

SYNTHESIS OF PLASMALEMMAL GLYCOPROTEINS IN INTESTINAL EPITHELIAL CELLS

Separation of Golgi Membranes from Villus and Crypt Cell Surface Membranes; Glycosyltransferase Activity of Surface Membrane

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ABSTRACT

The relationship between Golgi and cell surface membranes of intestinal cells was studied. These membranes were isolated from intestinal crypt cells and villus cells. The villus cell membranes consisted of microvillus membrane, a Golgi-rich fraction, and two membrane fractions interpreted as representing lateral-basal membranes. The villus cell microvillus membrane was purified by previously published techniques while the other membranes were obtained from isolated cells by differential centrifugation and density gradient velocity sedimentation. The two membrane fractions obtained from villus cells and considered to be lateral-basal membranes were enriched for Na⁺,K⁺-ATPase activity, but one also showed enrichment in glycosyltransferase activity. The Golgi membrane fraction was enriched for glycosyltransferase activity and had low to absent Na⁺,K⁺-ATPase activity. Adenylate cyclase activity was present in all membrane fractions except the microvillus membrane but co-purified with Golgi rather than lateral-basal membranes. Electron microscopy showed that the Golgi fraction consisted of variably sized vesicles and cisternalike structures. The two lateral-basal membrane fractions showed only vesicles of smaller, more uniform size. After ¹²⁵I labeling of isolated intact cells, radioactivity was found associated with the lateral-basal and microvillus membrane fractions and not with the Golgi fraction. Antibody prepared against lateral-basal membrane fractions reacted with the surface membrane of isolated villus cells. The membrane fractions from isolated crypt cells demonstrated that all had high glycosyltransferase activity. The data show that glycosyltransferase activity, in addition to its Golgi location, may be a significant property of the lateral-basal portion of the intestinal villus cell plasma membrane. Data obtained with crypt cells support earlier data and show that the crypt cell surface membrane possesses glycosyltransferase activity.

KEY WORDS glycosyltransferases · cell surface · Golgi · adenylate cyclase · differentiation

Studies with isolated intestinal epithelial cells have shown that the cell surface membrane of the differentiated cell is biochemically different from that of the undifferentiated crypt cell (41, 42). The villus cell demonstrated a high rate of incorporation of carbohydrate precursors into surface membrane glycoprotein while the crypt cell showed no incorporation (41). However, the isolated crypt cell surface was characterized by high glycosyltransferase: endogenous acceptor activities except for low sialyltransferase activity, while the villus cell surface had high sialyltransferase activity and no evidence for the other six glycosyltransferase activities tested (42). It was suggested that the high glycosyltransferase activities of the crypt cell surface may be a reflection of new surface membrane derived from Golgi membranes (42). Roseman (33) has suggested that cell surface membrane glycosyltransferases may function in cell adhesion, and the finding of high surface membrane sialyltransferase activity on the villus cell surface (42) seemed supportive of Roseman's hypothesis but it was not clear which part (microvilli, lateral or basal) of the villus cell surface membrane contained the sialyltransferase activity. This paper reports on the separation of villus cell surface membranes from Golgi membrane with glycosyltransferase activities detected in both membranes and also shows that isolated crypt cell surface membrane is characterized by glycosyltransferase activities.

MATERIALS AND METHODS

Tissue Preparation

Isolated intestinal epithelial cell fractions representing a gradient of cells from villus tip to crypt base were prepared from 160–180 g fasted (14 h) female rats (Holzman Co., Madison, Wis.) by the method of Weiser (41). Isolated cells from the villus (fractions 1–5) and from the crypt (fractions 8–10) were pooled and treated separately.

Subcellular Fractionation

The scheme illustrated in Fig. 1 was the one that gave the most reproducible results for separation of lateral and basal surface membranes from Golgi membranes (see Results). Microvilli were prepared by the method of Forstner et al. (10) and purified microvillus plasma membranes by the method of Hopfer et al. (16).

Iodination of Isolated Cell Surface Membrane

The method of Morrison (24) using glucose oxidase to generate peroxide was used (17). Isolated villus cells from three rats were prepared as above and then washed three times with phosphate-buffered saline (PBS) (0.01 M Na phosphate buffer, pH 7.2, 0.154 M NaCl) solution containing 5 mM glucose. The cell pellet was resuspended in this buffer, 4:1 (vol:vol), and divided into three fractions. To one was added lactoperoxidase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) to a final concentration of 0.1 μ M, 35 U of glucose oxidase (type V, Sigma Chemical Co., St. Louis, Mo., 1,400 U/ml) and 2 mCi of carrier-free Na¹²⁵I (New England Nuclear, Boston, Mass.). The other two cell suspensions served as controls, one with no lactoperoxidase or glucose oxidase and the other with lactoperoxidase present but no glucose oxidase. Iodination was performed at 23°C for 15 min. The reaction was stopped by dilution with PBS and cooled to 0°C. All subsequent operations were performed at 0–4°C. The cells were washed eight times, at which point the counts associated with the wash plateaued (0.00004% of total counts). The cells were then homogenized and subcellular fractions were obtained as described above.

Enzyme Assays

Sucrase was determined by the method of Messer and Dahlqvist (22). Alkaline phosphatase was determined as described by Weiser (41). Na⁺,K⁺-ATPase was determined according to Quigley and Gotterer (29) and adenylate cyclase as previously described (30) using the method of Gilman (12) to measure cAMP formed. NADPH-cytochrome *c* reductase was assayed by the method of de Duve et al. (5), and cytochrome oxidase was assayed according to Cooperstein and Lazarow (3). Galactosyltransferase activity was measured as previously described (27). The exogenous glycoprotein acceptor for galactosyltransferase was fetuin from which terminal sialic acid and penultimate galactose had been removed (28). Fetuin with only its terminal sialic acid residues removed was used as an exogenous acceptor for sialyl-transferase activity.

Slab Gel Electrophoresis

Membrane suspensions were diluted in 0.0625 M Tris-HCl buffer, pH 6.8, and centrifuged for 2 h in an SW-41 rotor at 200,000 *g*. The membrane pellets were resuspended in 0.0625 M Tris-HCl buffer, pH 6.8 to which had been added glycerol (10 g/100 ml), Na dodecyl sulfate (SDS) (2 g/100 ml), and dithiothreitol (to 100 mM). The membrane protein was further solubilized by heating in a boiling water bath for 2 min. Molecular weight protein standards were prepared by dissolving each (10 mg) in 0.5 ml of 6 M guanidine HCl and incubating for 4 h or more at 50°C under N₂. Carboxymethylation of these protein standards was then

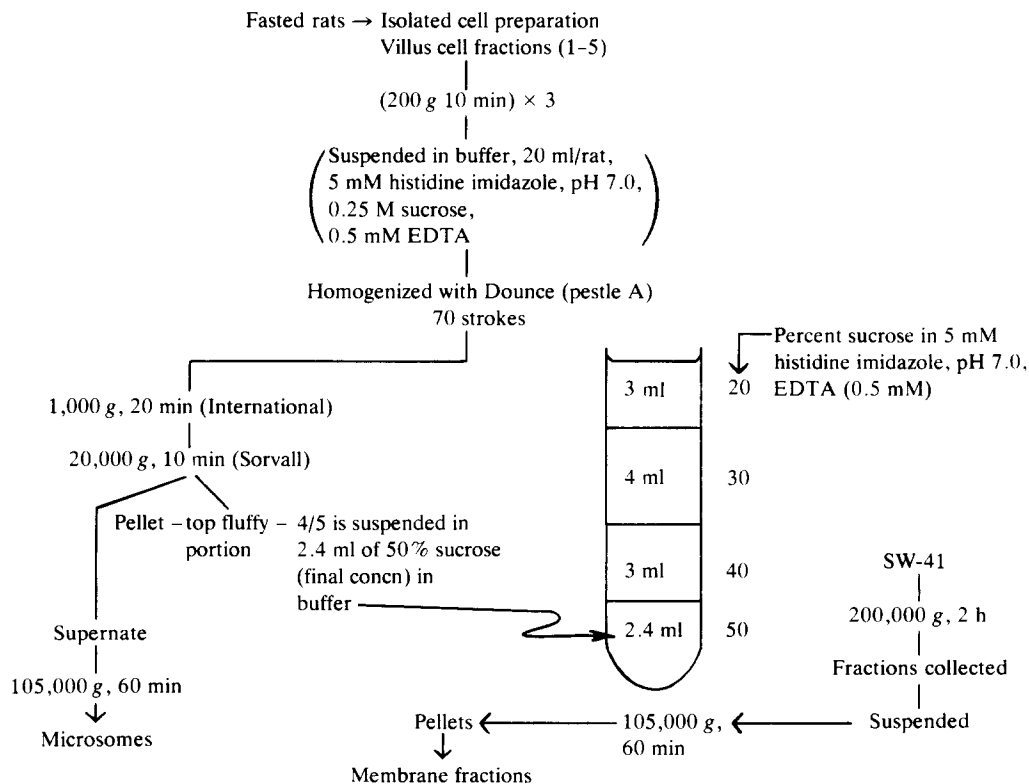


FIGURE 1 Schematic outline of the procedure used to isolate and separate epithelial cell surface membranes from Golgi membranes. See Materials and Methods and Results for further details. After isolating the cells, all procedures were done at 0°C.

initiated by adding 0.05 ml of iodoacetic acid (1.0 M in 1 N NaOH) and incubating for 90 min at 24°C. The solutions were then dialyzed overnight (4°C) against 0.0625 M Tris-HCl buffer, pH 6.8. SDS (2 g/100 ml) was then added, and the solutions were incubated for 1 h at 37°C and then dialyzed overnight (4°C) against SDS (0.1 g/100 ml) in 0.0625 M Tris-HCl buffer, pH 6.8. In the slab gel electrophoretic system, 20 μ g of each standard was usually applied. Slab gel electrophoresis was performed according to Laemmli (19), using the apparatus described by Reid and Bielecki (32), and 7.5% acrylamide gels containing SDS (0.1 g/100 ml). At the end of the electrophoresis, gels were stained for proteins with Coomassie Blue and for glycoproteins by the periodic-acid-Schiff stain (8).

Antibody Preparation

An antibody to isolated membranes was prepared by mixing the designated membranes (30/40 + 40 membrane fractions described below, 1.5 mg of membrane protein in 1 ml of PBS) with 5 ml of complete Freund's adjuvant and injecting this suspension into five or six areas of the skin of the back of New Zealand white rabbits. 2 wk later, the rabbits received booster injections (0.5 mg of membrane protein in 0.5 ml of PBS

suspended in 2 ml of complete Freund's adjuvant), and 1 wk later they received another similar booster injection. 1 wk after this final booster, the rabbits were bled and serum was prepared. Antibody was precipitated from the serum with $(\text{NH}_4)_2\text{SO}_4$ (to 50% saturation) and the precipitate was resuspended in PBS, dialyzed for 2 days against PBS, and then passed through a Millipore filter (Millipore Corp., Boston, Mass.) (0.25 μ m pore).

Isolated villus epithelial cells obtained by the method of Weiser (41) were fixed to glass slides with acetone (-70°C) and then overlaid with the above antibody preparation (diluted 1:20 with PBS). After washing three times with PBS, the cells were covered with goat-derived fluorescein-conjugated anti-rabbit immunoglobulin (Behring Diagnostics, American Hoechst Corp., Somerville, N. J.; 1:25 dilution with PBS) and again washed three times with PBS, counterstained with Evans blue (10 mg in 100 ml of PBS) and finally washed three times with PBS.

Electron Microscopy

The membrane fractions were prepared for positive staining by fixing in glutaraldehyde (3 g/100 ml) in 0.1 M cacodylate buffer, pH 7.5, and postfixed in Dalton's chrome osmium (4). The membranes were then soaked

in uranyl acetate (0.5 g/100 ml), dehydrated, and embedded in Epon-Araldite. Sections of 900 Å were obtained with an LKB ultramicrotome (LKB Instruments, Inc., Rockville, Md.), stained with lead citrate and uranyl acetate, and examined with a Philips 201 electron microscope.

Protein was determined by the method of Lowry et al. (21). All radioactive materials were purchased from New England Nuclear. Unlabeled UDP-galactose and *N*-acetylglucosamine were obtained from Sigma Chemical Co.

RESULTS

Method of Membrane Isolation

The method used for membrane isolation is similar to those described for separation of either surface membrane (7, 11, 14, 25, 31) or the Golgi apparatus (6, 23, 34, 37). It was necessary to use fasted (overnight) animals in order to get reproducible results; animals fed ad lib. or given a high-fat duodenal infusion did not permit adequate separation of surface and Golgi membranes. The use of the isolated cell preparation (41) permitted evaluation of the differentiated villus cell separate from the undifferentiated crypt cell. The usual number of cell fractions obtained by this method (41) is 9–10. Fractions 1–5 are villus cells with little or no admixture of crypt cells, while fractions 8–10 are mostly crypt cells. For isolation of villus cell lateral-basal membranes, only fractions 1–5 were used. All subsequent steps were done at 0°–4°C. Approximately 80% or more of the cells were homogenized with 70 strokes in the Dounce homogenizer (Kontes Co., Vineland, N. J.) as judged by observation with polarized light microscopy. This method of homogenization yielded reproducible results while Potter-Elvehjem, nitrogen cavitation, or Waring blender (Waring Prod-

ucts Div., Dynamics Corp. of America, New Hartford, Conn.) homogenizations were not satisfactory.

The 20,000 g 10-min precipitate consisted of a white fluffy upper layer which was easily separated by gentle swirling from a brown lower layer. As shown in Tables I and II, this top layer demonstrated an increased specific activity for those enzymes believed to be characteristic of Golgi and surface membranes.

The discontinuous density gradient system used was a sucrose gradient as illustrated in Fig. 1. SW-41 tubes (0.562 inch diam × 3.5 inch, no. 31411, Beckman Instruments, Inc., Fullerton, Calif.) were each loaded with $\frac{1}{5}$ the amount of material derived from one animal (9–11 mg of protein). Centrifugation for 2 h gave the most reproducible results, and overnight centrifugation obliterated the 40% membrane material. Fractions were collected by means of a sampling needle inserted from above, with a peristaltic pump. The volume of each fraction collected was: 20%, 2.5 ml; 20/30 interface, 1.3 ml; 30%, 2.9 ml; 30/40 interface, 1.2 ml; 40%, 1.9 ml; 40/50 interface, 1.4 ml; 50%, 1 ml; and pellet in remaining volume. All results reported refer to washed membrane pellets (105,000 g for 60 min) from the sucrose gradient fractions. The separated density gradient fractions, particularly the high density fractions, had to be extensively diluted in order to precipitate all of the suspended membranes. A representative distribution of protein is illustrated in Fig. 2.

Separation of Golgi from Plasma Membranes

As shown in Fig. 3, three membrane fractions could be separated from a homogenate of villus

TABLE I
Marker Enzyme Activities of the Fractions Obtained by Differential Centrifugation

Subcellular fraction	Na ⁺ .K ⁺ -ATPase <i>nmol/mg/15 min</i>	Adenylate cyclase <i>pmol/mg/10 min</i>	Sucrase <i>μmol/mg/15 min</i>	Cytochrome oxidase <i>pmol/mg/min</i>	NADPH cytochrome <i>c</i> reductase
Homogenate	280	415	118	16.5	1,586
20,000 g, 10 min Pellets					
Top	412	649	212	75.0	635
Bottom	106	250	490	92.0	1,269
105,000 g, 60 min Precipitate	198	316	706	7.8	36,266

The subcellular fractions were obtained by the method outlined in Fig. 1 and under Materials and Methods and Results. Enzyme assays were performed as described under Materials and Methods.

TABLE II
Golgi vs. Lateral-Basal Membrane

Subcellular fraction	Na ⁺ ,K ⁺ -ATPase activity <i>nmol/mg/15 min</i>	Glycosyltransferase:exogenous acceptor activities	
		Galactosyltransferase <i>cpm × 10⁻³/mg/60 min</i>	Sialyltransferase <i>cpm × 10⁻³/mg/60 min</i>
Homogenate, 20,000 g ppt.	420	22	31
Top layer	1,520	72	122
Bottom layer	670	33	75
Sucrose gradient fractions			
20/30	1,275	285	672
30/40	4,615	273	968
40	4,904	108	361

The subcellular fractions were obtained by the method outlined in Fig. 1 and under Materials and Methods and Results. Enzyme assays were performed as described under Materials and Methods.

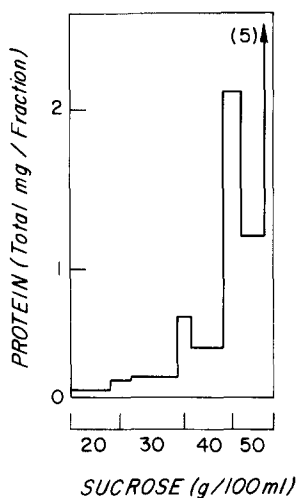


FIGURE 2. Sucrose density gradient separation of villus cell membranes. Protein distribution. Preparation and assay as described under Materials and Methods and Fig. 1.

cells. The material at the 20/30 interface was rich in galactosyltransferase activity and had low Na⁺,K⁺-ATPase activity. Sialyltransferase was also present in the 20/30 interface material. This fraction was considered most characteristic of the Golgi apparatus.

The material found at the 30/40 interface was high in Na⁺,K⁺-ATPase, galactosyltransferase, and sialyltransferase activities. The increase in specific activities with reference to the homoge-

nate was 11-, 12-, and 31-fold, respectively (Table II). Thus, this fraction had enzyme activities that have been considered markers for both Golgi and surface membrane. Another fraction equally as high in Na⁺,K⁺-ATPase activity was found in the 40% fraction. This membrane fraction had less glycosyltransferase activity and it had no activities characteristic of microvillus membrane, i.e., alkaline phosphatase and sucrase activities (Fig. 4). The 40% fraction appeared to be more characteristic of a surface membrane. However, adenylate cyclase did not co-purify with this fraction, but rather with the 20/30 fraction (Fig. 5). The endoplasmic reticulum marker enzyme NADPH cytochrome *c* reductase overwhelmingly co-purified with the microsomal precipitate (105,000 g precipitate of the 20,000 g 10-min supernate) (Table I). The mitochondrial enzyme marker, cytochrome oxidase, was detected on the sucrose gradient at the 40/50 interface and in the 50% layer and not in the other fractions.

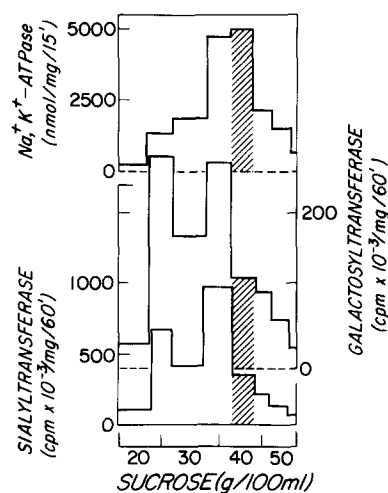


FIGURE 3. Sucrose density gradient separation of villus cell membranes. Enzyme profiles: Na⁺,K⁺-ATPase vs. glycosyltransferase:exogenous acceptor specific activities. Preparation as described under Materials and Methods and Fig. 1. Enzyme assays were done as described under Materials and Methods. All fractions of the sucrose gradient were assayed (ordinate values), and the abscissa represents eight fractions of the gradient (20, 20/30, 30, 30/40, 40, 40/50, 50, and pellet). Galactosyltransferase specific activity was highest in the 20/30 and 30/40 interfaces. Sialyltransferase specific activity peaked in the 30/40 interface. The 20/30 interface contained little Na⁺,K⁺-ATPase which was high in the 30/40 interface and in the 40% sucrose fraction (shaded area).

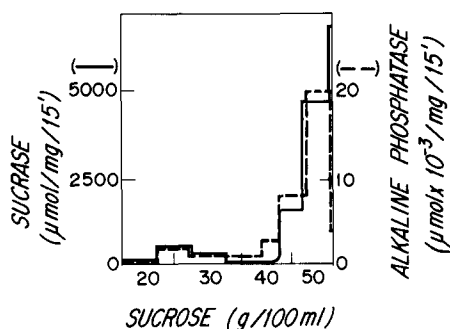


FIGURE 4 Sucrose density gradient separation of villus cell membranes. Enzyme profiles: alkaline phosphatase vs. sucrase specific activities. Preparation as in Fig. 3 and enzyme assays as described under Materials and Methods. These microvillus-associated enzymes were found in the 40/50 interface and the 50% sucrose fractions and do not appear to contaminate the other sucrose gradient fractions.

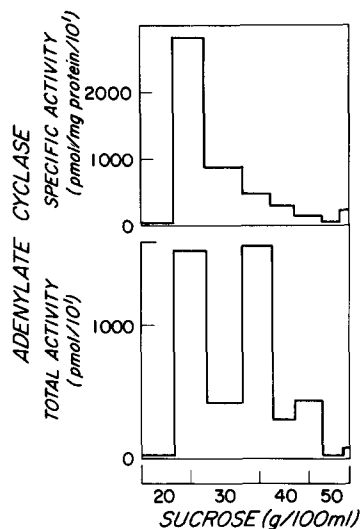


FIGURE 5 Sucrose density gradient separation of villus cell membranes. Enzyme profile: upper graph, adenylate cyclase specific activities; lower graph, adenylate cyclase total activity. Preparation similar to that of Fig. 3. Enzyme assays were done as described in Materials and Methods. Pattern of adenylate cyclase specific activity did not correlate with $\text{Na}^+.\text{K}^+.\text{ATPase}$ specific activity (compare with Fig. 3).

Triton X-100 (0.1%) increased galactosyltransferase activity (Table III) but had a greater effect on the activity present in the 20/30 fraction (three- to fourfold increase) than on the activity present in the 30/40 or 40 fraction (1.5- to 2.5-fold increase). The detergent had a markedly inhibitory effect on sialyltransferase activities. The pres-

ence or absence of Triton X-100 in the galactosyltransferase assay did not significantly change the distribution of galactosyltransferase activity on the sucrose gradient, and total recoveries with reference to the homogenate were also similar. The top fluffy layer of the 20,000 g precipitate contained 15–28% of the total glycosyltransferase activity. 80% of this was used in the sucrose gradient, with 50–87% recovered from the density gradient. The 20/30 fraction accounted for 2–4%, the 30/40 for 25–30%, and the 40 for 15–30%. Glycosyltransferase: endogenous acceptor activities represented 2–7% of the glycosyltransferase: exogenous acceptor activities and had a similar distribution during subcellular fractionation. Recovery of ATPase activity was similar except that most of the activity from the gradient was recovered in the 30/40 and 40 fractions.

When isolated crypt cells were used as the starting preparation, the sucrose gradient pattern was similar except that the 40% sucrose fraction was not enriched for $\text{Na}^+.\text{K}^+.\text{ATPase}$ but rather for galactosyltransferase activity (Fig. 6). The $\text{Na}^+.\text{K}^+.\text{ATPase}$ activity co-purified with the 30/40 interface material which was also high in galactosyltransferase activity.

Electron Microscope Appearance of Membrane Fractions

All three villus membrane fractions showed formation of membrane vesicles when viewed by electron microscopy (Fig. 7). The 20/30 fraction appeared to show greater variability in vesicle size as well as cisternalike structures (Fig. 7A). The 30/40 and 40 fractions showed fairly uniform membrane vesicles with no significant contamina-

TABLE III
Effect of Triton X-100 on
Galactosyltransferase: Exogenous Acceptor Activities

Subcellular fraction	Galactosyltransferase		Sialyltransferase	
	Without Triton X-100	With Triton X-100	Without Triton X-100	With Triton X-100
	<i>cpm/60 min</i>			
20/30	6,612	22,433	769	187
30/40	4,775	8,995	9,548	1,007

Final Triton X-100 concentration in enzyme assay was 0.1 mg/100 ml. The subcellular fractions were obtained by the method outlined in Fig. 1 and under Materials and Methods and Results. Enzyme assays were performed as described under Materials and Methods.

tion of particles or structures suggestive of other subcellular fractions (Fig. 7 B and C).

¹²⁵I-Labeling of Villus Cell

Surface Membrane

The method used for obtaining isolated villus cells may produce as many as 20% of cells that cannot exclude vital dyes (41). Therefore, it was important that two controls be done: (a) no glucose oxidase, and (b) no glucose oxidase or

lactoperoxidase. These controls were taken through all steps of the preparation and represented "nonspecific" labeling that was subtracted from the experimental preparation. These (a) and (b) controls showed similar nonspecific labeling levels, and amounted to 18–22% of the counts incorporated in the experimental preparation. The results, as shown in Fig. 8, indicate that the

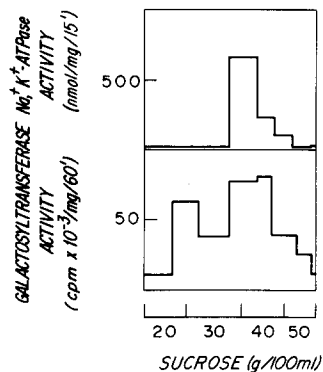


FIGURE 6 Sucrose density gradient separation of crypt cell membranes. Enzyme profiles: Na⁺,K⁺-ATPase vs. galactosyltransferase:exogenous acceptor specific activities. Isolated crypt cells, fractions 8–10 of the isolated intestinal cell preparations (41), were pooled, homogenized, and their membranes were isolated as in Fig. 1. Enzyme assays were done as described under Materials and Methods. Note the high galactosyltransferase specific activities in all the major membrane fractions (20/30, 30/40, and 40). The 40% sucrose membrane fraction, in contrast to the comparable villus cell membrane fraction, was not enriched for Na⁺,K⁺-ATPase but was enriched for galactosyltransferase activity.

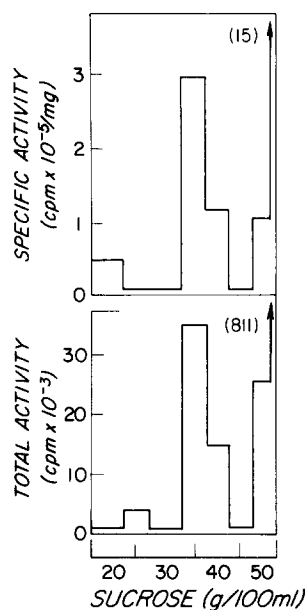


FIGURE 8 Sucrose density gradient profile after labeling isolated cells with ¹²⁵I by the method of Morrison (24); see Materials and Methods. Only the Na⁺,K⁺-ATPase-rich (30/40 interface and 40% sucrose fraction) and microvillus-rich fractions (50% sucrose fraction) were labeled.

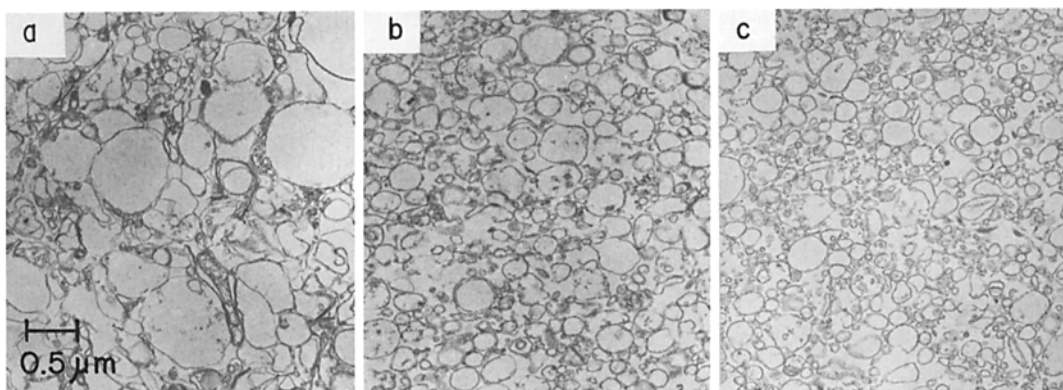


FIGURE 7 Electron microscope appearance of membrane fractions from sucrose density gradient separation of villus cell membranes. (a) 20/30 Interface membrane fraction. (b) 30/40 Interface membrane fraction. (c) 40% Sucrose membrane fraction. × 20,000.

microvillus-rich area was most prominently labeled (50% sucrose and the pellet fraction) as would be expected. The 20/30 Golgi-like fraction was not labeled but the 30/40 interface and 40 percent fractions were labeled, suggesting that these two latter fractions contain surface membrane components.

Immunofluorescence Identification of Surface Membrane

When isolated intestinal epithelial cells were overlaid with antibody produced by using the 30/40 and 40 membrane fractions as an antigen, the surface membrane was shown to be reactive

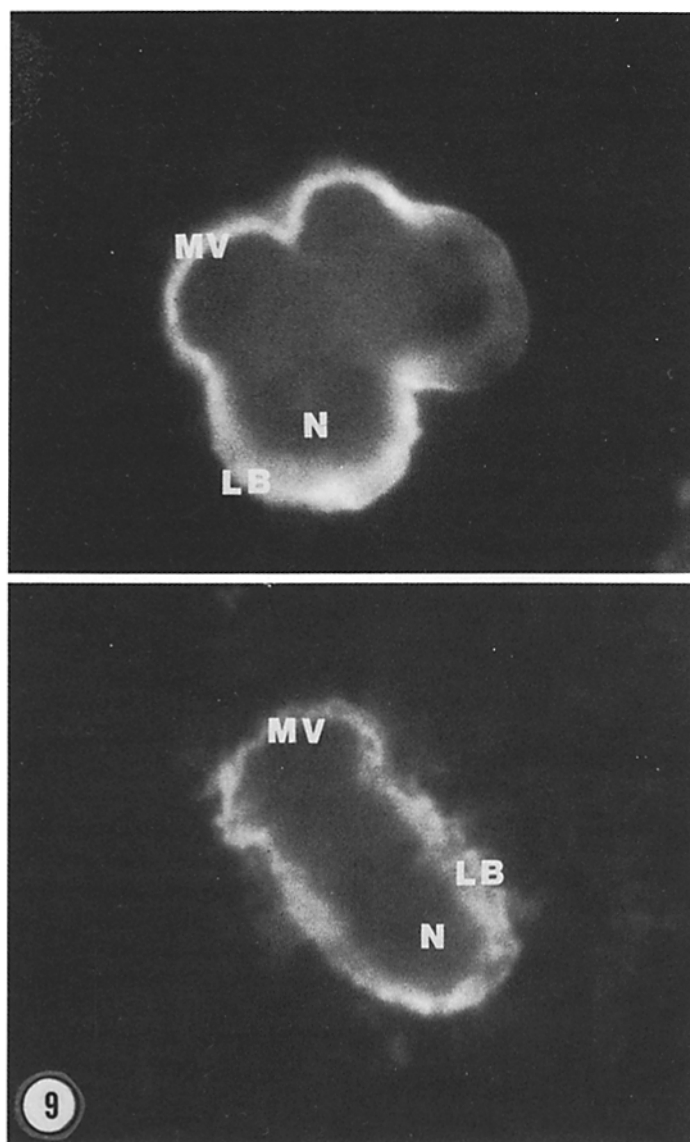


FIGURE 9 Indirect immunofluorescence labeling of the plasma membrane of isolated villus cells. An antibody was prepared to the 30/40 interface and 40% sucrose gradient membrane fractions as described under Materials and Methods. The antibody is seen to primarily react with the surface membrane of the cell, i.e., both lateral-basal (*LB*) and microvillus membrane (*MV*) portions. *N*, nucleus.

by indirect immunofluorescence (Fig. 9) with little fluorescence of intracellular membranes. Both lateral-basal and microvillus portions of the plasma membrane were labeled which is compatible with common antigen-proteins in these membrane fractions, although we cannot exclude a minimal microvillus contamination of the membrane fractions used as antigen. Nevertheless, the immunofluorescence data give further support that 30/40 and 40 fractions contain surface membranes.

SDS-Polyacrylamide Gel Electrophoresis of Golgi and Surface Membranes

The different membrane fractions were solubilized by SDS plus heat, and electrophoresed in an SDS-polyacrylamide slab gel system. The protein patterns are illustrated in Fig. 10. The various membrane fractions isolated by density gradient centrifugation appeared to have very similar protein patterns although minor differences can be seen. Since the 50% sucrose fraction did not represent a significant purification of microvilli, we prepared microvillus membranes purified according to Hopfer et al. (16) to compare its protein pattern on SDS-polyacrylamide electro-

phoresis. Fig. 9 demonstrates the the microvillus membrane showed significant differences in the protein pattern from that of the other membranes although there were a few common bands.

DISCUSSION

The intestinal differentiated villus epithelial cell is a long, columnar cell with a basal nucleus. At its luminal end, the plasma membrane is shaped into extensive microvilli with an actinlike core (38). This microvillus membrane has a thick glycocalyx extending out into the lumen (18) which, in part, represents the glycosylated portions of membrane-associated enzymes characteristic of this membrane including lactase, sucrase, peptidases, and alkaline phosphatase (41). The lateral membrane of the cell is often schematically drawn as a simple straight border, but as shown by Vial and Porter (40), it has extensive plicae and may exceed the microvillus membrane in total surface area. Vial and Porter suggest that the plicae may serve to "provide the cell surface with elongated channels." There does appear to be considerable evidence that the lateral membranes of adjacent cells form intercellular spaces of extensive volume capacity

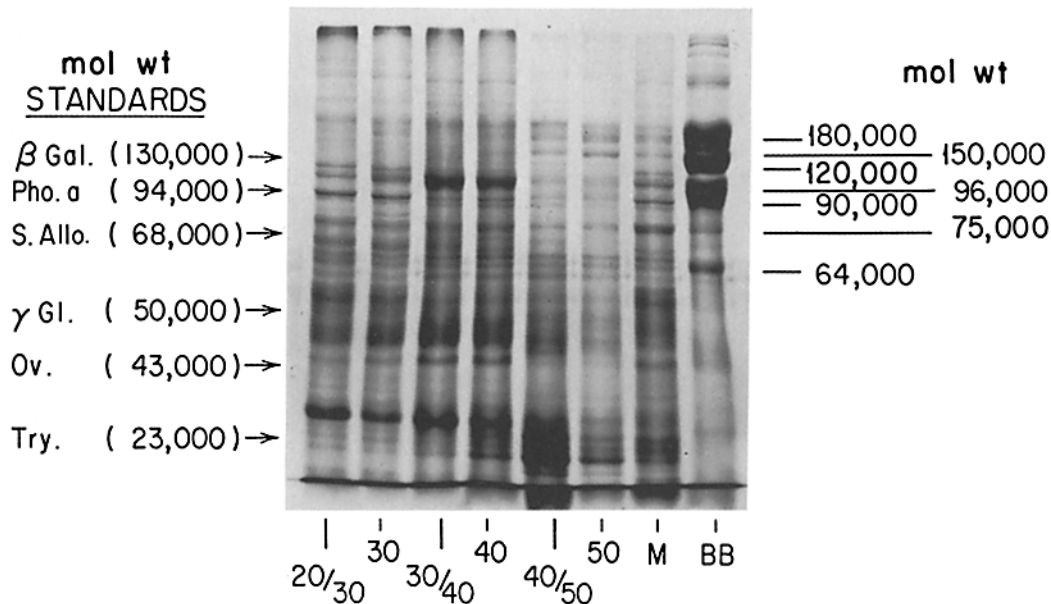


FIGURE 10 SDS-slab gel electrophoresis of villus cell membrane fractions. The membrane fractions derived from separation by sucrose density gradient centrifugation are indicated by percent sucrose and were prepared as described in Fig. 1. The microsomal fraction (*M*) was the 105,000 *g*. 60-min precipitate prepared from the 20,000 *g*. 10-min supernate as shown in the scheme of Fig. 1. The microvillus fraction (*BB*) was prepared by the method of Hopfer et al. (16). The methods of electrophoresis and preparation of molecular weight standards were as described under Materials and Methods.

responsive to solute flow and osmotic pressure (13, 20, 36).

However, the intestinal epithelial cell does appear to be well anchored to the villus structure through adherence to a basement membrane (36). A feature of intestinal epithelium is its constant renewal with movement of cells up the villus structure. The villus tip cells are continually being discharged into the lumen, suggesting that as the cells migrate up the villus, the bonds of adherence to the basement membrane must be altered and weakened. It is this latter feature which may be the explanation for the gradient of isolated cells (villus tip to crypt) that has been obtained by a number of methods, including one developed in this laboratory (41).

With this latter method of separating crypt from villus cells, it was possible to demonstrate that the differentiated villus cell had a very high rate of membrane glycoprotein synthesis whereas the undifferentiated crypt cell had a very low rate (41). This high rate of glycoprotein synthesis by the villus cell membrane appeared to be primarily directed toward glycosylation of microvillus membrane proteins. Another finding in the previous studies was that the crypt cell homogenate had higher glycosyltransferase activities than the villus cell homogenate (42). This was partly accounted for by the presence of these enzyme activities on the intact crypt cell surface. The presence of multiple glycosyltransferase activities on the crypt cell and not on the isolated cell surface was interpreted as indicating the need for a mitotically active cell to make new cell membrane rapidly and that this new cell membrane would have properties similar to those from which it was derived, possibly the Golgi apparatus (42). Others have recently suggested a similar explanation for the presence of these enzymes on surface membrane of cells in tissue culture (26). It appeared that isolation and purification of surface and Golgi membranes would be helpful in clarifying the relationship between the two membranes and the significance of surface membrane glycosyltransferases.

A number of methods have been described for the isolation of lateral-basal membranes (11, 29). The technique employed in the present report begins with isolated villus or crypt cells rather than scrapings and takes into account an enrichment for Golgi and surface membrane markers in an overlying white layer on the 20,000 g, 10-min precipitate. Enrichment for Golgi marker en-

zymes had also been observed in this layer by Morr  (23) in his isolation procedure for liver Golgi apparatus. His Golgi fraction was collected off of a sucrose gradient at 0.5-1.25 M sucrose (18/45%) interface. This would be equivalent to the material at the 30/40% interface in the system described here. Morr 's fraction was also enriched for the plasma membrane marker enzyme 5'-nucleotidase as well as for Golgi marker enzymes.

In the present study of intestinal epithelial cells, the separated membrane fractions, 20/30 and 30/40, may be similar to the GF₂ and GF₃ fractions obtained from ethanol-treated animals by Ehrenreich et al. (6) and Bergeron et al. (1). As with these latter authors, the major problem is that of interpretation since other investigators have isolated a fraction similar to GF₃ and called it the lateral-basal membrane (11). Our finding of another fraction, floating in the 40% sucrose, that is low in nondetergent stimulated glycosyltransferase activity and high in Na⁺,K⁺-ATPase suggests that this is a surface membrane fraction. We believe that Ehrenreich's GF₃ fraction is equivalent in our system to the 30/40 fraction which may be interpreted either as a mixture of lateral-basal and Golgi membranes, perhaps the latter being derived from Golgi vesicles near or about to fuse with lateral membranes, or as part of the surface membrane very similar to Golgi membrane. The ¹²⁵I-labeling experiments (Fig. 8) and the demonstration that an antibody made to 30/40 and 40 membrane preparations reacts primarily with the surface membranes of isolated intact intestinal epithelial cells (Fig. 8) provides further supportive data that 30/40 and 40 fractions contain surface membranes.

Other publications have suggested a structural-functional similarity between Golgi and surface membranes. Farquhar et al. (9) presented cytochemical data showing 5'-nucleotidase to be present in all three Golgi fractions that they isolated (i.e., GF₁, GF₂, and GF₃). They stressed that the enzyme was detected on the inside face of GF₁ and GF₂ vesicles but on the outside of GF₃, and suggested that GF₃ may represent secretory vesicles which fuse with the surface membrane on discharging their content. One can also interpret GF₃ as surface membrane, if the surface membrane is considered to have a patchwork composition (43) and include Golgi-like parts (43).

The 30/40 intestinal membrane fraction demonstrated less enhancement of galactosyltransferase activity by Triton X-100 when compared to

the 20/30 Golgi-rich membrane fraction (Table III). This was also true for the 40 fraction. This difference in latency may reflect a difference in the number of broken or inverted vesicles due to the isolation procedure. The electron microscope appearance (Fig. 7) suggests that the membrane fractions consisted mostly of closed vesicles. Alternatively, the data may be indicative of an intrinsic difference in the location of enzyme activity. Thus, as suggested by Farquhar et al. (9) for their similar fraction from liver, the Golgi-like 20/30 membrane has more enzyme activity on the inside face of the vesicle while the more surface membrane-like fractions, 30/40 and 40, have a greater amount of their enzyme activity on the outside of the vesicles.

Varga et al. (39) have recently demonstrated a relationship between hormone receptors (melanocyte-stimulating hormone receptors) on the cell surface and the near or contiguous Golgi membranes. They also demonstrated a Golgi-related accumulation of cAMP which would suggest that part of the Golgi apparatus must have adenylate cyclase activity. Recently, Cheng and Farquhar (2) have shown that adenylate cyclase co-purifies with liver Golgi apparatus. Our data with intestine also support a Golgi apparatus location for adenylate cyclase. Adenylate cyclase was found to co-purify with a Golgi fraction (20/30) to a greater extent than with Na^+,K^+ -ATPase-rich fractions (Fig. 5).

Hodson and Brenchley (15), using SDS-polyacrylamide electrophoresis, showed that there are marked similarities of protein patterns between rat liver plasma membrane and Golgi membranes. The major difference was an extra band in plasma membrane. Our data also indicate a marked similarity between intestinal surface and Golgi membranes, except for the highly specialized area of the intestinal villus cell—the microvilli (Fig. 10). The two surface membrane fractions (30/40 and 40) could not be distinguished from one another by protein patterns but the 40 fraction appeared to have less accessible glycosyltransferase activities (activity detected without detergent). The 30/40 membrane fraction could be interpreted in terms of Roseman's hypothesis (33) that surface membrane glycosyltransferases are the molecular mechanism for cell adhesion; under this hypothesis a membrane fraction rich in both Na^+,K^+ -ATPase and accessible glycosyltransferase activities could be considered the adhering part of the epithelial cell membrane, i.e., the basal mem-

brane, provided the isolated vesicles were right side out.

Finally, the fact that the 40 fraction isolated from crypt cells was high in galactosyltransferase activity but low in Na^+,K^+ -ATPase activity and that most of the Na^+,K^+ -ATPase activity was found at the 30/40 interface which also had high glycosyltransferase activities (Fig. 6), gives support to the previous finding that isolated crypt cells were characterized by accessible high glycosyltransferase activities on their cell surface (42). The previous finding that the isolated villus cell surface membrane did not show significant glycosyltransferase activities except for sialyltransferase activity (42) contrasts with the data on villus cell lateral-basal membranes described in this paper which do exhibit these activities. The results of the early experiments may be explained by the effects of glycosidase activities located on the microvilli of the intact villus cell which interfered with the detection of the glycosyltransferases on the lateral-basal membrane. Isolation of surface membrane without microvilli would then permit demonstration of glycosyltransferase activities without the effect of microvillus glycosidases. Shur and Roth (35) had initially suggested this possibility.

Glycosyltransferases were believed to be distinctive marker enzymes for Golgi membranes; whether they are also present in cell surface membranes is not easily proven. The development of a method of labeling these enzymes by autoradiography would be helpful if the label were specific and did not involve endogenous products. We have not been able, as yet, to make specific antibodies to galactosyltransferase that could be used for cellular localization. The accumulative evidence supports the idea of cell surface glycosyltransferases (35) for some cells, and we favor this interpretation of our data.

In summary, surface membrane-rich preparations have been separated from Golgi membranes of intestinal differentiated villus and undifferentiated crypt cells. A basic similarity between intestinal Golgi and surface membranes is suggested except for the distinctive microvillus membrane portion of the villus cell surface membrane. It is also suggested that glycosyltransferases are part of intestinal surface membranes but their role, if any, in this location has not been determined.

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