Mannose Efflux from the Cells
A POTENTIAL SOURCE OF MANNOSE IN BLOOD*

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All mammals have 50–100 μM mannose in their blood. However, the source of the dynamic pool of mannose in blood is unknown. Most of it is thought to be derived from glucose in the cells. We studied mannose uptake and release by various cell types. Interestingly, our results show that mannose taken up by the cells through transporters is handled differently from the mannose released within the cells due to glycan processing of protein-bound oligosaccharides. Although more than 95% of incoming mannose is catabolized, most of the mannose released by intracellular processing is expelled from the cells as free mannose predominantly via a nocodazole-sensitive sugar transporter. Under physiological conditions, incoming mannose is more accessible to hexokinase, whereas mannose released within the cells is protected from HK and therefore has a different fate. Our data also suggest that generation of free mannose due to the processing of glycoconjugates composed of glucose-derived mannose and its efflux from the cells can account for most of the mannose found in blood and its steady state maintenance.

Mannose is an important constituent of N-glycans. The addition of high mannose oligosaccharides to the newly synthesized polypeptide chain and their subsequent processing provide diversity to N-glycan structures. Mannose residues in N-glycans can be derived from either glucose or mannose in the blood. Blood has a metabolically active pool of 50–150 μM mannose in steady state; however, mannose homeostasis is not well understood (1). Dietary contribution is insignificant (2), arguing that most of it is probably derived from an endogenous source. Mannose enters the cells via hexose transporters present at the plasma membrane. It is immediately phosphorylated by hexokinase (HK)2 and then either catabolized via mannose phosphate isomerase (MPI) or diverted toward glycosylation through phosphomannomutase-2 (PMM2) (Scheme 1). Glycosylation precursors, such as mannose 1-phosphate, GDP-mannose, and dolichol phosphate mannose, are used to assemble lipid-linked oligosaccharides (LLO). The glycan portion is then transferred to proteins (3). Mannose, which is incorporated into N-glycans, is trimmed down by mannosidases within the endoplasmic reticulum (ER) and Golgi to generate free mannose. In this study, we show that although more than 95% mannose entering the cell is catabolized, surprisingly, more than 50% of mannose derived from glycan processing and degradation is protected from catabolism and exits the cells as free mannose, most probably via a transporter. This mannose efflux from the cells can account for the majority of mannose found in mammalian blood.

EXPERIMENTAL PROCEDURES

Materials—Most of the reagents were purchased from Sigma except BCA protein assay reagent (Pierce). α-Minimal essential medium, Dulbecco's modified Eagle's medium (DMEM) with and without glucose, DMEM/F-12 (1:1) medium, and equine serum were from Invitrogen. Fetal bovine serum was obtained from Hyclone Laboratories (Logan, UT). Concanavalin A-Sepharose was from Amersham Biosciences.

Radiolabels—[2-3H]Mannose was obtained from PerkinElmer Life Sciences. [6-3H]Glucose (60 Ci/mmole) and [14C]mannose (50 mCi/mmole) were from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

Cell Lines—C3a hepatoma cells, HK293 cells, CHO-K2 hamster ovary cells, SKNSH neuroblastomas, CRL2254 hepatocytes, and CRL1947 fibroblasts were obtained from American Type Culture Collection. Human umbilical vein endothelial cells were from Cell Application, Inc. (San Diego, CA). C2C12 skeletal muscle cells and CHO cell mutant, MI-54, were generous gifts from Drs. Eva Engvall and Sharon Krag, respectively. The cells were grown either in α-minimal essential medium or DMEM containing 2 mm glutamine, 10% fetal bovine serum, and penicillin/streptomycin. Human umbilical vein endothelial cells were grown in endothelial cell medium, from Cell Applications, Inc. Hepatocytes required DMEM/F-12 (1:1) with insulin/transferrin/selenium supplement, 40 ng/ml dexamethasone, and 10% fetal bovine serum for growth. C2C12 skeletal muscle cells were differentiated in high glucose DMEM containing 2% equine serum.

Pulse-Chase Labeling of Cells with [2-3H]Mannose—The cells were grown in individual 60-mm dishes or 6-well plates to 70% confluence and labeled for 1 h at 37 °C in DMEM containing 0.5 mm glucose and 16.6 μCi/ml [2-3H]mannose. Medium was collected, and the cells were washed twice to remove free radiolabel. This was followed by the addition of label-free DMEM containing 5 mm glucose, and the dishes were incubated at 37 °C. In some experiments, the chase medium con-
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Glycoproteins

LLO

Mannose-1-P

HK

PMM2

Man-6-P

MPI

Fru-6-P

SCHEME 1. Mannose metabolic pathway.

Obtained different monosaccharides, sugar analogues, and inhibitors as specified. At each time point, one dish was harvested. After removing the medium, the cells were washed and scraped into either phosphate-buffered saline (PBS) or chloroform/methanol (2:1), depending on the requirement of the experiment.

Analysis of $^{3}$H$_{2}$O in Chase Medium—An aliquot of the medium was used to determine radioactivity with and without evaporation to dryness. The difference was defined as the amount of $^{3}$H$_{2}$O in the medium.

Analysis of [2-3H]Mannose in Chase Medium—Free [2-3H]Mannose in the chase medium was determined by converting it to $^{3}$H$_{2}$O using HK and MPI, followed by evaporation. The difference between radioactivity with and without evaporation was defined as the amount of [2-3H]mannose in the medium.

Isolation of Glycosylation Intermediates—The cells scraped in chloroform/methanol (2:1) were extracted three times with 1 ml of chloroform/methanol (2:1) (fraction I) followed by three extractions with water (fraction II) and then with chloroform/methanol/water (10:10:3) (fraction III). Finally, the protein pellet was solubilized in 0.1% SDS. In some cases, N-linked oligosaccharides were released with PNGase F (4). Free oligosaccharides were separated from the rest of the water-soluble molecules by subjecting fraction II to concavalin A-Sepharose affinity chromatography.

Uptake of [2,3H]Mannose by Various Cells—[2-3H]Mannose radiolabel was dried down and resuspended in water just before the experiment to eliminate traces of $^{3}$H$_{2}$O. Seven different cell lines were grown to 70% confluence and labeled with [2-3H]mannose (33 μCi/ml) for 3 h in DMEM containing physiological concentration of glucose (5 mM) and mannose (50 μM) in a volume of 1.5 ml. 10-μl aliquots were withdrawn after every half hour. At the end of the labeling, the cells were washed and solubilized in 0.1% SDS. Radiolabel in the cell lysate and TCA-precipitated secreted protein in the medium was determined. The medium aliquots were analyzed for $^{3}$H$_{2}$O (as described for chase medium), and background at the zero time point was subtracted to obtain the actual values.

Immunostaining of C3a Cells in the Presence and Absence of Paclitaxel—The cells were grown on round glass coverslips and transferred to four different dishes. The cells were incubated in 0.5 mM glucose DMEM at 37 °C. After 30 min, indicated reagents were added and incubated for another 30 min. The medium was removed, and cells were incubated with fresh 5 mM glucose DMEM with the same additions as mentioned above for 90 min. The cells were fixed with 4% paraformalde-
glycoprotein synthesis. A small percentage of incoming man-
nose used for N-glycosylation is found in glycosylation precur-
sors, such as mannose 1-phosphate, Man-6-P, GDP-mannose,
and dolichol phosphate mannose, which make LLO (Scheme 1).
The oligosaccharide portion from LLO is then transferred to
newly synthesized proteins in the ER. Efficiency of this transfer
is incomplete; therefore, some of the LLO glycans are hydro-
lyzed to free glycans (6) and transported from the ER to the
cytoplasm (7). Glycans on improperly folded proteins exported
from the ER are cleaved by cytoplasmic endo- and exoglycosi-
dases, further adding to the free glycan pool (8). Incubation of
C3a hepatoma cells with [2-3H]mannose-labeled Man-6-P and
Man-1-P within a few seconds and GDP-mannose within 5 min
as determined by high pressure liquid chromatography
(HPLC). Labeled LLO and free glycans reached a steady state in
15 and 30 min, respectively (Fig. 1).

Post-steady State; Free Mannose inside the Cells Obtained
from Glyceran Processing Is Less Prone to Catabolism—C3a cells
were radiolabeled with [2-3H]mannose for 1 h to allow all pre-
cursors to reach steady state. When these labeled cells were
chased in fresh medium without radiolabel, LLO turned over
with a \( t_{1/2} \) of 15 min (Fig. 2a), which is consistent with previous
studies (9). Water-soluble oligosaccharides or free glycan pool
turned over much more slowly. This could be due to either a
slow degradation rate or an ongoing influx of free glycans.
Because HPLC profiles show continuous trimming of high
mannose oligosaccharides (data not shown), it is probably due
to continuous influx of oligosaccharides from LLO hydrolysis
labeled in the initial phase. Free glycans were then degraded
subsequently. More than 50% of the radiolabel from cell-asso-
ciated proteins decreased within the first 2 h, followed by a
much slower loss over the next 4 h (Fig. 2a).

We determined the loss of total \(^{3}\text{H}\) radioactivity from the cells
and its appearance in the chase medium containing 5 mM glu-
cose. The appearance of radiolabel in the medium corre-
sponded to the total radiolabel lost from the cells, and the total
radioactivity remained constant (Fig. 2b).

Next, we determined the identity of radiolabel in the chase
medium. Because our initial experiment showed that more
than 95% of the incoming [2-\(^{3}\text{H}\)]mannose was catabolized
and converted to \(^{3}\text{H}_{2}\text{O}\) (Table 1), we expected radiolabel in chase
medium to be predominantly \(^{3}\text{H}_{2}\text{O}\). Surprisingly, more than

50 – 60% of radioactivity was present as free mannose (Fig. 2c).
Only 5–10% was associated with secreted proteins, and the
remaining radioactivity was in \(^{3}\text{H}_{2}\text{O}\). This result suggests that
mannose released within the cells is not catabolized. This is not
unique to C3a hepatoma cells because we obtained similar
results with six other cell lines, including fibroblasts and muscle
cell lines (data not shown).

This observation raised three important questions. (i) What
is the major source of free mannose inside the cells? (ii) How
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FIGURE 3. Glycan processing contributes the majority of mannose secreted in the medium. C3a cells were labeled for 1 h with 50 μCi of [2-3H]mannose in DMEM containing 0.5 mM glucose and then chased in 5 mM glucose DMEM with a mixture of mannosidase inhibitors (MI) (50 μg/ml kifunensine, 20 μg/ml swainsonine, 20 μg/ml deoxymannojirimycin) and/or lysosomotropic agents (1 mM chloroquine (CHlQ) or 10 mM ammonium chloride (NH4Cl)) for 1 h. Radiolabel was measured in the medium. The data are an average of triplicate determinations.

TABLE 2

| Cell type            | Inhibition by CHlQ and MI |
|----------------------|---------------------------|
| 293 kidney cells     | 42 ± 3.0                  |
| C3a hepatoma cells   | 72 ± 2.5                  |
| CHO cells            | 45 ± 2.0                  |
| 42F fibroblasts      | 61 ± 1.7                  |
| HUVEC                | 66 ± 2.6                  |
| SKNSH neuroblastoma  | 51 ± 3.2                  |
| CRL1947 fibroblasts  | 79 ± 6.5                  |
| C2C12 muscle cells   | 65 ± 8.7                  |

Effect of mannosidase inhibitors and lysosomotropic agents on different cell lines

Seven other cell lines were treated as mentioned in Fig. 3. The data represent an average of triplicate determinations. MI, mannosidase inhibitor; HUVEC, human umbilical vein endothelial cells.

Our results indicated that free mannose in the chase medium was primarily contributed by the mannose released by N-glycan processing in ER and Golgi, which also explained rapid loss of more than 50% 3H radiolabel from cellular protein in the first 1–2 h followed by much slower loss afterward.

Mannose Efflux from the Cells Is Nocodazole-sensitive—To appear in the medium, mannose generated in ER and Golgi must cross ER/Golgi membranes as well as the plasma membrane. The appearance of mannose in the medium can be explained in several ways: (i) diffusion through ER/Golgi and plasma membranes; (ii) facilitated diffusion transporters; or (iii) vesicle-mediated route and fusion with the plasma membrane. With a diffusion constant of 10–10 cm/s for hexose sugar through the lipid bilayer, it would take ~50–150 min to cross a cell membrane of 3–10 nm (11). This time would double for crossing two membranes. In our experiments, we saw immediate release of free mannose within 2–5 min, which rules out simple diffusion as the mechanism of mannose loss from the cell.

To determine if mannose exits cells through a vesicular route, the cells were labeled with [2-3H]mannose and then chased in the presence of various inhibitors of vesicular transport, which block different steps of vesicle-mediated trafficking. Brefeldin A induces tubulation of secondary endosomes and lysosomes as well as merging of Golgi with ER, which disrupts trafficking of transport vesicles within the cell (12). Nocodazole and colchicine are microtubule-depolymerizing agents (13, 14), and bafilomycin A is an inhibitor of vacuolar H+-ATPase that prevents lysosomal acidification (15). None of the inhibitors had any effect on mannose transport (data not shown) except for nocodazole.

Nocodazole inhibited mannose efflux in a dose-dependent fashion (Fig. 4a). Although nocodazole is a microtubule-disrupting agent, it also inhibits glucose transporters, independent of its effect on microtubules (16). If the inhibition of mannose transport by nocodazole is due to microtubule disruption, then the addition of paclitaxel (taxol), a microtubule-stabilizing reagent (17), should reverse the inhibitory effect. When the chase was done in the presence of 40 μM paclitaxel and 25 μM nocoda-
Zole, inhibition of mannose efflux was still observed (Fig. 4b). We checked microtubule stabilization by immunofluorescence. Nocodazole-treated cells showed no staining of microtubules because of the depolymerization (Fig. 4e) as compared with untreated cells (Fig. 4c) and paclitaxel-treated cells (Fig. 4d), where microtubules are intact. Paclitaxel (40 μM) stabilized microtubules even in the presence of 25 μM nocodazole (Fig. 4f). Colchicine, which is another microtubule-depolymerizing agent, did not show any effect on mannose efflux (data not shown). All of these results clearly demonstrate that nocodazole is able to inhibit a sugar transporter that is responsible for mannose efflux, and the effect of nocodazole is not due to inhibition of vesicular transport.

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Mannose Pool Generated inside the Cells by Glycan Processing Is Shielded from Hexokinase—When we added phloretin, an inhibitor of facilitated hexose transporter to the chase medium, interestingly, we observed the same amount of radiolabel in the medium, but the proportion of [2-3H]mannose and 3H2O increased and [2-3H]mannose decreased (Fig. 5a). In other words, more endogenous free [2-3H]mannose obtained from glycan processing was catabolized ([2-3H]Man-6-P → fructose 6-phosphate + 3H2O) in the presence of phloretin. Phloretin blocks facilitative hexose transporters on the plasma membrane, which inhibits influx of glucose (5 mM) present in the chase medium. Incoming glucose competes for cellular enzymes with endogenous free mannose that is released within the cells. There are two enzymes involved in the production of 3H2O from [2-3H]mannose, HK and MPI (Scheme 1). Protection of endogenously released mannose from one of these enzymes could explain the differential fate of this pool from the fate of incoming mannose.

Pulse-chase experiments with human CDG-Ib cells deficient in MPI by 95% did not show any change in the proportion of [2-3H]mannose and 3H2O produced, making it unlikely that a limitation of MPI is responsible for the accumulation of [2-3H]mannose in the medium (data not shown). To determine

FIGURE 4. a, nocodazole inhibits mannose efflux. C3a cells were labeled for 1 h. After 30 min, during labeling, the indicated amount of nocodazole was added for preincubation. This was followed by a chase in fresh DMEM containing the indicated amounts of nocodazole. At different times, an aliquot of medium was collected, and [3H]mannose was estimated using hexokinase and MPI (as mentioned under “Experimental Procedures”). b, inhibition of mannose release by nocodazole is independent of microtubule depolymerization. The procedure is the same as mentioned above except that chase was done for 1 h with medium containing nocodazole either in the absence or presence of taxol. Free [3H]mannose was estimated in the medium using hexokinase and MPI (as mentioned under “Experimental Procedures”). c–f, microtubule stabilization by paclitaxel under experimental conditions. C3a cells were grown on glass coverslips and treated with nocodazole in the absence (e) and presence (f) of paclitaxel as described under “Experimental Procedures” and compared with no treatment (c) and paclitaxel alone (d). The cells were fixed, permeabilized, and stained for α-tubulin. Error bars, S.E.

FIGURE 5. Effect of phloretin, sugars, and sugar analogs. C3a cells were labeled with [2-3H]mannose for 1 h. This was followed by a chase in fresh DMEM containing the indicated amounts of the additives. a, phloretin-[1H]mannose and 3H2O were determined in the medium. Sugars (b) and sugar analogs (c) 3H2O were estimated in the medium. The data represent the average of triplicate determinations. Error bars, S.E.
if HK is the limiting enzyme, various sugars and sugar analogs were tested as extracellular competitors. Glucose, mannose, and 2-deoxyglucose are HK substrates, whereas fructose, galactose, and 6-deoxyglucose are not HK substrates. Increasing the concentration of exogenous glucose, mannose, and glucose analog, 2-deoxyglucose, in the chase medium decreased the proportion of $^3$H$_2$O in the medium, whereas fructose, galactose, and 6-deoxyglucose did not (Fig. 5, b and c). These results were consistent with glucose, mannose, and 2-deoxyglucose competing with endogenous mannose for either hexokinase or ATP. Fructose and galactose require ATP for activation and do not require hexokinase to phosphorylate the substrate to its 1-phosphate form. Thus, it is more likely that exogenous glucose and mannose compete with the released [2-3H]mannose for HK rather than competition for a limited amount of ATP.

In order to further confirm our hypothesis, we chose a CHO cell mutant, MI-54, with a 90% deficiency in hexokinase activity ($4.3 \pm 1.2$ nmol/min/mg) compared with wild type ($32.5 \pm 2.5$ nmol/min/mg). In a cell line that is already limited for HK, a lower concentration of exogenous sugar should be able to compete with endogenous [2-3H]mannose for HK as compared with wild type in reducing the proportion of $^3$H$_2$O in the medium. When labeled cells were chased with different concentrations of glucose, as low as 0.1 mM glucose could compete with hexokinase, resulting in decreased catabolism of [2-3H]mannose compared with wild type, which required 2.5 mM (Fig. 6). Our results clearly demonstrate that mannose released from glycan processing and degradation is much less accessible to hexokinase than mannose taken up by the cells through transporters. As a result, endogenous mannose released within the cell is released as free mannose. This return of endogenous mannose to the medium would probably contribute substantial amounts of mannose to mammalian plasma.

Most of the Mannose in N-Glycans Is Glucose-derived—Because most of the free mannose exiting the cells is contributed substantially by HK-deficient CHO and (MI-54) cells were labeled for 1 h. This was followed by chase in fresh DMEM containing the indicated amounts of glucose. $^3$H$_2$O formation was estimated by counting medium with and without evaporation. The data represent an average of triplicate determinations. Error bars, S.E.

TABLE 3
Glucose-derived mannose in N-glycans
The cells were dual labeled for 1 h with [6-3H]glucose and [14C]mannose in the medium containing 5 mM glucose and 50 mM mannose. Monosaccharides were analyzed by HPAEC-PAD on a CarboPac PA1 column. The contribution to mannose in glycoproteins from glucose and mannose was calculated. HUVEC, human umbilical vein endothelial cells.

| Cell lines tested | Contribution from glucose |
|-------------------|---------------------------|
| 293 kidney cells  | 99                        |
| C3a hepatoma cells| 99                        |
| Primary hepatocytes| 98                        |
| C2C12 muscle cells| 97                        |
| SKNSH neuroblastoma| 96                       |
| HUVEC             | 92                        |
| 42F fibroblasts   | 90                        |
| CRL 1947 fibroblasts| 80                      |

DISCUSSION
The source of mannose in plasma and its homeostasis remains to be fully elucidated. Glycogen breakdown has been proposed as a possibility, as shown in rats in response to epinephrine administration (19). Glycogen phosphorylase is activated upon induction of ER stress, which could provide glycosylation precursors during stress (20). These processes may account for a portion of mannose under certain metabolic states but do not explain the steady state of mannose in plasma under normal conditions. We propose a mechanism that is applicable under normal conditions to maintain mannose levels in plasma.

Interestingly, more than 95% of mannose entering various cells is catabolized, whereas mannose produced from glycan processing (endogenous mannose pool) is not metabolized but is expelled from the cells, presumably via a transporter. At a physiological concentration of 5 mM, glucose influx substantially competes for phosphorylation by HK and makes the enzyme less accessible to the endogenous pool of mannose. Substrate channeling through enzyme complexes might explain different handling of endogenous mannose from that of incoming mannose. There are many synthetic and degradation processes that occur in the same organelle and require compartmentalization of metabolic networks and pathways (21). There is a partitioning of glycolytic enzymes between cytomatrix-bound and aqueous cytoplasm free forms. High cytomatrix association concentrates the enzymes for most efficient substrate channeling (21). Glycolytic enzymes are bound to the plasma membrane and contractile apparatus of vascular smooth muscle cells and to the actin cytoskeleton, microtubules, and outer mitochondrial membranes of other cell types (22). Their localization depends on the metabolic state. Hardin and Finder (23) demonstrated that none of the glycolytic intermediates except fructose 6-phosphate is free in solution when studied in gently permeabilized vascular smooth muscle cells, implying a highly ordered assembly of the glycolysis machinery in the cytoplasm. The association of glycolytic enzymes with a variety of cytoplasmic structures results in strict channeling of glycolytic intermediates. Preferential substrate entry and exit from such complexes may be necessary for high metabolic efficiency. For example, fructose 6-phosphate lies at the intersec-
tion of several metabolic pathways, including glycosylation (Man-6-P, N-acetylglucosamine 6-phosphate), glycolysis (fructose 1,6-diphosphate), hexose monophosphate shunt, and gluconeogenesis (Glc-6-P). Channeling common substrates into different pathways may rely on subtle differences in the organization of multienzyme complexes.

Type III HK has perinuclear localization, whereas Type I and Type II HK have been shown to be associated with mitochondria (24). Such a location gives HK preferential access to ATP too. Under certain conditions, HK is mobilized to actin filaments, and its activity increases (25). In certain cells, hexose transporters are colocalized with HK (26). In pancreatic cells, enzyme to enzyme tunneling of hexose phosphates in the hexokinase/phosphoglucoisomerase/phosphofructokinase sequence has been postulated (27). In hepatoma cells, Glc-6-P generated by glucokinase is in a compartment that is accessible to several enzymes, whereas Glc-6-P produced by HK is directed to a compartment with restricted access (28). We propose similar regulated channeling of incoming mannose, which might explain why this mannose pool has more privileged access to hexokinase compared with endogenous mannose generated from glycan processing compared with endogenous mannose generated from glycan processing at various locations within the cell at physiological glucose concentration. Consequently, the majority of the endogenous mannose escapes catabolism and is exported from the cell.

Calculations based on our experiments suggest that efflux of endogenous mannose substantially contributes blood mannose levels. All of the cell lines we tested returned the majority of mannose derived from glycan processing directly to the medium. Previous in vivo studies in mice have shown that liver and intestines incorporate a substantial amount of [3H]mannose into glycoproteins, whereas muscle and heart incorporate 10–15-fold less radiolabel. Assuming that differentiated cultured muscle cells model the in vivo situation, we calculate that muscle cells release ~0.73 nmol of mannose/mg/h into the medium under physiological conditions. An average mouse of 30 g has a 12-g muscle weight and 1.32 g of muscle protein (29). Thus, mannose (derived primarily from glucose in muscle) is released at a rate of 0.96 µmol/h. Mouse plasma contains 100 µM mannose, which turns over at t½ = 28 min (18). Given the plasma and interstitial fluid volumes (7.8 ml), this would utilize ~0.85 µmol of mannose/h. Under physiological steady state conditions, muscle alone could provide the bulk of plasma mannose. Other cell types are also net producers of plasma mannose, and in each case, the majority is derived from glucose (Table 3).

The phenomenon of mannose efflux might also explain why CDG-I patients have lower blood mannose levels (5–40 µM) as compared with normal individuals (40–80 µM) (30). CDG-I patients have underglycosylated proteins, and if glycoprotein processing is the major source of mannose in the blood, then proteins in the patients would generate less endogenous mannose, resulting in a lower mannose concentration in the blood.

There are several reasons why cells may want to prevent intracellular accumulation of free mannose. Within the ER and Golgi, it could interfere with normal glycosylation processes by competing with glycosidases and proofreading lectins (31). Man-6-P in the cytosol could be detrimental because in vitro studies show that Man-6-P can reduce the amount of LLO available for protein N-glycosylation (32). Mouse embryonic fibroblasts lacking all MPI activity accumulate lethal concentrations of Man-6-P when exposed to exogenous mannose (33). Also, mannose is 5-fold more reactive than glucose in protein glycation (34); therefore, mannose could increase the amount of undesired intracellular glycated proteins. For mannose released from ER/Golgi/lysosomes to exit, cells would require a transport mechanism from the organelle to cytoplasm and across the plasma membrane. Rapid efflux can only be mediated by transporters and/or vesicles as opposed to simple diffusion. Facilitated diffusion of hexoses through glucose transporters is 50,000 times more rapid than simple diffusion (35).

Mannose is transported by most of the known glucose transporters. Lysosomes have a facilitated hexose transporter (Kₘ = 75 mM) for neutral monosaccharides, such as D-glucose, D-galactose, D-mannose, and D- and L-fucose (36). An ER-localized bidirectional glucose transporter distinct from plasma membrane GLUTs has been reported in HepG2 hepatoma cells (37). A recent study has described a novel class of transporters, SWEETs, which are distinct from the two already known classes (GLUTs and SGLT) and are present in several different species (38). They mediate glucose efflux. There is one copy of SWEET in humans (hsSWEET), which is ubiquitously expressed in all of the tissues. hsSWEET is mainly present in the Golgi and some at the cell surface (38). Although mannose transport was not specifically checked in mammalian cells, there is a possibility of involvement of this new class of transporter in mannose efflux from Golgi to cytoplasm and subsequently outside the cell. Our results suggest that rapid mannose efflux involves a nocoazol-e sensitive transporter either at the ER/Golgi or cell membrane or both. Nocodazole also inhibits [3H]deoxyglucose uptake in intact cells (16). Therefore, the same transporter at the cell surface could be involved in transporting endogenous mannose out of the cell. Facilitated sugar transporter inhibitors to the medium decreased transport of exogenous glucose into the cell and led to catabolism of endogenously generated [3H]mannose, producing more 3H₂O, which rapidly equilibrates with the extracellular milieu. This made it more difficult to provide direct evidence of a transporter for mannose efflux through the cell membrane in intact cells because endogenous [3H]mannose is no longer available in its free form.

In summary, we propose a mechanism by which mannose produced by glycan processing exits the cells, presumably by transporters. The extent of mannose flux is sufficient to account for mannose homeostasis in the blood. Maintenance of the blood mannose level is beneficial for CDG-Ib patients who are deficient in MPI. Mannose supplementation increases its concentration in the blood and provides enough substrate to reverse most of their pathological symptoms resulting from insufficient glycosylation.

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