Surface-membrane biogenesis in rat intestinal epithelial cells at different stages of maturation

Andrea QUARONI,* Katharina KIRSCH,* Annette HERSCOVICS†
and Kurt J. ISSELBACHER*

*Department of Medicine, Harvard Medical School, Medical Services (Gastrointestinal Unit), Massachusetts General Hospital, Boston, and †Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, MA 02114, U.S.A.

(Received 14 March 1980/Accepted 15 May 1980)

The biosynthesis of membrane proteins and glycoproteins has been studied in rat intestinal crypt and villus cells by measuring the incorporation of L-[5,6-³H]fucose, D-[2-³H]mannose and L-[3,4,5-³H]leucine, given intraperitoneally, into Golgi, lateral-basal and luminal membranes. Incorporation of leucine and mannose was approximately equal in crypt and villus cells, whereas fucose incorporation was markedly higher (3–4 times) in the differentiated villus cells. As previously reported [Quaroni, Kirsch & Weiser (1979) *Biochem. J.* **182**, 203–212] most of the fucosylated glycoproteins synthesized in the villus cells and initially present in the Golgi and lateral-basal membranes were found re-distributed, within 3–4 h of label administration, in the luminal membrane. A similar process appeared to occur in the crypt cells, where, however, only few fucose-labelled glycoproteins were identified. In contrast, most of the leucine-labelled and many mannose-labelled membrane components found in the lateral-basal membrane of both crypt and villus cells did not seem to undergo a similar re-distribution process. The fucosylated glycoproteins of the intestinal epithelial cells represent, therefore, a special class of membrane components, most of which appear with differentiation, that are selectively localized in the luminal portion of the plasmalemma. In contrast with the marked differences in protein and glycoprotein patterns between the luminal membranes of villus and crypt cells, only minor differences were found between their lateral-basal membrane components: their protein patterns on sodium dodecyl sulphate/polyacrylamide slab gels, and the patterns of fucose-, mannose- and leucine-labelled components (analysed 3–4 h after label administration) were very similar. Although the minor differences detected may be of importance, it appears that most of the surface-membrane changes accompanying cell differentiation in the intestinal epithelial cells are localized in the luminal portion of their surface membrane.

In adult animals, the intestinal epithelium represents a system of continuous renewal. Cell replication is limited to the crypts, and as the cells migrate from the crypts on to the villus they lose the capacity to divide as they differentiate into mature columnar cells and are eventually extruded into the intestinal lumen (Leblond & Stevens, 1948; Messier & Leblond, 1960). This progression of cells from the crypts to the villi occurs in adult rats within 48 h (Leblond & Stevens, 1948; Clarke, 1970) and is accompanied by marked changes in cell morphology, the appearance of many typical intestinal enzymes (Dahlqvist & Nordström, 1966; Imondi *et al.*, 1969; Webster & Harrison, 1969; Herbst & Koldovsky,

1972; Raul *et al.*, 1977; Simon *et al.*, 1979), changes in glycolipid composition and biosynthesis (Glickman & Bouhours, 1976; Bouhours & Glickman, 1976) and alterations in the patterns of reactivity of cell-surface components with various lectins (Etzler & Branstrator, 1974). Most of these changes result from alterations in the structure and composition of the luminal membrane of crypt and villus cells. Concomitant changes at the lateral-basal portion of the surface membrane of the intestinal epithelial cells have not yet been clearly documented. Yet, the constant migration of differentiated cells from the crypts towards the villus tip appears to be an intrinsic property of the epi-

thelial cells, and is likely to be regulated by changes in intercellular adhesive properties and in the interaction of the basal plasma membrane with the underlying basement membrane. When cell proliferation in the crypts is markedly decreased or completely blocked, differentiated cells continue to migrate along the villi and are lost at the tip with a resulting overall depletion of the epithelial cell population (Quastler, 1956; Williams *et al.*, 1958; Lamerton, 1966; Leshner, 1967; Galjaard & Bootsma, 1969; Devik, 1971; Rijke *et al.*, 1976). A gradient of adhesivity of the intestinal epithelial cells to the basement membrane, with the villus cells being the least adhesive, has been demonstrated by Weiser (1973a).

Surface-membrane components are rapidly synthesized and degraded in both villus and crypt cells (Alpers, 1972; Weiser, 1973a,b; Alpers, 1977; Hauri *et al.*, 1977; Quaroni *et al.*, 1979a). In our previous studies on the biosynthesis of fucosylated glycoproteins in intestinal villus cells, we have identified different routes for the incorporation of newly synthesized glycoproteins into either the lateral-basal or the luminal portions of the surface membrane (Quaroni *et al.*, 1979a,c). The re-distribution of newly inserted fucosylated glycoproteins from the lateral-basal to the luminal membrane was inhibited by colchicine, thereby suggesting that cytoplasmic microtubular structures may be important for the polarization of the intestinal villus cell and the biogenesis of luminal-membrane components (Quaroni *et al.*, 1979b). In the present work we have extended these studies to leucine- and mannose-labelled membrane proteins in both crypt and villus cells, to further characterize the selectivity of the re-distribution process and to determine if a similar process occurs in the proliferating crypt cells, contributing to their surface-membrane polarization.

We have also focused our studies on a comparison of the composition of crypt and villus lateral-basal membrane, in an attempt to identify the surface components involved in the migration of the intestinal cells along the basement membrane, and responsible for the changes in the cell's adhesive properties. Our results suggest that the re-distribution process is selective for the fucosylated glycoproteins, and occurs in both proliferating and differentiated cells. We have also demonstrated a considerable similarity between proteins and glycoproteins of the lateral-basal membrane of crypt and villus cells, where only a few differences could be identified.

Experimental

Materials and general methods

All radioactive materials were purchased from New England Nuclear: L-[5,6-³H]fucose (sp. radio-

activity 40–60 Ci/mmol; D-[2(n)-³H]mannose (sp. radioactivity 10–20 Ci/mmol); L-[3,4,5(n)-³H]leucine (sp. radioactivity >110 Ci/mmol). 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.

Villus and crypt cells were isolated from rat small intestine as described by Weiser (1973a): fractions 1–5 and fractions 7–9 were pooled as representing, respectively, villus- and crypt-cell fractions. From these two pools, Golgi and lateral-basal membranes were prepared by the method of Weiser *et al.* (1978). Sucrase, alkaline phosphatase and (Na⁺ + K⁺)-dependent ATPase activities were routinely measured in the cell homogenates and in all membrane fractions. Crypt-cell homogenates were used only if the sucrase and alkaline phosphatase specific activities were found to be at least 20 times lower than in the corresponding villus-cell fractions. The luminal membrane of the crypt cells was purified from the crypt-cell fraction by the procedure of Schmitz *et al.* (1973) as modified by Hauri *et al.* (1977). The luminal membrane of the villus cells (microvillus membrane) was prepared by the Forstner-Hopfer method (Hopfer *et al.*, 1973) and monitored for purification by measuring the increase in sucrase specific activity, which was 40–50 times higher in the final microvillus-membrane fraction than in the homogenate. Protein was determined by the method of Lowry *et al.* (1951), sucrase activity by the method of Messier & Dahlqvist (1966), (Na⁺ + K⁺)-dependent ATPase as described by Tashima (1975) and alkaline phosphatase by the method of Weiser (1973a). Enzyme units were defined in each case as the amount of enzyme transforming 1 μmol of substrate/min.

Radioactivity was determined by liquid-scintillation counting in Aquasol (New England Nuclear).

Labelling *in vivo*

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 250–500 μCi of labelled precursor (L-[5,6-³H]fucose, D-[2-³H]mannose or L-[3,4,5-³H]leucine) dissolved in 0.3 ml of 0.154 M-NaCl. At different intervals the rats were killed, and the desired membrane fractions were prepared. Acid precipitation (with 10% trichloroacetic acid) and chloroform/methanol extraction were performed as previously described (Quaroni *et al.*, 1979a). Metabolism of radioactive fucose and leucine incorporated into crypt- and villus-cell homogenates at different times after label administration was

analysed as follows. After fucose administration, homogenates were extensively dialysed against water, and the fraction of non-diffusible counts was determined; the non-diffusible material was freeze-dried, then hydrolysed in 15–30 ml of 1 M-HCl at 105°C for 60–90 min under N₂. After centrifugation, the soluble fraction (containing more than 90% of the incorporated counts) was taken to dryness by rotary evaporation, re-suspended in 10 ml of water and applied to a Dowex 1 (X2; HCO₃⁻ form) column (1.5 cm × 15 cm), which was subsequently washed with 100 ml of water. The eluate was concentrated to about 10 ml by rotary evaporation and applied to a Dowex 50 (X8) column (1.5 cm × 15 cm), which was then washed with 100 ml of water. The eluate, containing greater than 90% of the radioactivity present in the hydrolysate in all cases, was taken to dryness by rotary evaporation and subsequently analysed: (i) by liquid-scintillation counting; (ii) for fucose by the method of Dische & Shettles (1948); and (iii) by descending paper chromatography (on Whatman no. 1) with butanol/pyridine/water (6:4:3, by vol.) as eluent, for 18 h; radioactive areas were located

with a Packard radiochromatograph scanner, and their mobilities were compared with those of standard sugars (mannose, fucose, glucose and galactose), located with the silver nitrate reagent (Trevelyan *et al.*, 1950). After radioactive leucine administration, homogenates were extensively dialysed against water; the non-diffusible material, after freeze-drying, was hydrolysed in 6 M-HCl at 108°C for 24 h in tubes sealed under N₂, dried and portions were applied to the long column of a Beckman 120C amino-acid analyser run under standard conditions for acidic and neutral amino-acid analysis; portions of the column effluent were assayed for radioactivity by liquid-scintillation spectrometry. D-[2-³H]Mannose metabolism was analysed as described in the following paper (Herscovics *et al.*, 1980).

Slab-gel electrophoresis

Membrane proteins were separated by slab-gel electrophoresis in 7.5% acrylamide gels containing 0.1% sodium dodecyl sulphate as previously described (Quaroni *et al.*, 1979a). At the end of the electrophoresis gels were stained for protein with

Fig. 1. Marker enzyme activities of villus- and crypt-cell homogenates and of fractions obtained by differential centrifugation and density-gradient centrifugation

Villus and crypt cells were obtained from rat small intestines by the method of Weiser (1973a). Fractions 1–5 and fractions 7–9 of the cell gradient were pooled as representing respectively villus- and crypt-cell fractions. Subcellular fractionation was performed as described by Weiser *et al.* (1978). Enzyme assays and protein determinations were done as described in the Experimental section. Abbreviations used: Homo, cell homogenate; Sup, supernatant of 20 000g-10 min centrifugation; Micro, microsomal fraction isolated from the 20 000g-10 min supernatant by centrifugation at 105 000g for 60 min; White, white 'fluffy' upper layer of the 20 000g-10 min precipitate (see Weiser *et al.*, 1978), used for purification of Golgi- and lateral-basal-membrane fractions by sucrose-density-gradient centrifugation; ≤30, 30/40, 40, 40/50 and 50, sucrose-density-gradient fractions. Membranes present in the 20%/30% sucrose interphase and in the 30% sucrose layer (≤30) represent purified Golgi membranes, whereas membranes more characteristic of the lateral-basal part of the plasmalemma are present in the 30%/40% sucrose interphase (30/40) and in the 40% sucrose layer (40); luminal membrane components are concentrated in the 50% sucrose layer (50) (see Weiser *et al.*, 1978, and Quaroni *et al.*, 1979a, for details).

Coomassie Blue, photographed and processed for fluorography (Bonner & Laskey, 1974). Alternatively, radioactive proteins were detected by liquid-scintillation counting: sample lanes were individually frozen with solid CO₂ and cut in 1 mm slices with a Mickle gel slicer; each slice was dissolved in 100 μ l of 30% H₂O₂ at 50°C before addition of scintillation fluid (Aquasol) and the vials were kept in the dark until the radioactivity counts had stabilized.

Results and discussion

Isolation of Golgi, lateral-basal and luminal membranes from villus and crypt intestinal cells

The luminal membranes of villus and crypt cells were isolated by established techniques previously described and discussed in detail (Hopfer *et al.*, 1973; Schmitz *et al.*, 1973; Hauri *et al.*, 1977).

The subcellular fractionation of the intestinal epithelial cells, and the isolation of Golgi and lateral-basal membranes, have also been described previously (Weiser *et al.*, 1978). An important problem in the interpretation of the results obtained in the present work is the extent of separation of the villus and crypt cells, isolated by the method of Weiser (1973*a*). Fractions 1–5 of the cell gradient have been previously shown (Weiser, 1973*a,b*; Weiser *et al.*, 1978) to represent villus cells with little or no admixture of crypt cells. On the contrary, contamination of the crypt fractions (fractions 7–9) with villus cells was occasionally significant. In most cases, however, this contamination was shown to be negligible on the basis of the following criteria. (a) Sucrase and alkaline phosphatase activities, measured in the cell homogenates and in all membrane fractions, were very low or almost absent in

Fig. 2. *Comparison of protein patterns of villus (upper curve) and crypt (lower curve) lateral-basal membranes* Lateral-basal membranes were purified by the method of Weiser *et al.* (1978) from villus and crypt intestinal cells obtained as described by Weiser (1973*a*). Membranes present in the 30%/40% sucrose interphase and in the 40% sucrose layer were pooled, solubilized and analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis on 7.5% acrylamide gels. Gels were stained for protein and scanned with a Gilford spectrophotometer. The relative intensity of the bands was plotted on a transmission scale in arbitrary units. Protein migration was from right (–) to left (+). The patterns shown are representative of the results obtained in four different experiments (see the Experimental section for details).

crypt-cell fractions (Fig. 1). In particular, membranes present in the 50% sucrose fraction of the density gradient represented a useful marker of villus-cell contamination, since the luminal membrane of the villus cells, in which almost all the sucrase and a great portion of alkaline phosphatase are concentrated, is considerably enriched in this gradient fraction (Weiser *et al.*, 1978). The sucrase and alkaline phosphatase specific activities of the 50%-sucrose-density-gradient fraction obtained from crypt-cell homogenates was used therefore as an index of contamination by villus cells for all crypt cell Golgi and lateral-basal membrane preparations. (b) Golgi and lateral-basal membranes from villus cells isolated within 1–2 h of radioactive fucose injection (see below, Fig. 6) contained labelled glycoproteins not detected in corresponding crypt-cell fractions; since much more labelled fucose was incorporated in villus than in crypt-cell membranes (see Fig. 3), this result also excludes a significant contamination of the crypt-cell fractions with differentiated villus cells. The few crypt-cell preparations where relatively high sucrase and alkaline phosphatase activities were measured were excluded from our studies.

Golgi membranes obtained from both villus and crypt cells have been previously characterized and shown to be uncontaminated with surface-membrane components (Weiser *et al.*, 1978; Quaroni *et al.*, 1979a). Our previous studies have also demonstrated that the lateral-basal membranes purified from villus cells are little or not contaminated with luminal-membrane components. Similarly, the following results seem to exclude the possibility of a significant cross-contamination between luminal and lateral-basal membranes isolated from crypt-cell fractions. Lateral-basal membranes isolated from villus and crypt cells are very similar on the basis of several criteria. (a) They were isolated by the same technique, from the same density-gradient fractions (the 30%/40% sucrose interphase and the 40% sucrose layer). (b) They had very similar ($\text{Na}^+ + \text{K}^+$)-dependent ATPase specific activities (Fig. 1). (c) The protein patterns of lateral-basal membranes isolated from villus and crypt cells analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis were very similar, if not identical (Fig. 2). (d) Few differences could be detected between the [^3H]fucose- or [^3H]mannose-labelled-glycoprotein patterns of villus and crypt lateral-basal membranes isolated 3–4 h after label administration (see below, Figs. 7, 8 and 9). In contrast, luminal membranes isolated from villus and crypt cells were very different from each other, and contained [^3H]fucose- and [^3H]leucine-labelled glycoproteins and proteins never detected in the corresponding lateral-basal-membrane fractions (see Fig. 8, bands 6 and 8, and Fig. 10). Conversely,

contamination of crypt-cell luminal-membrane fractions with corresponding lateral-basal membranes appears unlikely, since they lacked at least one [^3H]fucose-labelled glycoprotein (peak 14, Figs. 6, 7 and 8) always found in crypt (and villus) lateral-basal membranes.

Gratecos *et al.* (1978) have recently isolated and characterized plasma membranes from crypt and villus rat intestinal cells by using a somewhat different technique, but with very similar results.

General characteristics of L-[5,6- ^3H]fucose, D-[2- ^3H]mannose and L-[3,4,5- ^3H]leucine incorporation into crypt and villus intestinal cells

Radioactive fucose, mannose and leucine were rapidly taken up by both intestinal villus and crypt cells. With all three substrates, incorporation of

Fig. 3. Kinetics of L-[5,6- ^3H]fucose incorporation into villus (●) and crypt (○) cells

Rats were injected intraperitoneally with 250 μCi of L-[5,6- ^3H]fucose dissolved in 300 μl of 0.9% NaCl. The animals were killed at different times after radioactive fucose injection, and villus and crypt cells were isolated from the entire small intestines by the method of Weiser (1973a). Incorporation of label into the cell homogenates was determined, and Golgi [the 20%/30% sucrose interphase and the 30% sucrose layer collected together (≤ 30)] and lateral-basal membranes [the 30%/40% sucrose interphase (30/40) or the 40% sucrose layer (40)] were isolated by the method of Weiser *et al.* (1978). Protein and radioactivity were determined as described in the Experimental section. Each point represents the mean for two separate determinations.

radioactivity reached a plateau value 45–60 min after injection and remained fairly constant for at least 3 h (Figs. 3, 4 and 5). At all times after label administration, following cell homogenization and separation of unbroken cells and nuclei, almost all the remaining radioactivity was associated with cell proteins and glycoproteins, since (a) at most 1–5% of the total radioactivity present in the homogenate was lost after extensive (2–3 days) dialysis, and (b) at least 90–95% of the radioactivity was precipitable with trichloroacetic acid. This finding is not surprising in the case of fucose, which is present in very low concentrations both extra- (Howard & Kelleher, 1971) and intra-cellularly (Yurchenco & Atkinson, 1977), but cannot easily be explained for leucine and mannose. Alpers (1972), in his studies of radioactive leucine incorporation into intestinal crypt and villus cells, has found a sizeable fraction of the incorporated counts as free leucine

in both cell types 45 min after label administration. This discrepancy may be at least in part related to a difference in the routes of radioactive leucine administration (intravenous or intraluminal versus intraperitoneal), but can be best explained by the difference in techniques used for cell isolation. Alpers (1972) separated the intestinal cells by cryostat sectioning of intestinal segments, quickly frozen at the end of the label administration, whereas we used the Weiser technique to obtain villus- and crypt-cell suspensions. It is likely that during the prolonged incubation of the tissue with different buffers (see Weiser, 1973a) required for cell dissociation any label not bound to macromolecules was lost in the buffers. This result, although not influencing the major conclusions of the present study, which focused mainly on differences in the incorporation of labelled precursors into membrane proteins and glycoproteins, did not allow an estimation of net cellular uptakes of radioactivity, and of the sizes

Fig. 4. Kinetics of D-[2-³H]mannose incorporation into villus (●) and crypt (○) cells

Rats were injected intraperitoneally with 500 μ Ci of D-[2-³H]mannose dissolved in 300 μ l of 0.9% NaCl. The animals were killed at different times after radioactive mannose injection, and villus and crypt cells were isolated from the entire small intestines by the method of Weiser (1973a). Label incorporation into the cell homogenates was determined, and Golgi [the 20%/30% sucrose interphase and the 30% sucrose layer, collected together (≤ 30)] and lateral-basal membranes [the 30%/40% sucrose interphase (30/40) or the 40% sucrose layer (40)] were isolated by the method of Weiser *et al.* (1978). Protein and radioactivity were determined as described in the Experimental section. Each point represents the mean for two separate determinations.

Fig. 5. Kinetics of L-[3,4,5-³H]leucine incorporation into villus (●) and crypt (○) cells

Rats were injected intraperitoneally with 250 μ Ci of L-[3,4,5-³H]leucine dissolved in 300 μ l of 0.9% NaCl. The animals were killed at different times after radioactive leucine injection, and villus and crypt cells were isolated from the entire small intestines by the method of Weiser (1973a). Label incorporation into the cell homogenates was determined, and Golgi [the 20%/30% sucrose interphase and the 30% sucrose layers, collected together (≤ 30)] and lateral-basal membranes [the 30%/40% sucrose interphase (30/40) or the 40% sucrose layer (40)] were isolated by the method of Weiser *et al.* (1978). Protein and radioactivity were determined as described in the Experimental section. Each point represents the mean for two separate determinations.

Fig. 6. Patterns of [^3H]fucose-labelled glycoproteins present in Golgi membranes of crypt and villus cells 30 min after fucose injection, and in the luminal membrane (LM) of villus cells 3 h after fucose injection.

Rats were injected intraperitoneally with 500 μCi of L-[5,6- ^3H]fucose, and killed 30 min or 3 h later. Golgi membranes were purified by the method of Weiser *et al.* (1978) from villus and crypt intestinal cells obtained as described by Weiser (1973a). The luminal membrane of the villus cells was prepared by the Forstner-Hopfer method (Hopfer *et al.*, 1973). The membrane fractions, containing 50000–70000 c.p.m. of radioactivity, were solubilized and analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis on 7.5% acrylamide gels. Each sample lane was individually frozen with solid CO_2 and cut in 1 mm slices with a Mickle gel slicer; each slice was dissolved in 100 μl of 30%

of the intracellular pools of fucose, leucine and mannose in villus and crypt cells. The differences in label incorporation between the two cell types, particularly significant in the case of fucose, could not therefore be directly related to differences in biosynthetic activity. Both villus and crypt cells incorporated fucose and leucine into cellular macromolecules without significant metabolic conversion: at all time points considered, after hydrolysis of a portion of the cell homogenates (see the Experimental section for details), greater than 90% of the label was recovered as fucose and leucine respectively. In contrast, in both cell types a significant portion of the labelled mannose (about 30%) was recovered in the form of fucose, the balance being represented by unmetabolized mannose (Herscovics *et al.*, 1980).

Kinetics of radioactive fucose, mannose and leucine incorporation into Golgi and lateral-basal membranes of villus and crypt cells

Radioactive fucose was rapidly incorporated into Golgi-membrane glycoproteins of intestinal villus cells. The specific radioactivity (d.p.m./mg of protein) was highest at the first two time points considered (15–30 min), and then decreased rapidly during the next 2 h to approx. 30% of the peak value (Fig. 3). Incorporation into the lateral-basal membrane fractions peaked at 40 min, somewhat later therefore than in the Golgi, and subsequently declined until the last time point considered (3 h). It should be noted that these kinetics are similar to, but not identical with, the ones previously described for the incorporation of fucose (Quaroni *et al.*, 1979a) and labelled sucrase-isomaltase precursor (Hauri *et al.*, 1979) into the same membranes. We attribute this slight difference (compare Fig. 3 in the present paper with Fig. 1 of Quaroni *et al.*, 1979a) to the different times required for the isolation of villus-cell fractions alone (20–25 min) or of villus and crypt cells from the same animal (40–50 min) by using the Weiser technique. It appears likely that during cell isolation intracellular processing of the labelled glycoproteins can at least partially progress, resulting in an apparent shift of the kinetics of fucose (and all other labelled substrates) incorporation to earlier times. Radio-

H_2O_2 at 50°C before addition of scintillation fluid (Aquasol) and determination of radioactivity by liquid-scintillation spectrometry. Counting efficiency in each vial was determined by internal standardization with [^3H]toluene; the d.p.m./slice were plotted against gel length in mm. Protein migration was from the left (–) to the right (+) of the Figure.

active fucose incorporation into Golgi and lateral-basal membranes of intestinal crypt cells was markedly lower (2–5 times; see Fig. 3) than in the same membranes from villus cells at all time points considered. A similar finding has been previously described by Weiser (1973a,b). It is tempting to attribute this difference to the fact that most of the fucosylated glycoproteins synthesized in the intestinal epithelium are specific for differentiated villus cells (see below), but this possibility could not be substantiated, since net fucose uptakes and the sizes of the intracellular pools of fucose in villus and crypt cells could not be determined.

The kinetics of mannose (Fig. 4) and leucine (Fig. 5) incorporation into Golgi and lateral-basal membranes were very similar, and almost identical in villus and crypt cells. The peak of incorporation in the Golgi was at 40–60 min, some 30–45 min later than the peak of fucose incorporation. This difference is most likely related to the different intracellular sites of label incorporation into macromolecules: fucose is added as a terminal sugar into newly synthesized glycoproteins in the Golgi complex, and represents therefore a late post-translational protein modification (Schachter *et al.*, 1979); in contrast, both leucine and mannose were probably incorporated mostly if not solely during protein synthesis in the rough endoplasmic reticulum, a much earlier step of glycoprotein synthesis (Whur *et al.*, 1969).

In contrast with the considerable difference found for fucose, mannose incorporation was approximately equal in crypt and villus cells, and leucine incorporation was about 50% higher in the crypt cells, as previously described by Alpers (1972).

Analysis of labelled proteins and glycoproteins in Golgi, lateral-basal and luminal membranes of villus and crypt cells

The membrane fractions obtained at various times after radioactive fucose, mannose and leucine injection were analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis. As in our previous work (Quaroni *et al.*, 1979a,b), we analysed, whenever possible, equal amounts of radioactivity and interpreted the results in a qualitative way, concentrating on differences in labelled proteins and glycoproteins between crypt- and villus-cell membranes, and on changes in intensity of radioactive bands relative to all the others in the same membrane at different times after label administration. Fucose- and leucine-labelled membrane proteins and glycoproteins were extensively analysed, and only the most representative results will be presented here.

The patterns of [^3H]fucose-labelled membrane glycoproteins in Golgi, lateral-basal and microvillus membranes from intestinal villus cells at different

times after fucose injection have been described previously (Quaroni *et al.*, 1979a,b), but a brief review is important in understanding the present work. The distribution of radioactivity among glycoproteins in the Golgi and lateral-basal membranes was very similar at all times. At the peak of incorporation into Golgi membranes (15–30 min) the radioactivity was distributed (see Fig. 6) among at

Fig. 7. Patterns of [^3H]fucose-labelled glycoproteins present in the lateral-basal membranes (LBM) of crypt and villus cells 3 h after fucose injection

Rats were injected intraperitoneally with 500 μCi of L-[5,6- ^3H]fucose, and killed 3 h later. Lateral-basal membranes were purified by the method of Weiser *et al.* (1978) from villus and crypt intestinal cells obtained as described by Weiser (1973a). Membranes present in the 30%/40% sucrose interphase and in the 40% sucrose layer were pooled together, solubilized and analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis on 7.5% acrylamide gels. Each sample lane was individually frozen with solid CO_2 and cut in 1 mm slices with a Mickle gel slicer. The radioactivity present in each slice, determined as described in the legend to Fig. 6, was plotted against the gel length in mm; protein migration was from the left (–) to the right (+) of the Figure.

least 12 glycoproteins (each peak of radioactivity detected by gel slicing and counting or each band of radioactivity detected by fluorography could and probably in some cases did correspond to more than one glycoprotein having similar or identical mobility on sodium dodecyl sulphate/polyacrylamide gels). At 3–4 h after fucose injection (see Fig. 7), a single peak of radioactivity was the outstanding component (no. 14, Figs. 7 and 8), all others having disappeared or being greatly decreased. All the peaks of radioactivity present in Golgi and lateral-basal membranes shortly after fucose injection (with the exception of peak 14) closely corresponded to peaks present in the luminal membrane at all times (see Fig. 6). It should be noted that two radioactive components present in the luminal membrane (peaks 6 and 8) were never detected in any other subcellular fraction: it has been recently shown that they represent the two subunits of the sucrose–isomaltase complex, and that they originate by proteolytic cleavage of a high-molecular-weight precursor present in peak 2 (Hauri *et al.*, 1979). This cleavage takes place only after incorporation of the precursor into the luminal membrane, probably under the action of pancreatic proteinases, explaining the absence of peaks 6 and 8 in all intracellular membranes and in the lateral-basal membranes. We have interpreted the rapid change in labelled glycoprotein patterns occurring in Golgi and lateral-basal membranes between 1 and 3 h after fucose injection as due to a selective re-distribution of newly synthesized membrane components into the luminal membrane, a process apparently mediated or controlled by cytoplasmic microtubular structures (Quaroni *et al.*, 1979b).

In Golgi and lateral-basal membranes isolated from crypt cells shortly after radioactive fucose injection, fewer labelled glycoproteins were present than in corresponding membranes from villus cells (Figs. 2, 6 and 8). Interestingly, all the peaks of radioactivity absent in crypt-cell Golgi membranes corresponded to glycoproteins that disappeared from Golgi and lateral-basal membranes of villus cells within 3 h of fucose injection, and are therefore likely to represent typical components of the luminal membrane of villus cells (peaks 3, 5, 9a, 10, 11 and 13; see Figs. 6 and 8). The patterns of labelled glycoproteins in lateral-basal membranes of crypt and villus cells isolated 2–4 h after fucose injection were very similar (see Fig. 7). The only significant exception was a major radioactivity peak (peak 9b, Figs. 7 and 8), which may represent glycoprotein(s) characteristic of the lateral-basal membrane of the crypt cells; this glycoprotein(s) was not detected in the luminal membrane of the same cells.

Although most of the components of the luminal membrane of the villus cells were labelled with

fucose, only few radioactive bands were detected in this membrane isolated from the crypt cells (Fig. 8). This result was expected, since most of the components of the luminal membrane of the villus cells represent well characterized enzymes, which have been extensively studied and shown to be absent in the crypt cells (Maestracci *et al.*, 1973,

Fig. 8. Scheme of $[^3\text{H}]$ fucose-labelled-glycoprotein patterns in Golgi, lateral-basal (LBM) and luminal (LM) membranes of villus (V) and crypt (C) cells. Rats were injected intraperitoneally with $500\mu\text{Ci}$ of $\text{L-}[5,6\text{-}^3\text{H}]\text{fucose}$, and killed at different times thereafter. Golgi and lateral-basal membranes were purified by the method of Weiser *et al.* (1978) from villus and crypt intestinal cells obtained as described by Weiser (1973a). The crypt-cell luminal membrane was purified from the isolated crypt cells by the procedure of Schmitz *et al.* (1973) as modified by Hauri *et al.* (1977). The luminal membrane of the villus cells was prepared by the Forstner–Hopfer method (Hopfer *et al.*, 1973). The different membrane fractions, containing 50000–70000 d.p.m. of radioactivity, 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). This scheme was derived from the results of four similar experiments.

1975; Weiser, 1973a; Hauri *et al.*, 1977; Raul *et al.*, 1977, 1978; Simon *et al.*, 1979). It was of interest, however, to detect bands 6 and 8 (see Fig. 8) in the luminal membrane of the crypt cells. In the villus cells these bands have been shown to correspond to the subunits of sucrase-isomaltase (Hauri *et al.*, 1979): although a similar positive identification has not been made for the crypt cells, their presence indicates that this enzyme may be present in at least a fraction of the crypt cells, a possibility that has been suggested previously on the basis of immunological and biochemical evidence (Silverblatt *et al.*, 1974; Dubs *et al.*, 1975; Raul *et al.*, 1977; Quaroni *et al.*, 1979d; Simon *et al.*, 1979). Other studies, however, have failed to detect any sucrase activity in the crypt cells (Dahlqvist & Nordström, 1966;

Nordström *et al.*, 1968; DeBoth & Plaisier, 1974).

The identification of mannose-labelled glycoproteins could not be carried out in all membrane fractions, due to the low net incorporation of radio-

Fig. 9. Patterns of [^3H]mannose-labelled glycoproteins present in the lateral-basal membranes (LBM) of crypt and villus cells 4 h after mannose injection

Rats were injected intraperitoneally with 1 mCi of D-[2- ^3H]mannose, and killed 4 h later. Lateral-basal membranes were purified by the method of Weiser *et al.* (1978) from villus and crypt intestinal cells obtained as described by Weiser (1973a). Membranes present in the 30%/40% sucrose interphase and in the 40% sucrose layer were pooled together, solubilized and analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis on 7.5% acrylamide gels. Each sample lane was individually frozen with solid CO_2 and cut in 1 mm slices with a Mickle gel slicer. The radioactivity present in each slice, determined as described in the legend to Fig. 6, was plotted against the gel length in mm; protein migration was from the left (–) to the right (+) of the Figure.

Fig. 10. Scheme of [^3H]leucine-labelled-protein patterns in lateral-basal (LBM) and luminal (LM) membranes of villus (V) and crypt (C) cells

Rats were injected intraperitoneally with 500 μCi of L-[3,4,5- ^3H]leucine and killed at different times thereafter. Lateral-basal membranes were purified by the method of Weiser *et al.* (1973a). The crypt-cell-luminal-membrane fraction was purified from the isolated crypt cells by the procedure of Schmitz *et al.* (1973) as modified by Hauri *et al.* (1977). The luminal membrane of the villus cells was prepared by the Forstner-Hopfer method (Hopfer *et al.*, 1973). The different membrane fractions, containing 60 000–70 000 d.p.m. of radioactivity, 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 proteins were subsequently detected by fluorography (see the Experimental section for details). This scheme was derived from the results of three similar experiments. *Indicates labelled bands present in villus-cell-membrane fractions not detected in corresponding crypt-cell fractions; † indicates labelled bands present in crypt-cell-membrane fractions not detected in corresponding villus-cell fractions.

activity observed with this label. We therefore focused our attention on the mannose-labelled glycoproteins present in the lateral-basal membranes at 3–4 h after mannose injection. At least seven to eight radioactive peaks were detected (Fig. 9), and almost identical patterns were obtained for membranes isolated from crypt and villus cells. It is likely that some of the mannose-labelled glycoproteins shown in Fig. 9 correspond to fucose-labelled glycoproteins observed in these membranes at 3 h after fucose injection (peaks 5, 7 and 8 of Fig. 9 had the same mobilities as peaks 7, 9a or 9b and 14 respectively, shown in Figs. 6, 7 and 8). Peaks 2, 3 and 4 (Fig. 9), however, must represent glycoproteins lacking fucose, since no corresponding peaks were observed after fucose labelling. This result suggests that glycoproteins with different oligosaccharide chains are present in the lateral-basal membrane of crypt and villus cells. In the following paper (Herscovics *et al.*, 1980) it is shown that both 'high-mannose' and 'complex' types of asparagine-linked oligosaccharides can be obtained by proteolytic digestion of mannose-labelled glycoproteins present in the lateral-basal membranes; in contrast, the mannose-labelled glycoproteins present in the luminal membrane of crypt and villus cells are greatly enriched in complex-type oligosaccharide chains.

All the proteins present in Golgi, lateral-basal and luminal membranes, and detected by Coomassie Blue staining after separation on sodium dodecyl sulphate/polyacrylamide slab gels, were labelled after leucine administration (Fig. 10). The complexity of the radioactive patterns of all membrane fractions prevented a detailed comparison of their labelled components, allowing only some general conclusions to be made. Thus: (a) at all times considered, the patterns of labelled proteins in lateral-basal membranes from villus and crypt cells were very similar and only few differences could be consistently detected; (b) on the contrary, the radioactive patterns of the luminal membranes isolated from the two cell types were quite different, and only a few common components could be observed; (c) the patterns of labelled proteins in lateral-basal membranes of both crypt and villus cells, isolated at either early (60–90 min) or late (3–4 h) times after leucine administration, were very similar, clearly contrasting the marked changes observed for the fucosylated glycoprotein patterns of the same membranes.

Conclusions

In our previous studies on the biosynthesis of membrane glycoproteins in the intestinal villus cells, we have obtained evidence suggesting that a continuous and selective re-distribution of newly synthesized membrane components is at least one of

the mechanisms by which these cells establish and maintain their marked polarization, despite a continuous turnover of their surface membrane. In the present work we have shown that this process of re-distribution is relatively specific for the fucosylated glycoproteins: many high-molecular-weight glycoproteins labelled with mannose but not with fucose appeared to be stable components of the lateral-basal membrane of the villus cells, and relatively minor changes were observed with time in the patterns of leucine-labelled proteins in this membrane. These results argue against the possibility of a much more rapid degradation of lateral-basal than of luminal-membrane components as a possible explanation for the observed changes in fucosylated glycoprotein patterns. It appears, therefore, that most fucosylated glycoproteins represent, in the intestinal villus cells, a special class of membrane components that (a) appear with cell differentiation, being absent in the undifferentiated crypt cells, and (b) are specifically localized in the luminal part of the plasmalemma.

Evidence for a similar re-distribution process in the crypt cells is more limited; however, a few labelled glycoproteins could be detected in these cells that appeared to be specifically localized in their luminal membrane.

In comparing the surface-membrane components of villus and crypt cells it is apparent that most changes occurring with cell differentiation are related to luminal-membrane components. The lateral-basal membranes of villus and crypt cells have very similar protein patterns, enzymic activities, and patterns of fucose-, mannose- and leucine-labelled components. However, a few differences were observed, and they may be of considerable importance in determining cell adhesion and cellular migration.

This research was supported by Grants AM-25956, AM-03014, CA-26675 and CA-14294 from the National Institutes of Health. This is publication no. 818 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School at the Massachusetts General Hospital, Boston, MA 02114, U.S.A.

References

- Alpers, D. H. (1972) *J. Clin. Invest.* **51**, 167–173
- Alpers, D. H. (1977) *Biochem. Biophys. Res. Commun.* **75**, 130–135
- Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88
- Bouhours, J. F. & Glickman, R. M. (1976) *Biochim. Biophys. Acta* **441**, 123–133
- Clarke, R. M. (1970) *J. Anat.* **107**, 519–529
- Dahlqvist, A. & Nordström, C. (1966) *Biochim. Biophys. Acta* **113**, 624–626

- DeBoth, N. J. & Plaisier, H. (1974) *J. Histochem. Cytochem.* **22**, 352–360
- Devik, F. (1971) *Acta Radiol. Ther. Phys. Biol.* **10**, 129–149
- Dische, Z. & Shettles, L. B. (1948) *J. Biol. Chem.* **175**, 595–603
- Dubs, R., Gitzelmann, R., Steinmann, B. & Lindenmann, J. (1975) *Helv. Paediatr. Acta* **30**, 89–102
- Etzler, M. E. & Branstrator, M. L. (1974) *J. Cell Biol.* **62**, 329–343
- Galjaard, H. & Bootsma, D. (1969) *Exp. Cell Res.* **58**, 79–92
- Glickman, R. M. & Bouhours, J. F. (1976) *Biochim. Biophys. Acta* **424**, 17–25
- Gratecos, D., Knibiehler, M., Benoit, V. & Sémériva, M. (1978) *Biochim. Biophys. Acta* **512**, 508–524
- Hauri, H.-P., Keding, M., Haffen, K., Freiburghaus, A., Grenier, J. F. & Hadorn, B. (1977) *Biochim. Biophys. Acta* **467**, 327–339
- Hauri, H.-P., Quaroni, A. & Isselbacher, K. J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5183–5186
- Herbst, J. J. & Koldovsky, O. (1972) *Biochem. J.* **126**, 471–476
- Herscovics, A., Bugge, B., Quaroni, A. & Kirsch, K. (1980) *Biochem. J.* **192**, 145–153
- 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
- Imondi, A. R., Balis, M. E. & Lipkin, M. (1969) *Exp. Cell Res.* **58**, 323–330
- Lamerton, L. F. (1966) *Radiat. Res.* **27**, 119–138
- Leblond, C. P. & Stevens, C. E. (1948) *Anat. Rec.* **100**, 357–377
- Leshner, S. (1967) *Radiat. Res.* **32**, 510–519
- 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
- Messier, B. & Leblond, C. P. (1960) *Am. J. Anat.* **106**, 247–285
- Messier, M. & Dahlqvist, A. (1966) *Anal. Biochem.* **14**, 376–392
- Nordström, C., Dahlqvist, A. & Josefsson, L. (1968) *J. Histochem. Cytochem.* **15**, 713–721
- Quaroni, A., Kirsch, K. & Weiser, M. M. (1979a) *Biochem. J.* **182**, 203–212
- Quaroni, A., Kirsch, K. & Weiser, M. M. (1979b) *Biochem. J.* **182**, 213–221
- Quaroni, A., Kirsch, K. & Weiser, M. M. (1979c) in *Glycoconjugate Research* (Gregory, J. D. & Jeanloz, R. W., eds.), vol. 2, pp. 1073–1075, Academic Press, London and New York
- Quaroni, A., Wands, J., Trelstad, R. L. & Isselbacher, K. J. (1979d) *J. Cell Biol.* **80**, 248–265
- Quastler, H. (1956) *Radiat. Res.* **4**, 303–320
- Raul, F., Simon, P., Keding, M. & Haffen, K. (1977) *Cell Tissue Res.* **176**, 167–178
- Raul, F., Simon, P. M., Keding, M., Grenier, J. F. & Haffen, K. (1978) *Enzyme* **23**, 89–97
- Rijke, R. P. C., Hanson, W. R., Plaiser, H. M. & Osborne, J. W. (1976) *Gastroenterology* **71**, 786–792
- Schachter, H., Narasimhan, S. & Wilson, J. R. (1979) in *Glycoconjugate Research* (Gregory, J. D. & Jeanloz, R. W., eds.), vol. 2, pp. 575–596, Academic Press, London and New York
- Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B. K., Cerda, J. J. & Crane, R. K. (1973) *Biochim. Biophys. Acta* **323**, 98–112
- Silverblatt, R., Conklin, K. & Gray, G. M. (1974) *J. Clin. Invest.* **53**, 76a
- Simon, P. M., Keding, M., Raul, F., Grenier, J. F. & Haffen, K. (1979) *Biochem. J.* **178**, 407–413
- Tashima, Y. (1975) *Anal. Biochem.* **69**, 410–414
- Trevelyan, W. E., Proctor, D. P. & Harrison, J. S. (1950) *Nature (London)* **166**, 444–446
- Webster, H. L. & Harrison, D. D. (1969) *Exp. Cell Res.* **56**, 245–253
- Weiser, M. M. (1973a) *J. Biol. Chem.* **248**, 2536–2541
- Weiser, M. M. (1973b) *J. Biol. Chem.* **248**, 2542–2548
- Weiser, M. M., Neumeier, M. M., Quaroni, A. & Kirsch, K. (1978) *J. Cell Biol.* **77**, 722–734
- Whur, P., Herscovics, A. & Leblond, C. P. (1969) *J. Cell Biol.* **43**, 289–311
- Williams, R. B., Toal, J. N., White, J. & Carpenter, H. M. (1958) *J. Natl. Cancer Inst.* **21**, 17–61
- Yurchenco, P. D. & Atkinson, P. H. (1977) *Biochemistry* **16**, 944–953