GT during development, Shur[2,256-258] employed morphogenetically arrested T/T embryos characterized by a generalized cell defect arresting the development of some tissues before others. By 9 days of development, T/T embryos had 1.8 to 2.0 times the surface GT activity of normal controls. This high activity was localized in normal mesenchymal tissues in T/T embryos. By 10 days, limb buds from T/T embryos which were morphologically abnormal, showed more than six times the endogenous GT activity of wild-type limb buds of similar size. Increased concentrations of galactosyl acceptor may have been due to the fact that normal galactosylation of extracellular glycoconjugates did not occur in T/T embryos owing to defective mesenchymal cell migration. This would result in higher residual levels of acceptors in T/T embryos than in wild-type embryos.

c. Shur's Model for Murine Fertilization and Mesenchymal Cell Migration

The data presented earlier indicate that sperm embryonic cells and teratocarcinoma cells are characterized by poly-*N*-acetyllactosamine glycoconjugates. This glycoconjugate is like a matrix on these cells. The cells may recognize, adhere to, and migrate on this matrix. Shur[2,256] has proposed a model based on the presence of this matrix, which is shown in Figure 4. The fresh sperm are coated with the glycoconjugate matrix, on the GT receptor of the surface. This coating is shed before sperm binds the egg through surface GT on sperm and GlcNAc acceptor on zona pellucida.[246] Thus, the matrix on the sperm acts as an inhibitor of its binding to egg. Accordingly, the fertilization capability of the sperm would be proportional to the number of exposed transferases, which are regulated by the degree of competing endogenous acceptors adsorbed on the surface. These endogenous acceptors can be removed by washing of the sperm. The sperm receptor on egg is likely to be a glycoprotein with terminal GlcNAc residue.[259] Using this model, migration of the mesenchymal cells on extracellular poly-*N*-acetyllactosamine substrate via surface GT can be explained. However, this model does not specify migrational direction, but rather, simply postulates which molecule may aid the cell to get where it is going. Further research in this area is required to understand the phenomena of directional behavior.

3. Role of GT in Cell Interaction and Adhesion

The role of surface glycosyltransferases in in vitro cellular adhesion is being explored in many laboratories.[2,3,260] The objectives are to determine the degree of adhesion specificities among various cell types during morphogenesis.[260]

a. Embryonic Neural Retina and Retinotectal Adhesion

Intact neural retina cells demonstrate surface GT activity toward both endogenous and exogenous acceptors.[261,262] It is suggested that these cells bind to the neighboring cells due to the presence of surface acceptor for the GT on them, thus, producing adhesion typical of these cells. Addition of exogenous acceptor to these cells, however, inhibited 20 to 50% of the initial retina cell adhesion. In control experiments, an oligosaccharide that does not act as galactosyl acceptor has no inhibitory effect on neural retina adhesion, indicating that GT is involved in the process of adhesion. Some of the observations suggesting the surface

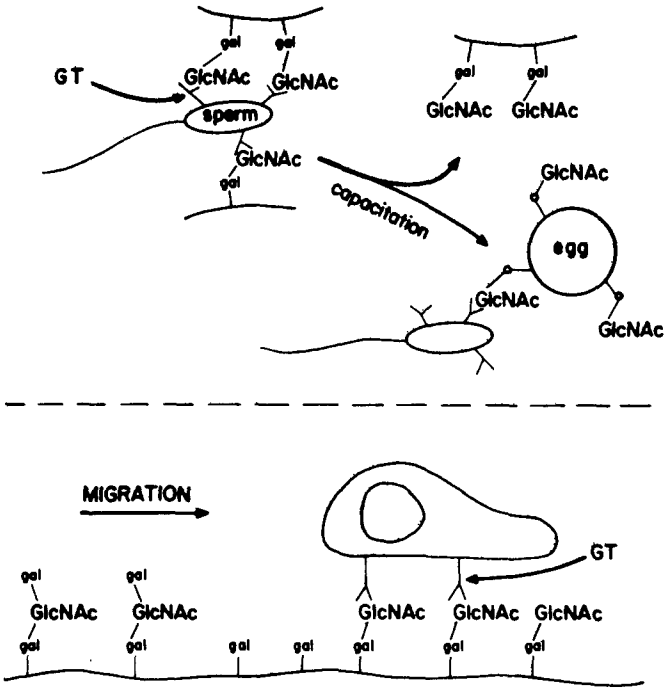


FIGURE 4. A specific model for murine fertilization and mesenchymal cell migration. In both panels, poly *N*-acetyllactosamine glycoconjugate interacts with the cell surface via terminal GlcNAc binding to surface GT. Only small portions of the oligosaccharide side chains are shown and consist partly of the traditional repeating keratan disaccharide, GalNAc. Some of these disaccharides may be fucosylated. On sperm, this glycoconjugate may be one class of "coating" factor, most of which is removable by washing the sperm and which inhibits sperm-egg binding. Terminal GlcNAc residues also occur in the sperm receptor on eggs, but this receptor has not yet been characterized. During early development, mesenchymal cells may migrate on keratan-like matrices via surface GT binding to terminal GlcNAc residues. Galactosylation would dissociate the cell from its substrate, similar to the effects of UDP-gal in vitro and allow the cell to advance to unglycosylated GlcNAc binding sites. (From Shur, B. D., in *The Glycoconjugates*, Vol. 3, Horowitz, M. I. and Pigman, W., Eds., Academic Press, New York, 1982, 145. With permission.)

locale of GTs are as follows: (1) acceptors such as mucin and glycoprotein are galactosylated by intact retina cells, (2) intact cells show GT activity, (3) when intact cells are incubated with UDP (^{14}C)-galactose and then cells are removed from the medium by centrifugation, the supernate(s) does not show GT activity using mucin glycoprotein and GlcNAc acceptors, (4) autoradiographic examination of neural retina suspension incubated with UDP (^{3}H)-gal shows that greater than 90% of the cells are galactosylated, (5) incubations with the excess of galactose-1-phosphate or free galactose failed to lower the radioactivity associated with the product, (6) when surface GT activity is studied during neural retina development using GlcNAc as an exogenous acceptor, the mitotically active marginal cells have highest GT activity. Moreover, the activity of GT declines as neural retinas age better.[261,262]

A cell-aggregating factor has been isolated from embryonic neural retina cells which promotes the cellular aggregation when added back to the culture.[263] One such aggregation promoting factor is believed to require terminal GalNAc residues for activity.[264,265] This aggregation-promoting factor is glycosylated by a specific GT present on the neural retina cell surface.[266] However, it is not clear from this study whether this surface GT functions

simply to add the required GalNAc residue to the aggregation promoting factor or the enzyme directly serves as the surface receptor for the aggregation factor. Although a similar aggregation factor has been reported by other investigators,[267] no one could detect enzyme or acceptor activity, indicating that neural retina factor might have some acceptor specificity for a particular glycosyltransferase(s).

GTs are reported to participate in retinotectal adhesions phenomenon.[268] To achieve adhesion of retina and tectum, dorsal-ventral axis is inverted onto the tectum and the ventral retina cells innervate the dorsal surface of the tectum, and the dorsal retina projects to the ventral tectum. The in vitro studies have shown that dissociated dorsal and ventral retina cells adhere to ventral and dorsal tectal surfaces, respectively.[268] The retinotectal adhesion may be specified by the complimentary interactions between protein and carbohydrates which are distributed in gradients of opposite polarity on both retina and tectum.[192,268] The dorsally located carbohydrate ligand requires terminal GalNAc residue for its activity and is postulated to be GM_2. This is due to GM_2's possession of a terminal GalNAc residue and preferential adherance to the proteinaceous receptor-rich ventral tecta.[192] However, no detectable difference in the levels of GM_2 is found between dorsal and ventral retinal sonicates. This data support the possible involvement of surface GT in retina cell adhesion.[261] In order to evaluate further, GT activities in ventral and dorsal retina towards GlcNAc were compared and found to be very similar.[192,269] However, GT activity toward the GM_2 ganglioside was 30% greater in ventral retina as compared to the GM_1 dorsal retina. Also, a marked increase in GM_1 GT activity occurs when adhesion specificity of ventral retina towards dorsal tectum becomes apparent. These results suggest that the ventral retina proteinaceous receptor may be GM_1 GT which participates in retinotectal adhesion by binding its dorsally concentrated substrate, GM_2.[268,270]

b. Cell Aggregation in Slime Mold and Sponges

Cellular interactions involving glycosyltransferases have been described in slime molds and sponges.[271] Cellular aggregation in the development of the slime mold *Dictyostelium discoideum* is thought to be mediated by specific carbohydrate-binding proteins, or lectins adhering to complex oligosaccharides on adjacent cells.[271] The levels of some of the surface transferases were found to be higher on aggregation competent cells than on incompetent cells, indicating the possible role of transferases.[272] A soluble aggregation factor(s) associated with glycosyltransferase(s) has been isolated from sponges[273] and receptors for the aggregation factor have been reported to be present on the cell surface.[274]

4. Surface GTs on Normal and Transformed Cells

Communication between adjacent cells occurs in embryonic, regenerating, and pathological tissues.[3,4] However, understanding of these interactions on a molecular level remains to be one of the central problems in developmental biology. Attempts to isolate specific "informational" molecules whose passage from cell to cell might account for such phenomena as embryonic induction and growth control between normal cells have failed.[275] A great deal of work is now being done on the comparison of GTs from normal and transformed tissues. The emphasis is whether or not, (1) GT from a transformed cell is different from normal, (2) is there excess amount of GT on transformed cells compared to normal cells, and (3) what is the role of GT in malignancy. Since the metastasizing ability of a transformed cell is ultimately manifested at the cell surface, many investigators are directing their efforts towards delineation of the differences in normal and transformed cell surface GT activities.

Studies on cell surface transferase(s) activities on chick embryo fibroblasts followed by their viral transformation indicated that activity of endogenous GT on transformed cells was about ten times higher than on either chick embryo fibroblast or on fibroblast infected with a nontransforming, Rous-associated virus.[276] The cells infected with a temperature-sensitive

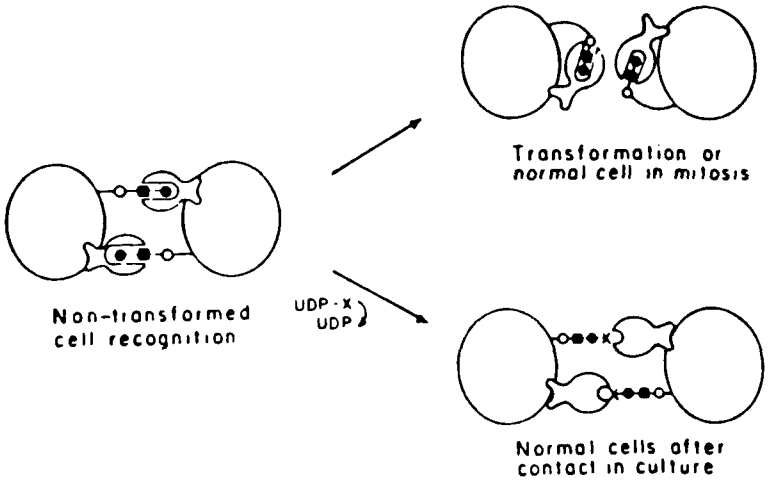


FIGURE 5. Model for cell-cell interactions between normal and transformed cells. Recognition between nontransformed cells is on left. Normal cells in mitosis, or transformed cells at all stages of their cell cycle, are able to bind their surface acceptors with their own surface transferases, rather than bind acceptors on adjacent cells. At lower right, nontransformed cells in culture have glycosylated each other's surface, upon contact, through the addition of monosaccharide. (From Shur, B. D. and Roth, S., *Biochim. Biophys. Acta*, 415, 473, 1975. With permission.)

virus exhibited enzyme levels comparable to the transformed cell-line at the permissive temperature. Morgan and Bosmann[277] employed four strains of Rous sarcoma virus to transform virally chick embryo fibroblasts. Increase in endogenous GT activity was observed. The authors suggested that increased endogenous transferase activity resulted from transformation rather than from viral infection. Some investigators, however, reported a decrease in transferase activity after viral transformation of hamster[278] and murine[279] fibroblasts. Meanwhile, two other reports[280,281] have reported an increase, decrease, or no change in transferase activities.

It has also been reported that surface GT activity of normal and transformed cells depends on culture density and cell contact.[282-284] Roth and White[282] have reported that normal 3T3 mouse fibroblasts taken from sparse cultures showed high endogenous GT activity, while confluent cultures showed reduced surface galactosylation. In contrast, transformed 3T12 mouse fibroblasts maintained a constant level of activity per cell independent of culture density. When assayed in sparse suspension, 3T3 cells showed less endogenous surface GT activity than when these cells were assayed in a pallet. Malignant 3T12 cells and mitotic 3T3 cells, however, gave similar levels of GT activities in both suspension and pellet incubations. Shur and Roth[3] have presented a model shown in Figure 5 to explain the behavior of normal and malignant cells in culture. According to this model, normal 3T3 cells glycosylate one another upon contact but are unable to glycosylate themselves because of a segregation of enzymes and acceptors within a relatively amobile plasma membrane. However, transformed cells and normal mitotic cells due to their increased plasma membrane fluidity can glycosylate themselves. Higher growth, and possibly malignancy, may result due to defect in either glycosylation or in the ability to respond to glycosylation.[3] Although the possible role of GT in growth control has been studied in many laboratories,[3,4,285] contact-dependent glycosylation[280] has not been clearly demonstrated.

D. Clinical Significance of GT

1. Tumor, Serum, and Plasma GT

Unlike many other molecules in the cell, enzymes are more specific in their function; hence, alteration (quantitative or qualitative) of enzyme(s) in blood, effusion, and body tissues may produce pathological conditions of clinical importance which may become a useful parameter in the diagnosis and prognosis of such metabolic disorders and diseases. A variety of enzymes, hormones, plasma proteins, protein degradation products, and immunologically related cellular and humoral substances have been used as markers for the diagnosis and management of human cancer.[286-297] The most studied tumor markers include carcinoembryonic antigen (CEA), alpha 1-acid glycoprotein, alpha-fetoprotein (AFP), phosphohexose isomerase, and beta$_2$-microglobulin among many others. Generally, the levels of such markers are elevated in blood and certain body fluids during malignancy.[298] Most of these markers suffer from having a significant rate of false positives and false negatives. Thus, a number of recent reports indicate the use of multiple markers towards diagnosis and follow-up of the neoplastic disease. Recently, Podolsky et al.[73,74] reported their findings on a carcinoma-associated GT II isoenzyme. The clinical data indicate that GT II isoenzyme is more sensitive than the CEA in the detection of malignant diseases, particularly pancreatic carcinoma,[73,74] and has a lower rate of false positives and false negatives.

Findings of altered glycoproteins on the surface of transformed and malignant cells may contribute towards change in social behavior of these cells from the normal cells. Accordingly, a number of investigators have examined biological fluids and cancer sera for soluble glycoproteins associated with malignancy.[299-301] In this section we will focus ourselves on the clinical usefulness of GT as a tumor marker.

Most of the earlier work on GT and its possible use as a tumor marker comes from Isselbacher and associates' laboratory at Massachusetts General Hospital.[1,33,61-63,73,74] A number of other investigators have also contributed towards the understanding of physicochemical aspects of glycosyltransferases in general, and GT in particular.[2,9,49] It is known that tumor cells secrete or shed a significant amount of glycoproteins into the surrounding media or fluid, followed by their entry through the lymphatic vascular system into the blood stream.[302,303] The increased levels of glycosyltransferases in general, and GT in particular, have been reported in serum,[46,60,95,304-306] plasma,[307] and malignant tissues[308-310] of cancer patients. Increase in total GT activity has also been reported in other animals bearing different kinds of tumors.[311-313]

a. GT in Serum and Plasma

It has been reported that total GT in serum of cancer patients was not found elevated unless liver was involved.[29,314] Kessel et al.[307] measured levels of GT in plasma of patients with metastatic liver tumors. Levels of GT were substantially elevated in cancer patients compared to normal controls. Although the source of elevated GT in plasma of their cancer patients was not clear, a number of other investigators have indicated liver,[29,314,315] tumor,[316,317] and erythrocyte[318] as the major contributors of plasma glycosyltransferase activity. Comparison of GT activities in metastatic tumor, peripheral uninvolved liver, and liver remote from tumor site indicated that enzyme concentrations were highest in liver tissue at the tumor periphery.[307]

Barlow and associates at Roswell Memorial Institute have been engaged for a number of years in determining the relationship between levels of GT and the stage of ovarian cancer.[60] Their results indicated that GT-EX activity (determined with an exogenously added acceptor DSG-fetuin) in the sera of ovarian cancer patients was elevated in comparison with age and blood-group matched normal controls.[306,309,316,319] By measurement of GT activity in serially drawn sera of a number of ovarian cancer patients, they demonstrated that the levels of GT-

Table 9
LEVELS OF SERUM GT IN NORMAL CONTROLS AND PATIENTS WITH VARIOUS TYPES OF CANCERS

Group	Number of subjects	GT activity[a] mean ± SEM	Significance ($p<$)[b]
Controls	55	50.5 ± 2.8	—
Prostatic	48	62.3 ± 6.7	NS[c]
Breast	126	70.8 ± 5.3	0.0001
Ovarian	21	68.9 ± 8.5	0.05
Respiratory tract	48	70.9 ± 7.1	0.01
Gastrointestinal tract	56	66.0 ± 6.3	0.02

[a] Expressed as nmol/hr/mℓ serum. Activity determined by radioactive method.[305]
[b] Significance of difference between means of normal controls and patients with various types of cancers.
[c] NS, not significant ($p > 0.05$).

EX correlated with the clinical status of the patients, and recurrence could be detected 3 to 7 months prior to clinical manifestations. Recently, these investigators extended their study and started serial measurement of GT-EN (GT activity without added acceptor) in addition to GT-EX in the sera of 30 patients undergoing treatment for ovarian epithelial cancer. The levels of GT-EN in the sera of these patients were significantly higher than age and blood-group matched controls. The patients with progressive disease despite therapy had percent elevations of GT-EN ranging from 18 to 440% of the initial value with a mean increase of 79%. In contrast, patients who responded to therapy, serum GT-EN decreased from 13 to 259% of the initial value, with a mean decrease of 59%. Although the values for GT-EX also followed a similar trend, the levels of GT-EN reflected more closely the clinical status of the patients than did GT-EX.[60] These investigators recommended that measurement of both GT-EN and GT-EX may be more useful in monitoring of patients with ovarian cancer than either of the two assays alone. It appears that the increased GT-EN activity in sera of cancer patients represents an indirect measurement of an endogenous glycoprotein acceptor present in the serum. Levels of some of these glycoprotein acceptors are known to increase under certain pathological conditions including cancer.[320] Podolsky and Weiser[61] have isolated a cancer-associated GT acceptor (CAGA) from sera and effusions of patients with extensive malignant disease.

Recently, Capel et al.[305] have reported the levels of total GT in sera of patients with various types of cancers. Their data presented in Table 9 shows that the GT activity is significantly higher in patients than in age-matched controls. The patients with breast and bronchial carcinomas have the highest levels of serum GT, followed by patients with ovarian and gastrointestinal cancers. In Table 10, the levels of serum GT have been compared in various cancer groups, particularly between patients with definite secondary involvement of the liver and those in whom metastases could not be detected. Unlike previous studies,[38,305,330,331] these data indicate that the increased tumor load associated with the secondary involvement of the liver are not accompanied by increased serum GT activity. Conversely, the breast cancer patients with terminal disease exhibited a steady decline in their serum GT activity in samples taken at monthly intervals over a 32-week period. No clear explanation for this decline has been put forth by these authors. In contrast, Ip and Dao[321] have found considerable overlap in serum GT levels of controls and cancer patients.

Table 10
LEVELS OF SERUM GT IN CANCER PATIENTS WITH AND WITHOUT METASTATIC INVOLVEMENT

Group	Number of subjects	GT activity[a] mean ± SEM	Significance ($p<$)[b]
Prostatic	28	59.2 ± 8.0	NS[c]
	16	70.9 ± 12.0	
Breast	88	68.0 ± 5.8	NS
	24	79.7 ± 12.8	
Respiratory tract	31	72.3 ± 7.5	NS
	15	67.9 ± 6.4	
Gastrointestinal tract	27	69.8 ± 10.1	NS
	21	61.9 ± 7.7	

[a] Expressed as nmol/hr/mℓ serum. Activity determined by radioactive method.[305] The upper values in each group indicate patients with metastases.
[b] Significance of difference between means of patients within the same tumor group.
[c] NS, not significant ($p > 0.05$).

b. GT in Tumor Tissue

Varying concentrations of GT in normal and tumor tissues have been reported.[309,310] Kijimoto-Ochiai et al.[310] conducted studies on GT in relation to the histology of carcinoma in human lung. The GT activities in tissue homogenates from adenocarcinoma and squamous cell carcinomas of the lung were higher than in adjacent normal lung tissue using DSG-fetuin as substrate. The activity of GT in adenocarcinomas (2 cases) was two times higher than in squamous cell carcinomas (19 cases) of the lung ($p < 0.001$). Using other acceptors, these investigators concluded that increased activity of GT(s) in lung carcinomas (especially in adenocarcinoma) is responsible for galactosylation of carbohydrate chains in *N*-glycoside-type but not *O*-glycoside-type glycoproteins.

In order to compare glycoprotein synthesis in normal and malignant ovarian tissues, Chatterjee et al.[309] determined the activities of GTs and other glycosyltransferases in tissue homogenates of ovarian epithelial carcinomas. Significantly high activities (more than normal mean ± 2 SD) of GT were found in these homogenates. Levels of GT in sera of these patients were also consistently elevated. Accordingly, these authors concluded that GT appears to be an excellent marker for ovarian carcinoma.[306,309]

Kiang et al.[322,323] have studied the levels of blood group synthetic enzymes in human colonic carcinoma. The GT activity in nonmalignant intestinal tissue was highest in patients with blood group B, intermediate in group AB, and was absent in group A or O. As compared to adjacent nonmalignant tissue, the GT activity in tumors increased by 1.6- to 6.9-fold in 4 of 5 patients with blood group B or AB. These investigators suggest that loss of ABH isoantigens in colorectal cancer are not due to deficiencies of GTs activities involved in the synthesis of blood group antigens.

c. Study of GT in Tumors Using Animal Model

Some of the information on the possible use of GT as a cancer marker in humans has come from the earlier studies conducted on laboratory animals. In an experiment, the levels of GT were compared at weekly intervals in the sera of rats bearing a metastasizing mammary tumor (SMT-2A), a nonmetastasizing mammary tumor (MT-W 9B), and controls, from the

day of tumor transplantation up to the sixth week of tumor development.[314] Compared to the control group of rats, the levels of GT in sera of rats bearing SMT-2A tumors for 3 weeks were significantly ($p < 0.05$) higher, while no GT elevation was observed in sera of rats bearing MT-W 9B tumors. Similar results were reported by Chatterjee and Kim,[311] who recovered fivefold GT activity in microsomal fraction of rat mammary carcinoma. When GT was assayed in purified plasma membrane fraction, 70% of the activity was associated with the plasma membrane vesicles, in which the enzyme was enriched 10- to 40-fold. Also, the number of galactose acceptor sites on the plasma membrane increased in parallel to the metastasizing capacity, indicating the presence of a larger number of incomplete glycopeptides on their cell surfaces. These findings suggest that the greater turnover of glycoproteins in the spontaneously metastasizing tumor cell surface was caused by the shedding of surface antigens into the systematic circulation of the host.[325]

Ip and Dao[326] reported GT results on tumors of rats with either 7,12-dimethylbenz (*a*) anthracene-induced mammary tumors that grew slowly or the transplantable Walker carcinomas 256 that grew rapidly. No change in the levels of serum and liver GT were detected in rats bearing the 7,12-dimethylbenz (*a*) anthracene-induced mammary tumors. However, an increase in serum sialyltransferase level was observed. The increase in both serum and liver GT and sialyltransferase in rats bearing the transplantable Walker carcinoma paralleled the weight of the growing tumor mass. The magnitude of increase of both enzymes in this group was significantly higher than that in rats carrying a similar load of 7,12-dimethylbenz (*a*) anthracene-induced mammary tumor. The authors suggest that tumor growth rate could be one of the factors in determining the levels of serum glycosyltransferases.[326]

Experimental liver carcinogenesis has long been considered as a useful model for the determination of the morphologic and biochemical characteristics of tumorigenesis. The character and number of events which take place between the initial exposure of the tissue to carcinogen and the appearance of malignant growth have been studied advantageously in the system due to the induction of slowly proliferating "hyperplastic areas" and "hyperplastic nodules". The glycolipid GT activity has been reported to correlate with development of nodule.[313,327]

Alterations in gangliosides and ganglioside biosynthetic enzymes in pooled nodules and in several grades of hepatocellular carcinomas induced by the carcinogen *N*-2-fluorenylacetamide have been reported.[328,329] A major alteration in ganglioside biosynthetic activity has been recognized in terms of a marked elevation in the levels of the monosialoganglioside GM_1, which correlated with loss of differentiation during tumorigenesis. The level of GM_1 was accompanied by an average tenfold elevation of the biosynthetic enzyme UDP-Gal·GM_2 GT; whereas the CMP-NAN:GM_1sialyltransferase which uses GM_1 as an acceptor was elevated only twofold. Thus, distinct populations of hepatic nodules with regard to GT may relate to specific classes of hepatomas, and accordingly, increases in GT activity have been found to correlate with development of the nodule from eosinophilic to basophilic type.[313]

2. GT Isoenzymes and Cancer

Thus far, the relationship of GT activity and tumor growth have been discussed in patients and animals. Although the result for a specific GT isoenzyme in cancer have been contradictory, a number of investigators, however, have found an elevation of total GT level in sera and tumor tissues of cancer patients.[33,62,73,74,139,330,331] Currently, many investigators are exploring other forms of GTs which may be more useful, and correlate quantitatively or qualitatively with cancer.[332] On the basis of kinetic and stability properties, Berger et al.[51] reported two different types of GT enzymes, GT A and GT B, in normal human serum. GT A incorporated galactose from UDP-gal into sialic acid-free ovine submaxillary mucin (asialo mucin), whereas GT B transferred galactose from UDP-gal to free GlcNAc or GlcNAc containing proteins. The relationship of GT A and/or GT B, if any, to cancer has not been indicated by these investigators.

Table 11
GT VARIANT (GT ℓ) IN HUMAN MALIGNANT EFFUSIONS

Diagnosis	Number tested	Number containing GT (ℓ)
Colon-rectal carcinoma	1	1
Breast carcinoma	5	4
Lung carcinoma	2	2
Thyroid adenocarcinoma	1	1
Lymphoma	5	3
Leukemia	1	1
Ovarian carcinoma	2	1
Renal carcinoma	1	1
Esophageal carcinoma	1	0
Bronchogenic carcinoma	1	0
Mesothelioma	1	1
Undifferentiated (primary site unknown) carcinoma	5	4
Total carcinoma cases	26	19 (73%)
Benign disease	25	8 (32%)

From Kim, Y. D., Weber, G. F., Tomita, J. R., and Hirata, A., *Clin. Chem.*, 28, 1133, 1982. With permission.

Recently, Kim et al.[139] reported the presence of a small amount of a GT variant (GT (ℓ)) in malignant pleural effusions. It appeared as a small peak (less than 3% of the total GT activity) on DEAE cellulose chromatography, compared to a large normal GT peak (GT (h)). The GT (ℓ) lost its activity quickly after separation from GT (h). It also moved more slowly on PAGE than the normal GT (h), indicating its similarity to carcinoma-associated GT II isoenzyme of Podolsky and Weiser.[33] Also, these investigators could not find any significant difference in the amount of total GT activity present in the pleural effusions from patients with benign or malignant disease. The results are given in Table 11. Of 26 specimens from cancer patients, GT (ℓ) was detected in 19, whereas only 8 of 25 specimens with nonmalignant diseases showed the presence of GT (ℓ), indicating its preferred association to malignancy. The authors postulate that the presence of GT (ℓ) may be restricted to physiological conditions emanating during the development of malignant disease and certain other abnormalities.[139]

Podolsky and Weiser[95] studied a large series of patients and reported that total GT activity was higher in patients with cancer. In 1975, they described an "isoenzyme" of serum GT which was associated with the presence of cancer, and later referred to it as "carcinoma-associated GT II isoenzyme". In 1979, they purified this cancer-associated GT II isoenzyme and the normal GT I isoenzyme from pooled pleural effusions of patients with various cancers. GT II isoenzyme was characterized to be a slower moving glycoprotein with a molecular weight of 76,000 daltons, higher than that of GTI's molecular weight of 54,000 daltons.[33]

Weiser et al.[333] assayed the GT activity in sera from 58 patients with various types of cancers. The results are presented in Table 12. The electrophoretically slow moving GT II isoenzyme was found to be present in the sera of 43 patients in addition to the major isoenzyme GT I. GT II isoenzyme was found only in 2 of the 38 patients with various nonmalignant disorders and was not detected in the sera of 22 normal controls. No correlation was found between the presence of these electrophoretically distinct isoenzymes and total serum GT activity. Patients with widespread metastases had significantly higher GT II levels than those

Table 12
GT II ISOENZYME IN MALIGNANT DISORDERS

Diagnoses	Number tested	Number demonstrating isoenzyme II
Bronchogenic carcinoma	8	7
Breast adenocarcinoma	6	4
Esophageal cancer (squamous cell)	4	3
Stomach adenocarcinoma	7	6
Pancreas adenocarcinoma	7	6
Colon-rectal adenocarcinoma	17	13
Gall bladder adenocarcinoma	1	1
Chronic lymphocytic leukemia	1	1
Polycemia rubra vera	1	1
Lymphomas	2	0
Undifferentiated (source unknown) carcinoma	2	0
Ovarian and testicular cancer	2	0
Total	58	43 (74.1%)

From Weiser, M. M., Podolsky, D. K., and Isselbacher, K. J., *Proc. Natl. Acad. Sci. U.S.A.*, 73, 1319, 1976. With permission.

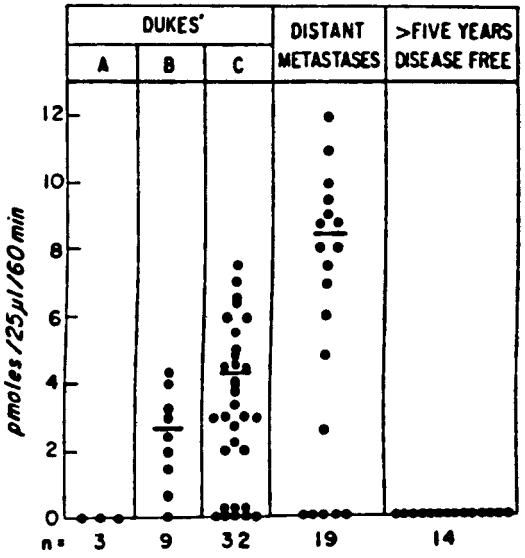


FIGURE 6. Correlation of serum GT II levels with Dukes' Classification in colonic carcinoma. (From Podolsky, D. K., Weiser, M. M., Isselbacher, K. J., and Cohen, A. M., *N. Engl. J. Med.*, 299, 703, 1978. With permission.)

with no metastases or with limited local disease. Later, Podolsky et al.[62] reported clinical evaluation of GT isoenzymes using sera from 232 patients with cancers of 14 different tissue types. Of these patients, 71% had measurable serum GT II activity. Of the 58 healthy laboratory personnel, none had GT II in their serum. In the study dealing with colorectal cancer patients, the levels of GT II isoenzyme were found to be increased with the tumor burden as shown in Figure 6. Of special interest was the finding that, of the nine patients

Table 13
FINAL DIAGNOSIS OF 270 PATIENTS EVALUATED FOR PANCREATIC DISEASE

Malignant disease	
Pancreatic carcinoma	61
Other gastrointestinal tumors or other intraabdominal primary	28
Other[a]	14
	103
Benign disease	
Cholelithiasis/biliary tract disease	24
Peptic ulcer disease	18
Acute pancreatitis	16
Hepatocellular disease	26
Chronic pancreatitis/pseudocyst	31
Functional pain/depression	27
Ampullary stenosis	6
Connective tissue disease	7
Miscellaneous[b]	27
	182[c]

[a] Includes 5 lung, 4 lymphoma, 2 prostate, 1 renal, 1 breast, 1 chronic myelogenous leukemia.

[b] Includes 8 osteoarthritis, 7 cardiovascular disease, 4 infectious gastroenteritis, 2 diabetic neuropathy, and 1 each of Crohn's disease, pyelonephritis, macroamylassemia, angiodysplasia, nontropical sprue, benign gastric polyp.

[c] 15 patients had two disorders.

From Podolsky, D. K., McPhee, M. S., and Isselbacher, K. J., *Trans. Assoc. Am. Phys.*, 94, 21, 1981. With permission.

with Duke's B lesions, seven had detectable GT II. In each of these cases, serum GT II became undetectable after patients underwent curative colectomy.

In a recent study, Podolsky et al.[73,74] have compared GT II determinations with currently available diagnostic modalities and other putative serologic markers in the diagnosis of pancreatic cancer. By using current radiologic and endoscopic techniques, 270 patients were suspected of having either benign or malignant pancreatic disease in whom definitive diagnostic information was available. As given in Table 13, 103 patients were found to have malignant disease. Of these 103 patients, 61 were ultimately proven to have cancers of the pancreas. An additional 28 patients were found to have other gastrointestinal cancers or to have hepatic metastases from an unknown primary site. The remaining 14 patients had various nonpancreatic and nongastrointestinal malignancies. Most of the patients with benign disorders (167) had either inflammatory pancreatic disease or benign biliary tract pathology.

Sera from each of 270 patients were screened for GT as well as other suggested serological markers of pancreatic cancer (CEA, AFP, ferritin, ribonuclease (RNase). As indicated in Table 14, GT II isoenzyme was more specific and more sensitive than all other serological markers studied. Of the 61 patients with pancreatic carcinoma, GT II levels were found increased in serum from 67%, and a similar frequency of positive GT II determinations were found in patients who had malignancies arising from an unknown primary lesion or other sites in the gastrointestinal tract. In contrast, of the 167 patients with nonmalignant diseases, only 3 patients had GT II activity, yielding a false positive rate of 1.8% and a specificity of 98.2% in discriminating between malignant and nonmalignant disease.

Radiologic and endoscopic examination (ultrasound, computer body tomography (CBT), endoscopic retrograde cholangiopancreatography (ERCP) were also conducted on these patients to see the accuracy and precision of GT II as a cancer marker. Results are presented

Table 14
RESULTS OF SEROLOGICAL MARKERS IN DETECTION OF CARCINOMA IN 270 PATIENTS

Final diagnosis	% Positive[a]				
	GT II	AFP	Ferritin	RNase	CEA
Pancreatic carcinoma (61)[b]	67.2	3.4	50	29.7	34.4
Other gastrointestinal cancer/primary unknown (28)	70.8	5.8	45.4	38.8	37.5
Nongastrointestinal malignancy (14)	21.4	7.1	64.3	8.3	7.1
Benign disease (167)	1.7	0.6	22.1	13.6	2.3

[a] Percentage of determinations above upper limit of normal (see text).
[b] Numbers in parentheses represent total number of determinations for each serologic marker in that disease category.

From Podolsky, D. K., McPhee, M. S., and Isselbacher, K. J., *Trans. Assoc. Am. Phys.*, 94, 21, 1981. With permission.

Table 15
COMBINATION OF GT II AND IMAGING TECHNIQUES IN THE DETECTION OF PANCREATIC CARCINOMA

Test(s)	Positive for pancreatic carcinoma
GT II alone (61)[a]	41/61 (67.2)[b]
GT II plus	
Ultrasound	46/50 (92)
ERCP(14)	14/14 (100)
CBT (34)	30/34 (88.2)

[a] Number in parentheses represents total number of pancreatic carcinoma patients studied by the test or procedure.
[b] Number of examinations suggestive of pancreatic carcinoma/total number of patients with pancreatic carcinoma studied. Percentage in parentheses indicates calculated sensitivity.

From Podolsky, D. K., McPhee, M. S., and Isselbacher, K. J., *Trans. Assoc. Am. Phys.*, 94, 21, 1981. With permission.

in Table 15. Only ERCP was more sensitive as an individual test than GT II. However, a combination of GT II with any of the radiologic or endoscopic tests yielded a sensitivity greater than any of the other single diagnostic tests listed above. Of 50 patients with pancreatic carcinoma undergoing both ultrasound and GT II determination, 46 were positive by this combination (92%). When either CBT (34 patients) or ERCP (14 patients) was combined with GT II measurements, pancreatic carcinoma was correctly detected in 88.2 and 100% of patients, respectively. There was no statistically significant difference in detection of pancreatic carcinoma when these three different combinations were compared. The combination of GT II with any of the other serologic markers did not result in an increase in cancer detection beyond that seen with GT II. Accordingly, these authors conclude that GT II isoenzyme appeared to be a good marker for malignancy in general, and pancreatic carcinoma in particular.[73,74]

Table 16
GT ISOENZYME II IN MALIGNANT DISORDERS

Type of carcinoma	No. of patients	Elevated (%)
Gastrointestinal carcinoma	49	78
Pancreatic carcinoma	22	73
Other sites carcinomas	61	52
Nonmalignant diseases	38	16

From Douglas, A. P. and Chandler, C., *Gastroenterology*, 74, 1120, 1978. With permission.

The presence of GT II isoenzyme has been shown in the sera of animals bearing induced tumors. In one such experiment, solid tumors were produced in hamsters using polyoma transformed BHK cells.[90] Tumor growth correlated with the appearance in serum of electrophoretically distinct GT activity on polyacrylamide gels. The slow moving band (GT II) was detected before solid tumors could be grossly observed and the amount of activity in this band was also found to be linearly related with growth of tumor. GT II isoenzyme was not detected in sera of control animals and was separable from the faster migrating GT band found in sera of tumor bearing animals.

To our knowledge, the results from only three other laboratories[334-336] have supported the earlier findings of GT II isoenzyme from Isselbacher's laboratory.[33,62] Data from one such report has been summarized in Table 16. Other investigators have not been able to identify GT II isoenzyme in sera and body fluids.[9,60,70,72] This may be partly due to the current procedure (running of PAGE, cutting and eluting the bands, and finally, performing radioactive procedure) which is complex and time consuming. Recently, a monoclonal antibody to GT has been developed.[48] Also, very recently, an enzyme immunoassay for GT has been published by Verdon et al.[337] Although the assay has been used to monitor GT levels in the sera of patients with ovarian carcinoma, it measures total GT activity and does not discriminate between the normal and carcinoma-associated GT isoenzymes. The development of monoclonal antibody for carcinoma-associated GT isoenzyme and its use in enzyme immunoassay will be more useful in the quantitation of cancer GT isoenzyme in serum, tissues, and other body fluids, and in radioimmunodetection of cancer at its site.

VI. CONCLUSIONS

Although GTs play an important role in the synthesis of complex glycoconjugates, their role on the surface of the cell and in malignancy has not yet been fully appreciated. The potential areas of research on glycosyltransferases in general, and GT in particular, include: (1) the transport of these enzymes onto the cell surface, (2) a comparison of the enzymes located inside and outside the cell, and (3) the improvement in immunochemical procedures for isolation of different GT isoenzymes. To achieve this, availability of monoclonal antibodies for an affinity purification of various GT isoenzymes will be helpful.

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ABBREVIATIONS USED

CDH:	galactosyl (β1-4) glucosylceramide
Cer:	Ceramide
Cer Gal:	galactocerebroside
CMH:	glucosylceramide or galactosylceramide
Con A:	Concanavalin A
DHase:	UDP-glucose dehydrogenase
DSG-fetuin:	desialylated degalactosylated fetuin
Fuc:	fucose
Fuco GA_1:	fucosyl (α1-2) galactosyl (β1-3) *N*-acetylgalactosaminyl (β1-4) galactosyl (β1-4) glucosylceramide
Fuco-GM_{1a}:	fucosyl (α1-2) galactosyl (β1-3)-*N*-acetylgalactosaminyl (β1-4) (*N*-acetylneuraminyl (α2-3) galactosyl (β1-4) glucosylceramide
GA_1:	galactosyl (β1-3) *N*-acetylgalactosaminyl (β1-4)-galactosyl (β1-4) glucosylceramide
GA_2:	*N*-acetylgalactosaminyl (β1-4) galactosyl (β1-4) glucosylceramide
Gal:	galactose
GalNAc:	*N*-acetylgalactosamine
Glc:	glucose
GlcNAc:	*N*-acetylglucosamine
$GlcNH_2$:	glucosamine
GM_{1a}:	galactosyl (β1-3) *N*-acetylgalactosaminyl (β1-4) (*N*-acetylneuraminyl (α2-3)) galactosyl (β1-4) glucosylceramide
GM_{1b}:	*N*-acetylneuraminyl (α2-3) galactosyl (β1-3)-*N*-acetylgalactosaminyl (β1-4) galactosyl (β1-4) glucosylceramide
GM_2:	*N*-acetylgalactosaminyl (β1-4)(*N*-acetylneuraminyl (α2-3)) galactosyl (β1-4) glycosylceramide
GM_3:	*N*-acetylneuraminyl (α2-3) galactosyl (β1-4) glucosylceramide
GT:	galactosyltransferase
Man:	mannose
NeuAc:	neuraminic acid
NPK:	nucleoside-5′-diphosphate kinase
OSM:	ovine submaxillary mucin
Paragloboside:	galactosyl (β1-4) *N*-acetylglucosaminyl (β1-3) galactosyl (β1-4) glucosylceramide
PPase:	UDP-glucose pyrophosphorylase
UDP-gal:	UDP-galactose

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