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Synthesis of Membrane Glycoproteins in Rat Small-Intestinal Villus Cells

REDISTRIBUTION OF L-[1,5,6-^{3}H]FUCOSE-LABELLED MEMBRANE GLYCOPROTEINS AMONG GOLGI, LATERAL BASAL AND MICROVILLUS MEMBRANES *IN VIVO*

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The biogenesis of plasmalemma glycoproteins of rat small-intestinal villus cells was studied by following the incorporation of L-[1,5,6-^{3}H]fucose, given intraperitoneally with and without chase, into Golgi, lateral basal and microvillus membranes. Each membrane fraction showed distinct kinetics of incorporation of labelled fucose and was differently affected by the chase, which produced a much greater decrease in incorporation of label into Golgi and microvillus than into lateral basal membranes. The kinetic data suggest a redistribution of newly synthesized glycoproteins from the site of fucosylation, the Golgi complex, directly into both lateral basal and microvillus membranes. The observed biphasic pattern of label incorporation into the microvillus membrane fraction may be evidence for a second indirect route of incorporation. The selective effect of the chase suggests the presence of two different pools of radioactive fucose in the Golgi complex that differ in (1) their accessibility to dilution with non-radioactive fucose, and (2) their utilization for the biosynthesis of membrane glycoproteins subsequently destined for either the microvillus or the lateral basal parts of the plasmalemma. The radioactively labelled glycoproteins of the different membrane fractions were separated by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis and identified by fluorography. The patterns of labelled glycoproteins in Golgi and lateral basal membranes were identical at all times. At least 14 bands could be identified shortly after radioactive-fucose injection. Most seemed to disappear at later times, although one of them, which was never observed in microvillus membranes, increased in relative intensity. All but two of the labelled glycoproteins present in the microvillus membrane corresponded to those observed in Golgi and lateral basal membranes shortly after fucose injection. The patterns of labelled glycoproteins in all membrane fractions were little affected by the chase. These data support a flow concept for the insertion of most surface-membrane glycoproteins of the intestinal villus cells.

A major question in the biosynthesis of membrane proteins is the nature of the intracellular route(s) by which newly synthesized proteins are processed after transcription and then inserted into the plasma membrane. It is likely that different pathways are followed by those membrane proteins destined for the exterior of the cell (or spanning the lipid bilayer), compared with the ones associated with the intracellular side of the plasma membrane (Rothman & Lenard, 1977). It is generally recognized that the glycosidic portion of glycoproteins is located at the extracellular side of the surface membrane. Many membrane glycoproteins represent well-characterized enzymes, as is the case for many components of the microvillus membrane of the intestinal villus cell, whereas others have been identified as hormone receptors (Kahn, 1976), or involved in cell aggregation (Hausman & Moscona, 1975; Vicker, 1976) and cell adhesion (Pearlstein, 1976; Grinnell *et al.*, 1977). According to the membrane flow theory, membrane biogenesis involves the physical transfer of membranes from one subcellular compartment to another, the endoplasmic reticulum being the precursor of the Golgi membrane, which in turn is processed into plasma membrane. Consequently, intrinsic membrane proteins and glycoproteins should follow an intracellular route analogous to the secretory process, and continuous plasma membrane renewal is achieved through fusion of Golgi-derived vesicles with the plasma membrane (Whaley *et al.*, 1972; Bennett *et al.*, 1974). The considerable differences in lipid and protein composition

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among the different cellular membranes, however, suggest that either different models have to be considered, or that specialized portions of the intracellular membranes are selectively involved in surface-membrane biogenesis (Palade, 1975). The structure of the intestinal villus cell, with its well-characterized morphological and functional polarity, introduces a new dimension to the problem: membrane proteins are present at both the luminal (microvillus) and lateral basal sides, but nothing is known of the process by which the cell is able to achieve a striking difference in protein composition between the two parts of the plasmalemma (Maestracci *et al.*, 1973, 1975; Weiser *et al.*, 1978), and to maintain this difference despite a continuous turnover of these proteins (Alpers, 1972, 1977). In addressing these problems, we have followed the redistribution among different membrane fractions of glycoproteins labelled *in vivo* with radioactive fucose. Methodologically, the intestinal villus cell is particularly appropriate for the present study, since its brush border can be isolated as a characteristic subcellular fraction (Forstner *et al.*, 1968), from which microvillus membranes can be purified (Hopfer *et al.*, 1973). Lateral basal and Golgi membranes can be isolated by a technique (Weiser *et al.*, 1978) that utilizes isolated cells and yields relatively well-characterized membrane fractions having no significant contamination by microvillus membranes. Fucose was chosen as the most convenient radioactive precursor because it is present in very low concentrations both extra- (Howard & Kelleher, 1971) and intracellularly (Yurchenco & Atkinson, 1977), it is not significantly metabolized, and it is incorporated in the Golgi complex as a terminal sugar into newly synthesized glycoproteins. Previous studies (Bekesi & Winzler, 1967) have shown that intestine and liver are the most active organs in the incorporation of labelled fucose into glycoproteins. In addition, extensive radioautographic studies by Bennett and Leblond (Bennett, 1970; Bennett & Leblond, 1971; Bennett *et al.*, 1974) of a variety of cells, including intestinal villus cells, have localized the distribution of fucose into the different subcellular compartments at different times after injection of the labelled sugar, providing a reference for the biochemical studies that are the subject of the present paper. According to Bennett and Leblond, fucose is incorporated in the intestinal villus cell, mainly if not solely into four subcellular structures: Golgi complex, lateral plasma membrane, microvillus membrane (where it accumulates preferentially) and uncharacterized dense and multivesiculated bodies. The lack of incorporation into other important cell structures, such as mitochondria, rough endoplasmic reticulum etc., simplifies the problem of contamination by these subcellular fractions. By following the kinetics of radioactive fucose incorporation into Golgi, lateral basal and microvillus membranes, and the redistribution of the labelled glycoproteins in the various membrane fractions at different times after injection, we have collected evidence supporting the membrane-flow theory. Newly synthesized glycoproteins, initially present in Golgi membranes, seem to be inserted into the surface membrane, both at the lateral basal and luminal sides. In addition, glycoproteins previously inserted into the lateral basal membrane may be subsequently redistributed to the microvillus membrane.

Experimental

Materials and general methods

All radioactive materials were purchased from New England Nuclear. Acrylamide and bisacrylamide were bought from Eastman Kodak, Rochester, NY, U.S.A., and sodium dodecyl sulphate (Sequanal grade) was from Pierce, Rockford, IL, U.S.A. All other chemicals were obtained from commercial sources and were of the highest purity available. Microvillus membranes were prepared by the Forstner–Hopfer method (Hopfer *et al.*, 1973) and monitored for purification by following the increase in sucrase specific activity, which was 40 to 50 times higher in the final microvillus membrane fraction than in the homogenate. Golgi and lateral basal membranes were prepared from villus intestinal cells by the method of Weiser *et al.* (1978); the villus cells were isolated from rat small intestine as described by Weiser (1973) and only fractions 1–5 of the cell gradient were used. Protein was determined by the method of Lowry *et al.* (1951), sucrase activity by the method of Messer & Dahlqvist (1966) and $(Na^+ + K^+)$-dependent ATPase as described by Tashima (1975). Galactosyltransferase was determined as previously described (Podolsky & Weiser, 1975); Triton X-100 was present in all incubations at a final concentration of 0.1% (Kim *et al.*, 1975). Radioactivity was determined by liquid-scintillation counting in Aquasol (New England Nuclear).

Labelling in vivo with L-[1,5,6-^3H]*fucose*

Female CD strain Sprague–Dawley rats (170–225 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.) and starved overnight before use. They were injected intraperitoneally with 200–500 μCi of L-[1,5,6-^{3}H]fucose (sp. radioactivity 5–12 Ci/mmol) dissolved in 0.3 ml of 0.154 M-NaCl; when a chase with unlabelled fucose was given 10 min after radioactive fucose injection, the rats received 7 mg of unlabelled fucose in 0.35 ml of 0.154 M-NaCl. At different intervals the rats were killed, the small intestine was removed, and either microvillus or Golgi and lateral basal membranes were isolated.

Slab-gel electrophoresis and fluorography

Membrane suspensions were diluted with 0.0624 M-

Tris/HCl buffer, pH6.8, and centrifuged at 200000*g* for 2h in an SW-41 rotor. Pellets were suspended in 0.0625M-Tris/HCl buffer, pH6.8, containing 10% glycerol, 2% sodium dodecyl sulphate, 50mM-dithiothreitol, and solubilized by immersion in boiling water for 2min. Electrophoresis was performed by the method of Laemmli (1970) in the apparatus described by Reid & Bieleski (1968), in 7.5% acrylamide gels containing 0.1% sodium dodecyl sulphate. At the end of the electrophoresis, gels were stained for protein with Coomassie Blue (Fairbanks *et al.*, 1971), photographed, and processed for fluorography (Bonner & Laskey, 1974).

Acid precipitation

Portions (50μl) of membrane suspensions containing a known amount of radioactivity were added to 1ml of cold (4°C) 10% trichloroacetic acid, and then the mixture poured onto glass-fibre filters, washed with cold water followed by cold ethanol and counted for radioactivity.

Chloroform/methanol extraction

Membrane suspensions (50μl) were extracted with 2ml of chloroform/methanol (2:1, v/v) and the protein was collected by filtration on glass-fibre filters. The test tube and filter were washed twice with 1ml of chloroform/methanol (2:1, v/v). The filter with the protein precipitate and the washes were combined and partitioned into two phases with 0.154M-NaCl (0.8ml). The water/methanol and the chloroform phases were warmed to 37°C while being concentrated under a stream of N_2, and then transferred quantitatively to glass-fibre discs. For reference, portions of the original membrane suspension were pipetted onto glass-fibre discs. All glass-fibre filters were dried under a stream of warm air. In a control experiment [U-^{14}C]palmitic acid was added to the initial solution and shown to be totally recovered in the chloroform phase.

Results

Although the subcellular fractionation of the intestinal epithelial cells, and the isolation of Golgi and lateral basal membranes were previously described (Weiser *et al.*, 1978), a brief review is important in understanding the present work. Intestinal villus cells, isolated by the method of Weiser (1973), were homogenized with 80 strokes of a hand-driven Dounce homogenizer. A low-speed centrifugation was used to remove unbroken cells and nuclei. The homogenate was then centrifuged at 20000*g* for 10min and part of the pellet fractionated on a discontinous sucrose density gradient. Golgi membranes were isolated in the 20%/30% sucrose interphase and in the 30% sucrose layer, whereas membranes more characteristic of the lateral basal part of the plasmalemma were isolated in the 30%/40% sucrose interphase and in the 40% sucrose layer. In the present study, these last two fractions were studied separately. Microsomal fraction was isolated from the 20000*g*-10min supernatant by centrifugation at 105000*g* for 60min. It is noteworthy that the brush border (microvillus) membranes were, instead isolated from total mucosal scrapings by an established technique (Hopfer *et al.*, 1973).

General characteristics of L-[1,5,6-^{3}H]*fucose incorporation into intestinal villus cells*

Radioactive fucose was rapidly taken up by the intestinal villus cells. Incorporation of radioactivity was half maximum at 15min, rose to a plateau value 30min after injection and remained fairly constant for at least 4h. Chase with non-radioactive fucose

Table 1. [^{3}*H*]*Fucose incorporation into intestinal membrane glycoproteins*

Rats were injected intraperitoneally with 500μCi of L-[1,5,6-^{3}H]fucose and killed 5min or 3h after injection. Golgi ($\leq$30%) and lateral basal (30%/40% and 40%) membranes were purified on a sucrose density gradient from villus cells by the method of Weiser *et al.* (1978). Portions of the membrane suspensions were treated with 10% trichloroacetic acid or extracted with chloroform/methanol as described in the Experimental section. Values in parentheses are percentages of the total radioactivity in the sample.

	^{3}H radioactivity (c.p.m.)					
Membrane fractions ...	$\leq$30%		30%/40%		40%	
Time of death after injection ...	5min	3h	5min	3h	5min	3h
Total	6072	1623	8082	6345	2496	4141
Trichloroacetic acid-precipitated material	4899 (79)	1269 (78)	6083 (75)	5234 (82)	2235 (90)	3582 (87)
Chloroform/methanol extraction						
On filter	3857 (63)	1096 (68)	6235 (78)	5234 (82)	2216 (89)	3495 (84)
Organic phase	96	87	149	151	46	261
Water phase	96	27	44	82	41	42

had no significant effect on the incorporation of radioactivity into the homogenate. After separation of unbroken cells, almost all the remaining radioactivity was associated with the crude membrane pellet (20000*g*, 10min) and with the microsomal fraction. At most 1–5% of the total radioactivity present in the homogenate was recovered in the postmicrosomal supernatant. In all membrane fractions studied, the great majority of the radioactivity appeared associated with membrane-bound glycoproteins or with glycoproteins tightly adhering to the membranes since: (*a*) trichloroacetic acid treatment of Golgi and lateral basal membranes obtained 5min or 3h (Table 1) after radioactive fucose injection showed that at least 70–80% of the radioactivity was acid precipitable; (*b*) after brief sonication, 80–90% of the radioactivity was still associated with the membrane pellets; (*c*) when the membranes were extracted with chloroform/methanol and partitioned in an organic and an aqueous phase, no significant amount of radioactivity was present in either phase, most of it being recovered on the filter (Table 1); and (*d*) more than 95% of the radioactivity present in the homogenate at all times after fucose injection was recovered associated with the non-diffusible material after extensive (2–3 days) dialysis. These results also indicate that no significant incorporation of radioactive fucose into glycolipids occurred.

Kinetics of L-[1,5,6-^3H]*fucose incorporation into Golgi, lateral basal and microvillus membranes*

The kinetics of radioactive-fucose incorporation into Golgi and plasmalemma membrane fractions were studied with and without chase (7mg of non-radioactive fucose given 10min after L-[1,5,6-^{3}H]-fucose). Without chase, radioactive fucose was rapidly incorporated into the Golgi membranes (Fig. 1); the specific radioactivity (d.p.m./mg of protein) was highest 30min after injection and then decreased rapidly during the next 2h to 15% of the peak value. A chase with non-radioactive fucose strongly decreased the incorporation of radioactivity found 15 min after injection of the radioactive fucose. However, the specific radioactivity of Golgi membranes subsequently rose to a peak at 30min as without chase and then similarly decreased almost linearly over 3h to the same plateau seen without chase.

Incorporation of radioactivity without chase into the lateral basal membrane fractions (Fig. 1) showed a broad peak between 15 and 60min after fucose injection, and later declined to a plateau of approx. 50% of the peak value. A chase with non-radioactive fucose caused a rapid decrease by 15min (as it was seen for Golgi fractions), then the specific radioactivity rose to a peak at 45min (15min later than the peak in Golgi) and subsequently declined to the same plateau value observed in the absence of chase.

In general, the chase had a smaller effect on the incorporation of radioactivity into lateral basal than into Golgi membranes. Peak values of radioactivity incorporation into lateral basal membranes were decreased by only 5–10% by the chase, in contrast with a 40% decrease in specific radioactivity into the Golgi peak (Fig. 1). However, chase with non-radioactive fucose had no effect on the specific radioactivity of both lateral basal and Golgi membranes found between 2 and 4h after radioactive fucose injection.

The incorporation of radioactive fucose into the microvillus membranes, with or without chase, exhibited a biphasic pattern (Fig. 2). A first peak of specific radioactivity was found at 60min (without chase) or at 30min (with chase). It was followed by a rapid decline, with low amounts of incorporation at 120min (without chase) or 60min (with chase), and by a subsequent new considerable increase in specific radioactivity. The most apparent effect of the chase was the significant decrease (40–60%) of incorporation at all time points considered, but particularly marked between 30 and 90min after radioactive fucose injection.

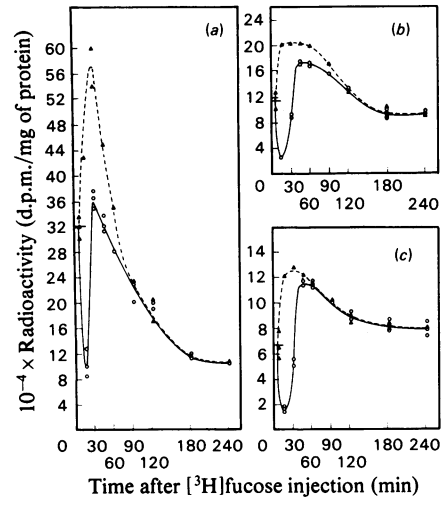

Fig. 1. *Kinetics of* L-[1,5,6-^{3}H]*fucose incorporation into Golgi and lateral basal membranes*

Rats were injected intraperitoneally with 250μCi of L-[1,5,6-^{3}H]fucose, followed by 0.9% NaCl (no chase; ▲) or 7mg of unlabelled fucose in saline (chase; ○) 10min later. The animals were killed at different times after radioactive-fucose injection, and Golgi (*a*) fractions of ≤30% sucrose and lateral basal membranes [the 30%/40% sucrose interphase (*b*) or the 40% sucrose layer (*c*)] were obtained from intestinal villus cells by the method of Weiser *et al.* (1978). Protein and radioactivity were determined as described in the Experimental section. Each point represents the result obtained with one rat in a separate experiment.

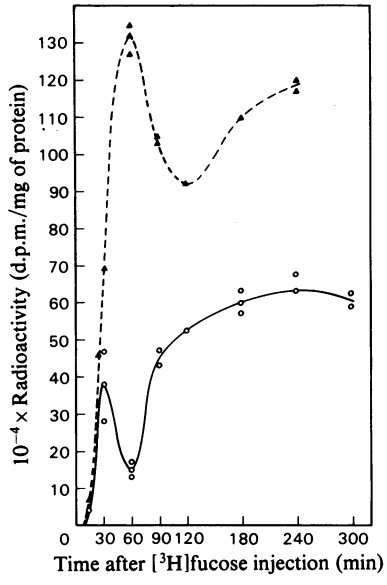

Fig. 2. *Kinetics of* L-[1,5,6-^{3}*H*]*fucose incorporation into microvillus membranes*

Rats were injected intraperitoneally with 250μCi of L-[1,5,6-^{3}H]fucose, followed by 0.9% NaCl (no chase; ▲) or 7mg of fucose in 0.9% NaCl (chase; ○) 10min later. They were killed at different times after radioactive-fucose injection, and microvillus membranes were obtained from intestinal mucosal scrapings by the method of Hopfer *et al.* (1973). Protein and radioactivity were determined as described in the Experimental section. Each point represents the result obtained with one rat in a separate experiment.

Identification of labelled glycoproteins

The membrane fractions obtained at various times after [1,5,6-^{3}H]fucose injection were analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis. Since the specific radioactivity of the membrane fractions considered was quite different, a choice was made between two alternatives, analysing either equal amounts of protein or equal amounts of radioactivity. Although the first alternative was likely to give more information about differences in labelling of individual glycoproteins with time, there was the possibility of missing many components due to the low specific radioactivity of some membrane fractions. The second alternative was therefore chosen and the results were interpreted in a qualitative way, concentrating on changes in intensity of radioactive bands relative to all the others in the same fraction. The difference in protein load between samples, however, resulted sometimes in artifactual slight differences in mobility of some bands: this was particularly evident comparing radioactive bands present in microvillus membranes in the 100000–150000mol.wt. range (bands 6–9; see Figs. 3, 4, 5 and 6) with bands corresponding to proteins of similar molecular weight present in Golgi and lateral basal membranes. Ambiguities were, in these cases, resolved by mixing experiments in which radioactively labelled Golgi and lateral basal membranes were co-electrophoresed with unlabelled microvillus membranes.

A study was carried out on all the membrane fractions available (including microsomal, 40%/50% and 50% sucrose-density-gradient fractions) and representative results are shown in Figs. 3, 5 and 6. A schematic drawing summarizing the major results of seven similar experiments is shown in Fig. 4.

The distribution of radioactivity among glycoproteins in the Golgi and lateral-basal-membrane fractions was, at all times, similar if not identical. Bands 1 and 2 were most prominently labelled in Golgi membranes 5min after fucose injection (Fig. 4). At the peaks of incorporation into Golgi (30min) and lateral basal (45min) membranes, the radioactivity was distributed among at least 12 bands with band 7 most prominent. At 3 and 4h after fucose injection, a single band was the outstanding component (band 14, Figs. 3 and 4), all the others having disappeared or being greatly decreased in intensity relative to band 14. The chase with non-radioactive fucose had little or no effect on the patterns of labelled glycoproteins in the various membrane fractions at each time point considered. However, in comparing the distribution of radioactivity among the different glycoproteins present in Golgi membranes at 5min (without chase) and at 30min (with chase), considerable quantitative differences were found (see the densitometric tracings in Fig. 5).

When microvillus membranes were evaluated, the same radioactive bands were present at all times, from 30min to 4h after fucose injection, with the exception of band 2 (Figs. 3 and 4). Band 2 glycoprotein was the most heavily labelled component in the microvillus membranes at the early peak of radioactive fucose incorporation (30min with chase, 60min without chase). It then disappeared (by 60min with chase and 120min without chase) and was not detected at later times. In comparing labelled glycoproteins in Golgi and lateral basal membranes with those present in microvillus membranes it is apparent that there is a close correspondence between bands present at early times in the Golgi and lateral basal fractions with those present at all times in microvillus membranes (Figs. 3, 4 and 6). However, there was one band, band 14, that concentrated in Golgi and lateral basal fractions and was never present in microvillus membranes. Two other glycoproteins (bands 6 and 8) were always present in the microvillus membranes, but were not detected in any other membrane fraction considered (Figs. 3, 4 and 6).

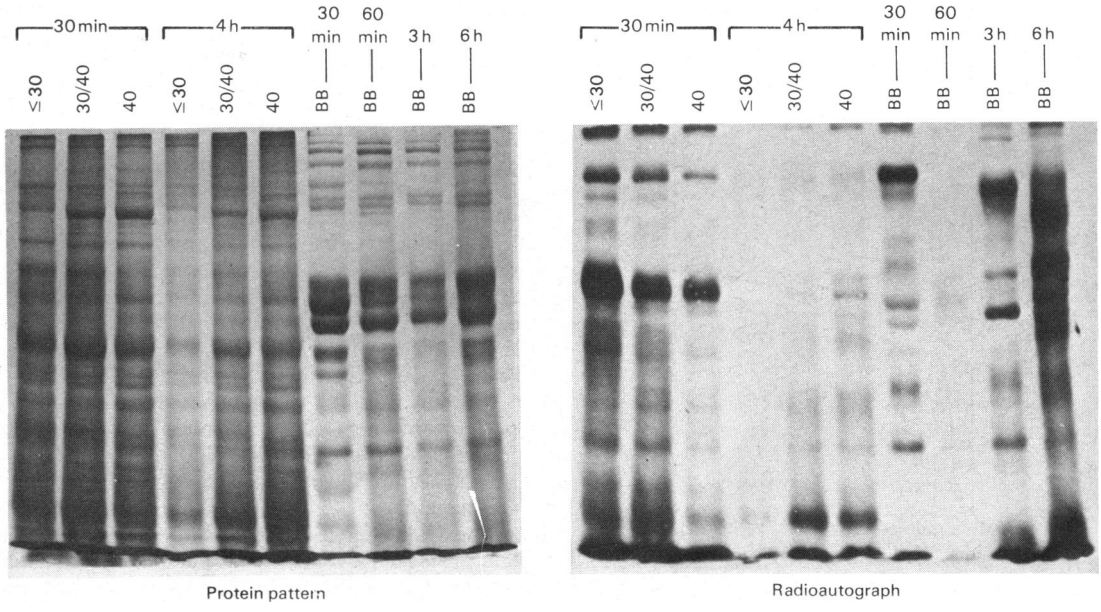

Fig. 3. *Distribution of radioactivity among different glycoproteins in Golgi, lateral basal, and microvillus membranes*
Rats were injected intraperitoneally with 500 μCi of L-[1,5,6-^{3}H]fucose, followed by 7 mg of unlabelled fucose 10 min later. They were killed at different times after radioactive-fucose injection, and either Golgi ($\leq$30) and lateral basal (30/40 and 40) [from isolated villus cells, by the method of Weiser *et al.* (1978)] or microvillus membranes (BB) [by the method of Hopfer *et al.* (1973)] were obtained. Membrane fractions, containing 60000 d.p.m. of radioactivity in all cases (other than $\leq$30, 4 h and BB, 60 min, which contained 35000 d.p.m.) were solubilized and analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis on 7.5% acrylamide gels. Gels were stained for protein, photographed, and the radioactive glycoproteins were subsequently detected by fluorography (see the Experimental section for details).

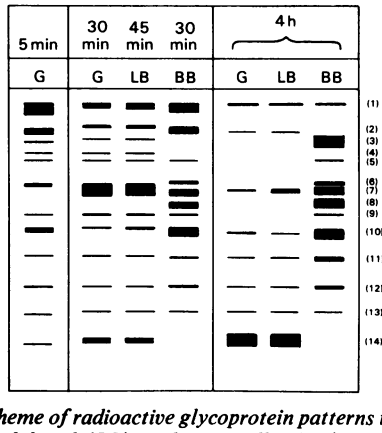

Fig. 4. *Scheme of radioactive glycoprotein patterns in Golgi (G), lateral basal (LB) and microvillus (BB) membranes*
This scheme was derived from results of many experiments of which those shown in Figs. 3, 5 and 6 are representative examples.

Discussion

The present study has followed the incorporation of radioactive fucose, administered intraperitoneally, into Golgi, lateral basal and microvillus membranes isolated from rat intestinal villus cells. A major problem in the interpretation of the results obtained is the definition of the different membrane fractions in terms of their purity and possible cross-contamination. The microvillus membrane, isolated by an established technique (Forstner *et al.*, 1968; Hopfer *et al.*, 1973) is a well-characterized membrane fraction and does not appear to be significantly contaminated by other subcellular components. It has a unique protein pattern (Maestracci *et al.*, 1973; Weiser *et al.*, 1978), and many of its components have been identified as characteristic brush-border enzymes (Maestracci *et al.*, 1975); enzymic activities characteristic of other subcellular fractions [such as ($Na^+ + K^+$)-dependent ATPase, glycosyltransferases and adenylate cyclase]

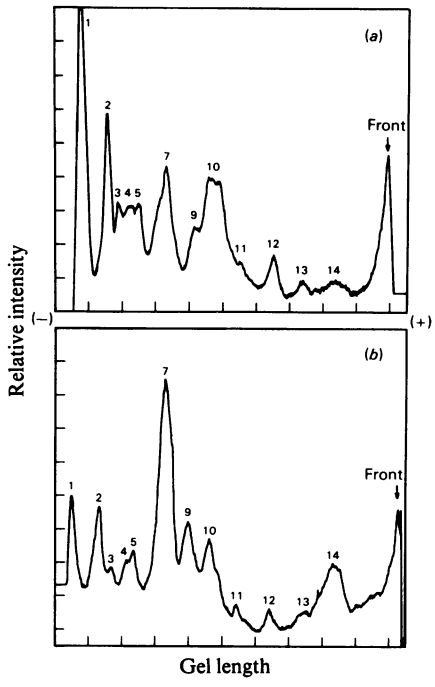

Fig. 5. *Densitometric scanning of radioautographs of labelled glycoproteins present in Golgi membranes 5min (without chase; a) and 30min (with chase; b) after fucose injection*

Rats were injected intraperitoneally with 500μCi of L-[1,5,6-^{3}H]fucose, and killed 5min later (*a*), or followed by 7mg of unlabelled fucose at 10min, and killed 30min later (*b*). Golgi membranes ($\leqslant$30% sucrose-density-gradient fractions) were obtained from isolated intestinal villus cells. The membrane fractions, containing 70000d.p.m. of radioactivity, were solubilized and analysed as described in the legend to Fig. 3. Migration was from left (−) to right (+). The radioautographs were scanned with a Gilford spectrophotometer. The relative intensity of the bands was plotted on a transmission scale in arbitrary units.

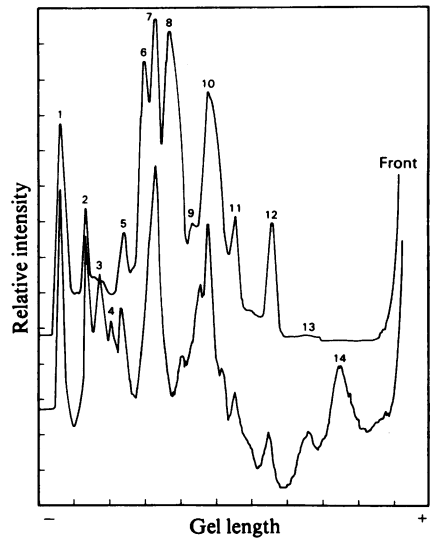

Fig. 6. *Comparison of radioactive bands present in brush border (upper curve) and Golgi (lower curve) membranes*

Rats were injected intraperitoneally with 500μCi of L-[1,5,6-^{3}H]fucose and killed 45min later. Golgi membranes ($\leqslant$30% sucrose-density-gradient fractions) were obtained from isolated intestinal villus cells (Weiser *et al.*, 1978). Brush-border membranes were obtained from intestinal mucosal scrapings (Hopfer *et al.*, 1973). The membrane fractions, containing 60000d.p.m. of radioactivity, were solubilized and analysed as in the legend to Fig. 3. Migration was from left (−) to right (+). The radioautographs were scanned with a Gilford spectrophotometer. The relative intensity of the bands was plotted on a transmission scale in arbitrary units.

have not been detected in this membrane (M. M. Weiser & A. Quaroni, unpublished observations). When injected into rabbits, this membrane has elicited antibodies having a striking specificity, exclusively reacting with the brush-border region of differentiated villus cells (Quaroni *et al.*, 1979*a*). The purification of Golgi and lateral basal membranes, and their characterization, have been described recently (Weiser *et al.*, 1978). A particularly important point for the interpretation of the results reported in the present study is the degree of cross-contamination between Golgi and lateral basal membrane fractions. Although the data presented in previous work (Weiser *et al.*, 1978) seem to exclude contamination of Golgi fractions with lateral basal membranes, the opposite has been more difficult to assess. Functionally, Golgi and lateral basal membranes are different with respect to Ca^{2+} uptake (Freedman *et al.*, 1977), and vitamin D repletion (J. A. MacLaughlin, M. M. Weiser & R. A. Freedman, unpublished work). Other data in the present report and the following paper (Quaroni *et al.*, 1979*b*) further support an effective separation of Golgi from lateral basal membranes. Thus: (*a*) the incorporation of radioactive fucose (d.p.m./mg of protein) into Golgi fractions was, in the first 2h, much higher than in fractions characterized as lateral basal portions of the plasmalemma (Fig. 1); (*b*) the kinetics of label incorporation into Golgi and lateral basal membranes were quite different (Fig. 1); (*c*) a chase with non-radioactive fucose had a marked effect on the incorporation into Golgi, but only slightly decreased the peak of specific radioactivity of lateral basal fractions (Fig. 1); (*d*) administration of colchicine (see following paper, Quaroni *et al.*, 1979*b*) affected the incorporation of radioactive

fucose quite differently in Golgi and lateral basal membranes. Although the presence of radioactivity in lateral basal membrane fractions already 5 min after fucose injection is evidence for some contamination of these fractions by Golgi elements, the above considerations seem to prove that this contamination was limited and is not a major factor in the interpretation of the results reported in the present paper. Significant contamination of Golgi and lateral basal membrane fractions with components of intestinal cells other than absorptive villus cells, for example goblet-cell mucins, also appears unlikely because: (*a*) the absorptive villus cells constitute the great majority of the cells present on the intestinal villus and isolated by the method of Weiser (1973); (*b*) all but one (band 14, Figs. 3, 4 and 6) of the labelled glycoproteins found in Golgi and lateral basal membrane fractions seemed to correspond to glycoproteins present in the microvillus membranes that, as discussed above, are derived exclusively from absorptive villus cells; (*c*) mucin-type glycoproteins synthesized by goblet cells are known to have a molecular weight much higher than the glycoproteins studied in the present report (Forstner *et al.*, 1973*a*,*b*), and are secreted at a slow rate (Forstner, 1970; Neutra & Leblond, 1966).

As expected from previous studies (Bennett, 1970; Bennett & Leblond, 1971; Bennett *et al.*, 1974), the radioactive fucose was initially incorporated into Golgi membranes (Fig. 1). The steep decline in specific radioactivity of this fraction, after the peak at 30 min, is consistent with a rapid redistribution of newly synthesized glycoproteins from the Golgi complex to other parts of the villus cell. A chase with non-radioactive fucose markedly lowered the amount of radioactivity incorporated into Golgi membranes, already at 15 min, presumably as a consequence of dilution of a labelled pool. However, the subsequent rise in specific radioactivity in these membranes to a peak at 30 min was unexpected. Little or no free fucose was found trapped inside Golgi membranes (Table 1) and most of the radioactivity appeared to be incorporated into chloroform-insoluble macromolecules. A probable explanation for the second peak of incorporation into Golgi membranes (with chase) is the existence of at least two different pools of fucose (or GDP-fucose) inside the villus cell, one rapidly diluted by chase, and another relatively inaccessible to chase. Considerable quantitative differences in the patterns of labelled glycoproteins present in Golgi membranes at 5 min (without chase) and at 30 min (with and without chase) were evident (Fig. 5). They may reflect differences in the participation in the glycosylation of membrane glycoproteins of two distinct enzyme systems, specifically utilizing one or the other pool of fucose.

The chase had no significant effect on the amount of radioactive fucose incorporated into both Golgi and lateral basal membranes 3–4 h after fucose injection (Fig. 1). In contrast, it caused a very marked decrease of incorporation into microvillus membranes at all time points, but particularly in correspondence with the first peak of incorporation (Fig. 2). This suggests that of the two distinct processes uncovered in the Golgi complex by the chase, the more sensitive to chase is involved in the synthesis of glycoproteins specifically destined for the microvillus membrane.

The incorporation of radioactive fucose into lateral basal membrane fractions followed closely that seen for Golgi (Fig. 1). Labelled glycoprotein patterns were also quite similar at all times (Figs. 3, 4 and 6). These results suggest a direct incorporation of Golgi-derived elements into the lateral basal part of the plasmalemma. The particular glycoprotein, band 14, which was never detected in microvillus membranes, concentrated in both Golgi and lateral basal membranes at late times (Figs. 3 and 4). All other labelled glycoproteins, present in these membranes shortly after fucose injection, tended to disappear at late times and seemed to correspond to labelled glycoproteins detected in the microvillus membrane. The period of their disappearance (1–3 h after fucose injection) corresponded to the second phase of incorporation of radioactivity into microvillus membranes (Fig. 2). These results, and the ones reported in the following paper (Quaroni *et al.*, 1979*b*) concerning the effects of colchicine on the incorporation of fucose into these membrane fractions, seem to suggest that newly synthesized glycoproteins, after being incorporated into the lateral basal portion of the plasmalemma, are redistributed into the microvillus membrane in an active and somewhat selective fashion (in that at least one protein, band 14, does not appear in the microvillus membrane). Our data do not, however, give any information on how this transfer might take place. In this respect, it is noteworthy that the tight junctions may indeed physically separate the luminal and the lateral sides of the plasmalemma, preventing any direct exchange of membrane proteins. Very different turnover rates between the two parts of the plasma membrane represent another explanation of the above mentioned results, which we cannot rule out. Endocytosis of entire portions of the membrane (Steinman & Cohn, 1976) or the action of proteolytic enzymes have been suggested as mechanisms for plasma-membrane degradation, a process that is not well documented. Both mechanisms are likely to take place at a faster rate at the luminal side of the intestinal villus cell, where absorption of nutrients is localized and where absorption of pancreatic proteolytic enzymes has been demonstrated. Alpers (1972) has provided evidence that proteins of the microvillus membrane are degraded fairly rapidly. In addition, turnover rates seem to be higher for villus cell microvillus proteins than for crypt-cell plasma-membrane components (Alpers, 1977). None of these studies, how-

ever, have shown that the proteins in the lateral basal membrane have a slower rate of turnover. Incorporation of radioactivity into microvillus membranes followed a biphasic pattern, whether or not a chase was given (Fig. 2). Similar kinetics of incorporation were found in previous studies of radioactive glucosamine incorporation into microvillus membranes (Forstner, 1970; Kim & Perdomo, 1974; Weiser, 1973). The first peak of incorporation most likely represents labelled glycoproteins coming directly from the Golgi complex, since it occurred at 30min (with chase) before the peak of incorporation in lateral basal membranes and, as with Golgi membranes, it was markedly affected by chase. The subsequent decline in specific radioactivity may be related to the disappearance, from microvillus membranes, of a glycoprotein (band 2, Figs. 3 and 4) that was the major labelled component of these membranes until 30min (with chase) or 60min (without chase) after fucose injection. A similar finding has been described for liver cells (Riordan *et al.*, 1974).

The second phase of incorporation of radioactivity into the microvillus membranes may represent an indirect route of incorporation, of proteins previously inserted into the lateral basal part of the plasmalemma (as discussed above), of cytoplasmic glycoproteins, or of glycoproteins associated with other cell organelles. Bennett and Leblond, in their radioautographic studies on the distribution of radioactive fucose in intestinal villus cells (Bennett, 1970; Bennett & Leblond, 1971), have localized a portion of the radioactivity in dense bodies and multilamellar vesicles, which appeared to concentrate near the microvillus membrane. These structures were not detected by electron-microscopic examination (Weiser *et al.*, 1978) in the Golgi and lateral basal membrane fractions studied in the present paper, and the lack of specific biochemical markers has prevented their localization in other subcellular fractions. It is possible that these structures are the means by which some of the newly synthesized glycoproteins are inserted into the microvillus membrane; they may represent either one or the two phases of incorporation found in the present study. In comparing labelled glycoprotein patterns of microvillus membranes with those of Golgi and lateral basal membranes, it is apparent that there are qualitatively more similarities than differences. Most of the labelled glycoproteins present in Golgi and lateral basal fractions shortly after fucose injection correspond to glycoproteins detected in the microvillus membrane at all times. There are three relevant exceptions: band 14 (Figs. 3 and 4) was never detected in microvillus membranes; bands 6 and 8 were only detected in microvillus membranes. These latter bands could have originated from: (*a*) a part of the Golgi complex selectively lost during subcellular fractionation; (*b*) another unidentified cell organelle, or (*c*) a post-translational modification after release from the Golgi complex. In conclusion, the results reported in the present paper suggest that, in the intestinal villus cell, newly synthesized membrane glycoproteins move from their site of final glycosylation, the Golgi complex, almost contemporaneously to both the lateral basal and the microvillus parts of the plasmalemma through two different routes. Subsequently, most of the glycoproteins newly inserted at the lateral surface membrane may be quickly destroyed or redistributed towards the microvillus membrane by a process that is likely to be actively directed by a subcellular structure.

Additional support for this hypothesis comes from the study of the effect of colchicine on the process of membrane glycoprotein biogenesis presented in the following paper (Quaroni *et al.*, 1979*b*).

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References

Alpers, D. H. (1972) *J. Clin. Invest.* **51**, 2621–2630
Alpers, D. H. (1977) *Biochem. Biophys. Res. Commun.* **75**, 130–135
Bekesi, J. G. & Winzler, R. J. (1967) *J. Biol. Chem.* **242**, 3873–3879
Bennett, G. (1970) *J. Cell Biol.* **45**, 668–673
Bennett, G. & Leblond, C. P. (1971) *J. Cell Biol.* **51**, 875–881
Bennett, G., Leblond, C. P. & Haddad, A. (1974) *J. Cell Biol.* **60**, 258–284
Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88
Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
Forstner, G. G. (1970) *J. Biol. Chem.* **245**, 3584–3592
Forstner, G. G., Sabesin, S. M. & Isselbacher, K. J. (1968) *Biochem. J.* **106**, 381–390
Forstner, J. F., Jabbal, I. & Forstner, G. G. (1973*a*) *Can. J. Biochem.* **51**, 1154–1166
Forstner, J. F., Taichman, N., Kalnins, V. & Forstner, G. (1973*b*) *J. Cell Sci.* **12**, 585–602
Freedman, R. A., Weiser, M. M. & Isselbacher, K. J. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3612–3616
Grinell, F., Hays, D. G. & Minter, D. (1977) *Exp. Cell Res.* **110**, 175–190
Hausman, R. E. & Moscona, A. A. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 916–920
Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248**, 25–32
Howard, C. B. & Kelleher, P. C. (1971) *Clin. Chim. Acta* **31**, 75–80
Kahn, C. R. (1976) *J. Cell Biol.* **70**, 261–286
Kim, Y. S. & Perdomo, J. M. (1974) *FEBS Lett.* **44**, 309–312

Kim, Y. S., Perdomo, J., Ochoa, P. & Isaacs, R. A. (1975) *Biochim. Biophys. Acta* **391**, 39–50
Laemmli, U. K. (1970) *Nature (London)* **227**, 680–684
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Maestracci, D., Schmitz, J., Preiser, H. & Crane, R. K. (1973) *Biochim. Biophys. Acta* **323**, 113–124
Maestracci, D., Preiser, H., Hedges, T., Schmitz, J. & Crane, R. K. (1975) *Biochim. Biophys. Acta* **382**, 147–156
Messer, M. & Dahlqvist, A. (1966) *Anal. Biochem.* **14**, 376–392
Neutra, M. & Leblond, C. P. (1966) *J. Cell Biol.* **30**, 137–150
Palade, G. (1975) *Science* **189**, 347–358
Pearlstein, E. (1976) *Nature (London)* **262**, 497–500
Podolsky, D. K. & Weiser, M. M. (1975) *Biochem. Biophys. Res. Commun.* **65**, 545–551
Quaroni, A., Wands, J., Trelstad, R. L. & Isselbacher, K. J. (1979*a*) *J. Cell Biol.* **80**, 248–265
Quaroni, A., Kirsch, K. & Weiser, M. M. (1979*b*) *Biochem. J.* **182**, 213–221
Reid, M. S. & Bieleski, R. L. (1968) *Anal. Biochem.* **22**, 374–381
Riordan, J. R., Mitranic, M., Slavik, M. & Moscarello, M. A. (1974) *FEBS Lett.* **47**, 248–251
Rothman, J. E. & Lenard, J. (1977) *Science* **195**, 743–753
Steinman, R. M. & Cohn, Z. A. (1976) in *Biogenesis and Turnover of Membrane Macromolecules* (Cook, J. S., ed.), pp. 1–14, Raven Press, New York
Tashima, Y. (1975) *Anal. Biochem.* **69**, 410–414
Vicker, M. G. (1976) *J. Cell Sci.* **21**, 161–173
Weiser, M. M. (1973) *J. Biol. Chem.* **248**, 2536–2541
Weiser, M. M., Neumeier, M. M., Quaroni, A. & Kirsch, K. (1978) *J. Cell Biol.* **77**, 722–734
Whaley, W. G., Dauwalder, M. & Kephart, J. E. (1972) *Science* **175**, 596–599
Yurchenco, P. D. & Atkinson, P. H. (1977) *Biochemistry* **16**, 944–953