Synthesis of Membrane Glycoproteins in Rat Small-Intestinal Villus Cells

EFFECT OF COLCHICINE ON THE REDISTRIBUTION OF L-[1,5,6-³H]FUCOSE-LABELLED MEMBRANE GLYCOPROTEINS AMONG GOLGI, LATERAL BASAL AND MICROVILLUS MEMBRANES

By Andrea QUARONI, Katherina KIRSCH and Milton M. WEISER*

*Department of Medicine, Harvard Medical School, Medical Services (Gastrointestinal Unit),
Massachusetts General Hospital, Boston, MA 02114, U.S.A.*

(Received 11 December 1978)

To define the role of cytoplasmic microtubules in the biogenesis of plasmalemma glycoproteins of rat small-intestinal villus cells, we studied the effect of colchicine on the incorporation of L-[1,5,6-³H]fucose into Golgi, lateral basal and microvillus membranes. Colchicine was administered intraperitoneally before or after injection of radioactive fucose. The incorporation of radioactivity into Golgi membranes was little affected by colchicine, which did not prevent the redistribution of most of the labelled glycoproteins from the Golgi complex into other parts of the villus cell. The incorporation of labelled glycoproteins into the microvillus membrane was greatly inhibited by colchicine given 2h or 10min before the radioactive fucose: all labelled glycoproteins present in this membrane were equally affected. In contrast, the administration of colchicine considerably increased the incorporation of radioactivity into the lateral basal part of the plasmalemma, and prevented the disappearance of most of the labelled glycoproteins from this membrane at late times after fucose injection. These results suggest that cytoplasmic microtubular structures are important for the polarization of the intestinal villus cell and the biogenesis of the microvillus membrane, although playing little or no role in the movement of membrane components from the Golgi complex to the lateral basal part of the plasmalemma.

Some of the major features that distinguish the differentiated intestinal villus cell from its undifferentiated progenitor cell in the crypt are those associated with the cell surface membrane. During maturation the intestinal epithelial cell assumes a long columnar shape with a basal surface membrane attached to a basement membrane, and a lateral membrane which is the site of the major 'active'-transport pump, (Na⁺+K⁺)-dependent ATPase (Douglas *et al.*, 1972), and has the potential to form large intercellular spaces (Smulders *et al.*, 1972) with many infoldings or plicae (Vial & Porter, 1975). The other major cell surface change is the formation of a distinct structure at the luminal portion of the cell, the brush border, which has a unique composition and is the site of many hydrolytic enzymes and transport carriers (Gardner *et al.*, 1970*a,b,c*). It has been shown that the villus cell, in contrast with the undifferentiated crypt cell, has a very rapid rate of glycoprotein synthesis, a large fraction of which is involved in the synthesis of microvillus membrane

glycoproteins (Weiser, 1973). This high rate of glycoprotein synthesis is apparently not related to mucus production by the goblet cells (Neutra *et al.*, 1977). Previous studies have suggested that the marked functional and morphological polarity of the small-intestinal villus cell may depend on a selective insertion of membrane components into different parts of the plasmalemma, and on its ability to redistribute newly synthesized membrane glycoproteins between lateral basal and microvillus membranes (Quaroni *et al.*, 1979). The mechanisms of this selective redistribution were not clear.

A considerable amount of evidence obtained with lymphocytes and cultured cells has suggested that microtubules and microfilaments are important in the movement of proteins within the surface membrane (Nicolson & Poste, 1976; Sundqvist & Ehrnst, 1976; Edelman, 1976). Anti-microtubular agents have also been shown to impair secretory functions (Le Marchand *et al.*, 1973; Ehrlich *et al.*, 1974; Stein *et al.*, 1974); the point of inhibition in these cases has been postulated to be in the movement of secretory vesicles from the Golgi complex to the plasma membrane (Ehrlich *et al.*, 1974; Stein *et al.*, 1974).

* Present address: Division of Gastroenterology and Nutrition, E. J. Meyer Memorial Hospital, 462 Grider Street, Buffalo, NY 14215, U.S.A.

In the intestinal villus cells, microtubules have been found localized in the apical and Golgi areas of the cytoplasm (Reaven & Reaven, 1977), suggesting a functional role in the exchange of material between the Golgi complex and the microvillus membrane. In most cases, information about the function of cytoskeletal structures has been obtained by the use of specific inhibitors, such as colchicine, vinblastine and cytochalasins.

Colchicine is known to have striking effects on small-intestinal epithelial cells. Chronic administration of colchicine to experimental animals and man produces a malabsorption syndrome (Race *et al.*, 1966). Colchicine given orally in relatively high doses over a period of days has been shown to cause a significant decrease in the activity of intestinal microvillus enzymes lactase, sucrase and alkaline phosphatase (Levin, 1966; Herbst *et al.*, 1970). Glickman *et al.* (1976) have shown that colchicine given intraperitoneally impairs lipid transport across the intestine, an effect that appeared to represent inhibition of the exit step of chylomicrons from the epithelial cells. In the latter studies, the general morphology of the intestinal cells, the mitotic activity in the crypts and the rate of cellular migration along the villus were not affected by colchicine. This suggested that the drug was mainly exerting its immediate effects through direct interaction with the differentiated intestinal villus cells.

In the present study, colchicine was given intraperitoneally to rats before or after injection of radioactive fucose, to investigate the role of cytoplasmic microtubules in the biogenesis of plasmalemma glycoproteins of the intestinal villus cell. Colchicine was shown to markedly inhibit the incorporation of labelled glycoproteins into the microvillus membrane, with a corresponding increase of incorporation into the lateral basal membrane. Release of labelled glycoproteins from the Golgi complex was little affected by colchicine.

These present results have been interpreted as suggesting a direct involvement of microtubules, or associated structures, in the polarization of the intestinal villus cell.

Experimental

Colchicine administration

Colchicine was dissolved in 0.154M-NaCl immediately before use and injected intraperitoneally (0.5mg/100g body wt.) 2h before, 10min before or 1h after radioactive fucose administration. Control animals were injected with an equal volume of 0.154M-NaCl only.

All other techniques were as previously described (Quaroni *et al.*, 1979).

Results

Colchicine has been shown to affect a number of intracellular processes in different tissues, between 1 and 3h after its administration (Le Marchand *et al.*, 1973; Ehrlich *et al.*, 1974; Stein *et al.*, 1974; Reaven & Reaven, 1977). A careful examination of its effects on the absorptive intestinal villus cells (Reaven & Reaven, 1977) has demonstrated a decrease of about 50% in the number of microtubules in the apical region 1h after injection of colchicine. The effects of colchicine are generally attributed to impairment of microtubule assembly, but any interpretation of results must exclude effects on other cell components or on the techniques used for subcellular fractionation. In the present study, colchicine had no significant effect on the total incorporation of radioactivity into intestinal homogenates (Table 1). This indicates that the drug did not interfere with transport or nucleotide activation of fucose.

Colchicine is also known to promote lipid accumulation in Golgi elements (Glickman *et al.*, 1976; Reaven & Reaven, 1977) with consequent changes in the density of these membranes. To be certain that the density-gradient fractions obtained from villus cell homogenates as previously described (Weiser *et al.*, 1978) contained similar membrane components when prepared from control or colchicine-treated rats, marker enzymes were re-evaluated. Galactosyl-transferase activity (total and specific) showed no difference between the two groups (Table 2). (Na^+ + K^+)-dependent ATPase and sucrase activities were also routinely measured in all Golgi and lateral basal or microvillus membrane preparations, and no

Table 1. *Lack of effect of colchicine on the total incorporation of L-[1,5,6- ^3H]fucose into intestinal homogenates*

Rats were injected intraperitoneally with either 0.5mg of colchicine/100g body wt. dissolved in 0.154M-NaCl (+C) or an equal volume of 0.154M-NaCl alone (−C), 2h before receiving 250 μCi of L-[1,5,6- ^3H]fucose. A chase with 7mg of unlabelled fucose (+chase) or an equal volume of 0.9% NaCl (−chase) was given 10min after radioactive fucose injection. The rats were killed 30–180min after radioactive fucose injection, and homogenates were obtained from intestinal villus cells isolated by the method of Weiser (1973). Radioactivity and protein were determined as previously described (Quaroni *et al.*, 1979). Values of radioactivity incorporation (d.p.m./mg of protein) represent the average $\pm$ S.E.M. for the number of experiments given in parentheses.

^3H radioactivity (d.p.m./mg of protein)

− C, − chase	30732 $\pm$ 3752 (11)
+ C, − chase	28898 $\pm$ 4112 (11)
− C, + chase	27018 $\pm$ 2250 (12)
+ C, + chase	28117 $\pm$ 1751 (12)

Table 2. *Lack of effect of colchicine on galactosyltransferase distribution in density-gradient fractions*

Rats were injected intraperitoneally with either 0.5 mg of colchicine/100 g body wt. dissolved in 0.154 M-NaCl (+C) or 0.154 M-NaCl alone (–C), followed by 500 μ Ci of L-[1,5,6- 3 H]fucose 10 min later, and 7 mg of unlabelled fucose 20 min later. They were killed 2 h after radioactive-fucose injection, and density-gradient fractions were obtained from intestinal villus cells by the method of Weiser *et al.* (1978). Galactosyltransferase activity was determined on the various fractions as previously described (Quaroni *et al.*, 1979). Radioactivity owing to [3 H]fucose has been subtracted from all samples.

Fractions (% sucrose)	Galactosyltransferase activity (c.p.m./mg per min)		Activity in density-gradient fractions (% of total)	
	–C	+C	–C	+C
≤ 30	62484	42589	14.4	14.9
30/40	26974	17256	32.1	32.7
40	22412	17734	19.1	23.8
40/50	5107	5073	28.0	28.7
50	3625	4040	6.3	8.5

Table 3. *Lack of effect of colchicine on the (Na⁺+K⁺)-dependent ATPase activity in isolated lateral basal membranes and on the sucrase activity in isolated microvillus membranes*

Rats were injected intraperitoneally with either 0.5 mg of colchicine/100 g body wt. dissolved in 0.154 M-NaCl (+C) or an equal volume of 0.154 M-NaCl (–C) 2 h before receiving 250 μ Ci of L-[1,5,6- 3 H]fucose. They were killed 15–240 min after radioactive fucose injection, and either lateral basal membranes [from isolated villus cells by the method of Weiser *et al.* (1978)] or microvillus membranes [by the method of Hopfer *et al.* (1973)] were obtained. Protein, (Na⁺+K⁺)-dependent ATPase activity (in lateral basal membranes) and sucrase activity (in microvillus membranes) were determined as previously described (Quaroni *et al.*, 1979). Enzyme specific activities represent the average $\pm$ S.E.M. for the numbers of experiments given in parentheses.

	Enzyme specific activity	
	–C	+C
(Na ⁺ +K ⁺)-dependent ATPase activity (nmol/mg of protein per min)	312 $\pm$ 46 (11)	327 $\pm$ 85 (11)
Sucrase activity (units/mg of protein)	1.59 $\pm$ 0.22 (10)	1.55 $\pm$ 0.18 (8)

differences were found between controls and colchicine-treated rats (Table 3). When Golgi, lateral basal and microvillus membranes were solubilized with sodium dodecyl sulphate and analysed by polyacrylamide-slab-gel electrophoresis, no differences were found between the protein patterns of membranes obtained from controls or colchicine-treated rats.

These observations suggest that the injection of colchicine had no significant effect on the subcellular-fractionation techniques used in the present work. It is also noteworthy that all the present experiments were done on rats starved overnight, and that a normal ultrastructural appearance of the Golgi complex, without endogenous lipid accumulation, has been demonstrated for intestinal villus cells of rats given colchicine without an accompanying lipid infusion (Glickman *et al.*, 1976).

Effect of colchicine on L-[1,5,6- 3 H]fucose incorporation into Golgi membranes

The injection of colchicine 2 h before radioactive

fucose produced two changes in the kinetics of radioactivity incorporation into Golgi membranes (Fig. 1): (a) the peak of incorporation, with or without chase, was found at 15 min, contrasting with the low specific radioactivity of control membranes (with chase) at this time point, and the peak of incorporation at 30 min for controls without chase (see Fig. 1 in Quaroni *et al.*, 1979); (b) 2–3 h after injection of fucose, the specific radioactivity of Golgi membranes from rats given colchicine was 3 times higher than for controls. Without chase (and with colchicine) the incorporation of radioactivity was considerably higher than with chase (Fig. 1), but followed similar kinetics and attained the same plateau value as with chase at 2–3 h after injection of fucose.

The distribution of radioactivity among different glycoproteins in Golgi membranes at 30 min was, with and without colchicine, quite similar (Fig. 2), suggesting that the drug did not significantly alter the types of glycoproteins fucosylated in the Golgi complex. Most of the labelled bands seen in the Golgi membranes shortly after fucose injection tended to

Fig. 1. Effect of colchicine injected 2h before radioactive fucose on the kinetics of L-[1,5,6-³H]fucose incorporation into Golgi and lateral basal membranes

Rats were injected intraperitoneally with 0.5mg of colchicine/100g body wt. dissolved in 0.154M-NaCl (○ and ●) or an equal volume of 0.154M-NaCl (△) 2h before receiving 250 μ Ci of L-[1,5,6-³H]fucose. A chase with non-radioactive fucose (7mg; ○ and △) or an equal volume of 0.9% NaCl (●) were injected 10min after radioactive fucose. The rats were killed at different times after radioactive-fucose injection, and Golgi (a from fractions $\leq 30\%$ sucrose) and lateral basal membranes from the 30%/40% sucrose interphase (b) or the 40% sucrose fraction (c) were obtained from intestinal villus cells by the method of Weiser *et al.* (1978). Protein and radioactivity were determined as previously described (Quaroni *et al.*, 1979).

Fig. 2. Lack of effect of colchicine on the patterns of labelled glycoproteins of Golgi membranes 30min after radioactive fucose injection

Rats were injected intraperitoneally with 0.5mg of colchicine/100g body wt. dissolved in 0.154M-NaCl,

Fig. 3. Densitometric scanning of radioautographs of labelled glycoproteins present 3h after fucose injection in Golgi membranes (a) and lateral basal membranes (b) obtained from rats injected with colchicine 2h before fucose

A rat was injected intraperitoneally with 0.5mg of colchicine/100g body wt. in 0.9% NaCl and, subsequently, 500 μ Ci of L-[1,5,6-³H]fucose 2h later and 7mg of unlabelled fucose 2h and 10min later. Golgi and lateral basal membranes were obtained from isolated villus cells, solubilized and analysed by slab-gel electrophoresis as described in the legend to Fig. 2.

or an equal volume of 0.9% NaCl 2h before receiving 500 μ Ci of L-[1,5,6-³H]fucose. A chase with non-radioactive fucose (7mg) was given 10min after radioactive fucose. Golgi membranes were obtained from isolated villus cells as previously described (Weiser *et al.*, 1978). The membrane fractions, containing 60000 d.p.m. of radioactivity, were solubilized and analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis on 7.5% acrylamide gels. The radioactive proteins were detected by fluorography (for details see Quaroni *et al.*, 1979). Densitometric scanings of the patterns of labelled glycoproteins in membranes from colchicine-treated rats (upper curve) or controls (lower curve) were obtained with a Gilford spectrophotometer. The relative intensity of the bands was plotted on a transmission scale in arbitrary units.

disappear or were considerably decreased in intensity at 2–3 h (Fig. 3), in the presence of colchicine, as found in its absence (see Figs. 3 and 4 in Quaroni *et al.*, 1979).

When colchicine was injected 10 min before or 1 h after the radioactive fucose, it had no significant effect either on the kinetics of radioactivity incorporation or on the patterns of labelled glycoproteins of Golgi membranes (results not shown).

Effect of colchicine on L-[1,5,6-³H]fucose incorporation into lateral basal membranes

The kinetics of radioactive fucose incorporation into lateral basal membranes were greatly affected by colchicine injected 2 h before fucose (Fig. 1). The specific radioactivity (d.p.m./mg of protein) of these membrane fractions continuously increased until the latest time point considered (3 h), and was much higher (about 3 times) than in controls. Without chase, a peak of incorporation was found at 15–30 min, but it was followed by only a slight decline in specific radioactivity (Fig. 1). Incorporation at 3 h, with and without chase, was quite similar.

When colchicine was injected 10 min before radioactive fucose (with chase), the peak of radioactivity incorporation was found at 45 min, and at this time no effect of colchicine was evident (Fig. 4). Thereafter, a slow decrease in specific radioactivity was observed in membranes from control rats (Figs. 1 and 4; see also Fig. 1 in Quaroni *et al.*, 1979); in contrast, the specific radioactivity of membranes from rats treated with colchicine was consistently higher at times after 45 min (Fig. 4), although this effect was considerably smaller than when colchicine was administered 2 h before fucose (Fig. 1).

Lateral basal membranes from rats given colchicine 1 h after radioactive fucose had, at 3 h, a specific radioactivity indistinguishable from controls (Fig. 4).

The most striking effect of colchicine on the incorporation of radioactive fucose into lateral basal membranes was on the patterns of labelled glycoproteins observed 2–3 h after fucose injection. Membranes from control animals, isolated at these times, showed the previously described (Quaroni *et al.*, 1979) change of pattern from the one of 45 min, i.e., disappearance or decrease in intensity of bands corresponding to higher-molecular-weight glycoproteins and increase in intensity of the band corresponding to the lowest-molecular-weight glycoprotein(s) [Fig. 5, (i) and (j) against (a) and (b); see also Figs. 3 and 4 in Quaroni *et al.*, 1979]. In contrast, no such change was observed in membranes from animals given colchicine 10 min (Fig. 5) or 2 h (results not shown) before radioactive fucose. The patterns of labelled glycoproteins of lateral basal and Golgi membranes isolated 3 h after fucose injection from rats given colchicine 10 min or 2 h (Fig. 3) before

Fig. 4. Effect of colchicine, injected 10 min before fucose, on the kinetics of L-[1,5,6-³H]fucose incorporation into lateral basal membranes

Rats were injected intraperitoneally with 0.5 mg of colchicine/100 g body wt. dissolved in 0.154 M-NaCl 10 min before (●) or 1 h after (▲) receiving 500 μ Ci of L-[1,5,6-³H]fucose. Unlabelled fucose (7 mg) was injected 10 min after radioactive fucose. Controls (○) received 0.154 M-NaCl alone. The animals were killed at different times after radioactive-fucose injection, and lateral basal membranes from the 30%/40% sucrose interphase (a) and 40% sucrose-density-gradient fraction (b) were obtained from intestinal villus cells by the method of Weiser *et al.* (1978). Protein and radioactivity were determined as previously described (Quaroni *et al.*, 1979).

fucose were also quite different. In contrast, similar patterns of labelled glycoproteins for these two membrane fractions were observed at all times after fucose injection in the absence of colchicine (Quaroni *et al.*, 1979).

Effect of colchicine on L-[1,5,6-³H]fucose incorporation into microvillus membranes

The injection of colchicine greatly inhibited the incorporation of radioactive fucose into microvillus membrane (Fig. 6). When the drug was given 2 h before fucose, the inhibition was evident at all time points, although greatest 2–4 h after fucose injection (at 4 h after fucose injection, the specific radioactivity

Fig. 5. Effect of colchicine on radioactivity distribution among different glycoproteins in lateral basal membranes

Rats were injected intraperitoneally with either 0.5 mg of colchicine/100 g body wt. dissolved in 0.154 M-NaCl (+C), or the same volume of 0.154 M-NaCl (-C), followed by 500 μ Ci of L-[1,5,6- 3 H]fucose 10 min later, and 7 mg of unlabelled fucose 20 min later. The animals were killed 45 min, 2 h or 3 h after radioactive-fucose injection, and lateral basal membranes from the 30%/40% sucrose interphase (30/40) and 40% sucrose-density-gradient fraction (40) were obtained from intestinal villus cells by the method of Weiser *et al.* (1978). Proteins in the different membranes, containing 60000 d.p.m. of 3 H radioactivity in all cases, were solubilized with sodium dodecyl sulphate and separated by slab-gel electrophoresis on 7.5% polyacrylamide gel. Radioactivity was detected by fluorography as previously described (Quaroni *et al.*, 1979).

of microvillus membranes from rats given colchicine was 5–6-fold lower than for controls). When colchicine was given 10 min before fucose, no significant difference in specific radioactivity was found at 30 min between microvillus membranes obtained from colchicine-treated and control rats. Later, however, the specific radioactivity of microvillus membranes from colchicine-treated animals increased (at 60 min) instead of decreasing (as for controls), slightly decreased at 90 min and remained fairly constant over the next 3 h. During this last period the incorporation of radioactivity in microvillus membranes from colchicine-treated rats was 40–50% of the incorporation in membranes from control animals.

When colchicine was given 1 h after radioactive fucose injection, the specific radioactivity of microvillus membranes at 3 and 4 h after fucose was given was indistinguishable from that of membranes from control animals (Fig. 6).

Fig. 6. Effect of colchicine on the kinetics of L-[1,5,6- 3 H]-fucose incorporation into microvillus membranes

Rats were injected intraperitoneally with 0.5 mg of colchicine/100 g body wt. dissolved in 0.154 M-NaCl 2 h before (○), 10 min before (▲) or 1 h after (□) receiving 500 μ Ci of L-[1,5,6- 3 H]fucose. Unlabelled fucose (7 mg) was injected 10 min after radioactive fucose. Controls (●) received 0.154 M-NaCl alone. The animals were killed at different times after radioactive-fucose injection and microvillus membranes were obtained from mucosal scrapings by the method of Hopfer *et al.* (1973). Protein and radioactivity were determined as previously described (Quaroni *et al.*, 1979). Each point represents the result obtained with one rat in a separate experiment. These results have been reproduced in two other sets of experiments.

Despite these changes in the kinetics of radioactivity incorporation into microvillus membranes, no major qualitative differences were found at any time between 30 min and 4 h between the patterns of labelled glycoproteins of membranes obtained from controls and from rats injected with colchicine 10 min before fucose. The specific radioactivity of microvillus membranes from rats given colchicine 2 h before fucose was too low, and a similar comparison of labelled glycoprotein patterns could not be carried out.

Discussion

In the preceding paper (Quaroni *et al.*, 1979), 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 injected intraperitoneally into Golgi, lateral basal and micro-

Fig. 7. Effect of colchicine on radioactivity distribution among different glycoproteins in microvillus membranes

Rats were injected intraperitoneally with either 0.5 mg of colchicine/100 g body wt. dissolved in 0.154 M-NaCl (+ Colchicine) or 0.154 M-NaCl alone (- Colchicine), followed by 500 μ Ci of L-[1,5,6- 3 H]fucose 10 min later, and 7 mg of unlabelled fucose 20 min later. They were killed at the different times indicated after radioactive-fucose injection, and microvillus membranes were obtained from mucosal scrapings by the method of Hopfer *et al.* (1973). Proteins in the different membranes, containing 60000 d.p.m. of radioactivity in all cases except for (b) (- Colchicine, 60 min, which contained 33000 d.p.m.) were solubilized with sodium dodecyl sulphate and separated by slab-gel electrophoresis on 7.5% polyacrylamide gel. Proteins were stained with Coomassie Blue, and radioactivity was detected by fluorography.

villus membranes. The results obtained are summarized in Scheme 1. Its main features are: (a) labelled glycoproteins are transferred from the Golgi complex to both the luminal and lateral basal parts of the plasmalemma via two different routes; and (b) most of the glycoproteins newly inserted into the lateral basal membrane are either rapidly destroyed or redistributed into the microvillus membrane (our results did not allow us to distinguish between these two alternatives).

In the present study we have investigated the role of cytoplasmic microtubules in the regulation of these processes. The relative times of colchicine and radioactive fucose injection were varied in an attempt to better define the microtubule-mediated events and to exclude, if possible, effects of colchicine independent from inhibition of microtubule polymerization. Thus: (a) colchicine injected 2 h before fucose was considered capable of affecting even the earliest events, i.e., the absorption of fucose by the villus cells, its utilization in the Golgi complex, and the release of labelled glycoprotein from this organelle; (b) colchicine given 10 min before fucose was considered capable of mostly affecting later events, in particular

a possible redistribution of labelled glycoproteins from the lateral basal to the microvillus membrane; and (c) administration of colchicine 1 h after fucose represented the control monitoring effects of colchicine not mediated through impairment of microtubule assembly.

Under all three conditions, colchicine appeared to have no inhibitory effect on the uptake of radioactive fucose by the intestinal villus cells and on the fucosylation of glycoproteins in the Golgi complex, since (a) the total incorporation of radioactivity into villus-cell homogenates was not significantly affected by the drug, (b) the specific radioactivity of Golgi membranes obtained from rats given colchicine 2 h before fucose was generally higher than that of Golgi membranes prepared from control rats (Fig. 1), and (c) the electrophoretic pattern of labelled glycoproteins of Golgi membranes obtained 30 min after fucose injection was unaffected by colchicine administered 2 h before fucose (Fig. 2).

The release of most of the labelled glycoproteins detected in Golgi membranes shortly after fucose injection was also apparently not affected by colchicine, since quite different patterns of labelled glycoproteins

Scheme 1. Membrane glycoprotein movement in rat small-intestinal villus cells

The proposed Scheme summarizes results obtained in the present investigation and in the preceding paper (Quaroni *et al.*, 1979). The values of 40% and 60% for the relative amount of glycoprotein incorporation into microvillus membrane, by direct movement from the Golgi complex or via the lateral basal membrane respectively, are derived from Fig. 5 and intended to be only an approximate estimate. The results of the present work suggest that colchicine inhibited the movement of labelled glycoproteins from the Golgi complex to the microvillus membrane only when given 2h before fucose (■); colchicine inhibited the redistribution of newly inserted glycoproteins from the lateral basal membrane to the microvillus membrane when given both 2h and 10min before fucose (■).

were found at 30min and at 3h after fucose (Figs. 2 and 3), similar to what had been found in the absence of colchicine (Quaroni *et al.*, 1979). Colchicine given 2h before radioactive fucose did, however, cause a significant change in the kinetics of radioactive-label incorporation into Golgi membranes (Fig. 1) and a 3-fold increase in specific radioactivity of this membrane fraction 2–3h after fucose injection. These effects were apparently due to an impairment of microtubule polymerization, since they were not observed when the drug was given 10min before or 1h after fucose injection, but cannot, at present, be interpreted.

The effects of colchicine on the incorporation of labelled glycoproteins into the different parts of the plasmalemma (microvillus and lateral basal membranes) were much more remarkable.

Colchicine injected 2h before fucose produced a considerable increase in the specific radioactivity of lateral basal membranes, and identical patterns of labelled glycoproteins in this membrane at 45min and at 3h after fucose injection (Fig. 5). This last result is particularly noteworthy since it suggests that the changes in patterns detected in controls (Fig. 5;

Figs. 3 and 4 in Quaroni *et al.*, 1979) are not due to a rapid extracellular release of newly inserted glycoproteins, a process that should be unaffected by colchicine. These changes more probably represent a redistribution of glycoproteins to other part(s) of the villus cell mediated or controlled by structures sensitive to colchicine.

The effects of colchicine on the incorporation of labelled glycoproteins into the microvillus membrane were opposite to those found for the lateral basal membrane. When the drug was given 2h before fucose, incorporation of radioactivity was strikingly decreased at all times after fucose injection, whereas when it was given 10min before fucose only the second phase of incorporation appeared to be affected (Fig. 6). All labelled glycoproteins present in the microvillus membrane were equally affected (Fig. 7).

These results can be interpreted as suggesting: (a) an effect of colchicine on the movement of labelled glycoproteins from the Golgi complex to the microvillus membrane, occurring between 15 and 90min after fucose injection and therefore likely to be inhibited only when colchicine was given 2h before fucose; and (b) a similar inhibition by colchicine of the redistribution of newly inserted glycoproteins from the lateral basal to the microvillus membrane. Since this process is likely to take place between 1 and 3h after fucose injection (Quaroni *et al.*, 1979), it could be affected by colchicine given both 2h and 10min before fucose.

Considering the decreased incorporation of labelled glycoproteins into the microvillus membrane caused by colchicine, the corresponding increase in incorporation into the lateral basal membrane might simply be explained in terms of contamination of the latter membrane fractions with subcellular components normally destined for, or associated with, the microvillus membrane. This does not appear a likely possibility: with colchicine, as found without it (Quaroni *et al.*, 1979), a chase with unlabelled fucose had no effect on the specific radioactivity of lateral basal membranes at 2 and 3h after fucose injection (Fig. 1). In contrast, the specific radioactivity of glycoproteins destined for the microvillus membrane was found to be considerably decreased by a chase (Quaroni *et al.*, 1979).

In conclusion, the results presented seem to support Scheme 1 for the biogenesis of membrane glycoproteins of the intestinal villus cell, and suggest that microtubular structures are an important factor in the generation and maintenance of the morphological and functional polarity of the villus cells.

We especially thank Dr. Kurt J. Isselbacher for his support and helpful criticisms of this work, as well as his critical review of the manuscript. This research was supported by USPHS grant CA-16703 and American Cancer Society grant BC-93.

References

- Douglas, A. P., Kerley, R. & Isselbacher, K. J. (1972) *Biochem. J.* **128**, 1329–1338
- Edelman, G. M. (1976) *Science* **192**, 218–226
- Ehrlich, H. P., Ross, R. & Bornstein, P. (1974) *J. Cell Biol.* **62**, 390–405
- Gardner, J. D., Brown, M. S. & Laster, L. (1970a) *N. Engl. J. Med.* **283**, 1196–1202
- Gardner, J. D., Brown, M. S. & Laster, L. (1970b) *N. Engl. J. Med.* **283**, 1264–1271
- Gardner, J. D., Brown, M. S. & Laster, L. (1970c) *N. Engl. J. Med.* **283**, 1317–1324
- Glickman, R. M., Perrotto, J. L. & Kirsch, K. (1976) *Gastroenterology* **70**, 347–352
- Herbst, J. J., Hurwitz, R., Sunshine, P. & Kretchmer, N. (1970) *J. Clin. Invest.* **49**, 530–536
- Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248**, 25–32
- Le Marchand, Y., Singh, A., Assimacopoulos-Jeannet, F., Orci, L., Rouiller, C. & Jeanrenaud, B. (1973) *J. Biol. Chem.* **248**, 6862–6870
- Levin, R. J. (1966) *Gut* **7**, 250–257
- Neutra, M. R., Grand, R. J. & Trier, J. S. (1977) *Lab. Invest.* **36**, 535–546
- Nicolson, G. L. & Poste, G. (1976) *N. Engl. J. Med.* **295**, 197–203; 253–258.
- Quaroni, A., Kirsch, K. & Weiser, M. M. (1979) *Biochem. J.* **182**, 203–212
- Race, T. F., Paes, I. C. & Falcon, W. W. (1966) *Clin. Res.* **14**, 480
- Reaven, E. P. & Reaven, G. M. (1977) *J. Cell Biol.* **75**, 559–572
- Smulders, A. P., Tormey, J. McD. & Wright, E. M. (1972) *J. Membr. Biol.* **7**, 164–197
- Stein, O., Sanger, L. & Stein, Y. (1974) *J. Cell Biol.* **62**, 90–103
- Sundqvist, K. G. & Ehrnst, A. (1976) *Nature (London)* **264**, 226–231
- Vial, J. & Porter, K. R. (1975) *J. Cell Biol.* **67**, 345–360
- 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