

GALACTOSYL TRANSFERASE OF A GOLGI FRACTION FROM CULTURED NEOPLASTIC MAST CELLS

LAWRENCE S. FREILICH, RICHARD G. LEWIS, ALBERT C. REPPUCCI, JR.,
and JEREMIAH E. SILBERT

From the Connective Tissue—Aging Research Laboratory, Veterans Administration Outpatient Clinic, the Connective Tissue Research Laboratory, Boston Veterans Administration Hospital, and the Departments of Medicine and Anatomy, Tufts University School of Medicine and Dental Medicine, Boston, Massachusetts 02108. Dr. Freilich's present address is the Department of Periodontics, Tufts University School of Dental Medicine, Boston, Massachusetts, 02111.

ABSTRACT

Suspension cultures of neoplastic mouse mast cells were used to obtain large quantities of a homogeneous cell population as starting material for cell fractionation. A Golgi fraction was prepared by slight modification of established techniques and identified by electron microscopy. Assay of galactosyl transferase activity using ovalbumin, desialylated degalactosylated orosomucoid, and *N*-acetylglucosamine as galactose acceptors showed that the Golgi fraction was enriched in specific activity over the homogenate. The Golgi galactosyl transferase was examined in detail. Acceptor concentrations for optimal galactose incorporation were determined, and substrate inhibition effects were shown with higher concentrations of all three acceptors. Manganese was shown to be necessary for galactose incorporation. A higher concentration of manganese afforded some protection from substrate inhibition by acceptors, but at the same time was itself inhibitory. All three acceptors competed with one another for galactose incorporation, indicating that a single enzyme catalyzed the transfer of galactose for all acceptors.

Formation of the carbohydrate portion of glycoproteins involves the sequential addition of sugars onto a protein core. The subcellular site for addition of at least some of these sugars has been localized to the Golgi apparatus (20, 24), and in particular, the transfer of galactose to glycoprotein has been examined with this organelle. Galactose transfer to glycoprotein or *N*-acetylglucosamine (5, 6, 10, 15) is now considered to be the best marker for the Golgi apparatus, at least in tissues such as liver that produce glycoprotein primarily for export. Although transfer to *N*-acetylglucosamine has been shown with milk enzyme (21) to reflect the same enzymatic activity as transfer of galactose to glycoproteins, this identity for Golgi fractions has not been fully established.

Golgi fractions from whole tissues such as liver (5, 6, 10, 16, 20, 24), pancreas (19), kidney (7), or testis (4), represent preparations derived from a heterogeneous mixture of cells, including diverse connective tissue elements in addition to the major functional cells of the organ. Thus, even those fractions composed largely of Golgi elements would contain Golgi material derived from several different cell types, and the metabolic activities (such as galactosyl transferase)¹ of these fractions could not be ascribed with certainty to the Golgi apparatus of any one particular cell. In order to

¹ The term galactosyl transferase in this paper refers to UDP-galactose: *N*-acetylglucosaminide galactosyl transferase.

obtain information concerning the Golgi apparatus of a specific cell type by fractionation techniques, a pure population of cells would be advantageous.

The growth of large volumes of cells in tissue culture provides a convenient means of obtaining homogeneous cell populations for subsequent cell fractionation. We have prepared an enriched Golgi fraction from a population of cultured neoplastic mast cells,² and have studied the galactosyl transferase of this Golgi fraction. These cells were chosen because they offer several advantages in growth characteristics and also display interesting facets of specialized metabolism (8). The cells can be grown in large volume suspension cultures so that gram quantities can be obtained free of extracellular material (13).

MATERIALS AND METHODS

Cell Sources and Growth Conditions

Cells originally derived from a solid Dunn-Potter P815 mouse mastocytoma have been carried in this laboratory for several years (12). Maintenance of cell cultures and techniques for production in large volume have been described (12, 13). Harvests on a single run have been as high as 14 ml of packed cells from a 12-liter spinner culture.

Incorporation of [³H]leucine by the growing cells was used to label cell protein, allowing assay of the ³H-protein radioactivity in the various subcellular fractions. For this purpose a relatively small volume of cells was labeled by growth for 3–4 days in separate 250-ml cultures with the addition of [³H]leucine (0.2 mCi/250 ml) to the medium. These cells were collected, washed several times, and pooled with the larger cell harvest just before homogenization.

Fractionation Procedure

Several established methods for preparing liver Golgi fractions were first attempted on the cultured cell system. When the fractionation methods of Morré et al. (16); Leelavathi et al. (10), and Fleischer and Fleischer (6) were each applied individually and essentially without modification, the Golgi fractions obtained were of low yield and appeared to be highly contaminated with other cell material. The technique ultimately devised to yield the highest recovery and purity of the Golgi fraction represented a combined version of these methods. All fractionation procedures were conducted at 4°C.

For a typical experiment, 4–8 ml of packed cells (including [³H]leucine-labeled cells) were suspended to-

gether in 1 vol of a homogenization medium composed of 0.5 M sucrose in 0.1 M potassium phosphate buffer, pH 6.65, plus 0.005 M MgCl₂. All subsequent sucrose gradient solutions were made in this same buffer. The viscous suspension was transferred to a tight-fitting glass Dounce homogenizer (Kontes Company, Vineland, N. J.), and the cells were disrupted with 40–50 strokes. The progress of homogenization was assessed by phase-contrast microscopy. The homogenate was then centrifuged for 10–15 min at 2,000 g (Sorvall RC2-B centrifuge, SS-34 rotor [Du Pont Instruments, Sorvall Operations, Newtown, Conn.]), resulting in a cloudy supernate and a dense pellet. The yellowish upper one-tenth of the pellet (designated by Morré et al. [16] as the "Golgi-rich" region in liver fractionation procedures) was aspirated and combined with the entire 2,000 g supernate. The resulting suspension was diluted by an equal volume of homogenization medium, and 4-ml portions were then layered over discontinuous gradients composed of 4.0 ml each of 1.7 M and 2.1 M sucrose solutions in 12.5-ml cellulose nitrate centrifuge tubes. These gradients were centrifuged for 60 min at 105,000 g (Beckman L2-75B ultracentrifuge, SW-41 rotor; Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) to form a two-layered band at the 0.5 M:1.7 M interface and a single band at the 1.7 M:2.1 M interface. The yellowish upper layer of the band at the 0.5 M:1.7 M interface was aspirated separately and designated as "membrane fraction 1". This was used to prepare the "Golgi" and "mixed membrane" pellets. The lower layer of this band was pooled with the entire band at the 1.7 M:2.1 M interface and was designated "membrane fraction 2". Although the latter fraction was used for correlated experiments, it was not fractionated further. Membrane fraction 1 was then adjusted to 1.1 M in sucrose and was diluted by the addition of 1 or 2 vol of a 1.1 M sucrose solution. Portions of this fraction (4 ml each) were layered over 4 ml of 1.5 M sucrose, followed by 4 ml of homogenizing medium in 12.5-ml centrifuge tubes. The resulting gradients were centrifuged at 105,000 g for 90 min to form a "Golgi" band (0.5 M:1.1 M interface) and a "mixed membrane" band (1.1 M:1.5 M interface). These bands were aspirated, suspended in homogenizing medium, and pelleted by centrifugation at 105,000 g for 30 min. Portions of each pellet were processed for electron microscopy, and the remaining portions were used for determination of protein content and enzyme activity.

Electron Microscopy

Whole cells or pelleted cell fractions were fixed 2–4 h in a solution of 2.0% glutaraldehyde (Polysciences, Inc., Warrington, Pa.) in 0.05 M phosphate buffer, pH 7.2, washed overnight in 0.1 M phosphate buffer plus 0.2 M sucrose, pH 7.2, and post-fixed in 1.0% OsO₄ in 0.1 M phosphate buffer, pH 7.2, 1–2 h. Specimens were dehydrated in ascending grades of ethanol and finally infiltrated and embedded in Spurr (Polysciences, Inc.).

² A preliminary communication has been published which includes the preparation and use of this Golgi fraction (8).

Sections of both the pelleted fractions and the whole cells were stained with uranyl acetate (25) and lead citrate (18). For negative staining, a small aliquot (0.05–0.1 ml) of the Golgi band was suspended for 1–2 min in 1–2 drops of 2.0% phosphotungstic acid at pH 6.8 over parlodion-coated grids. All specimens were examined and photographed on a Phillips EM-300 electron microscope.

Analytical Procedures

Protein content of the homogenate and the various fractions was assayed by the Lowry procedure (14) and by determination of the amount of [³H]leucine-labeled protein.

Galactosyl transferase assays were performed with two different glycoproteins and a monosaccharide as [¹⁴C]galactose acceptors. Incubation mixtures contained the following ingredients in a total volume of 0.025 ml: 0.05 M 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer, pH 6.5, (Calbiochem, San Diego, Calif.); 0.01 M MnCl₂; 0.1% Triton X-100 (Fisher Scientific Co., Pittsburgh, Pa.); 0.0007 M UDP[¹⁴C]galactose, 2.5 × 10⁶ cpm (New England Nuclear Corp., Boston, Mass.); 0.001–0.008 ml of cell homogenate or subcellular fraction plus one of the following acceptors: (a) 0.25 mg ovalbumin (Sigma Chemical Co., St. Louis, Mo.); (b) 0.05 mg desialylated, degalactosylated orosomucoid³ (a generous gift of Dr. H. Schachter, University of Toronto, Toronto, Ont.); (c) 0.02 M *N*-acetylglucosamine (Calbiochem).

The incubation mixtures for the measurement of 5'-nucleotidase activity contained the above MES buffer, MnCl₂, Triton X-100, and aliquots of homogenate or subcellular fraction in the same concentrations as in the galactosyl transferase assays with the addition of 0.0008 M [³H]AMP, 1.7 × 10⁵ cpm, (New England Nuclear Corp.). It was possible to assay 5'-nucleotidase in the same reaction mixtures used for the ovalbumin or orosomucoid galactosyl transferase assay as previously described (9).

Reaction mixtures for both assays were incubated at 37°C, and at 15 min and 45 min, 0.005-ml aliquots were removed and spotted on Whatman #1 filter paper (VWR Scientific Div. Univar, San Francisco, Calif.) and chromatographed in ethanol:1 M ammonium acetate, pH 7.8 (5:2), as previously described (9). Appropriate radioactive areas of the chromatogram were assayed for determination of galactose transfer and adenosine formation (9). Various permutations and combinations of acceptors and components were used in assays investigating pH optimum, cofactor requirements, substrate concentration optima, and competition for galactose transfer.

³ The term orosomucoid in this paper refers to desialylated, degalactosylated orosomucoid.

RESULTS

Morphology and Fractionation

Low magnification electron microscopy (Fig. 1) revealed that the size, shape, and nucleocytoplasmic ratio of the cultured cells were similar to those of normal mast cells, but there was an absence of cytoplasmic storage granules. This was consistent with the known loss of heparin-producing ability by this particular line of cultured cells (12). Mitochondria were present as were several structures representing secondary lysosomes. Although the rough endoplasmic reticulum was distributed only sparsely as branching tubules and cisternae, there was an abundance of free ribosomes throughout the cytoplasm. (Aggregates of these free ribosomes proved to be a major contaminant during the initial attempts at isolation of a Golgi fraction.) The Golgi apparatus usually appeared as stacks or irregular arrays of cisternae in the cell center, relatively close to the nucleus.

Electron microscopy of the Golgi fraction is shown in Fig. 2, which represents a typical field from upper, middle, or lower levels of the Golgi pellet. Membranous structures present included what appeared to be cross-sections through stacks of curved, parallel Golgi cisternae (10, 16). Much of the remaining field contained membranous elements which may represent cisternae sectioned in other planes, plus some membranous fragments derived from plasma membrane. Fields at both low and high magnification appeared indistinguishable from similar published views of liver Golgi fractions (16).

Negatively-stained Golgi elements (Fig. 3) resembled comparable structures from negatively-stained liver Golgi fractions (16). The fraction designated mixed membranes appeared to be composed of rough endoplasmic reticulum, plasma membrane, and some free ribosomes.

Representative values for the protein content of the homogenate and the various fractions are shown in Table I. Routinely, protein values determined by the technique of Lowry were compared with ³H-protein assays in order to calculate a value representing the ³H-protein cpm/mg protein for the homogenate. Protein recovery in the various fractions was then calculated by dividing the ³H-protein assay values by the figure for ³H-protein cpm/mg protein of the homogenate. In order to preserve fraction material, Lowry protein determinations were not performed routinely on all the fractions. When performed, the Lowry determina-

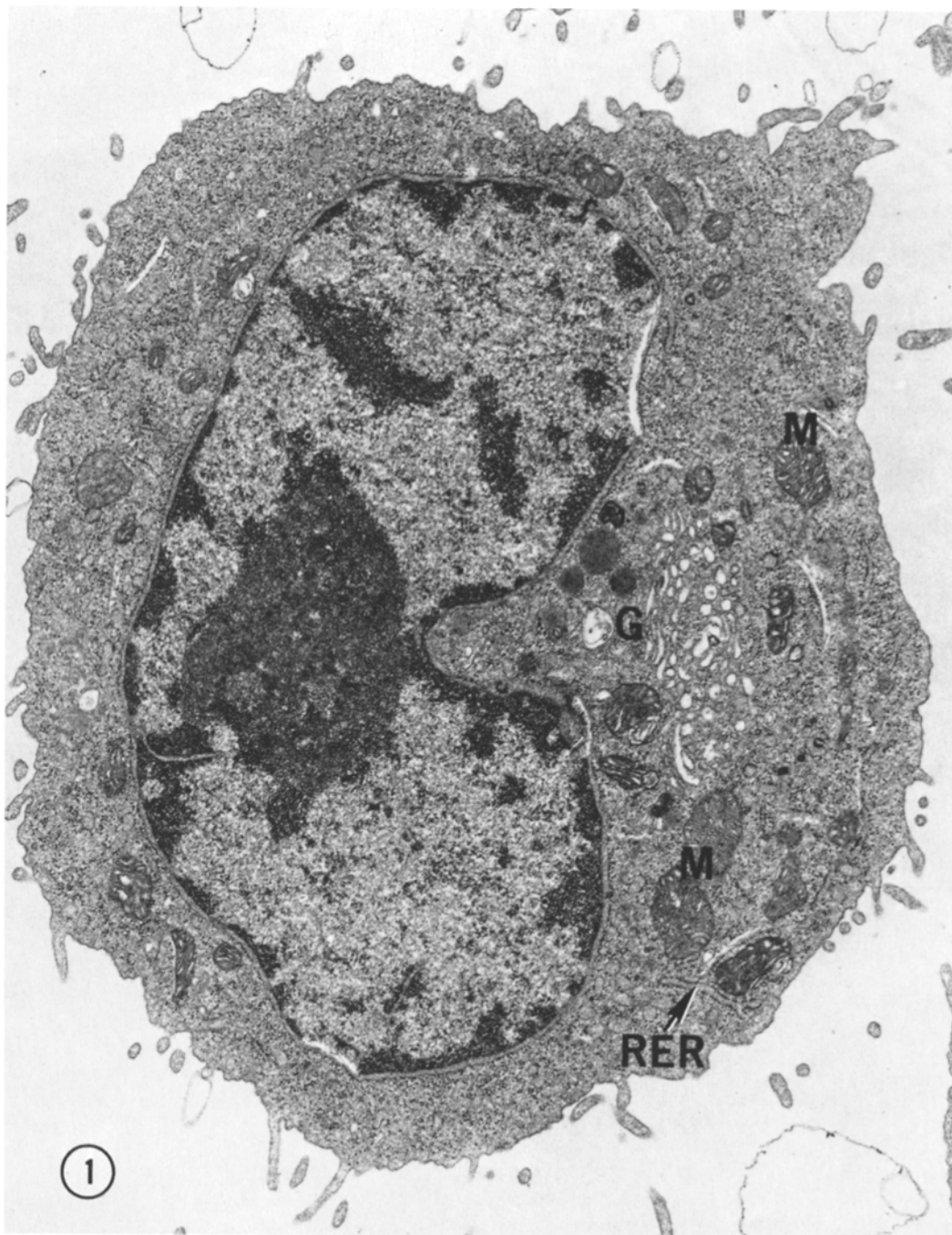


FIGURE 1 Cultured neoplastic mast cell. Mitochondria (*M*) show a normal morphology and distribution. Cisternae of the rough endoplasmic reticulum (*RER*) are relatively sparse while close inspection reveals abundance of free ribosomes throughout the cytoplasm. Portions of the Golgi apparatus (*G*) in this cell appear as irregular groups of cisternae. $\times 24,500$.

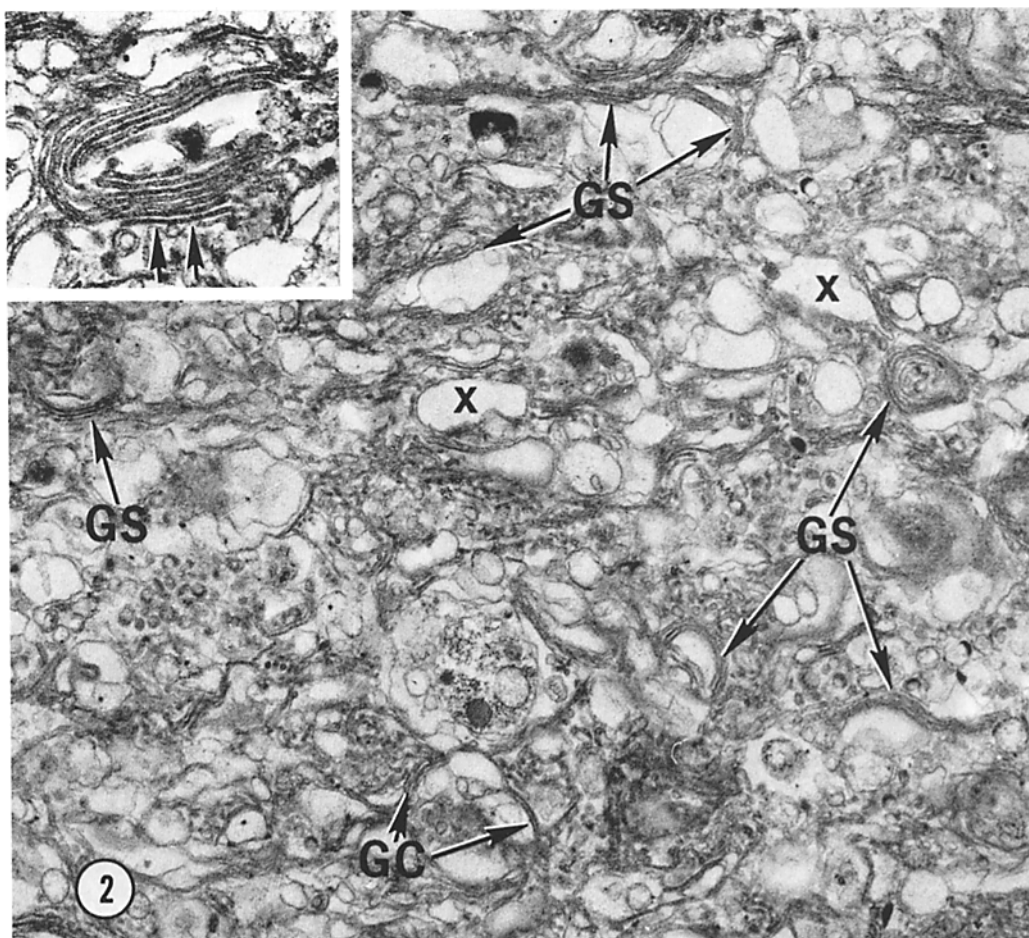


FIGURE 2 Section from a typical Golgi pellet. The field contains many small Golgi stacks (GS) consisting of several parallel cisternae. In addition, there are numerous single Golgi cisternae (GC) throughout the field. The irregular membrane-bounded spaces (X) seen adjacent to many Golgi cisternae represent dilations of individual cisternae. $\times 19,000$. Inset shows higher magnification of a Golgi stack (double arrows) from a neighboring field. $\times 33,000$.

tions were similar to those derived by the above method.

The recovery of protein in the Golgi pellet amounted to approx. 0.4% of the total homogenate protein. This is somewhat less than the usual yield for Golgi fractions isolated from liver (1, 10). However, it should be noted that, in the present procedure, only the upper portion of the band at the 0.5 M:1.7 M interface was used to prepare the final Golgi fraction so that the yield of Golgi particles was sacrificed for the sake of fraction purity.

Enzyme Activities

Table I shows the results of galactosyl transfer-

ase assays performed after a typical fractionation procedure. Assays with each of the three different acceptors were performed on fractions from a single preparation, although each set of assays with any one of the acceptor types was carried out on separate days. The 15-min and 45-min samples of all assays indicated that the incorporation of [14 C]galactose into the various acceptors under our conditions was linear within this time interval. Routinely, four or more concentrations of Golgi preparation and two concentrations of homogenate were utilized in the assays. Incorporations were always proportional to the sample 3 H-protein. Two concentrations of the other cell fractions were utilized on occasion. Activities were always

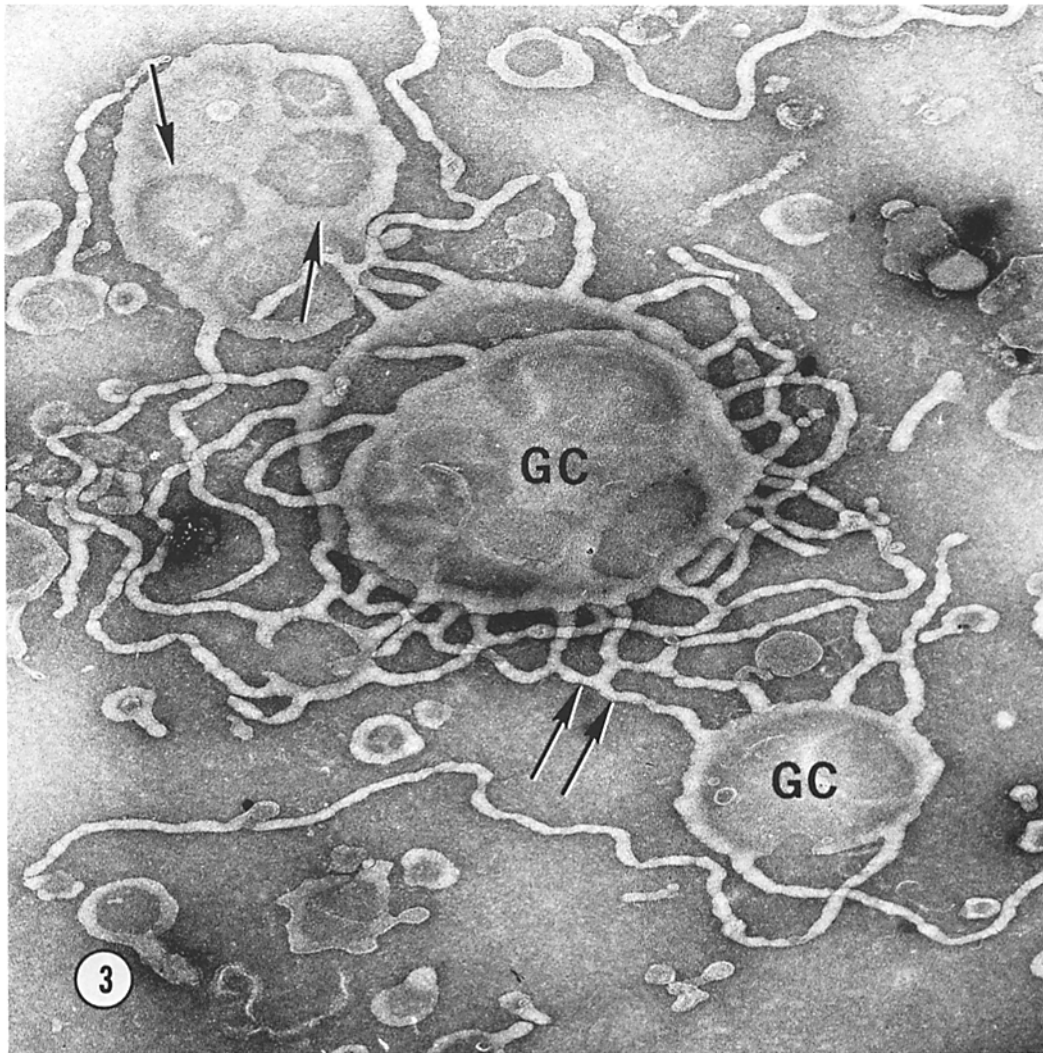


FIGURE 3 Golgi fraction, negatively stained. Flattened, platelike Golgi cisternae (GC) of various sizes display attached peripheral tubules which in one area (double arrow) connect adjacent cisternae. Certain cisternae show evidence of possible intracisternal channels (single arrows), although this appearance could be due to shrinkage or staining effects. $\times 60,000$.

proportional to protein. In this experiment the specific activity of galactosyl transferase in the Golgi pellet showed an enrichment of 11- to 14-fold over membrane fraction 1 with all acceptor types. Enrichments over homogenate were less consistent, due to problems in sampling related to viscosity of the homogenate. Ovalbumin was the acceptor employed after most fractionation procedures, and the specific activity of galactosyl transferase of four other Golgi preparations was 35-50 times that of the homogenate. As noted previously, recoveries were limited by a sacrifice of

Golgi protein to permit isolation of a relatively pure fraction. In this regard, the assay employing ovalbumin indicated that as much as 25% of the total homogenate galactosyl transferase activity was located in membrane fraction 2, which was not further utilized for preparation of the Golgi fraction. The specific activity of galactosyl transferase was not enriched in the mixed membrane fraction, suggesting that galactosyl transferase may be a specific Golgi-marker enzyme in this cell type.

The results of the 5'-nucleotidase assays are also

TABLE I
Galactosyl Transferase and 5'-Nucleotidase Activities

Cell preparation		[¹⁴ C]Galactose incorporation into acceptors						[³ H]Adenosine formed	
Fraction	Total Protein	Ovalbumin*		Orosomuroid		N-Acetyllactosamine		Protein	Total
		Protein	Total	Protein	Total	Protein	Total		
	mg	μmol/mg	μmol	μmol/mg	μmol	μmol/mg	μmol	μmol/mg	μmol
Homogenate	1,540	0.0017	2.56	0.012	17.7	0.012	17.7	0.014	21.6
2,000 g supernate	985	0.0021	2.05	—	—	0.013	12.8	0.014	13.8
Membrane fraction									
1	153	0.011	1.60	0.039	5.9	0.047	7.2	0.012	1.8
2	390	0.0017	0.65	—	—	—	—	0.017	6.6
Pellets									
Golgi	6	0.155	0.95	0.430	2.5	0.600	3.6	0.190	1.1
Mixed membrane	4	0.0038	0.02	—	—	0.013	0.15	0.100	0.4

* These assays were performed with an ovalbumin concentration less than maximal; total amounts incorporated are lower than can be optimally achieved with this acceptor.

shown in Table I. The Golgi pellet contained about 5% of the total homogenate 5'-nucleotidase activity and showed an increase in specific activity of about 13-fold over the homogenate. The 5'-nucleotidase enrichment in the mixed membrane pellet was about one-half that of the Golgi pellet, probably reflecting a proportion of rough endoplasmic reticulum and free ribosomes greater than plasma membranes in this fraction.

Characteristics of Galactosyl Transferase

Galactosyl transferase activity with ovalbumin as the acceptor had an absolute manganese requirement which could not be satisfied by the substitution of magnesium. Omission of Triton X-100 resulted in a 75% loss of activity. Incorporation into endogenous material was too low to be measurable, so that omission of ovalbumin essentially eliminated incorporation. The pH optimum for [¹⁴C]galactose incorporation into ovalbumin was at pH 6.5, with approx. 50% of the activity remaining at pH 5.5 and 8.0.

Incorporation of [¹⁴C]galactose with orosomuroid, ovalbumin, and N-acetylglucosamine as acceptors over a range of acceptor concentrations is shown in Fig. 4. There was a sharp maximum for orosomuroid and N-acetylglucosamine as acceptors and a rapid falling off of incorporation with higher acceptor concentrations. This was particularly marked in the case of orosomuroid. Ovalbumin also appeared to be a poorer acceptor at higher concentrations, but the decrease in incorporation was minimal. This orosomuroid preparation contained 0.4 μmol of terminal N-acetylglucosamine per milligram (H. Schachter; personal

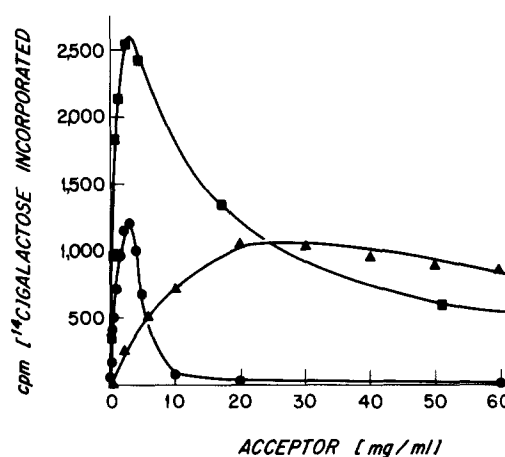


FIGURE 4 Incorporation of [¹⁴C]galactose at varying acceptor concentrations. Incubation mixtures contained the following in a total vol of 0.025 ml: 0.05 M MES (pH 6.5); 0.01 M MnCl₂; 0.1% Triton X-100; 0.0007 M UDP[¹⁴C]galactose (2.5 × 10⁸ cpm); 0.0012 mg of Golgi fraction; plus either (a) ovalbumin, ▲; (b) orosomuroid, ●; or (c) N-acetylglucosamine, ■. Incubation and assay were carried out as described in Materials and Methods. Results show incorporation with a 0.005-ml aliquot after 45-min incubation at 37°C.

communication). From this, the concentration of terminal N-acetylglucosamine acceptor at the 2 mg/ml concentration needed for maximum [¹⁴C]galactose incorporation can be calculated to be 0.0008 M. The peak of [¹⁴C]galactose incorporation with free N-acetylglucosamine as acceptor occurred at 3.3 mg/ml which is equal to 0.015 M, a much higher concentration. The terminal N-acetylglucosamine content of ovalbumin has been

estimated as 0.02–0.05 $\mu\text{mol}/\text{mg}$ (3), much less than that of orosomuroid. From this, the concentration of acceptor *N*-acetylglucosamine at maximal [^{14}C]galactose incorporation (20 mg/ml ovalbumin) can be calculated as 0.0004–0.001 M. When K_m values were estimated by reciprocal plots, the value for orosomuroid was 3.5×10^{-4} M while that for the free *N*-acetylglucosamine acceptor was 12×10^{-4} M. The K_m for ovalbumin was 8 mg/ml or approx. $1.6\text{--}4 \times 10^{-4}$ M for hexosamine acceptor. These K_m values must be considered to be approximate because of the indeterminate degree of substrate inhibition at lower substrate concentrations. In any case, it would appear that orosomuroid (and probably ovalbumin as well) is a better galactose acceptor than free *N*-acetylglucosamine. Even though the maximum amount of [^{14}C]galactose incorporation with free *N*-acetylglucosamine as acceptor was higher, substrate inhibition by orosomuroid was much more marked, and it is probable that this inhibition prevented a higher [^{14}C]galactose incorporation by orosomuroid.

On several occasions, a ^{14}C -labeled origin (after incubation of UDP[^{14}C]galactose with orosomuroid or ovalbumin) was incubated with a highly specific β -galactosidase from *Charonia lampus* (Miles Laboratories Inc., Miles Research Products, Kankakee, Ill.). This treatment quantitatively released ^{14}C -radioactivity, while incubation of ^{14}C -labeled origins in similar mixtures lacking β -galactosidase resulted in less than 5% release of radioactivity. The radioactivity released by β -galactosidase chromatographed as a monosaccharide on Sephadex G-10. The ^{14}C -labeled material chromatographing in the *N*-acetylglucosamine area was also incubated with β -galactosidase. Before incubation, it chromatographed as a disaccharide on Sephadex G-10, while after β -galactosidase treatment the radioactivity chromatographed as a monosaccharide.

The effects of manganese concentration on [^{14}C]galactose incorporation into orosomuroid at optimal (2 mg/ml) and inhibitory (10 mg/ml) concentrations of orosomuroid are shown in Fig. 5. At optimal orosomuroid concentration, manganese was inhibitory at a concentration greater than 0.01 M. In contrast, when inhibitory levels of orosomuroid (10 mg/ml) were used, higher concentrations of MnCl_2 became necessary for optimal activity.

In order to investigate the effects of manganese further, the incorporation of [^{14}C]galactose into

varying concentrations of orosomuroid was examined at several manganese concentrations (Fig. 6). At 0.1 M manganese concentration, there was a slight shift to a higher orosomuroid concentration for maximum incorporation and a partial protection from inhibitory effects of the higher orosomuroid concentration. At still higher manganese levels (0.3 M MnCl_2), there appeared to be even

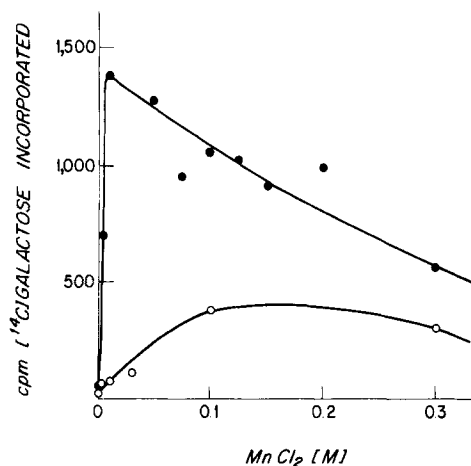


FIGURE 5 The effects of manganese on [^{14}C]galactose incorporation. Incubation and assay were carried out as described in Fig. 4 with MES buffer, Triton X-100, UDP[^{14}C]galactose, and Golgi fraction, plus MnCl_2 as indicated, and orosomuroid 2 mg/ml, ●, and 10 mg/ml, ○.

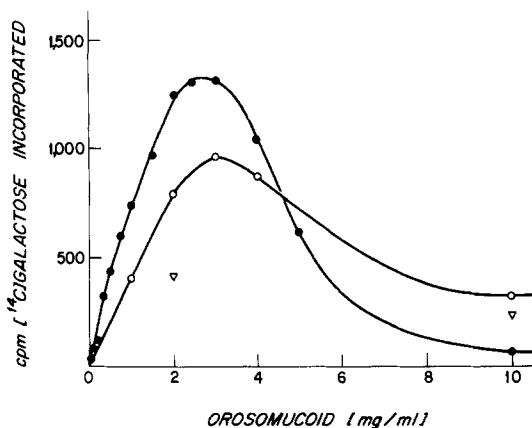


FIGURE 6 Incorporation of [^{14}C]galactose into varying concentrations of orosomuroid at varying manganese concentrations. Incubation and assay were carried out as described in Fig. 4 with MES buffer, Triton X-100, UDP[^{14}C]galactose, and Golgi fraction, plus orosomuroid as indicated, and 0.01 M MnCl_2 , ○; 0.1 M MnCl_2 , ●; 0.3 M MnCl_2 , ▽.

greater protection from orosomucoid inhibition. It should be noted, however, that despite the protection from orosomucoid substrate inhibition afforded by manganese, the maximum [^{14}C]galactose incorporation at noninhibitory orosomucoid concentrations was less with increasing MnCl_2 concentrations. Thus, the MnCl_2 itself seemed to be inhibitory at higher concentrations at the same time that it partially reversed the orosomucoid substrate inhibition.

Incorporation of [^{14}C]galactose into varying concentrations of orosomucoid in the absence and presence of *N*-acetylglucosamine (0.005 M) is shown in Fig. 7. *N*-acetylglucosamine inhibited incorporation of galactose into orosomucoid. Furthermore, incorporation of galactose into *N*-acetylglucosamine was markedly reduced with increasing concentrations of orosomucoid. The two substrates would thus appear to be mutually competitive. However, problems regarding substrate inhibition (Fig. 4) immensely complicate any detailed analysis of the kinetics of these reactions. Incorporation of [^{14}C]galactose into ovalbumin in the absence and presence of *N*-acetylglucosamine (0.005 M) is shown in Fig. 8. The competition was similar to that seen with orosomucoid. Incorporation of [^{14}C]galactose with varying concentrations of *N*-acetylglucosamine as acceptor in the absence and presence of orosomucoid (2 mg/ml) is shown

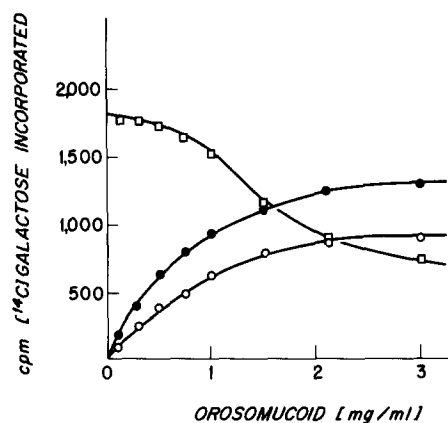


FIGURE 7 Effect of *N*-acetylglucosamine on the incorporation of [^{14}C]galactose into orosomucoid. Incubation and assay were as described in Fig. 4 with MES buffer, MnCl_2 , Triton X-100, UDP[^{14}C]galactose, and Golgi fraction, plus orosomucoid as indicated, with or without *N*-acetylglucosamine. Incorporation into orosomucoid without *N*-acetylglucosamine, ●, with 0.005 M *N*-acetylglucosamine, ○, incorporation into *N*-acetylglucosamine, ▲, □.

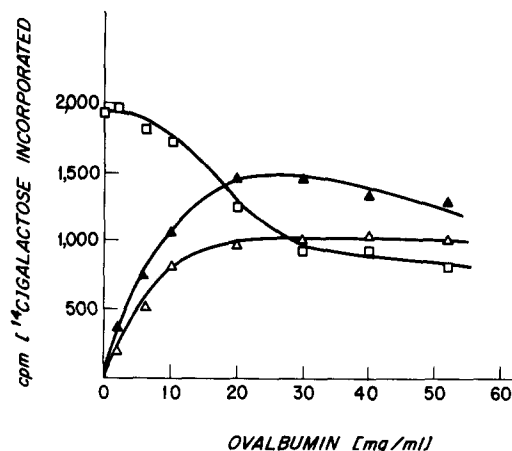


FIGURE 8 Effect of *N*-acetylglucosamine on the incorporation of [^{14}C]galactose into ovalbumin. Incubation and assay were as described in Fig. 4 with MES buffer, MnCl_2 , Triton X-100, UDP[^{14}C]galactose, and Golgi fraction, plus ovalbumin as indicated, with or without *N*-acetylglucosamine. Incorporation into ovalbumin without *N*-acetylglucosamine, ▲, with 0.005 M *N*-acetylglucosamine, △, incorporation into *N*-acetylglucosamine, □.

in Fig. 9. Orosomucoid inhibited incorporation into *N*-acetylglucosamine, and incorporation into orosomucoid was markedly reduced with increasing *N*-acetylglucosamine concentrations. The results obtained using ovalbumin (20 mg/ml) in place of orosomucoid were essentially the same. Here again, either of the two glycoprotein acceptors and *N*-acetylglucosamine was mutually inhibitory. When orosomucoid and ovalbumin were utilized together in the same incubation mixture (Table II), the incorporation of [^{14}C]galactose into the two acceptors was not additive, implying that the transfer of galactose to both glycoproteins was catalyzed by the same enzyme.

DISCUSSION

The results of this study indicate that fractionation techniques previously applied to solid tissues can be applied with modification for successful use with a cultured cell system. The recovery of an enriched Golgi fraction was confirmed by electron microscopy, the elements composing the fraction being quite similar in appearance to Golgi preparations isolated from liver, the tissue used most widely for the isolation of Golgi fractions. The density and sedimentation characteristics of the Golgi apparatus from these neoplastic mast cells must also be quite similar to that of liver Golgi

apparatus, since the centrifugal forces and sucrose densities used to obtain the Golgi preparations in the present study were essentially identical to those used in studies on liver tissue.

As with the Golgi apparatus prepared from solid tissues, the Golgi fraction from the cultured mast cells was enriched in galactosyl transferase activity which could thus be used as a marker enzyme. It is noteworthy that the specific activities of galactosyl transferase in both the purified Golgi fraction (0.4–0.6 μmol galactose transferred per milligram protein per 45 min) and the mast cell homogenate (approx. 0.012 μmol galactose transferred per milligram protein per 45 min) were higher than the values reported for liver Golgi fractions (0.03–0.24 $\mu\text{mol}/\text{mg}$ protein/h [1, 6, 10]) or liver homogenates (0.0017–0.0022 $\mu\text{mol}/\text{mg}$ protein/h [1, 6]). If it is assumed that liver

Golgi fractions are derived mainly from hepatic parenchymal cells, then our results might indicate that the Golgi apparatus of neoplastic mast cells may have a significantly higher galactosyl transferase activity than the Golgi apparatus of liver parenchymal cells. However, the comparison may be misleading, since many of the reported assays for liver galactosyl transferase have been performed at inhibitory concentrations of MnCl_2 or acceptor while our assays were performed at optimal concentrations.

The 5'-nucleotidase enrichment in the mast cell Golgi fractions was greater than the enrichment reported for liver Golgi fractions (2). However, the actual specific activity of our Golgi preparation (~ 0.19 $\mu\text{mol}/\text{mg}$ protein/45 min) was only a fraction of that reported for liver Golgi preparations (1.5–6 $\mu\text{mol}/\text{mg}$ protein/h [1, 2, 10]) and was insignificant in comparison to values for liver plasma membranes (ca. 40 μmoles per mg protein per hr: ref. 2).

Although galactosyl transferase has been described in Golgi fractions isolated from several tissue sources (5–7, 10, 11, 19, 20, 24), generally, a single galactose acceptor has been used and no systematic investigations of the galactosyl transferase activity have been performed. For this reason, it has not been demonstrated previously whether this enzymatic activity is present within the organelle as a family of galactosyl transferases or as a single enzyme. Studies on galactosyl transferase from milk have indicated that a single enzyme is involved (21). It now appears from the present inhibition studies that in the mast cell Golgi apparatus, a single enzyme transfers galactose to two different *N*-acetylglucosamine-containing glycoprotein acceptors and to free *N*-acetylglucosamine.

The galactosyl transferase of Golgi apparatus isolated from neoplastic mast cells is strikingly similar to galactosyl transferase isolated from other tissues. Included in the similarities are pH

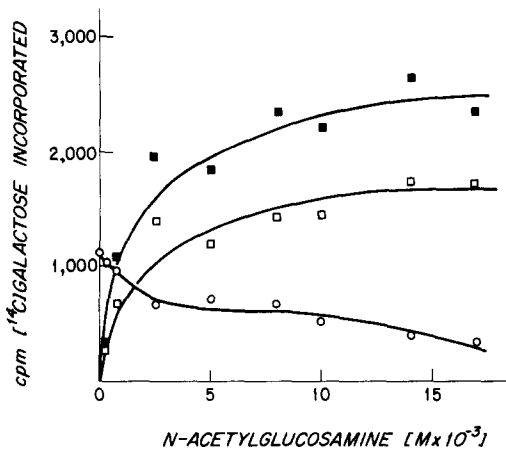


FIGURE 9 Effect of orosomucoid on the incorporation of [^{14}C]galactose into *N*-acetylglucosamine. Incubation and assay were as described in Fig. 4 with MES buffer, MnCl_2 , Triton X-100, UDP[^{14}C]galactose, and Golgi fraction plus *N*-acetylglucosamine as indicated with or without orosomucoid. Incorporation into *N*-acetylglucosamine without orosomucoid, ■, with 2 mg/ml orosomucoid, □, incorporation into orosomucoid, ○.

TABLE II
Competition Studies With Glycoprotein Acceptors

Acceptors	Galactosyl transferase activity*	
	cpm	$\mu\text{mol}/\text{mg}$ protein/45 min
Orosomucoid (2 mg/ml)	930	0.25
Ovalbumin (20 mg/ml)	750	0.19
Orosomucoid (2 mg/ml) + ovalbumin (20 mg/ml)	910	0.24

* Incubation mixtures were similar to those described in Figure 4. Incorporations represent assay of 0.005-ml aliquots after 45-min incubations.

optima (6, 22), requirements for manganese (6, 22, 24), inhibition by higher manganese concentrations (17, 22), and substrate inhibition (17, 23). In particular, the concentrations of *N*-acetylglucosamine and orosomucoid that produce substrate inhibition in our experiments are almost identical to those described for liver Golgi preparations (23).

The purified galactosyl transferase from milk and mammary gland has been examined in detail (17) and the complex kinetics have been shown to involve such phenomena as substrate and cofactor stimulations and inhibitions. An ordered sequence of cofactor addition has been proposed, and substrate inhibition by *N*-acetylglucosamine is thought to be caused by its binding to the enzyme at high concentrations without a concomitant binding of manganese to the enzyme. Protection against substrate inhibition with high manganese concentrations in our experiments might be related, in that manganese at high concentration might effectively compete with substrates to allow ordered incorporation to occur. Alternatively, our results might suggest that higher concentrations of substrates could be effectively removing manganese, resulting in a decreased galactose incorporation. This could then be overcome in part by higher manganese concentrations.

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