Kasetsart J. (Nat. Sci.) 33 : 234 - 242 (1999)

Lectin Histochemical Characterization of Glycoconjugates Present in Abomasal Epithelium of the Goat

Apinun Suprasert[1], Urai Pongchairerk[1], Pakawadee Pongket[1], and Takao Nishida[2]

ABSTRACT

In the abomasal epithelium of the goat, glycoconjugates have been studied by means of selective histochemical methods. The staining procedures employed were alcian blue (AB) pH 1.0 AB pH 2.5, Periodic acid Schiff (PAS) and AB pH 2.5-PAS. In addition twelve lectins, *Triticum vulgaris* WGA, *Concana valin* A, *Glycine max* SBA, *Dolichos biflorus* DBA, *Arachis hypogea* PNA, *Solanum tuberosum* STL, *Datura stramonium* DSA, *Ulex europeus* UEA-I, *Bauhinia purpurea* BPA, *Ricinus communis* RCA-I, *Lotus tetragonolobus* LTA, and *Limulus polyphemus* LPA were applied to detect saccharides residues. According to the results obtained, the nature of glycoconjugates in mucous epithelium was found to change during cell migration from deep to superficial part. The surface mucous cells contained primarily neutral glycoconjugates with vicinal diol groupings and terminal fucose residues. By contrast, the mucous cells in deep pits and in pyloric glands contained acidic sulfated glycoconjugates with terminal sialic acid residues. The α-D-mannose, α-D-glucose, β-D-galactose, N-acetylglucosamine and N-acetylgalactosamine were also found at the pyloric gland cells while they were smaller in amount at the surface mucous cells. The data suggested that changes of epithelial cells were closely correlated with cellular maturation.

Key word: lectin, glycoconjugates, abomasum, goat

INTRODUCTION

Investigations in many laboratories have revealed that oligosaccharides of cell glycoconjugates have a major influence over developmental and differentiative processes, intercellular recognition, and thus also in many pathological processes, including malignancy (Taatjes and Roth, 1991; Bourillon and Aubery, 1989). A voluminous literature exists concerning the histochemistry of glycoconjgates in gastrointestinal epithelium of various mammals (Ito *et al*., 1985; 1983; Sato and Spicer, 1980; Sheahan and Jervis, 1976) Recently, lectin has been used as a potent probe for the demonstration of sugar residues in mammalian gastrointestinal mucosa and for discrimination of specific carbohydrate unit alterations, where conventional histochemical technique cannot perform (Aoki *et al*., 1993; Spicer and Schulte, 1992). Cellular and regional differences in lectin binding to mammalian gastrointestinal cells have been reported (Fischer *et al*., 1984; Boland *et al*., 1982) However, little knowledge is available concerning abomasum of

1 Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
2 Department of Anatomy and Physiology, College of Agriculture and Veterinary Medicine, Nihon University, Kameino 1866, Japan.

the goat.

In view of interesting structures and functions of goat stomach and the physiological important of glycoconjugates in this organ, glycoconjugates involved in the abomasal epithelium of this animal have been studied histochemically, employing a wealth of currently available methods of lectin histochemical staining procedures. The results obtained here are believed to involve hitherto unknown aspects of histochemical architecture of the goat stomach and give a clue to thorough recognition of the histophysiological functions performed by goat abomasal epithelium in general.

MATERIALS AND METHODS

A total of 3 goats of different ages and sexes were sacrificed by exsanguination under deep ether anesthesia. From the donor animals, pyloric part of abomasum has dissected out and then fixed by immersion in any one of the following fixatives.

1). 10% formalin containing 2% calcium acetate (Leppi, 1968) for 24 hrs at 4°C

2). Bouin's fluid for 24 hrs at 4°C.

The tissue specimen was then dehydrated and embedded in paraplast. On sliding microtome, sections were cut at a thickness of 1-2 μm, deparaffinized in xylene, hydrated in grade ethanol series and then subjected to the following histological and histochemical staining procedures.

Conventional staining procedures

1). Haematoxylin and eosin for the general observation of histological structures.

2). Periodic acid-Schiff (PAS) for vicinal diol containing glycoconjugates (Pearse, 1968).

3). Alcian blue (AB) pH 1.0 for sulfated glycoconjugates (Lev and Spicer, 1964).

4). AB pH 2.5 for acidic glycoconjugates (Spicer *et al*., 1967).

5). AB pH 2.5-PAS for demonstrating of acidic and neutral glycoconjugates (Spicer *et al*., 1967).

Lectin staining procedures

Briefly, deparaffinized sections were treated with 1% bovine serum albumin (BSA) in 10 mM phosphate buffer saline (PBS) pH 7.4 and then incubated with biotinyl lectins (25 μg/ml, Vector Lab Inc. USA) in0.1% BSA-PBS for 30 min. After rinsing with PBS, the sections were incubated in avidin-biotinyl peroxidase complex (ABC Vector Lab Inc. USA) for 30 min. After briefly rinsing in PBS, the sections were immersed in 3,3-diaminobenzidine (DAB, 0.2 mg/ml)-H_2O_2 (0.005%) for 10 min, rinsed with distilled water, dehydrated and mounted.

Control experiments

for lectin staining, control procedures were performed: Tissue sections were preincubated in appropriated hapten sugars for each lectin (sucrose for ConA, lactose for RCA-I and PNA, N-acetylglucosamine for WGA and STL, fucose for UEA-I and LTA, and N-acetylgalactosamine for DBA, DSA and SBA) and then incubated in lectin solutions containing the hapten sugars. Non specific staining was also checked by incubation in the ABC and DAB-H_2O_2 solutions.

RESULTS

In the pyloric part of the goat abomasum, the epithelium of the mucosa invaginated into the lamina propria, forming tubular structure (gastric pit). Pyloric glands are exclusively mucous type and empty into the bottom of gastric pit. The epithelial cells lining the surface and gastric pits are also found to be mucous cells. When the abomasal epithelium was reacted with PAS (Figure1), surface mucous cells and pyloric glands stained intensely

and moderately respectively. By contrast, the AB pH 1.0 and AB pH 2.5 (Figure 3) staining procedures resulted in weak or negative reactions in the surface mucous cells and resulted in strong positive reactions in the pyloric glands. Furthermore the surface mucous cells stained deep red or red purple with AB pH 2.5-PAS (Figure 2). Pyloric glands, on the other hand, stained deep purple at the upper part and primarily blue at the deep part.

Intensity of positive reactions were distinguished in the surface mucous cells and the pyloric gland cells according to the results of the lectin staining method. Mucous granules, striated border and golgi zone of both structures exhibited moderate to intense positive reaction to STL (Figure 4), RCA-I (Figure 5), WGA (Figure 7), BPA , ConA , SAB (Figure 8) and DBA. When the pyloric abomasal epithelium reacted with LTA (Figure 6), the staining intensity gradually decreased from surface epithelium to gastric pit whereas pyloric gland was found to exhibit negative reaction. However, this was not true for the STL (Figure 4), SBA (Figure 8), ConA and RCA-I (Figure 5) in which the binding affinity tended to increase from surface epithelium to pyloric glands. In addition, only the pyloric gland cells showed affinity against LPA while the surface mucous cells stained negatively. The DSA, PNA and UEA-I reactions of the both part were nearly negative.

The connective tissue in lamina propria stained weakly with ConA And RCA-I and negatively with all other lectins. In the control experiment for the lectin staining procedures, the addition of appropriate sugar to lectin solution greatly diminished the lectin reactions in all histological tested. Endogenous peroxidase activeity and other on specific binding such as those caused by HRP were mostly undetectable. All the results obtained from conventional and lectin staining procedures are summarized in Table 2.

DISCUSSION

Although the histology of the goat abomasum has been studied by many authors, the

Table 1 List of lectins employed histochemical studies and their binding specificities according to references cited.

Lectins	Bindind specificities	References
Triticum vulgaris WGA	β-D GlcNAC.	Goldstein and Hayes, 1978
Concana valin A	α-D Man., a-D-glu.	Kiernan, 1975
Glycine Max SBA	α-D GaINAc.	Sharon and Lis, 1982
Dolichos biflorus DBA	α-D-GaINAc.	Goldstein and Hayes, 1978
Arachis hypogea PNA	Gal β1-3 GaINAc.	Stoward et al., 1980
Solanum tuberosum STL	β-D-GlcNAc.	Goldstein and Hayes, 1978
Datura stramonium DSA	β-D-GluNAc.	Sharon and Lis, 1982
Ulex europeus I UEA-I	α-L-Fuc.	Goldstein and Hayes, 1978
Bauhinia purpurea BPA	α-D-GaINAc.	Goldstein and Hayes, 1978
Ricinus communis RCA-I	β-D-gal.	Yamada and Shimizu, 1977
Limulus polyphemus LPA	NeuAc.	Goldstein and Hayes, 1978
Lotus tetragonolobus LTA	α-L-fuc.	Goldstein and Hayes, 1978

Table 2 Histochemical reactions of glycoconjugates in the goat pyloric abomasal epithelium.

Staining methods	Pyloric gland cells	Surface mucous cells
PAS	2R	3R
AB pH 1.0	2B	0
AB pH 2.5	3B	0-1B
AB pH 2.5-PAS	3BP	3RP
WGA	2Br	2Br
ConA	2-3Br	1Br
SBA	3Br	2Br
DBA	2-3Br	2Br
PNA	0-1Br	0
STL	3Br	1Br
DSA	0	0
UEA-I	0	0
BPA	3Br	2Br
RCA-I	3Br	1-2Br
LPA	1Br	0
LTA	0	1-2Br

Key to symbols in table

R : PAS positive, B : AB positive, Br : Lectin positive

BP : A mixture of AB-and PAS-positive glycoconjugates with AB-positive glycoconjugates predominanting.

RP : A mixture of PAS-and AB-positive glylycoconjugates with PAS-positive glycoconjugates predominanting.

O : Negative reaction.

1-3 : Numerical values correspond to increasing intensity of staining.

distribution of histochemically detectable glycoconjugates in the mucous epithelium has been poorly investigated. In the present study, the histological structure of the pyloric abomasal epithelial cells and the pyloric gland cells was found to be similar without any particular alterations during migration along the pits. In contrast, changes in histochemical reactivities during the migration were a finding of particular note.

In view of the mechanism underlying the conventional staining procedures (PAS, AB pH 1.0, AB pH 2.5, AB pH 2.5-PAS) applied to our control samples, the present results would seem to indicate that the surface mucous epithelium contains predominantly neutral glycoconjugates whereas the pyloric gland contains a mixture of acidic and neutral glycoconjugates with sulfate and vicinal diol groupings. At the deep part of the pyloric glands are furthermore found to involve predominant acidic sulfated glycoconjugates. From the results obtained, glycoconjugates are believed to vary in a cell during its maturation. Since cationic dyes demonstrated production of sulfated secretory product by immature cells in pyloric gland. In contrast, the mature cells in surface epithelium produced a non-sulfated glycoprotein.

The quantitative and quanlitative changes in the mucous cells secretions were confirmed by

the binding patterns with lectins as the mucous cells matured and moved toward the luminal surface. The most obvious and dramatic difference was noted in the binding pattern of LTA, which moderately stained surface mucous cells but weakly to negatively stained at the pyloric gland cells. Conversely STL, SBA, ConA and RCA-I reactivity decreased in mucous secretion during cell aging and upward migration. The staining pattern with LTA, STL, SBA, ConA and RCA-I can be explained on the basis of the presence of α-L-fucose, N-acetylglucosamine, N-acetylgalactosamine, α-D-mannose or α-Dglucose and β-D-galactose residues. The presence of N-acetylglucosamine and N-acetylgalactosamine was confirmed by the positive reaction of WGA and DBA or BPA respectively. Furthermore, glycoconjugates with terminal sialic acid residues were also presence in the pyloric glands as judged from their positive staining with LPA. The negative reaction with PNA lectin at the pyloric gland cells suggested that the binding site for PNA lectin was masking by negative charge of sialic acid residues. All the results obtained would seem to indicate that the surface epithelium contains primarily complex type of N-linked glycoproteins while the pyloric gland involves a mixture of N-linked (high mannose forms) and 0-linked glycoproteins.

Comparison of reactivity of the UEA-I and LTA in the mucous epithelium of the goat abomasum, each having a nominal binding specificity for α-L-fucose residues, revealed some interesting findings. UEA-I binding was restricted to the presence of 0-glycosidically-linked secretory glycoproteing whereas LTA binding was restricted to the presence of N-linked glycoprotein (Schulte and Spicer, 1983). The positive LTA and negative UEA-I reactions confirm the different binding affinity for the same terminal sugar having different glycosidic linkage. In addition, differentiation-related changes in cell glycosylation pattern were strongly confirmed during cell migration from pyloric gland to surface epithelium. Since the positive staining with LPA, sialic acid-specific lectin, was restricted to the mucous cells in lamina propria. In contrast, binding of fucose-specific LTA was restricted to mucous cells at the surface epithelium. All of these results taken together support the hypothesis that as epithelial cells undergo normal differentiation and migration to surface, new metabolic activities emerge (fucosyltransferae) while some other enzymes become unactive (for example sialyltransferase) (Weiser, 1973)

According to the previous studies, neutral glycoconjugates were found in surface epithelium of pyloric part of simple stomach of most mammals while sulfated glycoconjugates were found in pyloric glands of mouse, rat and rabbit (Spicer, 1960; Sheahan and Jervis, 1979). In addition, carboxylated glycoconjugates were found in pyloric gland of guinea pig (Sato and Spicer, 1980) whereas both sulfated and carboxylated glycoconjugates were involved in baboon (Sheahan and Jervis, 1976). In the present study, all surface mucous cells of goat pyloric abomasum contain predominantly neutral glycoconjugates whereas pyloric gland cells involve a mixture of neutral and acidic glycoconjugates with sulfate groups and terminal sialic acid residues. Our finding conforms with earlier observations that these seem to be interspecies variation together with regional variation.

In a similar way to that described in many mammalian species, the epithelial cells of the goat abomasum contain both sialo-and sulfomucins. This acidic in mucus may play an important role in resisting the invasion of potential pathogens (Schauer, 1982). Sialic acid and sulfated groups are also believed to play an essential role of lubrication and protection in digestive tract (Werner *et al*., 1982). In addition, they may provide a

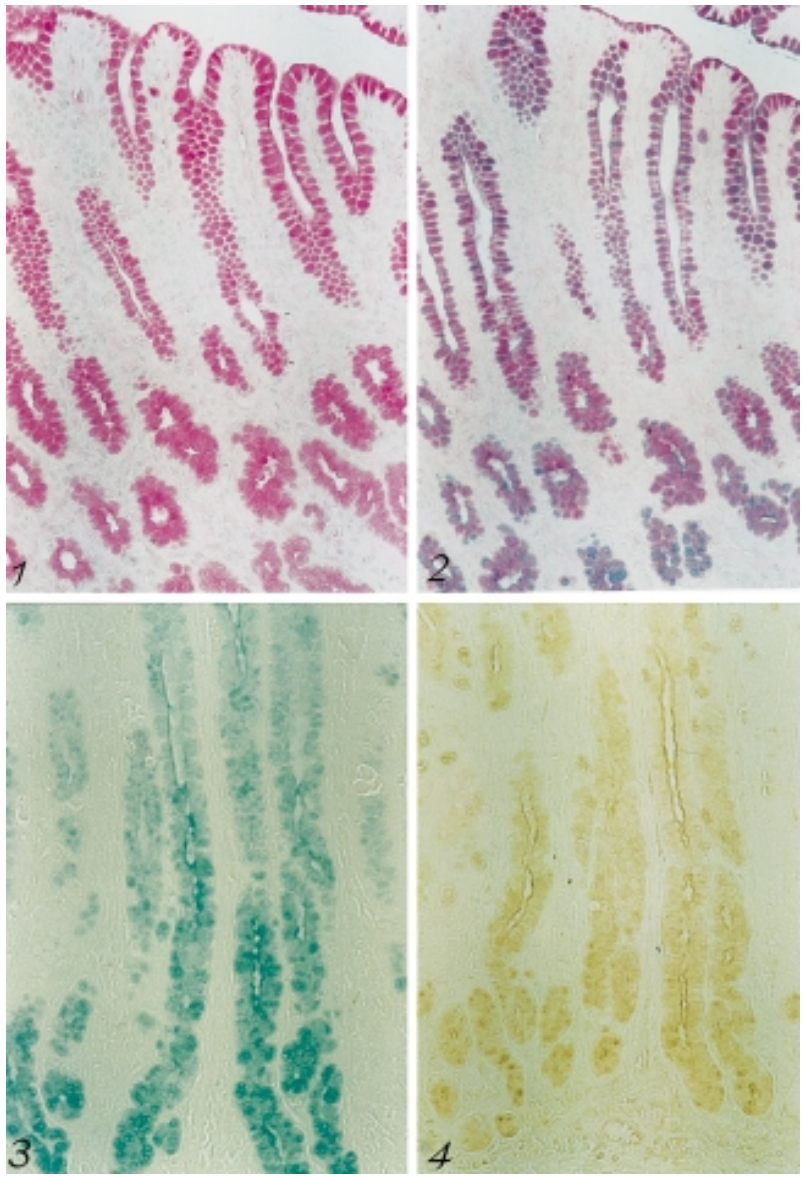

Figure 1 In the epithelium of the goat abomasum, surface mucous cells and pyloric gland cells stained intensely and moderately respectively. PAS x 264.

Figure 2 The dual staining with AB pH 2.5-PAS result in deep red or red purple at surface mucous cells but stain deep purple or primarily blue at pyloric gland cells. AB pH 2.5-PAS. x 264

Figure 3 Mucous granules of pyloric gland cells exhibit strong positive reactions. However, the positive reactions of the mucous granules at the deep part are stronger in intensity than those at the superficial part. AB pH 2.5 staining. X 525.

Figure 4 Shows the same results as in Fig. 3. STL staining. X 525.

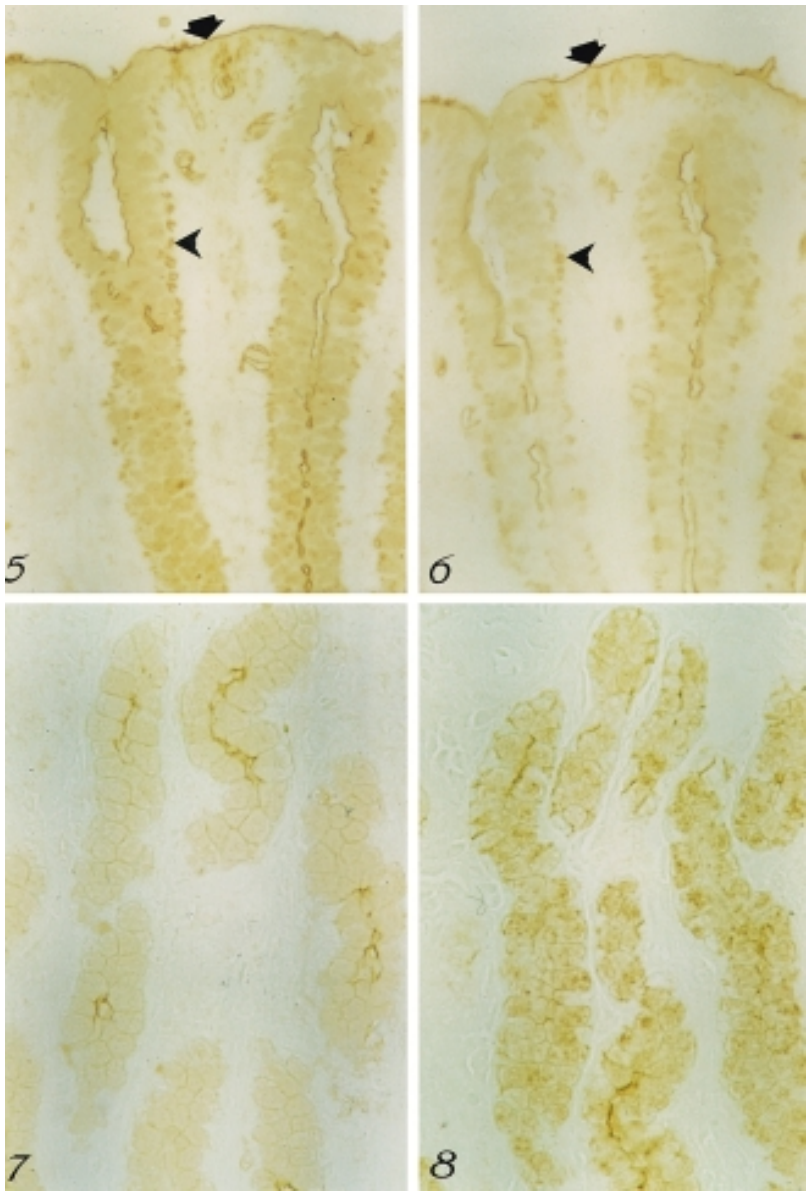

Figure 5 Mucous granules, striated boder (arrow) and supranuclear region (arrow head) of surface epithelium stain moderately. RCA-I. x 1000

Figure 6 Mucous granules, striated border (arrow) and supranuclear region (arrow head) of surface epithelium stain moderately. However, the positive reactions of the mucous granules at superficial part are stronger in intensity than that at deep part. LTA staining. X 1000.

Figure 7 Mucous granules in pyloric gland cells exhibit weakly positive reaction. WGA. X 1000.

Figure 8 Mucous granules in pyloric gland cells are strongly reactive SBA x 1000.

conduit from the gastric gland for secretions rich in pepsin and HCL (Sato and Spicer, 1980). The importance of glycosylation is mostly unknown at this point, although the shift of glycoconjugates from sialation to fucosylation during cellular migration has been attributed to represent the change in physiological functioning of the goat pyloric abomasum (Taatjes and Roth, 1991). Further critical studies are need to elucidate the importance of cellular glycosylation in a myriad functions.

LITERATURE CITED

Aoki, T., J. Kawano, T. Oinuma, Y. Haraguchi, T. Eto, and T. Suganuma. 1993. Human colorectal carcinoma specific glycoconjugates detected by pockeweed mitogen lectin. J. Histochem. Cytochem. 41: 1321-1330

Boland, C.R., C.K. Montgomery, and Y.S. Kim. 1982. Alterations in human colonic mucin occuring with cellular differentiation and malignant transformation. Proc. Natl. Acad. Sci. 79: 2051-2055.

Bourrilon, R., and M. Aubery. 1989. Cell surface glycoproteins in embryonic development. Inter. Rev. Cytol. 116 : 257-338

Fischer, J., P.J. klein, M. Vierbuchen, B. Skutta, G. Uhlenbruck, and R. Fischer. 1984. Characterization of glycoconjugates 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 neoplasm. J. Histocem. Cytochem. 32 : 681-689

Goldstein, I.J. and C.E. Hayes. 1978. The lectins : Carbohydrate-binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem. 35 : 127-340.

Ito, M., K. Takata, S. Saito, T. Aoyaki, and H. Hirano. 1985. Lectin binding pattern in normal human gastric mucosa : A light and electron microscopy study. Histochemistry 83: 189-193.

Kiernan, J.A. 1975. Localization of α-D-glucosyl and α-D-mannosyl groups of mucosubstances with concanavalin A and horseradish peroxidase. Histochemistry. 44 : 39-45.

Leppi, T.J. 1968. Morphological analysis of mucous cells in the skin and slime glands of hagfishes. Histochemie. 15 : 68-78.

Lev, R. and S.S. Spicer. 1964. Specific staining of sulphate groups with alcian blue at low pH. J. Histochem. Cytochem. 12 : 309.

Pearse, A.G.E. 1968. The periodic acid-Schiff technique, pp.659-660 *In* Histochemistry Theorectical and applied. Vol 1. 3rd. ed. J and A Churchil, Edinburgh.

Sato, A. and S.S. Spicer. 1980. Ultrastructural cytochemistry of complex carbohydrates of gastric epithelium in the guinea pig. Am J. An at. 159 : 307-329.

Schauer, R. 1982. Chemistry, metabolism and biological functions of sialic acids. Adv. Carbohyd. Chem. Biochem. 40 : 131-234.

Schulte, B.A. and S.S. Spicer. 1983. Light microscopic detection of sugar residues in glycoconjugates in salivary glands and the pancreas with lectin-horseradish peroxidase conjugates. I. Mouse. Histochem. J. 15 : 1217-1238.

Sharon, N., and H. Lis. 1982. Glycoprotein : Research booming on long-ignored ubiquitous compounds. Mol. Cell Biochem. 42 : 167-187

Sheahan, D.G., and H.R. Jervis. 1976. Comparative histochemistry of gastrointestinal mucosubstances. Am. J. Anat. 146 : 103-132

Spicer, S.S. 1960 A correlative study of the histochemical properties of rodent acid mucopolysaccharides. J. Histochem. Cytochem. 8 : 18-36

Spicer, S.S. and B.A. Schulte. 1992. Diversity of cell glycoconjugates shown histochemically :

A perspective. J. Histochem. Cytochem. 40 : 1-38

Stoward, P.J., S.S. Spicer, and R.T. Miller. 1980. Histochemical reactivity of peanut lectin-horseradish peroxidase conjugate. J. Histochem. Cytochem. 28 : 987-1018.

Taatjes, D.J. and J. Roth. 1991. Glycosylation in intestinal epithelium. Inter. Rev. Cytol. 126 : 135-193.

Weiser, M.M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. J. Biol. Chem. 248:2536-2541.

Werner, R., K. Eckart, B. Christian, and G. Wolfgang. 1982. Biological significance of sialic acid, pp.263-305. *In* R. Schauer (ed.) Sialic acid : Chemistry, Metabolism and Function, Cell Biology Monograph. Vol. 10 Springer Verlag, Vien, New York.

Yamada, K. and S. Shimizu. 1977. The histochemistry of galactose residues of complex carbohydrates as studies by peroxidaselabeled Ricinus Communis agglutinin. Histochemistry. 53:143-156.

Received date : 20/11/98
Accepted date : 20/04/99