ULTRACYTOCHEMISTRY OF GLYCOSYLATION SITES OF RAT COLONIC MUCOUS CELLS WITH LABELED LECTINS

SHINICHIRO TSUYAMA, TATSUO SUGANUMA, KAORI IHIDA
AND FUSAYOSHI MURATA

*Department of Anatomy, Faculty of Medicine, Kagoshima University
1208-1 Usuki-cho, Kagoshima 890*

Received for publication May 12, 1986 and revised form August 27, 1986

The mucous glycoproteins of mucous cells in the lower half of the rat proximal colon were examined ultracytochemically to study the intracellular sites of glycosylation in relation to the cell differentiation and the functional polarity of the cell organelles using a combination of a hydrophilic resin embedment and a postembedding staining technique with lectin gold complexes.

In the immature mucous cells located near the branching point of the glandular crypt, the Golgi stacks and mucous granules were stained with soybean agglutinin (SBA), *Ricinus communis* agglutinin-I (RCA-I) and *Helix pomatia* agglutinin (HPA). In the mature mucous cells at the bottom of the crypt, the *cis* region of the Golgi stack was stained with SBA, a few intermediate saccules were stained with RCA-I, and both the *cis* and *trans* sides of the cisternae and mucous granules, but not the intermediate lamellae, were stained with HPA. After neuraminidase digestion of ultrathin sections, the RCA-I binding sites were greatly increased. *Ulex europaeus* agglutinin-I (UEA-I) and *Limax flavus* agglutinin (LFA) positively stained the *trans* side lamellae and mucous granules of the mucous cell at the bottom of the glandular crypt.

The processing of the glycosylation of the mucous glycoprotein in the lower half crypt of the rat proximal colon was partly clarified electron microscopically.

The main function of the mucus that covers the luminal surface of the alimentary tract is the protection of the epithelial cell layer from physical damage, the filtration of absorptive substances and the lubrication of the luminal surface. The nature of the mucus differs among segments in the alimentary tract and among species of animals.

The mucus is a biochemically heterogeneous polymer composed of high molecular weight glycoproteins with various structural features. The sequence of sugar residues from each mucous glycoprotein is destined genetically and is modified by the microenvironment of the cells that constitute each organ (7, 9, 29).

Cytochemistry by means of the labeled lectin has been employed recently to examine the glycoprotein composition in the intestine, including the colonic mucous cell (8, 12, 20-23, 36, 37). These studies showed that several lectins are useful

to label the compartment of the Golgi apparatus and/or mucous granules (25–27, 35). Moreover, lectins labeled with colloidal gold are a better probe to study the intracellular glycosylation sites especially in relation to the Golgi apparatus (13, 14, 28).

The epithelial cells of the colonic crypt in the rat proximal colon proliferate at the branching point of the crypt. Those epithelial cells migrate bidirectionally upward or downward along the crypt of the gland (36). In this report, the epithelial mucous cells which differentiate downward from the branching point were studied.

The mucous cell located deeper in the glandular crypt of the rat proximal colon has a Golgi apparatus composed of five to ten well developed cisternae. This cell is a suitable model cell to study the glycosyl processing at an electron microscopic level, with special reference to the Golgi apparatus (36). The major type of the mucin in the rat proximal colon is the sialomucin containing N-acetyl-neuraminic acid (27, 31), which is biochemically incorporated at the terminal portion of the saccharide chain (4, 30). Glucose, galactose, fucose, N-acetyl-galactosamine and N-acetylglucosamine are also incorporated in the Golgi apparatus for the synthesis of mucous glycoproteins.

We examined the mucous glycoprotein electron microscopically using colloidal gold labeled lectins to clarify the intracellular sites of the oligosaccharide glycosylation in relation to the cell differentiation and cell organelles of the rat colonic mucous cells.

MATERIALS AND METHODS

Chemicals

Soybean *Glycine max* agglutinin (SBA), castor bean *Ricinus communis* agglutinin-I (RCA-I), snail *Helix pomatia* agglutinin (HPA) and gorse seed *Ulex europaeus* agglutinin-I (UEA-I) were obtained from E-Y Laboratories Inc. (San Mateo, CA, U.S.A.). Slug *Limax flavus* agglutinin (LFA) and fetuin (type III & IV, from fetal calf serum) were purchased from Calbiochem-Behring Co. (La Jolla, CA, U.S.A.) and from Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. Neuraminidase from *Arthrobacter ureafaciens* was a product of Marukin Shoyu Co. (Uji, Kyoto, Japan).

Preparation of the tissues

Tissues from the proximal colon of Wistar strain rat were fixed by immersion in a solution containing 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hr at 4°C. Afterwards, the tissue blocks were dehydrated through a graded series of ethanol and embedded in low temperature embedding resin, Lowicryl K4M (Polysciences, Inc. Warrington, PA, U.S.A.) at –20°C, for 24 hr essentially as reported previously (22). Ultrathin sections of Lowicryl K4M embedded tissues were cut with glass knives and mounted onto nickel grids with formvar supporting film.

Preparation of colloidal gold

The colloidal gold solution (mean particle diameter, 15 nm and 20 nm) was prepared according to Frens (11) by reducing a boiling solution of aqueous 0.01% tetrachloroauric acid with a 1% aqueous solution of trisodium citrate (32).

Preparation of lectin-gold complexes and fetuin-gold complex

The lectin-gold complexes and fetuin-gold complex were prepared as described by Roth (25, 27). The pH of the colloidal gold solution was adjusted with 0.1 M K_2CO_3 to pH 6.1 for SBA, pH 8.0 for RCA-I, pH 7.7 for HPA, pH 6.3 for UEA-I and pH 4.0 for fetuin. Lectin or fetuin in 10% excess of the amount necessary to stabilize a gold solution were added to the gold solution by stirring, then, after a few minutes, a 1% aqueous polyethylene glycol solution (Carbowax 20-M, Union Carbide, U.S.A.) was added and centrifuged at 4°C, 60,000 g, for 60 min. The sedimented lectin/fetuin-gold complexes were resuspended in phosphate buffered saline (PBS) and recentrifuged in the same condition. The resulting pellets of lectin/fetuin-gold complexes were diluted with PBS containing 0.02% NaN_3 , 0.01% polyethylene glycol. The final concentration of lectin/fetuin-gold complexes in the stock solution was about 100 $\mu g/ml$ in PBS.

Cytochemical staining procedure

Lectin staining: Ultrathin sections mounted onto nickel grids were incubated with each lectin-gold complex solution at room temperature in a moist chamber for 60 min. Each lectin-gold complex was used at a concentration of between 25 and 50 $\mu g/ml$ in PBS. For the LFA staining, the two step method was employed. Briefly, the ultrathin sections were first incubated with LFA (100 $\mu g/ml$ in PBS), washed in PBS and then stained with a fetuin-colloidal gold complex at a concentration of 10 $\mu g/ml$ in PBS.

Enzyme digestion method: The effect of sialic acid removal on the pattern of RCA-I binding to the Golgi stack and mucous granules was investigated by exposing thin sections to the neuraminidase solution before incubation in the RCA-I gold complex (37). Before enzyme digestion, sections were saponified with 0.5% KOH in 70% ethanol for 3 min and then incubated for 15 hr at 40°C, in a moist chamber, on a drop of 1 unit of neuraminidase/ml in 0.05 M phosphate buffer at pH 5.0, and then stained with an RCA-I gold complex as described elsewhere (35).

RESULTS

Lectins labeled with colloidal gold are very excellent probes for studying the glycosylation processing of colonic mucous glycoproteins.

Soybean lectin (SBA) which binds specifically to the N-acetyl-galactosamine (GalNAc) residue (16, 17), stained two or three saccules in the *cis* region of the Golgi stack of the mucous cell at the bottom of the crypt (Fig. 1). Sometimes, a certain type of dilated cistern adjacent to the *cis* region of the Golgi stack was also stained (Fig. 2). Occasionally, in the mucous cells located at the branching point, whole cisternae of the Golgi stacks and mucous granules were evenly stained with this lectin-gold complex (Fig. 3).

Castor bean lectin (RCA-I) which binds specifically to the D-galactose (D-Gal) residue (15) stained only intermediate lamellae of the Golgi stack and not the *cis* or *trans* lamellae and mucous granules of the cell at the bottom of the crypt (Fig. 4). After neuraminidase digestion followed by RCA-I staining, the pattern of lectin-binding to the Golgi stacks was greatly modified, compared to the narrow distribution of the RCA-I binding sites found in non-digested sections. The Golgi stacks

from intermediate saccules to immature mucous granules were stained continuously through the *trans* side lamellae region (Fig. 5).

Snail lectin (HPA) which binds specifically to the Gal-GalNAc residue sequence (1) stained both the *cis* and *trans* side lamellae of the Golgi apparatus, and mucous granules of the mucous cell at the bottom of the crypt, while intermediate lamellae of the Golgi stack were virtually unstained (Fig. 6).

Gorse seed lectin (UEA-I) which binds specifically to L-fucose (Fuc) (18, 24) stained a few *trans* side lamellae of the Golgi stack and mucous granules of the mucous cell at the bottom of the crypt (Fig. 7). Compared to other lectins, the labeling density of saccules and mucous granules was somewhat weak.

Slug lectin (LFA) which binds specifically to N-acetyl neuraminic acid (NeuNAc) and N-glycolyl neuraminic acid (NeuNGc) (19) stained the *trans* saccules of the Golgi stack and mucous granules through the whole lower half of the crypt (Fig. 8). Many of the mucous cells in the bottom of the crypt were stained with the LFA/fetuin-gold complex, while some cells were not (Fig. 9). These two types of cells were found together. In the most positively stained mucous cells, a few saccules of *trans* side cisternae were intensely stained, while mucous granules showed a heterogeneous staining pattern (Fig. 10). The results of the stainability comparison of immature cells and mature cells is shown in Table 1.

FIG. 1. A mucous cell at the bottom of the crypt of the proximal colon stained with an SBA-gold complex. Gold particles are found at the *cis* side lamellae of the Golgi stack. $\times 31,000$

FIG. 2. A mucous cell of the proximal colon at the same segment as in Fig. 1, stained with the SBA-gold complex. On the same material, in addition to the labeling of *cis* side lamellae, a certain type of dilated cistern next to the Golgi lamellae is stained (arrows). $\times 24,000$

FIG. 3. A mucous cell of the proximal colon stained with the SBA-gold complex. In the immature cell (lower left hand corner of the figure) at the branching point of the intestinal crypt, the mucous cell, not only the *cis* lamellae of the Golgi stack but also mucigen granules, are stained, whereas in the mature type cell (upper right hand corner of the figure) only the *cis* lamellae is labeled. $\times 17,000$

FIG. 4. A Golgi apparatus of a mucous cell of the proximal colon at the bottom of the crypt labeled with the RCA-I-gold complex. Gold particles are only seen in the intermediate region of the cisternal stacks, and not in the other compartments of the Golgi saccules. $\times 22,000$

FIG. 5. The mucous granules and Golgi apparatus of the mucous cell at the bottom of the crypt of the proximal colon are saponified by the ethanolic KOH treatment followed by neuraminidase from *Arthrobacter ureafaciens* and stained with the RCA-I-gold complex. Golgi lamellae from the medial to *trans* side cisternae and mucous granules show strong labeling. $\times 23,000$

FIG. 6. Labeling of the Golgi stack and mucous granules from the mucous cell at the bottom of the proximal colonic crypt with HPA-gold complex. Many gold particles are observed on the *cis* and *trans* side lamellae of the Golgi cisternae and mucous granules. $\times 22,000$

FIG. 7. The UEA-I-gold complex stains a few *trans* cisternae of the Golgi stack and mucous granules of the mucous cell which is located at the bottom of the colonic crypt. $\times 26,000$

FIG. 8. Staining pattern of the mucous cell at the bottom of the crypt of the proximal colon with the LFA/fetuin-gold complex. The LFA/fetuin-gold binds intensely to the *trans* cisternae of the Golgi stack and mucous granules. $\times 18,000$

FIG. 9. A cell intensely stained with the LFA/fetuin gold and a cell essentially not stained. The unstained cell is distributed mosaically at the bottom of the crypt of the proximal colon. $\times 5,000$
FIG. 10. In the mucous cells at the bottom of the crypt, LFA intensely stains the *trans* cisternae of the Golgi stack but the mucous granules show a heterogeneous labeling pattern. $\times 13,000$

TABLE 1. *Stainability of the Golgi apparatus subcompartment with lectin-gold complex (+: positive staining, -: negative staining).*

IMMATURE CELL				
	cis-side cisternae	intermediate cisternae	trans-side cisternae	mucous granule
SBA	+	+	+	+
HPA	+	+	+	+
RCA-I	+	+	+	+
MATURE CELL				
	cis-side cisternae	intermediate cisternae	trans-side cisternae	mucous granule
SBA	+	+	-	-
HPA	+	-	+	+
RCA-I	-	+	-	-
RCA-I with neuraminidase treatment	-	+	+	+

DISCUSSION

The mucous glycoproteins from the colonic mucous cells of the rat were examined electron microscopically to study the glycosyl processing of the saccharide chain in relation to the cell differentiation and the functional polarity of cell organelles, using the combination of Lowicryl K4M embedding and postembedding staining with colloidal gold labeled lectins.

It is generally thought that the colonic glandular epithelia of the rat proliferate at the bottom of the crypt and they migrate upwards to the luminal surface and that the maturation of mucous cells is completed as those cells migrate from the bottom of the crypt to the surface of mucosa (5, 6, 34). These observations are adaptable about the epithelial cells in the distal colon. Cell proliferation in the rat proximal colon is not well-known. As previously described in our report, on the HRP labeled UEA-I staining pattern and conventional histochemical staining such as AB-PAS and HID-AB staining, the goblet cell mucin at the lower half of the proximal colon contains sialic acid and fucose residues (36). It is said that those sugar residues link to the terminal position of the carbohydrate chain (2, 7). It means that the carbohydrate chain containing those residues is a complete form and so that the sugar chain does not alter any further. The goblet cell mucin at the lower half crypt of the proximal colon, in our observation, is equivalent to this form. Although the mitotic figures were often found in the neighborhood of the branching point, those figures were seldom found at the bottom (36). For those reasons, we have concluded that the cell proliferation in the proximal colon of the rat occurs near the branching point of the crypt.

The epithelial cell in the rat proximal colon proliferates at the branching point of the crypt, and then migrates bidirectionally upward or downward along the crypt of the gland. Accordingly, the mucous cells located near the branching point are considered to be immature cells and those at the bottom of the crypt or at the luminal surface as well differentiated cells. In this report the mucous cells which differentiate in the direction of the bottom of the crypt were studied.

In the immature cells located at the branching point, whole Golgi stacks and mucous granules were labeled with SBA (Fig. 3), RCA-I and HPA gold complexes, although in the mature cells at the bottom, the *cis* side saccules of the Golgi stack were stained with SBA (Figs. 1, 2) and a few intermediate lamellae were stained with RCA-I (Fig. 4). On the other hand, HPA stained both the *cis* and *trans* side lamellae and the mucous granules but not the intermediate lamellae. The immature cell is thought to synthesize a sequence of the saccharide chain different from that of the mature one. According to the cell maturation, the compositions of the saccharide chains seem to be altered.

These phenomena suggest that the glycosyl processing in the *cis*, intermediate and *trans* cisternae of the Golgi stack is different (10, 31) and bring forward a very interesting problem in relation to the functional polarity of the Golgi apparatus.

Soybean lectin strongly binds to N-acetyl-galactosamine and binds only weakly to galactose. The N-acetyl-galactosamine residue is glycosylated at the initial stage of saccharide processing (7, 26). N-acetyl-galactosamine residue is usually located at the position nearest to the protein core. The preferential localization of SBA-gold complex at the *cis* side of the Golgi apparatus and the sparse labelling of

the intermediate lamellae with the same conjugates agrees with the presence of the O-glycosidically linked N-acetyl-galactosamine. In addition, positive reaction with SBA-gold complex was discernible in the dilated cisternae near the Golgi apparatus, which suggests the possibility that the glycosylation of N-acetyl-galactosamine residue occurs at those dilated cisternae, not at the Golgi lamellae.

The RCA-I reactions show that the galactose residue is detectable only at the intermediate cisternae. When the galactose residue occupied the terminal position of the elongating glycoprotein, the labeling intensity with RCA-I was high (Fig. 4). However, the addition of other sugar residues causes a shift of the galactose to a penultimate position, and then the resultant binding affinity with RCA-I is reduced. This means that RCA-I is not bound at the *trans* Golgi cisternae or the mucous granules. On the other hand, by the removal of the terminal N-acetyl-neuraminic acid with neuraminidase, the RCA-I binding intensity is increased at the *trans* side of the Golgi stack and over the mucous granules (33, 35, 37). This also indicates that the binding activity of RCA-I is inhibited by the sialic acid, and is attributable to the steric hindrance or other factors (26). It seems that initial glycosyl processing of D-galactose occurs at the intermediate cisternae of Golgi lamellae.

Snail lectin HPA binds to N-acetyl galactosamine as does SBA lectin. This lectin stained both the *cis* and *trans* lamellae of the Golgi stack and mucous granules. This observation has been reported by Roth (26). For this reason, it is considered that the binding capacity of HPA lectin not so influenced by adjacent sugar residues as that of SBA (26).

UEA-I demonstrated selective binding sites of *trans* lamellae of the Golgi stack and mucous granules even through weak labeling. Incorporation of the alpha-L-fucose to the terminal position of the developing saccharide chain, which has been confirmed by electron microscopic radioautography (3, 29) and biochemical methods, was further verified here using a simpler, less time consuming and more reliable staining method with high resolution.

Other investigators have shown that LFA-lectin binds to the *trans* lamellae of the Golgi stack and mucous granules but not to the *cis* and intermediate cisternae of the stack (27, 28). In our experiment, many mucous cells located at the bottom of the crypt of the proximal colon were positively stained with the LFA-gold complexes. Two or three saccules at the *trans* side of the stack and mucous granules were stained with this lectin, although the density of staining and pattern of staining in the mucous granules were heterogeneous. Cells which were hardly stained with LFA were found next to the stained cells. Such a staining pattern is also often observed when UEA-I is used (36). Biochemically, both fucose and sialic acid residues are ultimate terminal sugars (24, 30). Our findings also verified this view ultracytochemically.

ACKNOWLEDGMENT

The authors wish to thank Mr. J. Uehara for his technical assistance. This study was supported in part by a grant-in-aid (No. 60010064) from the Ministry of Education, Science and Culture, Japan, and the Kodama Foundation for Research of Medical Science.

REFERENCES

1. Baker D., Sugii S., Kabat E. A., Ratcliffe R. M., Hermentin P. and Lemieux U.: Immunochemical studies on the combining sites of forssman hapten reactive hemagglutinins from *Dolichos biflorus*, *Helix pomatia* and *Wistaria Floribunda*. *Biochemistry* 22; 2741-2750, 1983.
2. Bennett G. and Leblond C. P.: Formation of cell coat material for the whole surface of columnar cells in the rat small intestine, as visualized by radioautography with L-fucose-³H. *J. Cell Biol.* 46; 409-416, 1970.
3. Bennett G. and Leblond C. P.: Migration of glycoprotein from the Golgi apparatus to the surface of various cell types as shown by radioautography after labeled fucose injection into rats. *J. Cell Biol.* 60; 258-284, 1974.
4. Berger E. G. and Hesford F. J.: Localization of galactosyl- and sialyltransferase by immunofluorescence; Evidence for different sites. *Proc. Natl. Acad. Sci. USA* 82; 4736-4739, 1985.
5. Chang W. W. L. and Leblond C. P.: Renewal of the epithelium in the descending colon of the mouse. I. Presense of the three cell populations; Vacuolated-columnar cell, mucous and argentaffin. *Am. J. Anat.* 131, 73-100, 1971.
6. Chang W. W. L. and Nadler N. J.: Renewal of the epithelium in the descending colon of the mouse. IV. Cell population kinetics of vacuolated-columnar and mucous cells. *Am. J. Anat.* 144; 39-56, 1975.
7. Dawson P. A. and Filipe M. I.: Changes in [³H] galactose uptake in human colonic mucosa with carcinoma: an ultrastructural study. *Histochem. J.* 14; 361-383, 1983.
8. Ellinger A. and Pavelka M.: Postembedding localization of glycoconjugates by means of lectins on thin sections of tissues embedded in LR white. *Histochem. J.* 17; 1321-1336, 1985.
9. Fischer J., Klein P. J., Vierbuchen H., Skutta B., Uhlenbruck G. and Fischer R.: Characterization of glycoconjugate of human gastrointestinal mucosa by lectins. I. Histochemical distribution of lectin binding sites in normal alimentary tract as well as in benign and malignant gastric neoplasms. *J. Histochem. Cytochem.* 32; 681-689, 1984.
10. Fleischer B.: Mechanism of glycosylation in the Golgi apparatus. *J. Histochem. Cytochem.* 31; 1033-1040, 1983.
11. Frens G.: Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature (Phys. Sci.)* 241; 20-22, 1973.
12. Gorelick F. S., Sarraz M. P. Jr. and Jamieson J. D.: Regional differences in lectin binding to colonic epithelium by fluorescent and electron microscopy. *J. Histochem. Cytochem.* 30; 1097-1108, 1982.
13. Horisberger M.: Evaluation of colloidal gold as a cytochemical marker for transmission and scanning electron microscopy. *Biol. Cellulaire* 36; 253-258, 1979.
14. Horisberger M. and Rosset J.: Colloidal gold, a useful marker for transmission and scanning electron microscopy. *J. Histochem. Cytochem.* 25; 295-305, 1977.
15. Irimura T., Kawaguchi T., Terao T. and Osawa T.: Carbohydrate binding specificity of the so-called galactose-specific phytohemagglutinins. *Carbohydr. Res.* 39; 317-327, 1975.
16. Lotan R., Cacan R., Cacan M., Bebray H., Carter W. G. and Sharon N.: On the presence of two types of subunit in soybean agglutinin. *FEBS Lett.* 57; 100-103, 1975.
17. Lotan R., Lis H. and Sharon N.: Aggregation and fragmentation of soybean agglutinin. *Biochem. Biophys. Res. Commun.* 62; 144-150, 1975.
18. Matsumoto I. and Osawa T.: Purification and characterization of an anti-H(O) phytohemagglutinin of *Ulex europaeus*. *Biochem. Biophys. Acta* 194; 180-189, 1969.
19. Miller R. L., Collawn J. F. Jr. and Fish W. W.: Purification and macromolecular properties of a sialic acid-specific lectin from the slug *Limax flavus*. *J. Biol. Chem.* 257; 7574-7580, 1982.
20. Murata F., Suganuma T., Tsuyama S., Ihida K. and Funasako S.: Glycoconjugates cytochemistry of the rat small intestine using *Helix pomatia* agglutinin and colloidal gold conjugates. *J. Electron Microsc.* 35; 29-37, 1986.
21. Murata F., Suganuma T., Tsuyama S. and Suzuki S.: A simplified embedding method of

- Lowicryl K4M and its application to postembedding staining method. *Saibo* 17; 217-220, 1985 (in Japanese).
22. Murata F., Suganuma T., Tsuyama S. and Suzuki S.: Glycoconjugates of the rat gastrointestinal tracts. *Abst. 7th Int. Cong. Histochem. Cytochem.* 281, 1984.
 23. Pavelka M. and Ellinger A.: Localization of binding sites for Concanavalin A, *Ricinus communis* I and *Helix pomatia* lectin in the Golgi apparatus of rat small intestinal absorptive cells. *J. Histochem. Cytochem.* 33; 905-914, 1985.
 24. Pereira M. E. A., Kisailus E. C., Gruezo F. and Kobat E. A.: Immunocytochemical studies on the combining site of the blood group H-specific lectin I from *Ulex europaeus* seeds. *Arch. Biochem. Biophys.* 185; 108-115, 1978.
 25. Roth J.: Application of lectin-gold complexes for electron microscopic localization of glycoconjugates on thin sections. *J. Histochem. Cytochem.* 31; 987-999, 1983.
 26. Roth J.: Cytochemical localization of terminal N-acetyl-D-galactosamine residues in cellular compartments of intestinal goblet cell: Implications for the topology of O-glycosylation. *J. Cell Biol.* 98; 399-406, 1984.
 27. Roth J., Lucocq J. M. and Charest P. M.: Light and electron microscopic demonstration of sialic acid residues with the lectin from *Limax flavus*: A cytochemical affinity technique with use of fetuin-gold complexes. *J. Histochem. Cytochem.* 32; 1167-1176, 1984.
 28. Roth J., Taatjes J., Lucocq J. M., Weinstein J. and Paulson J. C.: Demonstration of an extensive trans-tubular network continuous with the Golgi apparatus stack that may function in glycosylation. *Cell* 43; 287-295, 1985.
 29. Sandoz D. and Roland J.-C.: Radioautographic study of glycoproteins and polyglycans. *J. Microscopie Biol. Cell.* 27; 207-214, 1976.
 30. Schauer R.: Chemistry and biology of the acylneuraminic acids. *Angew. Chem. internat. Edit.* 12; 127-138, 1973.
 31. Schulte B. A., Spicer S. S. and Miller R. L.: Histochemical localization of sialoglycoconjugates with a sialic acid-specific lectin from the slug *Limax flavus*. *Histochem. J.* 16, 1125-1132, 1984.
 32. Slot J. W. and Geuze H. J.: Sizing of protein A-colloidal gold probes for immunoelectron microscopy. *J. Cell Biol.* 90; 533-536, 1981.
 33. Spicer S. S. and Warren L.: The histochemistry of sialic acid containing mucoproteins. *J. Histochem. Cytochem.* 8; 135-137, 1960.
 34. Tsubouchi S. and Leblond C. P.: Migration and turnover of enteroendocrine and caveolated cell in the epithelium of the descending colon, as in fusion of ³H-thymidine into mice. *Am. J. Anat.* 156; 431-452, 1979.
 35. Tsuyama S., Suganuma T. and Murata F.: Mucin histochemistry of colonic mucous cells with lectin-colloidal gold complex. *J. Electron Microsc.* 34; 174-178, 1985.
 36. Tsuyama S., Suzuki S. and Murata F.: The histochemical differences of intestinal gland epithelia in the rat colon with special reference to their glycoconjugates. *Acta histochem. cytochem.* 16; 456-471, 1983.
 37. Uchida Y., Tsukada Y. and Sugimori T.: Enzymatic properties of neuraminidases from *Arthrobacter ureafaciens*. *J. Biochem.* 86; 1573-1585, 1979.