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Glycosylation of VSV Glycoprotein Is Similar in Cystic Fibrosis, Heterozygous Carrier, and Normal Human Fibroblasts

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The single envelope glycoprotein of vesicular stomatitis virus was used as a specific probe of glycosyltransferase activities in fibroblasts from two cystic fibrosis patients, an obligate heterozygous carrier and a normal individual. Gel filtration of pronase-digested glycopeptides from both purified virions and infected cell-associated VSV glycoprotein which had been labeled with [^{3}H]glucosamine did not reveal any significant differences in the glycosylation patterns between the different cell cultures. All 4 cell lines were apparently able to synthesize the mannose- and glucosamine-containing core structure and branch chains terminating in sialic acid which are characteristic of asparagine-linked carbohydrate side chains in cellular glycoproteins. Analysis of tryptic glycopeptides by anion-exchange chromotography indicated that the same 2 major sites on the virus polypeptide were recognized and glycosylated in all 4 VSV-infected cell cultures. These studies suggest that the basic biochemical defect(s) in cystic fibrosis is not an absence or deficiency in enzymes responsible for the biosynthesis of complex carbohydrate side chains.

Key words: virus glycoprotein, cystic fibrosis, glycosyltransferases

Cystic fibrosis is the most common lethal genetic disease among Caucasian children, and is presumed to be transmitted as an autosomal recessive trait (1, 2). This disease is characterized by chronic pulmonary disease and pancreatic insufficiency, both of which may be secondary to a general abnormality in mucous secretions. The basic biochemical defect(s) of cystic fibrosis is unknown. Although the abnormalities are thought by a number of investigators to be expressed in all body tissues including skin fibroblasts in tissue culture (1, 2), there are no reproducibly detected abnormal phenotypes characteristic of cystic fibrosis fibroblasts. The phenomenon of elevated sweat electrolytes is utilized as a diagnostic test for cystic fibrosis, but this "sweat test" is not always reliable or easily interpreted. At present there is no methodology for either detection of "heterozygote carriers" or for prenatal diagnosis of cystic fibrosis.

Abbreviations: VSV – vesicular stomatitis virus; C.F. – cystic fibrosis

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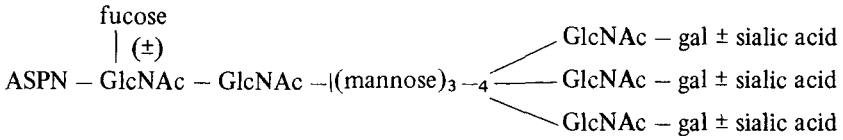
Because of possible abnormalities in mucous secretion and cell membrane function, a defect in glycoprotein metabolism has been suggested as a possible site of the biochemical defect(s) (1,3). There have been several conflicting reports concerning differences in glycoprotein glycosyltransferases activities between normal and cystic fibrosis tissue (3–5), but there is little definitive evidence for differences in glycoprotein metabolism between normal and cystic fibrosis cells.

We have used the envelope glycoprotein of vesicular stomatitis virus, a lipid-containing animal virus, as a specific in vivo probe of glycosyltransferase activities in normal, "heterozygous carrier," and "homozygous" cystic fibrosis fibroblast cells. Fibroblasts were used even though the expression of the cystic fibrosis defect(s) in these cells is questionable because: i) they can be easily grown in tissue culture, and ii) possible differences observed between normal and cystic fibrosis fibroblasts could potentially be used in a prenatal detection assay. VSV matures by budding through the host cell membranes which have been modified by the insertion of the virus glycoprotein (G) and a nonglycosylated matrix protein (M) into the lipid bilayer composed of host cell lipids and glycolipids (6–12). The virus RNA genome codes for the G polypeptide chain, but host enzymes are responsible for the glycosylation of the virus glycoprotein. The carbohydrate moiety of the G protein has been well characterized for VSV grown in a number of tissue culture lines, and consists of 2 major N-glycosidically linked oligosaccharide side chains per glycoprotein, with the following structure (13–17):



METHODS

Human fibroblast cell lines were obtained from the Human Genetic Mutant Cell Repository at the Institute for Medical Research in Camden, New Jersey: "normal" (GM123), "obligate heterozygous carrier" (GM849), and "homozygous" cystic fibrosis (GM770 and GM1012). Cells were grown in monolayer culture in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum, nonessential amino acids, glutamine, and penicillin/streptomycin (all from Flow Laboratories, Rockville, Maryland).

Stock preparations of VSV (Indiana serotype) were grown in HeLa S3 suspension cultures, purified, and assayed as described previously (18, 19). Confluent cultures of human skin fibroblasts were infected with 10 plaque forming units of VSV per cell. VSV-infected cells were radiolabeled from 4 to 16 h postinfection with 10 μCi/ml D-[6-^{3}H] glucosamine (5–15 Ci/mmole), 10 μCi/ml D-[1-^{3}H] galactose (5–10 Ci/mmole) or 1 μCi/ml D-[1-^{14}C] glucosamine (45–55 mCi/mmole) (all from New England Nuclear Corporation, Boston, Massachusetts) in MEM with 2% serum and one third the normal concentration of unlabeled glucose. Alternatively, cultures were labeled with 10 μCi/ml [^{35}S] methionine (New England Nuclear Corporation; 100 Ci/mmole) in MEM with 2% serum but lacking unlabeled methionine. Virus was harvested from the supernatant medium and purified by equilibrium and velocity sedimentation in preformed sucrose gradients (18).

Radioactive proteins from infected cell membranes (12) or purified virions were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (11), and dried slab gels were subjected to fluorography (20).

Radioactive sugar-labeled glycoprotein from purified virions or infected cell membranes was digested with pronase (B grade, Calbiochem) and chromatographed on Bio Gel P-4 (Bio Rad Laboratories) as previously described (12). Alternatively, virus glycoprotein was digested with trypsin (TPCK-treated; Worthington) and analyzed by anion exchange chromatography on DEAE-cellulose (Whatman DE-52) (16). Prior to exoglycosidase treatment, pronase glycopeptides were chromatographed on Sephadex G15/G50 (Pharmacia) to remove undigested protein and salts present in the pronase digest mixture. Pronase- or trypsin-digested glycopeptides were treated with neuraminidase (Clostridium perfringens) as described earlier (12).

RESULTS

Analysis of purified virions and the membrane fraction of VSV-infected cells by SDS-polyacrylamide gel electrophoresis indicated that the VSV G protein was the only major glycoprotein which was synthesized in infected cells and assembled into mature virions (Fig. 1), as expected from the shut-off of host protein synthesis by VSV (18). Slight differences in the mobility of G protein from VSV grown in different cell lines (compare C.F. (GM1012) to "carrier" (GM849) and C. F. (GM770)] can be attributed to heterogeneity in the amount of terminal sialic acid (J. Robertson, personal communication). The nonglycosylated VSV proteins had electrophoretic mobilities identical to those of the [^{35}S] methionine-labeled proteins in VSV grown on HeLa cells, based upon the pattern of methionine-labeled proteins from VSV grown on cell line GM849 (Fig. 1) and the pattern of Coomassie Brilliant Blue-stained polypeptides (not shown) for VSV grown on all 4 cell lines.

Detailed analysis of [^{3}H] glucosamine-labeled virus glycopeptides was undertaken to determine possible differences in the carbohydrate moieties which could reflect abnormalities of glycosyltransferases of either "heterozygous carrier" or "homozygous" cystic fibrosis fibroblasts. Pronase-digested glycopeptides from VSV grown on each cell type were subjected to gel filtration on Bio Gel P-4 (Fig. 2). In all 4 cases, the glycopeptides were resolved into 3 major peaks [designated S_1, S_2, and S_3 (12)] and several minor peaks corresponding to undigested material in the void volume and "S_0" glycopeptides eluting just before the S_1 glycopeptides.

Treatment with neuraminidase to remove terminal sialic acid resulted in a single major peak comigrating with the S_3 glycopeptides of untreated glycopeptides with all 4 samples (Fig. 2). Therefore, heterogeneity in terminal sialic acid (see postulated structure in Introduction) was responsible for the multiple glycopeptide peaks, as previously observed for VSV grown in HeLa cells (12) and BHK cells (17). This elution pattern was due to a negative charge exclusion property of Bio Gel resins. These results suggested that the cystic fibrosis cells (both "heterozygous carrier" and "homozygous" cystic fibrosis) have the enzymatic capacity to add both the oligomannosyl core and the branch chains terminating in sialic acid. The differences in the relative amounts of [^{3}H] glucosamine label in the 3 major peaks between the 4 samples were not considered significant with respect to the cystic fibrosis genotype(s) because: i) the difference between the VSV glycopeptides from the 2 cystic fibrosis cell lines (GM1012 and GM770, Fig. 2) was as great as the differences between the "normal" cell line (GM123) and the cystic fibrosis "heterozygous carrier" or "homozygous" cell lines, and ii) these differences were minor compared to the major differences in terminal sialic acid content and glycopeptide distribution on Bio Gel columns previously reported between HeLa-grown VSV and BHK-grown VSV (12–14, 17).

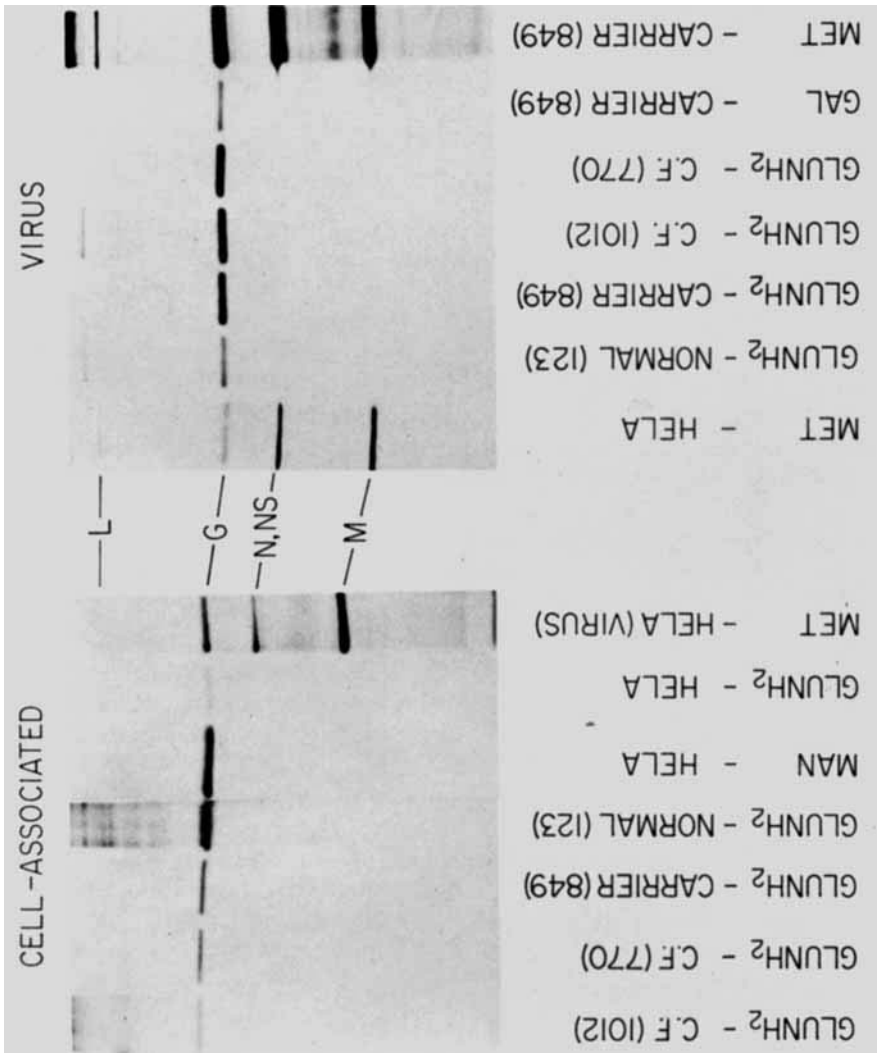


Fig. 1. SDS-polyacrylamide gel profile of residual cell-associated membrane protein and purified vesicular stomatitis virus grown in normal, "homozygous" cystic fibrosis, and "heterozygous carrier" human fibroblasts. The approximate molecular weights of the VSV (HeLa grown) marker proteins are: L, 200,000; G, 67,000; N, 50,000; M, 25,000–30,000 (8).

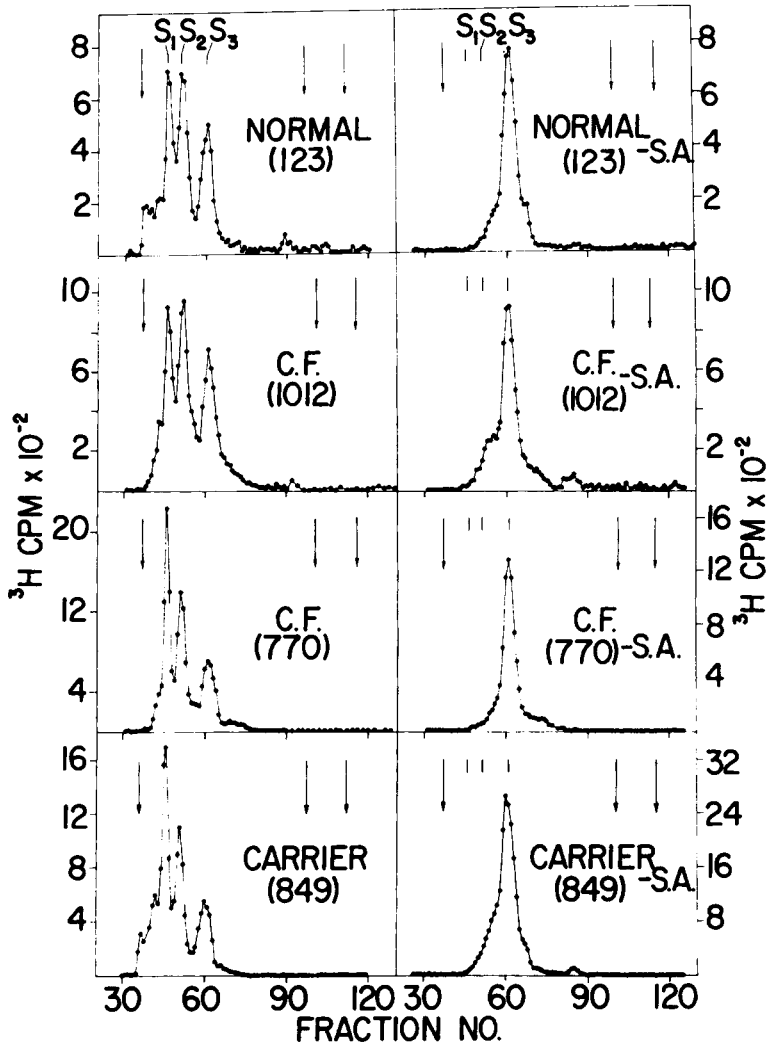


Fig. 2. BioGel P-4 gel filtration of pronase-digested glycopeptides from [^{3}H] glucosamine-labeled VSV grown in normal, "homozygous" cystic fibrosis, and "heterozygous carrier" human fibroblasts. The column markers are blue dextran (void volume, approximately 4,000 mol. wt.), stachyose (666 mol. wt.), and mannose (180 mol. wt.). S_0, S_1, S_2, and S_3 refer to glycopeptide peaks which differ in their content of terminal sialic acid (probably 3, 2, 1, and 0 residues), which can be enzymatically removed by neuraminidase.

The differences in the relative amounts of S_1–S_3 glycopeptides from VSV grown in GM1012, GM770, and GM849 correlated with the slight electrophoretic mobility differences observed for the undigested glycoproteins in Fig. 1.

In these human fibroblast cell lines, [^{3}H] glucosamine did not seem to be a significant precursor of sialic acid, since a major radioactive peak eluting in the position of sialic acid (approximately fraction No. 85) was not detected after treatment of glycopeptides with neuramidinidase. This was in contrast to studies with uninfected Chinese hamster cells in tissue culture (21) and with VSV grown in other mammalian tissue culture cells (12, 16, 17).

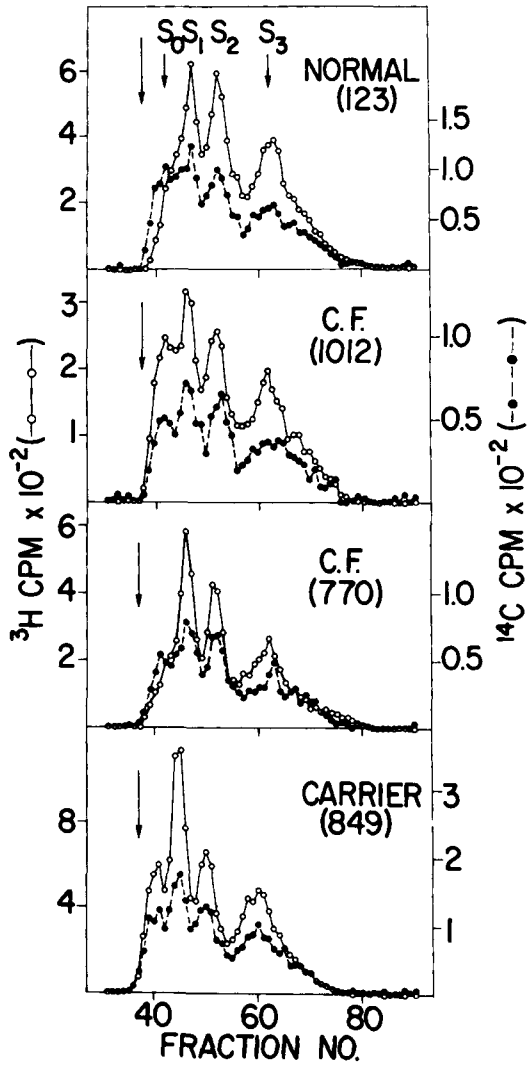


Fig. 3. BioGel P-4 gel filtration of pronase-digested glycopeptides from [^{3}H]glucosamine-labeled cell-associated glycoprotein from VSV-infected normal, cystic fibrosis, and carrier human fibroblasts. The [^{14}C]glucosamine glycopeptides are from VSV-infected normal cells (GM849).

Pronase-digested glycopeptides from residual cell-associated glycoprotein were also examined by gel filtration to rule out the possibility that a major portion of the total glycoprotein was not assembled into virions because it exhibited some deficiency or alternation in glycosylation, whereas the glycopeptides from released VSV were a specific subset of the total which were processed normally. The pattern of glycopeptides of cell-associated G protein was found to be similar to that of virion glycopeptides, with all 4 cell types exhibiting the major S_1-S_3 peaks in addition to variable amounts of S_0 material (Fig. 3). Neuraminidase treatment of the residual cell-associated glycopeptides converted the multiple peaks to the single major S_3 peak (not shown). The decreased resolution of the sialic acid containing peaks (S_0-S_2) and the broader S_3 peak in Fig. 3 compared to

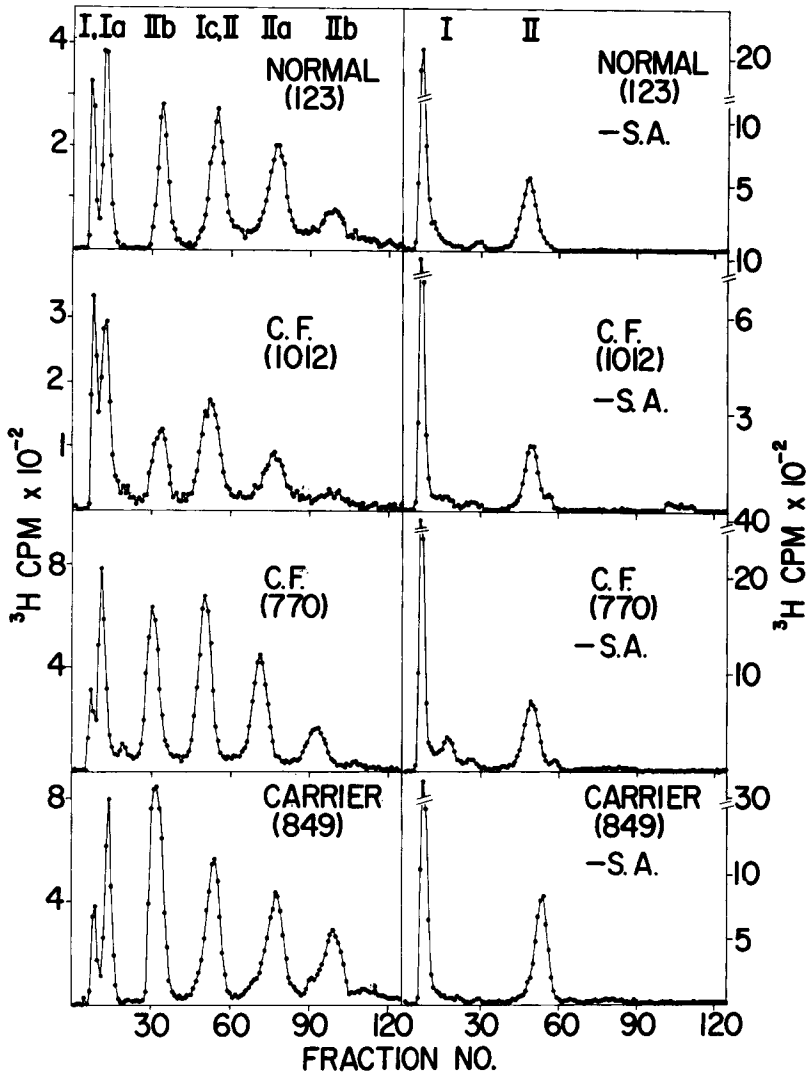


Fig. 4. DEAE-cellulose chromatography of trypsin-digested glycopeptides from [^{3}H]glucosamine-labeled VSV grown in normal, cystic fibrosis, and carrier human fibroblasts. Peptides were eluted with a 0.0–0.1 M NaCl gradient (in 10 mM Tris, pH 8.5) starting at fraction No 21. Neuraminidase treatment eliminates the charge heterogeneity due to terminal sialic acid [converting Ia, Ib, and Ic to I; and IIa, IIb, and (IIc) to II (16)].

virion glycopeptides in Fig. 2 probably could be attributed to the presence of oligosaccharide chains which lack all or part of the branch sugars, as previously described for VSV-infected HeLa cells (12). Other contributing factors could have been the presence of small amounts of heterogeneous, non-VSV glycopeptides and incomplete pronase digestion because of the large amount of unlabeled cellular protein in these samples.

The possibility of a defect in the recognition of the proper glycosylation sites on the VSV G polypeptide was examined by anion exchange chromatography of trypsin-digested glycopeptides before and after neuraminidase treatment (Fig. 4). There were 2 major

glycosylated tryptic peptides (designated I and II) regardless of the cell line on which the VSV was grown, as reported earlier for VSV grown in HeLa suspension culture cells (16). Multiple peaks were obtained before neuraminidase treatment because the terminal sialic acid contributed to the charge heterogeneity of the glycopeptide mixture. The amounts of glucosamine label in peak I and peak II were not equal after neuraminidase treatment, and the reason for this inequality is not known.The ratio of radioactivity in peak I versus peak II was approximately 1.5 (ranging from 1.34 to 1.64 for the different cell lines), which was similar to the published value for VSV grown in HeLa suspension cells (16).

DISCUSSION

No significant differences in the oligosaccharide side chains of the VSV envelope glycoprotein have been detected with virus grown in normal human skin fibroblasts or fibroblasts of "heterozygous carrier" and "homozygous" cystic fibrosis patients. Since the VSV glycoprotein is processed by cellular enzymes and has oligosaccharide side chains with structures similar to complex, N-glycosidically linked side chains on cellular membrane and exported glycoproteins, the uninfected cystic fibrosis fibroblasts can be presumed to be normal with respect to the complex oligosaccharides on their own complement of glycoprotein.

If fibroblasts in tissue culture actually exhibit the metabolic defect(s) of cystic fibrosis, then the enzymes involved in the addition of these complex oligosaccharides are apparently not the site of the biochemical defect(s). These studies did not examine the biosynthesis of oligosaccharides which are O-glycosidically linked to threonine or serine, because they are not found in significant amounts in the membrane glycoproteins of enveloped animal viruses. This class of oligosaccharides is found in a large fraction of the mucous glycoproteins, and may be more important in cystic fibrosis than asparagine-linked, complex oligosaccharides (1, 2).

Although these studies were unable to detect any differences which might be correlated with the biochemical defect(s) in cystic fibrosis, the membrane glycoproteins of the lipid-enveloped animal viruses are powerful experimental tools in the investigation of altered glycoprotein metabolism in a wide variety of tissue culture cell lines. The advantages of the virus-infected cell system are twofold: elimination of the problems of i) a heterogeneous mixture of cellular glycoproteins and oligosaccharide side chains, and ii) the nonphysiological nature of cell-free glycosyltransferase assays. For example, the phenotype of tissue culture cells which have been selected for resistance to various plant lectins were reflected in the structure of the oligosacharide side chains which were smaller and lacked part or all of the branch chain sugars (–GlcNAc–gal±sialic acid) (22, 23; M. Robertson et al., manuscript in preparation). Sindbis virus was also used to examine possible defects in fibroblasts from patients with I-cell disease (mucolipidosis II), but no significant differences in virus glycopeptides were observed between Sindbis virus grown on normal or I-cell disease fibroblasts (24).

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