Terminal glycosylation in rat hepatic Golgi fractions: heterogeneous locations for sialic acid and galactose acceptors and their transferases

J.J M Bergeron, Jacques Paiement, M N Khan and C E Smith

Departments of Anatomy, Oral Biology, and Medicine, McGill University, and Department of Anatomy Universite de Montreal, Montreal Quebec, H3A 2B2 (Canada)

Endogenous acceptors for *N*-acetylglucosamine (GlcNAc), galactose (Gal) or sialic acid (NeuAc) transfer were labeled to high activities when purified hepatic Golgi fractions were incubated with the corresponding radiolabeled nucleotide sugar in the absence of detergent. The in vitro conditions which were optimal for the endogenous glycosylation of GlcNAc and Gal acceptors (Mn^{2+}, ATP) also promoted fusion within a subset of Golgi membranes. Electron microscope radioautography revealed that the majority of NeuAc acceptors were associated with unfused Golgi membranes, whereas the majority of Gal acceptors were localized to fused membranes. GlcNAc acceptors were approximately equally distributed between fused and unfused membranes. Under conditions in which Golgi membrane fusion was absent ($-Mn^{2+}$), only NeuAc transfer was active. The majority of endogenous NeuAc acceptors were consequently assigned to the more trans regions of the hepatic Golgi apparatus as concluded from a combination of radioautography (NeuAc transfer) and acid NADPase cytochemistry (reactive medial and trans Golgi saccules) The distribution of NeuAc and Gal transferases was assessed after Percoll gradient centrifugation of disrupted Golgi fractions. The median density of NeuAc transferase was lower than that of Gal transferase. The studies are indicative of distinct Golgi components harboring the majority of acceptors and enzymes for terminal glycosylation

Introduction

The Golgi apparatus consists of an anatomically polarized aggregate of stacked and flattened cisternae in continuity with a variety of anastomosing tubular and bulbous extensions at the periphery of the saccules (cf Refs 1-9) The localization of different phosphatase activities to distinct saccules has been established for some time [1,3,10-13] More recently, specific glycosyl transferases and associated glycosidases have been assigned to distinct Golgi subcompartments as defined by subcellular fractionation [14-17], in situ lectin binding [18-21], sugar uptake as assessed by radioautography [22,23] as well as in situ immunocytochemistry of GlcNAc [24] and galactose transferases [25,26] These studies as well as observations on the transport of secretory [27-29] and membrane proteins [30-32] have all indicated subcompartmentation of the Golgi apparatus

In the present study we define the optimal conditions for the glycosylation of endogenous glycopeptide acceptors in purified Golgi fractions

using detergent-free conditions We also attempt to exploit our previous observations on Mn^{2+}- and ATP-induced fusion of Golgi membranes [43] to determine by EM radioautography whether all acceptors for GlcNAc, Gal and NeuAc transfer are localized within the same fused membrane domain The approach required that we also define the distribution of terminal sugar transferases by analytical subfractionation of Golgi components on Percoll gradients

Materials and Methods

Materials Guanosine diphosphate [^{3}H]mannose (GDP-1-[^{3}H]Man, 10 4 Ci/mmol), uridine diphosphate [^{3}H]galactose (UDP-1-[^{3}H]Gal, 11 6 Ci/mmol), uridine diphosphate N-acetyl[^{3}H]glucosamine (UDP-6-[^{3}H]GlcNAc, 24 Ci/mmol) and cytidine monophosphate [^{3}H]sialic acid (CMP-9-[^{3}H]NeuAc, 18 9 Ci/mmol) were obtained from New England Nuclear (Boston, MA) Tunicamycin was a generous gift from Dr R S Dolman (Eli Lilly and Co (Canada) Ltd, Toronto, Ontario) or was purchased from Calbiochem (Calbiochem-Behring Co, La Jolla, CA) NADP was obtained from Boehringer, Mannheim (Montreal) All other chemicals were ordered from Fisher Scientific (Montreal, Quebec) or Sigma Chemical Co (St Louis, MO)

Subcellular fractionation Intact Golgi fractions were isolated directly from liver homogenates by a one-step procedure [33] The Golgi intermediate fraction was purified from liver microsomes isolated from normal rats (no alcohol intoxication) [34,35] For some experiments, the Golgi intermediate fraction was subfractionated in continuous Percoll gradients [35] The enzymic constituents of these Golgi fractions and their morphological properties have been described in detail previously [33,35]

Endogenous glycosylation The incorporation of radiolabeled sugar from the nucleotide sugar substrate UDP-[^{3}H]GlcNAc, UDP-[^{3}H]Gal and CMP-[^{3}H]NeuAc was based on the endogenous galactosylation assay [33] Thus biochemical studies were carried out immediately after the isolation of the subcellular fractions [36] For GlcNAc transfer, the assay consisted of 30 mM sodium cacodylate buffer, pH 6 5, 20 mM $MnCl_2$, 2 mM

ATP 1 μCi of UDP-[^{3}H]GlcNAc (spec act 24 Ci/mmol) and Golgi fractions (20–60 μg) in a final volume of 0 1 ml Incubations were carried out for 10 min at 37°C and stopped by the addition of 1 ml of ice-cold 1% phosphotungstic acid in 0 5 M HCl Bovine serum albumin (100 μg) was added to serve as protein carrier After 30 min at 0°C the pellets were washed twice in phosphotungstic acid-HCl and once with ice-cold absolute ethanol Pellets were dissolved in 0 5 ml of Protosol (New England Nuclear, Boston, MA) and treated with H_2O_2 before determination of radioactive content in a Packard 460 CD liquid scintillation spectrometer For Gal transfer the incubation mixture was identical except that 1 μCi of UDP-[^{3}H]Gal (spec act 11 6 Ci/mmol) was used as substrate For NeuAc transfer, $MnCl_2$ was usually omitted although some experiments were carried out with 5 mM $MnCl_2$ 1 μCi of CMP-[^{3}H]NeuAc (18 9 Ci/mmol) was added to each 0 1 ml assay mixture All incubations were done in duplicate and averaged Furthermore, experiments were carried out on two or more separate occasions and the resultant data were averaged

Exogenous glycosylation Galactosyltransferase was assayed with ovomucoid as acceptor [33,35] Sialyltransferase was determined as described by Bretz et al [37] using asialofetuin as acceptor Asialofetuin was prepared from fetuin by hydrolysis in 0 003 N sulfuric acid at 80°C for 1 h followed by neutralization and dialysis and lyophilization of the purified acceptor

Lipid extraction Organic extractions were carried out as described previously [43] Thin-layer chromatography was done with silica gel G-coated glass plates (Analtech, Inc, Montreal, Quebec) and chloroform/methanol/water (60 25 4, v/v) as solvent [43,38] Mild acid hydrolysis of lipids was carried out as described by Tkacz et al [39] with 0 01 M HCl added to N_2 dried aliquots of the glycolipid extracts DEAE-cellulose chromatography of glycolipids was carried out after preparing columns as described by Rouser et al [40] After the addition of glycolipid the columns were eluted with chloroform/methanol (4 1) followed by the same solvent containing 50 mM ammonium acetate and finally by chloroform/methanol (4 1) containing 250 mM ammonium acetate

SDS-polyacrylamide gel electrophoresis and fluo-

rography Protein samples were electrophoresed in discontinuous SDS polyacrylamide gradient gels (5–15%) modified from the method described previously [33] Fluorography of dried gels was carried out as described by Laskey and Mills [41]

Electron microscopy, radioautography and cytochemistry Unpelleted samples (i e, directly off the continuous sucrose gradient) were fixed in 2 5% glutaraldehyde in 0 05 M sodium cacodylate (pH 7 4) then recovered onto Millipore membranes (Millipore Corp, Bedford, MA) using the Baudhuin et al procedure [42] The samples were postfixed and processed routinely for electron microscopy and electron microscope radioautography [42–44] Quantitation of radioautographs was done using techniques described previously [44]

For the cytochemical studies, intact Golgi fractions were fixed for 20 min in 1% glutaraldehyde at 4°C then recovered onto Millipore membranes [42] The samples were then washed at pH 5 0 in three changes of 0 1 M acetate buffer with 6% sucrose and then incubated for 4 h at 37°C at pH 5 0 in media prepared with 4 mM NADP as substrate [45,46] The fractions were postfixed and processed routinely for electron microscope radioautography [44]

Results

Optimization of glycosylation

Sugar transfer increased temporally for up to 10 min with NeuAc and for 15 min with GlcNAc and Gal (Fig 1a) with approximately linear transfer observed between 200 and 600 μg protein/ml (Fig 1b) The concentration of $MnCl_2$ optimal for glycosylation was 10 mM for GlcNAc, 20 mM for Gal and 5 mM for NeuAc (Fig 1c) using the intact Golgi fraction The absence of $MnCl_2$ resulted in negligible transfer of Gal but 12% of maximal transfer of GlcNAc and 80% for NeuAc ATP was required for the transfer of GlcNAc and Gal, with only 31% and 22%, respectively, of the transfer noted in the absence of ATP (data not shown) The presence of a nucleotide-regenerating system had no effect on Gal transfer in the presence of 2 mM ATP (Fig 1a) Transfer of NeuAc was less dependent on ATP than was the case for GlcNAc or Gal, with 79% of NeuAc transfer observed in the absence of ATP as compared to that

Nucleotide sugar	K_m (μM)	V_{max} (nmol/10 min per mg protein)
UDP-GlcNAc	0 72 ± 0 12	0 52 ± 0 04
UDP-Gal	0 80 ± 0 11	1 48 ± 0 33
CMP-NeuAc	0 36 ± 0 14	0 27 ± 0 08

in the presence of 2 mM ATP (data not shown) The optimum pH for transfer was approx 6 5 (Fig 1d) with a slightly more acidic profile evident for NeuAc transfer than for either Gal transfer or GlcNAc transfer Apparent Michaelis-Menten kinetics were observed when incubations were carried out under optimal conditions (Table I)

Incorporation into proteins

Analysis of the radiolabeled acceptor peptides by SDS-polyacrylamide gel electrophoresis fluorography (Fig 2) indicated that radioactive glycopeptides were similar but not identical in molecular weight for the three labeled sugars Several labeled bands were displaced upwards by 2000 in M_r in the order GlcNAc, Gal, NeuAc Addition of unlabeled sugars (e g unlabeled UDP-Gal or CMP-NeuAc) did not alter appreciably the distinctive profiles for labeled GlcNAc or Gal acceptors

Incorporation into lipids

Approx 3% of the incorporated label was recovered from the $CHCl_3/CH_3OH/MgCl_2$ extractions after GlcNAc transfer, 10% after Gal transfer and 10% after NeuAc transfer Silica gel G chromatography revealed one major GlcNAc lipid, one major Gal lipid but two major NeuAc labeled glycolipid acceptors Mild hot acid hydrolyzed the GlcNAc and NeuAc labeled lipids but
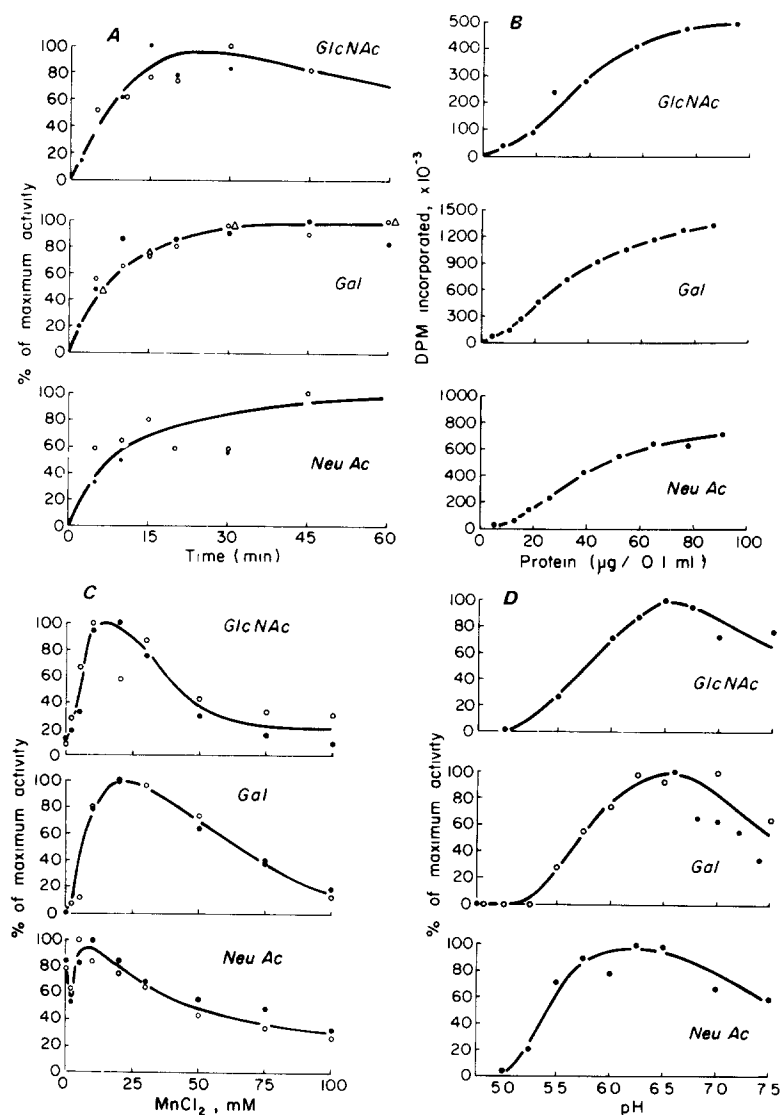
Fig 1 Parameters for endogeous glycosylation Each point represents an average of two determinations and is based on two separate fractionations (open and closed symbols) carried out for each parameter assayed (A) Time course of glycosylation with intact Golgi fractions and UDP-[^{3}H]GlcNAc, UDP-[^{3}H]Gal or CMP-[^{3}H]NeuAc as nucleotide sugar donors In a separate experiment, the effect of the nucleotide-regenerating system consisting of creatine phosphokinase (0 14 mg/ml) and creatine phoshate (1 4 mg/ml) was evaluated (△) for Gal transfer with no effect noted (B) Endogenous glycosylation as a function of Golgi fraction protein concentration for sugar transfer from UDP-[^{3}H]GlcNAc, UDP-[^{3}H]Gal and CMP-[^{3}H]NeuAc nucleotide sugar donors The reaction mixtures were assayed in a volume of 0 1 ml and the times of incubation were 10 min for GlcNAc and NeuAc and 15 min for Gal The specific activities of the nucleotide sugar substrates were 24 Ci/mmol for UDP-[^{3}H]GlcNAc, 11 6 Ci/mmol for UDP-[^{3}H]Gal and 18 9 Ci/mmol for CMP-[^{3}H]NeuAc The concentration of UDP-GlcNAc was 0 57 μM, UDP-Gal, 0 62 μM, CMP-NeuAc, 0 76 μM (C) Effect of MnCl$_2$ concentration on endogenous glycosylation with intact Golgi fraction (D) Endogenous glycosylation as a function of pH with sodium cacodylate as buffer (30 mM)

the galactolipid generally was resistant to acid treatment Both the GlcNAc and NeuAc glycolipids were bound to DEAE-cellulose This was not observed for the galactolipid, 95% of the galactolipid did not bind to DEAE-cellulose and of the remaining 5% of the label, 2% was eluted by 50 mM ammonium acetate and 3% by 250 mM ammonium acetate Tunicamycin inhibited the transfer of GlcNAc and Gal at high concentrations but had no noticeable effect on NeuAc transfer (Fig 3)

Localization of labeled acceptors

EM radioautography was carried out after endogenous glycosylation in the presence and absence of Mn^{2+} (Fig 4) Silver grains were more numerous for GlcNAc or Gal transfer when Mn^{2+}, the Golgi fusogen, was present Comparison of the grain distributions under optimal endogenous transfer conditions (Fig 4b,d,f) indicated an almost equal distribution of GlcNAc acceptors in both fused and unfused membranes (Fig 4b) In contrast, the majority of Gal acceptors were found
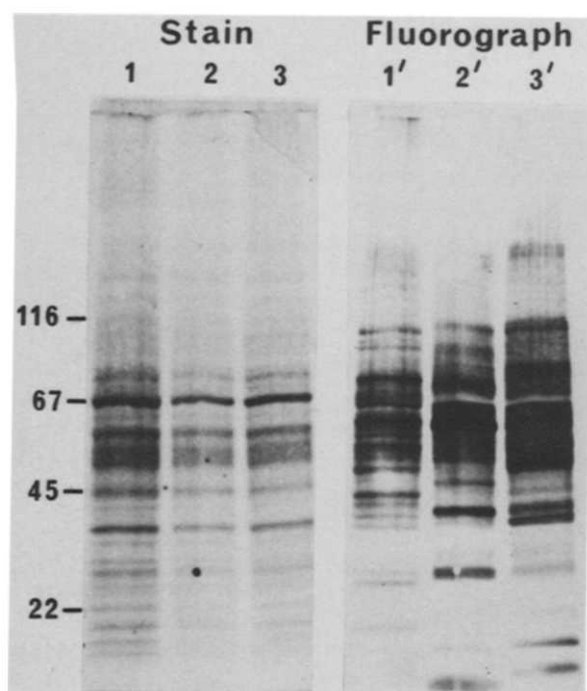
Fig 2 Fluorography of labeled proteins from intact Golgi fraction after incubation in the presence of various radiolabeled nucleotide sugar precursors Intact Golgi fractions (2 4 mg) were incubated 10 min in 0 5 ml of medium containing 25 μCi UDP-[^{3}H]GlcNAc (24 Ci/mmol), 25 μCi UDP-[^{3}H]Gal (11 6 μCi/mmol) and 25 μCi CMP-[^{3}H]NeuAc (18 9 Ci/mmol), respectively After incubation (10 min at 37°C), equal amounts of radioactivity were electrophoresed (1 5 10^6 dpm) Major protein constituents are shown on the left after electrophoresis and staining (1, 2, 3) The major band corresponds to the mobility of albumin On the right is shown the corresponding fluorogram Radioactivity is associated with several Golgi peptides and the profiles are similar but not identical to when fractions were incubated with UDP-[^{3}H]GlcNAc (1'), UDP-[^{3}H]Gal (2') or CMP-[^{3}H]NeuAc (3') The relative mobilities of protein standards are indicated on the extreme left Fluorographic exposure was 2 weeks

in fused membranes (Fig 4d), whereas the majority of NeuAc acceptors were found in unfused membranes (Fig 4f) Quantitation confirmed the qualitative observations (Table II) Since the interpretation of Golgi membrane structure after Mn^{2+}-induced fusion was far from obvious, we focused on NeuAc acceptors which could be labeled to near maximum in a structurally unperturbed Golgi apparatus (Fig 4e) Combined acid NADPase cytochemistry and [^{3}H]NeuAc transfer was carried out (Fig 5), with silver grains observed
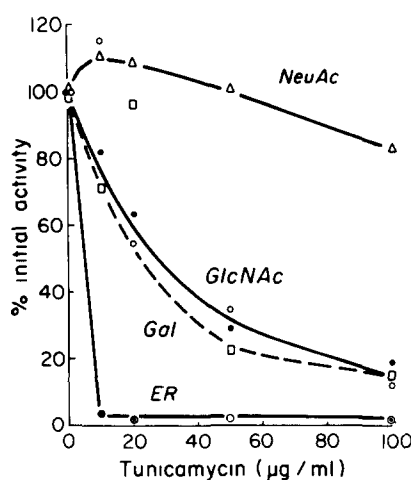

Fig 3 Effect of tunicamycin on endogenous glycosylation with the intact Golgi fraction incubated as described in Materials and Methods with UDP-[^{3}H]GlcNAc ($\square$), UDP-[^{3}H]Gal ($\bullet$, $\bigcirc$) and CMP-[^{3}H]NeuAc ($\triangle$) as nucleotide sugar substrates Control experiments were carried out with purified rough endoplasmic reticulum fractions incubated with UDP-[^{3}H]GlcNAc as described in Ref 44 Tunicamycin was dissolved in dimethyl sulfoxide (20 mg/ml) and added to the incubation media at the concentrations indicated Control experiments with equivalent amounts of dimethyl sulfoxide alone revealed no effect on the transfer of labeled nucleotide sugars under these conditions

primarily over NADPase-reactive components (Table III)

Subcompartmentation of glycosyltransferases

Percoll gradient subfractionation of the dis-

TABLE II

DISTRIBUTION OF GlcNAc Gal, NeuAc ACCEPTORS IN GOLGI FRACTIONS

Silver grains (five analyses) were scored over vesicles and tubules with a lipoprotein content or over empty fused Golgi membranes by direct counting with a resolution boundary circle of 1 HD in radius Values presented are means $\pm$ S D
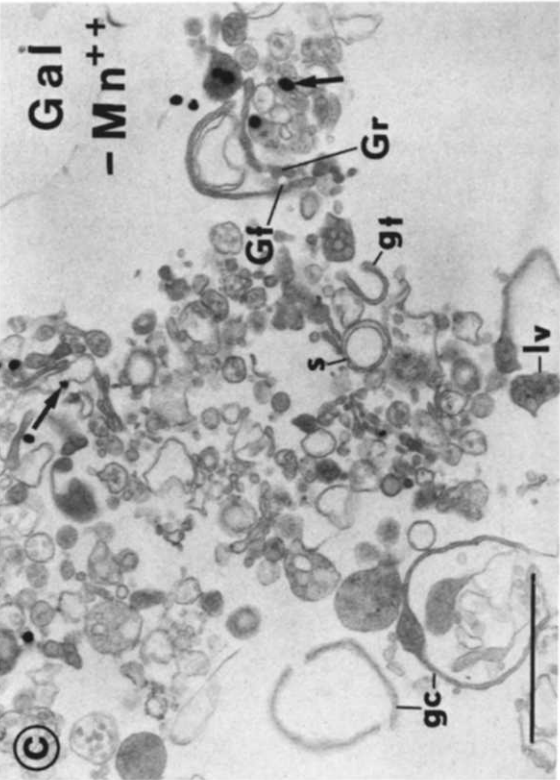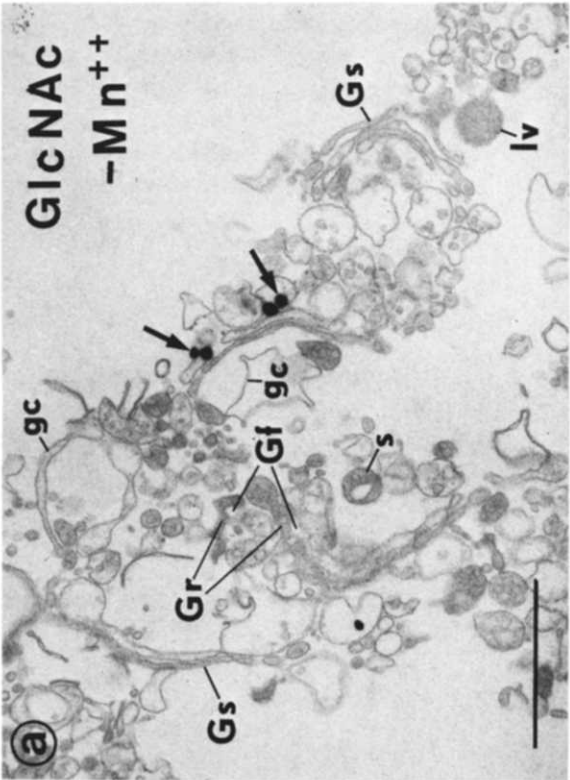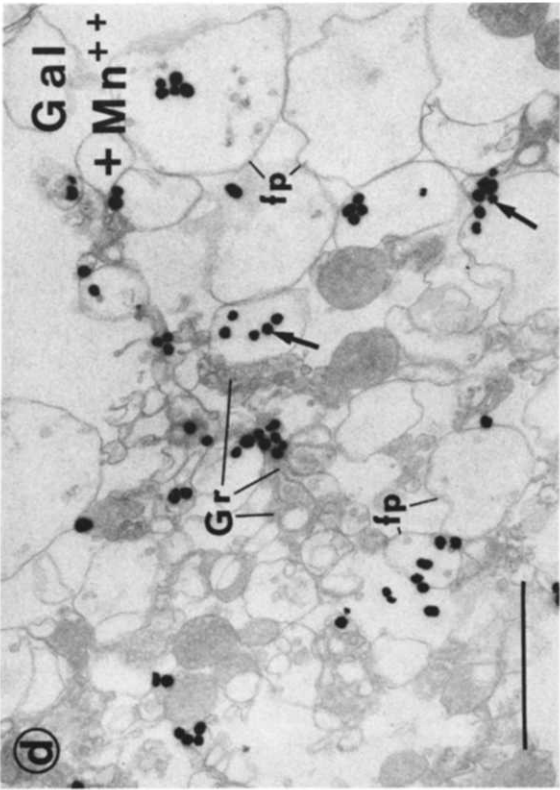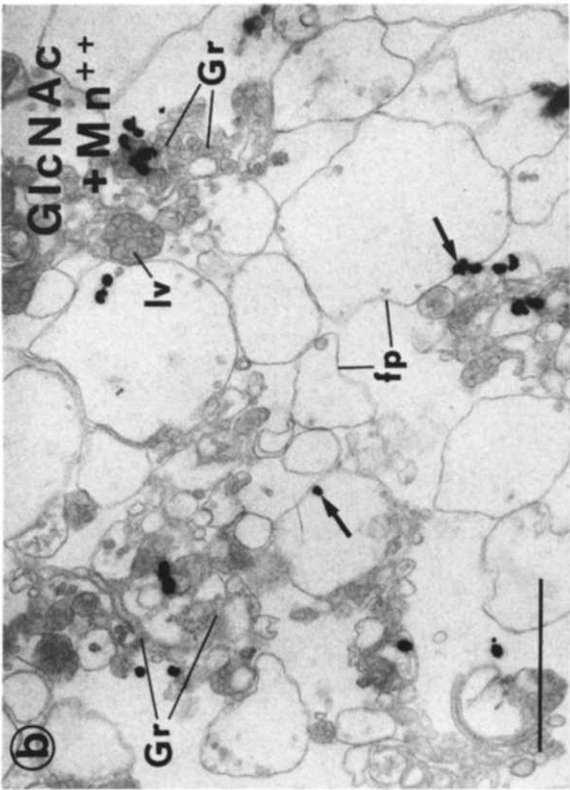
Nucleotide sugar	Distribution of silver grains (%)	
	lipoprotein content	electronlucent content
UDP-GlcNAc (1332) [a]	55 8 $\pm$ 6 2 [b]	44 1 $\pm$ 6 4
UDP-Gal (2675)	39 4 $\pm$ 9 9 [c]	60 6 $\pm$ 9 8
CMP-NeuAc (2781)	68 8 $\pm$ 8 7 [d]	31 1 $\pm$ 8 7

[a] Number of silver grains
[b] GlcNAc vs Gal, $P < 0 009$
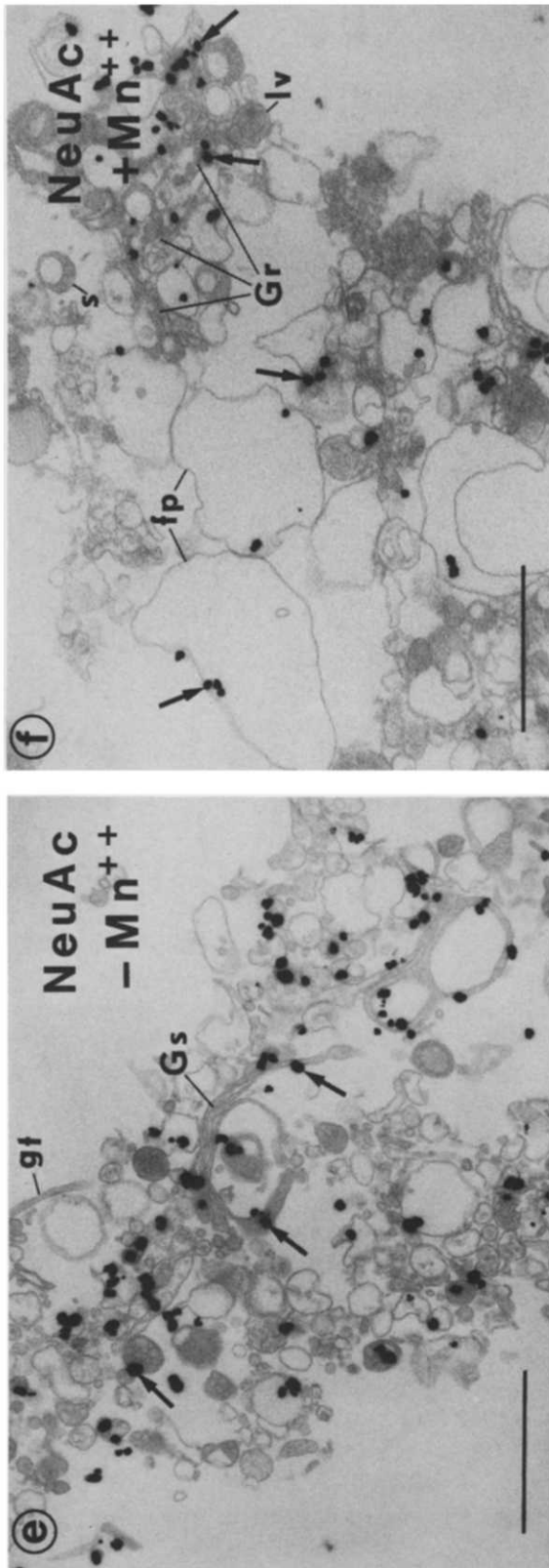[c] Gal vs NeuAc, $P < 0 001$
[d] NeuAc vs GlcNAc, $P < 0 013$

Fig 4 Location of endogenous acceptors by electron microscope radioautography in Golgi fractions after incubations with labeled nucleotide sugar donors Purified Golgi apparatus (36 µg) protein taken directly from the sucrose gradient was incubated for 15 min at 37°C in 0 5 ml of medium containing 30 mM sodium cacodylate buffer, 2 mM ATP in the absence (left side) or presence (right side) of 30 mM $MnCl_2$ and with 30 µCi of UDP-[^{3}H]GlcNAc, 6 6 Ci/mmol (panels a, b) or 20 µCi of UDP-[^{3}H]Gal, 11 6 Ci/mmol (panels c, d) or 30 µCi of CMP-[^{3}H]NeuAc, 18 9 Ci/mmol (panels e, f) After incubation, the fractions were fixed and processed for electron microscope radioautography Despite the 15 min incubation at 37°C, the morphology of the Golgi apparatus in the incubations minus $MnCl_2$ (a, c, e) was almost identical to that of freshly prepared Golgi fractions with characteristic stacks of Golgi saccules (Gs) some of which reveal fenestrations (Gf oblique view) The fenestrations are surrounded by a lipoprotein particle-filled reticulum of interconnecting tubules (Gr) Individual Golgi tubules (gt) and Golgi cisternae (gc) containing low and variable amounts of lipoprotein particles were also revealed as well as signet ring structures (s) and various-sized lipoprotein-containing vesicles (lv) The arrows indicate radioautographic 'fine' silver grains and mark the location of labeled endogenous acceptors Few GlcNAc or Gal labeled acceptors are present in incubations minus $MnCl_2$ (a, c) In contrast, silver grain density is markedly increased with $MnCl_2$ addition (arrows, b d) Large membrane fusion products are generated (fp) coincident with the loss of characteristic Golgi stacks, individual Golgi tubules and individual cisternae Grains from labeled GlcNAc acceptors are particularly evident over lipoprotein-filled elements (Gr, b), whereas grains from labeled Gal acceptors are mainly over the fused Golgi membranes (d) NeuAc acceptors are heavily labeled in the absence (e) or presence (f) of $MnCl_2$, with grains observed preferentially over unfused lipoprotein particle-filled elements (panels e, f) and only few grains over fused membranes (f) Bar 1 µm exposure, 19 days

TABLE III

DISTRIBUTION OF NeuAc ACCEPTORS IN INTACT GOLGI FRACTION

108 μg protein of intact Golgi fraction were incubated at 37°C for 10 min in 1 5 ml of medium containing 15 7 μCi of CMP-[^{3}H]NeuAc (18 9 Ci/mmol) and 30 mM sodium cacodylate A small aliquot was taken and total incorporation was determined to be 8 964 10^6 dpm The remaining fraction was then fixed and treated for NADPase cytochemistry and electron microscope radioautography as indicated in Materials and Methods 22 radioautographs (20 day exposure) having a final magnification of ×38000 were used Area analysis (total area = 660 μm^2) was carried out by the point-hit method Grain distribution was done using a resolution circle having a radius equal to 1 HD The proportion of grains within 1 HD of NADPase-positive and NADPase-negative structures was determined and compared to that over intermembrane spaces In total 2329 grains were analysed Chi-squared analysis indicated a significantly higher (P < 0 001) labeling over NADPase-reactive structures

	NADPase-positive	NADPase-negative	Intermembrane space
Percent volume	29 8 ± 9	18 5 ± 6	51 5 ± 10
Percent grains	76 0 ± 6	17 9 ± 5	6 1 ± 4
Relative grain concentration	2 6	1 0	0 1

rupted Golgi intermediate fraction was carried out and fractions were assayed, in the presence of detergent, for NeuAc transfer to asialofetuin and Gal transfer to ovomucoid (Fig 6) The distribu-

tion of sialyltransferase activity was shifted to lower density values than that for Gal transferase

Discussion

Despite the multiplicity of endogenous acceptors and glycosyltransferases present in Golgi fractions, only a single rate-limiting event of low K_m was observed for each nucleotide sugar donor during endogenous glycosylation This was presumably related to the transport of nucleotide sugars through Golgi membranes Most of the other biochemical properties of endogenous glycosylation were as expected [37,47–49] except for the glycolipid acceptors and the relatively high requirement for Mn^{2+} for endogenous GlcNAc (10 mM Mn^{2+}) and Gal (20 mM Mn^{2+}) transfer

We had previously shown a correlation between Mn^{2+}-dependent membrane fusion and Gal transfer [43] The present study confirmed by EM radioautography that the majority of Gal acceptors were found in fused membranes Braell et al [50] have suggested that fusion of cis and medial Golgi saccules containing acceptors with trans cisternae containing transferases may explain the data The following lines of evidence argue against this (i) the majority of GlcNAc and NeuAc acceptors were not correlated with fused Golgi membranes, (ii) near-maximal transfer of NeuAc was observed
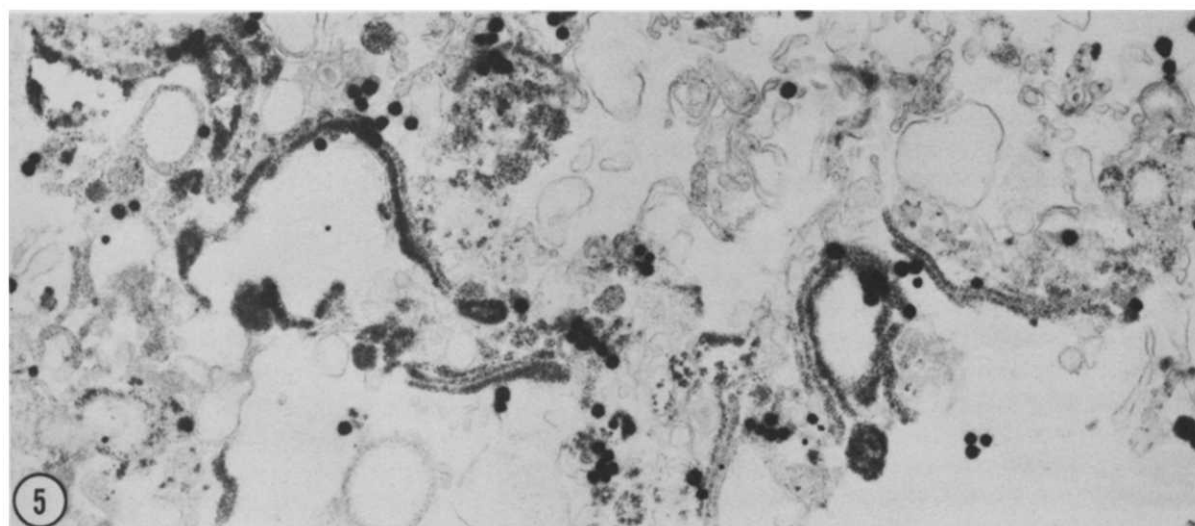

Fig 5 Dual localization of NeuAc acceptors and NADPase activity in the intact Golgi fraction Silver grains indicative of sites of incorporation of NeuAc are mainly distributed over NADPase-positive elements ×32000
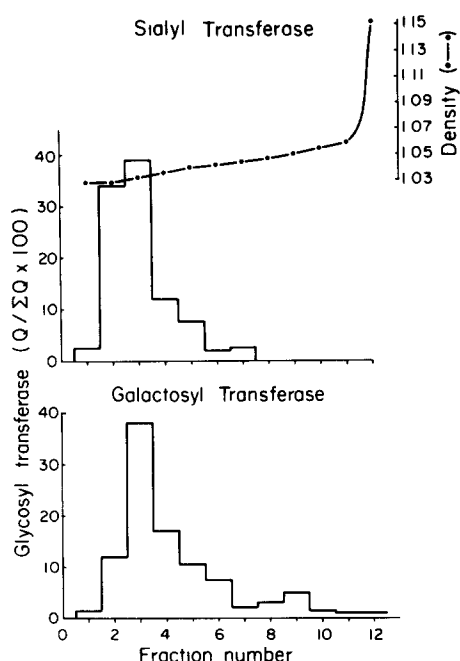
Fig 6 Distribution of sialyl- and galactosyltransferase activities to defined exogenous substrates (asialofetuin and ovomucoid, respectively) A parent Golgi intermediate fraction was subjected to Percoll gradient centrifugation with glycosyltransferase assays and density determinations were carried out on each fraction The parent Golgi intermediate fraction was 35-fold enriched over the homogenate in sialyltransferase activity and 49-fold enriched in galactosyltransferase activity After Percoll gradient subfractionation, peak sialyltransferase activity) was 71-fold enriched over the homogenate, while peak galactosyltransferase activity was 210-fold enriched over the homogenate

under non-fusogenic conditions ($-Mn^{2+}$), (iii) the spectrum of labeled acceptors, as evaluated by SDS-polyacrylamide gel electrophoresis, was unaltered by co-incubation with complementary unlabeled nucleotide sugars As several labeled bands were displaced by approx 2000 in M_r for labeled GlcNAc, Gal or NeuAc acceptors, respectively, then such shifts should have been observed if transport of acceptors had occurred during the conditions of endogenous glycosylation

No evidence for an acid-labile Gal lipid was found The small proportion of charged galactolipid probably corresponded to that previously identified on SDS-polyacrylamide gel electrophoresis [43] The GlcNAc lipid was identified as a dolicholglycophospholipid but did not serve as a

intermediate in terminal glycolysation as based on the tunicamycin studies Very high concentrations of tunicamycin inhibited both GlcNAc and Gal transfer This observation confirms and extends to UDP-GlcNAc, but not CMP-NeuAc, the studies of Yusuf et al [51] on the tunicamycin sensitivity of nucleotide sugar transport through Golgi membranes

We elected to use NADPase as a cytochemical marker since cleaner and more consistent results were obtained with this enzyme compared to thiamine pyrophosphatase in our intact Golgi fractions Angermuller and Fahimi [55] reported strong NADPase activity within medial and trans Golgi saccules of rat hepatocytes We have observed similar Golgi localizations in whole rat liver and intact Golgi fractions [52] Since contiguous NADPase-reactive saccules occupied between 100 and 150 nm at the trans side of the Golgi stack, and considering that the resolution boundary circle for ^3H under our conditions was approx 70 nm for 1 half-distance [53], it seemed reasonable to conclude from the data in Table III that few NeuAc acceptors were associated with the two NADPase-negative saccules comprising the cis side of the stack The exact location of NeuAc acceptors in relation to NADPase-positive medial and trans regions of the Golgi stack could not be resolved by the present study Furthermore, it was not possible to extend these observations to GlcNAc or Gal acceptors because of the loss of normal structural relationships within the Golgi stack that results when high Mn^{2+} concentrations induce membrane fusion

The significantly different proportions of GlcNAc, Gal and NeuAc acceptors in fused vs unfused Golgi membranes was indicative of distinct regions of the Golgi apparatus harboring the majority of their respective acceptor glycopeptides in situ Morre et al [54] have also made similar conclusions based on Golgi apparatus subfractionation using an electrophoretic protocol These observations imply distinct domains in hepatic Golgi apparatus containing the corresponding glycosyltransferases Dunphy et al [15] and Goldberg and Kornfeld [17] observed different peak densities for GlcNAc and Gal/NeuAc transferases by analytical sucrose gradients Using higher-resolution Percoll gradients, we have docu-

mented different median densities for Gal and NeuAc transferases As for the acceptors (vide supra), a complete separation of transferases was not observed, thereby leaving open the possibility of some overlap in situ

These studies therefore extend the concept of intra-Golgi specialization of function [13] The reasons for this subcompartmentation are not clear to us but the wide variety of structural components observed in detailed morphological studies of the in situ Golgi apparatus (e g Refs 2, 4–9, 12) leaves considerable scope for functional differentiation within this organelle

Acknowledgements

References

1 Bennett, G (1984) In Cell Biology of the Secretory Process (Cantin, M , ed), pp 102–147, Karger, Basel

2 Clermont, Y , Lalli, M F and Rambourg, A (1981) Anat Rec 201, 613–622

3 Farquhar, M G and Palade, G E (1981) J Cell Biol 91 775–1035

4 Hermo, L , Clermont, Y and Rambourg, A (1980) Am J Anat 157, 357–373

5 Mollenhauer, H H and Morre, D J (1966) J Cell Biol 29 373–376

6 Mollenhauer, H H , Morre, D J and Bergmann L (1967) Anat Rec 158, 313–317

7 Morre, D J , Hamilton, R L , Mollenhauer H H Mahley, R W Cunningham, W P, Cheetham, R D and Lequire, V S (1970) J Cell Biol 44, 484–491

8 Rambourg, A , Segretain, D and Clermont, Y (1984) Am J Anat 170, 163–179

9 Rambourg, A , Clermont Y and Hermo, L (1979) Am J Anat 154, 455–476

10 Novikoff, A B and Novikoff, P M (1977) Histochem J 9 525–551

11 Novikoff, A B and Goldfischer, S (1961) Proc Natl Acad Sci USA 47, 802–810

12 Novikoff, P M , Novikoff, A B , Quintana, N and Hauw, J J (1971) J Cell Biol 50, 859–886

13 Tartakoff, A M (1983) Int Rev Cytol 85, 221–252

14 Dunphy, W G and Rothman, J E (1983) J Cell Biol 97 270–275

15 Dunphy W G Fries F Urbani L J and Rothman J E (1981) Proc Natl Acad Sci USA 78 7453–7457

16 Elhammer E A and Kornfeld S (1984) J Cell Biol 95 327–331

17 Goldberg, D E and Kornfeld, S (1983) J Biol Chem 258 3159–3165

18 Griffiths G , Brands R Burke, B Louvard D and Warren, G (1982) J Cell Biol 95, 781–792

19 Roth J (1983) J Histochem Cytochem 31 987–999

20 Sato A and Spicer S S (1982) Histochem J 14 125–138

21 Sato A and Spicer S S (1982) Histochemistry 73, 607–624

22 Kramer, M F and Geuze J J (1980) J Histochem Cytochem 28 381–387

23 Bennett G and O'Shaughnessy D (1981) J Cell Biol 88 1–15

24 Dunphy, W G Brands, R and Rothman J E (1985) Cell 40 463–472

25 Roth, J and Berger E G (1982) J Cell Biol 92 223–229

26 Slot, J W and Geuze, H J (1983) J Histochem Cytochem 31, 1049–1056

27 Bergeron, J J , Borts, D and Cruz, J (1978) J Cell Biol 76 87–97

28 Redman C M Banerjee, D , Manning, C Huang, C Y and Green K (1978) J Cell Biol 77 400–416

29 Morre, D J Creek K E Matyas, G R , Minnifield N Sun, I Baudhuin, P , Morre D M and Crane, F L (1984) Biotechniques 2 224–233

30 Bergmann, J E and Singer, S J (1983) J Cell Biol 97 1777–1787

31 Fries E and Rothman, J E (1981) J Cell Biol 90 697–704

32 Rothman J E Miller R L and Urbani L J (1984) J Cell Biol 99 260–271

33 Bergeron, J J M , Rachubinski, R A , Sikstrom R A Posner B I and Paiement, J (1982) J Cell Biol 92, 139–146

34 Bergeron J J M (1979) Biochim Biophys Acta 555 493–503

35 Khan, M N Posner, B I , Khan, R J and Bergeron J J M (1982) J Biol Chem 257, 5969–5976

36 Howell, K E Ito A and Palade G E (1978) J Cell Biol 79 581–589

37 Bretz, R Bretz, H and Palade G E (1980) J Cell Biol 84 87–101

38 Behrens, N H and Tabora E (1978) Methods Enzymol 50, 402–435

39 Tkacz J S , Herscovics, A , Warren, C D and Jeanloz R W (1974) J Biol Chem 249, 6372–6381

40 Rouser G Kritchesky, G , Yamamoto A , Simon, G , Galli C and Bauman, A J (1969) Methods Enzymol 14 272–317

41 Laskey R A and Mills, A D (1975) Eur J Biochem 56 335–341

42 Baudhuin P Evrard, P and Berthet, J (1967) J Cell Biol 32, 181–191

43 Paiement J , Rachubinski, R Ng Ying Kin N M K Sikstrom R and Bergeron, J J M (1982) J Cell Biol 92 147–154

44 Paiement J and Bergeron J J M (1983) J Cell Biol 96 1791–1796

45 Smith C E (1980) J Histochem Cytochem 28, 16–26

46 Parsons, S M and Smith, C E (1984) J Histochem Cytochem 32 989–997

47 Powell J T and Brew K (1976) J Biol Chem 251, 3645–3652

48 Powell, J T and Brew, K (1976) J Biol Chem 251, 3653–3663

49 Smith, C E and Brew, K (1977) J Biol Chem 252 7294–7299

50 Braell W A, Balch W E, Dobbertin, D C and Rothman J E (1984) Cell 39, 511–524

51 Yusuf, H K M, Pohlentz, G and Sandhoff, D (1983) Proc Natl Acad Sci USA 80, 7075–7079

52 Smith, C E Paiement, J and Bergeron, J J M (1984) J Cell Biol 99, 361a (Abstract)

53 Kopriwa, B M, Levine, G and Nadler, N J (1984) Histochemistry 80, 519–522

54 Morre, D J, Morre, D M and Heidrich H G (1983) Eur J Cell Biol 31 263–274

55 Angermuller, S and Fahimi, H D (1984) J Histochem Cytochem 32, 541–546