Characterization of glycopeptides labelled from D-[2-³H]mannose and L-[6-³H]fucose in intestinal epithelial cell membranes during differentiation

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The labelled glycopeptides obtained by Pronase digestion of rat intestinal epithelial cell membranes were examined by gel filtration after injection of D-[2-³H]mannose and L-[6-³H]fucose. Three labelled fractions were eluted in the following order from Bio-Gel P-6. Fraction I, which was excluded from the gel, was labelled mostly with [³H]fucose and slightly with [³H]mannose. Fraction II contained 'complex' asparagine-linked oligosaccharides since it was labelled with [³H]mannose and [³H]fucose, was stable to mild alkali treatment, and resistant to endo- β -N-acetylglucosaminidase H. Fraction III contained 'high-mannose' asparagine-linked oligosaccharides, which were labelled with [³H]mannose, but not with [³H]fucose; these were sensitive to endo- β -N-acetylglucosaminidase H, and were adsorbed on concanavalin A-Sepharose and subsequently eluted with methyl α -D-mannopyranoside. The time course of incorporation of [³H]mannose into these glycopeptides in microsomal fractions showed that high-mannose oligosaccharides were precursors of complex oligosaccharides. The rate of this processing was faster in rapidly dividing crypt cells than in differentiated villus cells. The ratio of radioactively labelled complex oligosaccharides to high-mannose oligosaccharides, 3 h after [³H]mannose injection, was greater in crypt than in villus-cell lateral-basal membranes. Luminal membranes of both crypt and villus cells were greatly enriched in labelled complex oligosaccharides compared with the labelling in lateral-basal membranes. These studies show that intestinal epithelial cells are polarized with respect to the structure of the asparagine-linked oligosaccharides on their membrane glycoproteins. During differentiation of these cells quantitative differences in labelled membrane glycopeptides, but no major qualitative change, were observed.

The carbohydrate groups on glycoproteins play an important role in a variety of biological recognition phenomena both at the cell surface and in intracellular processes (Hughes, 1975; Ashwell & Morell, 1974, 1977; Jeanloz & Codington, 1976; Kaplan *et al.*, 1977). In addition, alterations in the carbohydrate structure of glycoproteins are somehow related to growth control in a manner that is still poorly understood (Atkinson & Hakimi, 1980). It has been found that the membranes of a variety of transformed cells treated with Pronase are enriched in high-molecular-weight glycopeptides compared with normal cells. These large glycopeptides have extra sialic acid residues, and appear to be more highly branched (Ogata *et al.*, 1976; Tuszynski

et al., 1978; Glick & Santer, 1978). It has also been shown that membrane glycoproteins of growing fibroblasts have a higher ratio of 'high-mannose' to 'complex' asparagine-linked oligosaccharides than non-growing cells (Muramatsu *et al.*, 1976; Ceccarini & Atkinson, 1977). Furthermore, it has been observed more recently that embryonic carcinoma cells contain a high-molecular-weight glycopeptide fraction that may be a marker of the embryonic phenotype since it disappears with differentiation (Muramatsu *et al.*, 1978, 1979).

In view of these growth-dependent and developmentally induced changes, we decided to examine the glycopeptides obtained from intestinal epithelial cells to determine if any changes occur during

differentiation of these cells. Although several studies have described differences between crypt- and villus-cell membranes (Weiser, 1973*a,b*; Bouhours & Glickman, 1976; Quaroni *et al.*, 1980), little is known about the structure of the carbohydrate groups present on intestinal cell membrane glycoproteins. In the previous paper, Quaroni *et al.* (1980) compared the pattern of labelling of membrane glycoproteins by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis in villus and crypt cells after administration of [^3H]mannose and [^3H]fucose, and showed that the major change with differentiation is the appearance of specific fucosylated glycoproteins destined for the brush border of villus cells. In the present work we have examined the labelled glycopeptides obtained by Pronase digestion of intestinal cell membranes after injection of [^3H]mannose and [^3H]fucose. The glycopeptides were separated by gel filtration before and after treatment with endo- β -*N*-acetylglucosaminidase H, an enzyme from *Streptomyces plicatus* that cleaves asparagine-linked high-mannose oligosaccharides between the two *N*-acetylglucosamine residues of the inner core, but does not affect asparagine-linked complex oligosaccharides (Tarentino *et al.*, 1974; Trimble *et al.*, 1978). A preliminary report of this work has been presented previously (Herscovics *et al.*, 1979).

Experimental

Materials

The following compounds were obtained from the companies indicated: Pronase (B grade), Calbiochem, La Jolla, CA, U.S.A.; Bio-Gel P-6 and Bio-Gel P-30 (200–400 mesh), Bio-Rad Laboratories, Richmond, CA, U.S.A.; endo- β -*N*-acetylglucosaminidase H and bovine serum albumin, Miles Laboratories, Elkhart, IN, U.S.A.; L-fucose, cycloheximide and chloramphenicol, Sigma, St. Louis, MO, U.S.A.; D-mannose, Pfanstiehl Laboratories, Waukegan, IL, U.S.A.; concanavalin A–Sephadex, Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A. Radioactive compounds were purchased from New England Nuclear, Boston, MA, U.S.A.

Tissue preparation

Female Sprague–Dawley rats, CD strain, weighing 130–250 g were obtained from Charles River Co., Wilmington, MA, U.S.A., and were starved overnight before use. They were injected intraperitoneally with either D-[2- ^3H]mannose (sp. radioactivity, 10–20 Ci/mmol) or L-[6- ^3H]fucose (sp. radioactivity, 10–15 Ci/mmol), and then killed at different intervals by a blow on the head. The small intestine was removed, and intestinal epithelial cells were collected as described by Weiser (1973*a*). Villus cells (fractions 1–5) and crypt cells

(fractions 6–9) were pooled separately. The assays for marker enzymes on these cell fractions are reported in the previous paper (Quaroni *et al.*, 1980). Microsomal fraction ('microsomes') and lateral-basal membranes (material sedimenting at the 30%/40% sucrose interphase and in 40% sucrose) were prepared as described previously (Weiser *et al.*, 1978; Quaroni *et al.*, 1980). Villus-cell luminal membranes were prepared by the Forstner–Hopfer method (Hopfer *et al.*, 1973), and crypt-cell luminal membranes were purified by the procedure of Schmitz *et al.* (1973) as modified by Hauri *et al.* (1977). Characterization of these membrane fractions has been discussed previously (Weiser *et al.*, 1978; Quaroni *et al.*, 1980).

Preparation of glycopeptides

Each membrane suspension was mixed with 5 vol. of chloroform/methanol (2:1, v/v) by using a Vortex mixer. After 20 min at room temperature, the suspension was mixed again, and centrifuged. Two phases were obtained with an insoluble precipitate at the interphase. First, the lower chloroform/methanol phase, and then the upper aqueous phase were removed with a Pasteur pipette. The insoluble residue was washed twice with 4 vol. of n-butanol, and then three times with 4 vol. of ice-cold water. The precipitate was extracted twice with 4 vol. of chloroform/methanol/water (4:4:1, by vol.) for 20 min at room temperature. After centrifugation and removal of the chloroform/methanol/water supernatants, the precipitate was allowed to dry at room temperature.

For Pronase digestion, a solution of 1% Pronase in 0.05 M-Tris/HCl buffer, pH 7.8, containing 5 M-CaCl₂ was first preincubated at 37°C for 2 h to inactivate glycosidases. The delipidated precipitate was suspended in 250 μl of 0.05 M-Tris/HCl, pH 7.8, containing 5 mM-CaCl₂, boiled for 5 min, and 25 μg each of cycloheximide and chloramphenicol were added. Preincubated Pronase (1 mg) was added at the start of incubation and after 24 h of incubation, and another 0.5 mg of Pronase was added after 48 h of incubation. At the end of the incubation (minimum of 72 h at 37°C), the mixture was boiled for 5 min to inactivate the Pronase.

Gel filtration

Glycopeptides obtained after Pronase digestion were chromatographed on a column (1 cm $\times$ 113 cm) of Bio-Gel P-6 (200–400 mesh) in 0.1 M-pyridine acetate buffer, pH 5.0, with bovine serum albumin to determine V_0 and with either D-mannose or L-fucose as standard. Fractions (1 ml) were collected and assayed for radioactivity.

Treatment with endo- β -*N*-acetylglucosaminidase H

Glycopeptide fractions obtained by gel filtration

on Bio-Gel P-6 were freeze-dried and treated for 16–18 h at 37°C with 2 m-units of endo- β -*N*-acetylglucosaminidase H in 100 μ l of 0.15 M-citrate buffer, pH 5.5, containing 25 μ g each of cycloheximide and chloramphenicol. One unit of this enzyme is defined by the manufacturer as the amount that hydrolyses 1 μ mol of (Man)₅-(GlcNAc)₂-Asn-[¹⁴C]acetyl per min at 37°C. After incubation the products were chromatographed on the same column of Bio-Gel P-6, as described above.

Concanavalin A–Sephrose chromatography

The labelled fractions were freeze-dried and dissolved in 0.1 M-Tris/HCl, pH 7.5, containing 0.1 M-NaCl, and were then passed through a column (0.5 cm $\times$ 2 cm) of concanavalin A–Sephrose previously equilibrated with the same buffer. The column was first washed with 10 ml of 0.01 M-Tris/HCl, pH 7.5, containing 0.1 M-NaCl, and then with 10 ml of 0.1 M-methyl α -D-mannopyranoside in the same solution. Fractions of 1 ml were collected and assayed for radioactivity.

Alkali treatment

Glycopeptides were treated with either 0.05 M-KOH in 1 M-NaBH₄ at 45°C for 18 h (Carlson, 1968), or 0.1 M-KOH in 2 M-NaBH₄ at 45°C for 48 h. After neutralization with acetic acid, the samples were chromatographed on Bio-Gel P-6.

Acid hydrolysis

Samples were hydrolysed with 0.5 M-H₂SO₄ or 0.05 M-H₂SO₄ for 7.5 h at 100°C. The hydrolysates were then passed through coupled columns of Dowex AG 50W (X8; H⁺ form; 200–400 mesh) and Dowex AG1 resin (X8; formate form; 200–400 mesh). The resins were eluted with water, and the eluates were concentrated and chromatographed with standards of D-galactose, D-glucose, D-mannose and L-fucose on Whatman no. 1 paper in ethyl acetate/pyridine/water (8:2:1, by vol.) for 48 h. Guide strips with standards were stained with the silver nitrate reagent (Trevelyan *et al.*, 1950). Radioactivity on the chromatograms was determined by suspending 1 cm strips in 1 ml of water for at least 20 min, followed by the addition of 8 ml of Aquasol. The amount of radioactivity that migrated as L-fucose was the same under both conditions of hydrolysis, showing that no significant destruction occurred in 0.5 M-H₂SO₄. With 0.05 M-H₂SO₄, however, a significant proportion of the radioactive products remained at the origin, indicating that hydrolysis was incomplete under these conditions. Therefore, the results obtained with 0.5 M-H₂SO₄ are reported.

Radioactivity was determined with a Packard liquid-scintillation spectrometer, model 3255, using

8 ml of Aquasol (New England Nuclear). Protein was determined by Lowry's method with bovine serum albumin as standard (Lowry *et al.*, 1951). Carbohydrate was estimated with phenol/H₂SO₄ (Dubois *et al.*, 1956).

Results

Incorporation of radioactivity into microsomes after injection of D-[2-³H]mannose

The biosynthesis of the carbohydrate groups of glycoproteins was compared in crypt- and villus-cell microsomes after injection of D-2-³H]mannose. In both cell populations, labelling of the microsomes reached a maximum about 1 h after injection (Fig. 1). The total incorporation was much lower in crypt cells than in villus cells because of the smaller yield of cells, but the specific radioactivity of microsomal glycoproteins was only slightly lower in crypt cells than in villus cells. The specific radioactivity remained constant in villus cells, and decreased gradually in crypt cells between 1 and 3 hours after injection.

The microsomes were extracted with chloroform/methanol (2:1, v/v) and chloroform/methanol/water (4:4:1, by vol.) by using a procedure that removes dolichol-linked saccharides (Herscovics *et al.*, 1977). The incorporation of radioactivity into these lipid fractions was too small to allow further characterization. The delipidated residues were completely solubilized by treatment with Pronase, and the glycopeptides were chromatographed on a column of Bio-Gel P-6 (Fig. 2a). Three labelled glycopeptide fractions were obtained: fraction I was eluted at the *V*₀ of the column, and fractions II and III were retarded, and eluted in that order. The proportion of radioactivity in fraction I remained constant with time (17–18%), and was the same in both crypt and

Fig. 1. *Incorporation of radioactivity into microsomes after injection of D-[2-³H]mannose*

For each time interval two rats were each injected with 250 μ Ci of D-[2-³H]mannose, and microsomes isolated from crypt and villus cells were assayed for radioactivity and protein.

villus cells, whereas the relative proportions of radioactivity in fractions II and III were variable. Shortly after injection (15 min), fraction III was the major labelled glycopeptide fraction. It accounted for about 85% and 70% of the radioactivity in the included glycopeptides in villus- and crypt-cell microsomes respectively. With time, the proportion of labelled glycopeptides in fraction III decreased, whereas that in fraction II increased. This change in the relative distribution of labelled glycopeptides was much more rapid in crypt than in villus cells (Fig. 2). This difference was most clearly observed 2 h after injection; at that time there was already a large amount of radioactivity in fraction II of crypt-cell microsomes and relatively little in villus-cell microsomes (Fig. 2). At 3 h after injection, the proportion of labelled glycopeptides in fraction II compared with fraction III was still greater in crypt-cell than in villus-cell microsomes. Since the specific radioactivity of microsomal glycoproteins (Fig. 1), and of other membrane glycoproteins (Quaroni

et al., 1980), did not increase beyond the first hour after injection, these results indicate that the labelled glycopeptides in fraction III are precursors of those in fraction II.

Characterization of glycopeptides labelled from D-[2-³H]mannose

The labelled products in fraction I and those in fractions II and III were combined, and freeze-dried. Each of these samples was then incubated with endo- β -N-acetylglucosaminidase H, and re-chromatographed on the same column of Bio-Gel P-6. This treatment did not affect the elution profile of the labelled glycopeptides of fraction I (results not shown). However, the enzyme caused a shift of the labelled glycopeptides in fraction III toward lower-molecular-weight species. The peak of radioactivity in this fraction moved from about fraction 45 (Fig. 2a) to fraction 50 (Fig. 2b). The elution of the glycopeptides in fraction II was not significantly affected by the endoglycosidase, and the peak was

Fig. 2. Gel filtration of glycopeptides obtained from microsomes after injection of D-[2-³H]mannose. Villus- and crypt-cell microsomes obtained from the experiment described in Fig. 1 were treated with Pronase. The resulting glycopeptides were chromatographed on Bio-Gel P-6 (a). Fraction I (nos 25–32) and combined fractions II and III (nos. 32–52) were pooled, freeze-dried, and treated with endo- β -N-acetylglucosaminidase H. The samples were then re-chromatographed on Bio-Gel P-6. Only the profiles resulting from treatment of fractions II and III are shown (b) since the elution of fraction I was unaffected. Some of the labelled products in fraction II of the 40 min crypt sample were lost before treatment with endo- β -N-acetylglucosaminidase H.

found in tubes 38–39 before and after treatment with endo- β -*N*-acetylglucosaminidase H. The labelled products in fraction II and those in fraction III obtained after treatment with endo- β -*N*-acetylglucosaminidase H were pooled and studied separately.

The labelled products in fraction III were retained on a column of concanavalin A–Sephadex, and subsequently eluted with methyl α -D-mannopyranoside, whereas none of the labelled products in fractions I and II were adsorbed on this column. Alkali treatment (0.05 M-KOH in 1 M-NaBH₄ at 45°C for 18h), which causes β -elimination of *O*-glycosidically linked carbohydrate groups (Carlson, 1968), did not affect the elution pattern of the glycopeptides in fractions I and II. Additional treatment of the isolated products in fraction I with Pronase did not alter their elution volume, showing that their relatively large size was not due to incomplete Pronase digestion.

Each of the labelled fractions obtained 3h after injection was hydrolysed with acid and the hydrolysates were subjected to paper chromatography. Most of the radioactivity in fraction I was present as fucose (82–84%), and a small proportion as mannose (13–18%). The radioactivity in fraction II was recovered in both mannose (43–57%) and fucose (40–48%), whereas almost all the radioactivity in fraction III was found as mannose (96–98%). There was no significant difference between crypt- and villus-cell microsomes in the distribution of label in the various fractions.

These results show that fraction III contains labelled asparagine-linked oligosaccharides of the high-mannose type since they are sensitive to endo- β -*N*-acetylglucosaminidase H, whereas fraction II contains labelled asparagine-linked oligosaccharides of the complex type, labelled with [³H]mannose but resistant to the endoglycosidase. The identity of the labelled products in fraction I, which are mostly labelled with [³H]fucose, is unclear.

Incorporation of radioactivity into surface membranes after injection of D-[2-³H]mannose

The glycopeptides obtained from surface-membrane glycoproteins were examined 3h after injection of D-[2-³H]mannose. Lateral-basal membranes contained three labelled fractions with the same elution characteristics on Bio-Gel P-6 as those obtained from microsomes (Fig. 3). Treatment of the glycopeptides in fractions II and III with endo- β -*N*-acetylglucosaminidase H caused a shift in the elution of the labelled compounds in fraction III similar to that observed for microsomal glycopeptides, but did not affect the elution profile of labelled glycopeptides in fraction II (Fig. 3). For both villus and crypt cells a similar proportion (16%) of the total radioactivity in the glycopeptides

Fig. 3. Incorporation of radioactivity into glycopeptides of lateral-basal membranes after injection of D-[2-³H]mannose

Two rats were each injected with 1mCi of [2-³H]mannose. The lateral-basal membranes of crypt and villus cells were prepared 3h after injection, and treated with Pronase. (a) and (b), Bio-Gel P-6 chromatography of lateral-basal membrane glycopeptides from villus and crypt cells respectively; (c) and (d), Bio-Gel P-6 chromatography of combined fractions II and III from (a) and (b) respectively, after treatment with endo- β -*N*-acetylglucosaminidase H. The elution volumes of fractions II and III in (a) are slightly larger than usual due to a small change in behaviour of the column as evidenced by the larger elution volume of mannose.

was recovered in fraction I. However, there was some difference between the two cell populations in the relative proportion of radioactivity recovered in fractions II and III. In crypt cells, about 70% of the radioactivity in the included glycopeptides of lateral-basal membranes was found in fraction II compared with about 55% in the corresponding villus-cell membranes (Fig. 3).

The profile of glycopeptides obtained from luminal membranes of both crypt and villus cells was different from that obtained from microsomes and from lateral-basal membranes at the same time interval (Fig. 4). For luminal membranes, radioactivity was incorporated mostly into fractions I and II, and only a small amount of radioactivity was found in fraction III, which appeared as a shoulder to fraction II (Figs. 4a and 4b). Treatment with endo- β -*N*-acetylglucosaminidase H did not affect the elution of glycopeptides in fraction II, although it caused a shift in the elution of labelled products in fraction III. In fact, fraction III became clearly apparent only after this treatment (Figs. 4c and 4d).

Fig. 4. Incorporation of radioactivity into glycopeptides of luminal membranes after injection of D-[2-³H]mannose

Four rats were each injected with 500 μ Ci of D-[2-³H]mannose. At 3 h after injection, two of these were used for the preparation of villus-cell luminal membranes (brush-border membranes), and two were used for the preparation of crypt-cell luminal membranes. These membranes were then treated with Pronase. (a) and (b), Bio-Gel P-6 chromatography of glycopeptides obtained from luminal membranes of villus and crypt cells respectively; (c) and (d), Bio-Gel P-6 chromatography of fractions II and III from (a) and (b) respectively, after treatment with endo- β -N-acetylglucosaminidase H.

Crypt and villus cells had a similar proportion of radioactivity in the various glycopeptide fractions, with 24–27% being recovered in fraction I.

Characterization of glycopeptides labelled after injection of L-[6-³H]fucose

The labelled glycopeptides formed from [³H]-fucose were examined, 3 hours after injection, in microsomes and in the top 'fluffy' portion of the 20000g pellet, which contains surface membranes (Weiser *et al.*, 1978). In both cases, two labelled glycopeptide fractions were obtained on Bio-Gel P-6, corresponding in elution to fractions I and II, which were labelled after [³H]fucose injection (Fig. 5). The elution profile of these glycopeptides was not affected by treatment with endo- β -N-acetylglucosaminidase H, and neither of these fractions were retained on concanavalin A-Sepharose. The profiles obtained for both membrane fractions of crypt and villus cells were similar; about 40–55% of the radioactivity was recovered in fraction I. When fraction I was chromatographed on a column of Bio-Gel P-30, about 40% of the labelled products

Fig. 5. Incorporation of radioactivity into glycopeptides of intestinal cell membranes after injection of L-[6-³H]fucose

Two rats were each injected with 500 μ Ci of L-[6-³H]fucose. At 3 h after injection, microsomes and the membranes present in the top 'fluffy' portion of the 20000g pellet were prepared (Weiser *et al.*, 1978) and treated with Pronase. The resulting glycopeptides were chromatographed on Bio-Gel P-6. (a) Villus-cell microsomes; (b) crypt-cell microsomes; (c) villus-cell 20000g pellet; (d) crypt-cell 20000g pellet.

was excluded from the gel, and the rest were retarded, indicating that some of the fucose-labelled glycopeptides in fraction I have a relatively high molecular weight. Treatment with alkali (0.05 M-KOH in 1 M-NaBH₄ at 45°C for 18 h), under conditions that usually cause β -elimination of O-glycosidically-linked carbohydrate groups (Carlson, 1968), did not change the elution pattern of the labelled glycopeptides in fraction I on either Bio-Gel P-6 or on Bio-Gel P-30. Stronger and longer alkali treatment (0.1 M-KOH in 2 M-NaBH₄ at 45°C for 48 h) resulted in 40–50% degradation of the labelled products in fraction I to lower-molecular weight species.

Discussion

These results show that membranes of intestinal epithelial cells treated with Pronase contain three groups of glycopeptides separated by gel filtration: (1) asparagine-linked oligosaccharides of the high-mannose type (fraction III), which are endo- β -N-acetylglucosaminidase H-sensitive, are adsorbed specifically to concanavalin A-Sepharose, and are not labelled with fucose; (2) asparagine-linked oligosaccharides of the complex type (fraction II),

which can be labelled with mannose and fucose; these are resistant to endo- β -*N*-acetylglucosaminidase H, and to mild alkali treatment, and are not retained on concanavalin A-Sepharose; (3) high-molecular-weight glycopeptides (fraction I), which can be labelled with fucose and to a small extent with mannose; these glycopeptides are resistant to the action of endo- β -*N*-acetylglucosaminidase H and are relatively resistant to mild alkali treatment.

We have demonstrated a marked difference between the asparagine-linked carbohydrate groups present on the lateral-basal membrane glycoproteins and those obtained from luminal membranes. This difference is not related to differentiation of the cells, since it was observed in both crypt and villus cells. After injection of [3 H]mannose, the lateral-basal membranes contain both labelled high-mannose and complex oligosaccharides, whereas the luminal membranes contain mostly complex and relatively little high-mannose oligosaccharides. The small amount of labelled high-mannose oligosaccharides that was obtained from luminal membranes could be due to contamination by lateral-basal membranes that remain attached to the luminal membranes during the isolation procedure, as demonstrated by Forstner *et al.* (1968) for villus-cell membranes. These results demonstrate that intestinal epithelial cells are polarized with respect to the structure of the asparagine-linked oligosaccharides on their membrane glycoproteins. The mechanism whereby polarization of cell membranes occurs is unknown, but it is possible that the structure of the carbohydrate groups serves as a recognition signal to determine the final destination of the membrane glycoproteins.

The formation of asparagine-linked oligosaccharides was studied by using [3 H]mannose as precursor, since this sugar is mostly found in this type of carbohydrate side chain. However, unlike previous work with cells *in vitro* (Yurchenco *et al.*, 1978), we observed a significant conversion of the labelled mannose into labelled fucose *in vivo*. Most of the unchanged labelled mannose was recovered in the glycopeptides of fractions II and III. The time course of incorporation of radioactivity into these glycopeptides showed that high-mannose oligosaccharides are labelled earlier than complex oligosaccharides. The small amount of radioactivity observed in fraction II at early times is probably present as labelled fucose, but too little radioactivity was available to establish this possibility. The kinetics of labelling of the glycopeptides indicate that high-mannose oligosaccharides are precursors of complex oligosaccharides in intestinal cells *in vivo*. This conclusion is in agreement with previous work on other cells in tissue culture (Robbins *et al.*, 1977; Kornfeld *et al.*, 1978; Hubbard &

Robbins, 1979). In those studies it was proposed that asparagine-linked oligosaccharides are synthesized from a common dolichyl pyrophosphate oligosaccharide precursor containing di-*N*-acetylchitobiose at the reducing end, mannose and glucose. After transfer of the oligosaccharide from the lipid to protein, processing of the carbohydrate moiety begins with the removal of glucose and a variable number of mannose residues. Some of the oligosaccharides remain as high-mannose oligosaccharides containing five to nine mannose residues, whereas others are modified further to yield complex oligosaccharides that contain only three mannose residues and *N*-acetyl-lactosamine branches. Complex oligosaccharides may also have additional fucose and sialic acid residues. Little is known about the factors that control processing and that determine the type of oligosaccharide formed at specific asparagine residues.

In the present work we have shown that the rate of processing of high-mannose to complex asparagine-linked oligosaccharides changes during differentiation of intestinal epithelial cells. It is greater in rapidly dividing crypt cells than in differentiated villus cells. During glycoprotein biosynthesis key steps in processing of the carbohydrate occur during passage of the glycoproteins through the Golgi. The difference in the rate of processing between the two cell populations could be due to a difference in the rate of transfer of newly synthesized glycoproteins from rough endoplasmic reticulum to Golgi. This possibility is unlikely since it was shown in the previous paper (Quaroni *et al.*, 1980) that the peak of appearance of mannose-labelled glycoproteins in the Golgi occurs at about the same time in both cell types, namely 40–60 min after injection. The difference between the two cell populations is probably due to changes in the activity of the glycosidases and glycosyltransferases involved in processing of the carbohydrate moiety.

We have also found that, after [3 H]mannose injection, the lateral-basal membranes of crypt cells have a higher ratio of labelled complex to high-mannose asparagine-linked oligosaccharides than the corresponding villus-cell-membrane fractions. This difference is probably due to the higher rate of processing of the carbohydrate side chains in the crypt cells. These results suggest that some decrease in complex oligosaccharides of lateral-basal membrane glycoproteins probably occurs during differentiation. This type of change could be partly responsible for the decreased adhesiveness of intestinal epithelial cells to the basement membrane as they differentiate and migrate toward the tip of the villi, since it has been shown that a deficiency in complex oligosaccharides on the cell surface of ricin-resistant fibroblasts is associated with decreased adhesion to fibronectin and collagen (Pena & Hughes, 1978; Hughes, 1979).

A large proportion of the labelled fucose incorporated into intestinal cell membranes was recovered in fraction I, which contains a heterogeneous mixture of high-molecular-weight glycopeptides. This fraction may contain glycopeptides with a large number of *O*-glycosidically linked oligosaccharides, which would render them inaccessible to digestion with Pronase. Although this type of glycopeptide is usually sensitive to mild alkali treatment, resistance to β -elimination has been demonstrated for some *O*-glycosidically-linked oligosaccharides (Swann *et al.*, 1977). This fraction may also contain glycopeptides similar to the high-molecular-weight alkali-stable glycopeptides isolated from erythrocytes (Jarnefelt *et al.*, 1978; Krusius *et al.*, 1978) and Chinese-hamster ovary cells (Li *et al.*, 1980). These glycopeptides may have blood-group activity and consist of a large polyglycosyl moiety (20–70 sugar residues) with a repeating disaccharide of galactose and *N*-acetylglucosamine attached to asparagine through a mannose-containing inner core. High-molecular-weight glycopeptides have also been described in teratocarcinoma cell membranes (Muramatsu *et al.*, 1978, 1979), in human colonic adenocarcinoma and foetal intestinal cells in culture (Kim *et al.*, 1979). Additional work is necessary to determine the structure of the large glycopeptides of intestinal cells.

No major qualitative change in the glycopeptide profiles obtained from intestinal cell membranes was detected during differentiation. However, each glycopeptide fraction obtained by gel filtration probably contains a mixture of oligosaccharides exhibiting microheterogeneity. The present results, therefore, do not rule out the possibility that changes in the fine structure of the oligosaccharides occur during differentiation.

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