Kasetsart J. (Nat. Sci.) Vol. 24 : 116 - 124 (1990)

Histochemical Characterization of Glycoconjugates Present in Cloacal Epithelium of the Chicken

Apinun Suprasert, Boontham Chongcharoen and Suppaluk Romrattanapun [1]

ABSTRACT

The distribution of glycoconjugates in the chicken cloaca was studied by means of light microscopic histochemical methods. The staining procedures employed were horseradish peroxidase conjugated lectin, Alcian blue (AB) pH 1.0, AB pH 2.5, periodic acid-Schiff (PAS), AB pH 2.5-PAS in combination with enzyme neuraminidase digestion. The lectin used in the present study were peanut agglutinin (PNA), wheat germ agglutinin (WGA), *Dolichos biflorus* agglutinin (DBA), and *Lotus tetragonolobus* agglutinin (LTA)

The mucosa of both coprodeum and urodeum is covered by simple columnar epithelium in which 2 type of cells are encountered-columnar and goblet cells. In contrast, one layer of only columnar cells is found in the mucosa of proctodeum. Tubular glands of proctodeum are commonly provided at submucosal layer. All the goblet cells in coprodeum and urodeum and tubular glands of proctodeum were coloured blue and purple with AB pH 1.0, and AB pH 2.5-PAS, respectively. They were furthermore stained moderately with WGA. Neuraminidase digestion changed the blue coloration with AB pH 2.5 to pale blue. Removal of sialic acid with neuraminidase imparted weak to strong affinity of PNA.

Histophysiological significances of glycoconjugates involved in the chicken cloaca were discussed with special reference to the functional activities of the carbohydrates.

INTRODUCTION

Terminal portions of three organ systems come into contact in the area of the avian cloaca. The digestive system terminates in the first of these three regions, the coprodeum. The next region in sequence is the urodeum into which opened the ducts of urinary and genital organs. The third region, the proctodeum, lies between urodeum and the vent. The functions of these organ systems are expressed in different epithelia that line them such as storage, resorption, excretion and secretion (Dahm *et al.*, 1980 ; Turk 1982).

In contrast to some physiological data, glycoconjugate informations on epithelial cells of the avian cloaca is lacking. A series of previous histoand biochemical studies on mammalian (Freeman *et al.*, 1980 ; Poddar and Jacob 1981 ; Schulte *et al.*, 1985 ; Thomopolous *et al.*, 1983) and avian (Suprasert and Fujioka, 1988. Suprasert *et al.*, 1987) digestive and urogenital tract has revealed that glycoconjugates are one of the most important key substances as lubricative and protective functions.

In view of the circumstance mentioned, glycoconjugates involved in the cloacal epithelium of normal chicken as been studied histochemically. The results obtained here are thought to involve either to unknown aspects of the histochemical architecture of the chicken cloaca and

[1] Dept. of Veterinary Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.

to give a clue to thorough recognition of the histophysiological functions performed by avian cloaca in general.

MATERIALS AND METHODS

Ten young White Leghorn Chickens were the donors of the cloaca examined. With the animals under pentobarbitone sodium anesthesia, cloaca was dissected out and fixed immediately in either Carnoy's fluid for 4-6 hours at room temperature or 10% formalin containing 2% calcium acetate (Leppi, 1968) for 12 - 24 hours at 4°C, Thereafter, the tissue specimens were dehydrated in graded ethanol series and embedded in paraffin wax.

Sections were cut at a thickness of 4 μm on a sliding microtome, deparaffinized in xylene, hydrated through graded ethanol series and then representative slides were routinely stained using hematoxylin and eosin. For the histochemical investigation of glycoconjugates, the following staining procedures were used :

Staining procedures

1. The periodic acid-Schiff (PAS) reaction for studying vicinal diol groups of glycoconjugates (Pearse, 1968)

2. Staining with 1% alcian blue (AB) 8 GX, pH 2.5 for demonstration of acidic glycoconjugates (Spicer *et al.*, 1967)

3. Staining with AB pH 1.0 for demonstration of sulfate glycoconjugates (Lev and Spicer, 1964)

4. A combination of AB pH 2.5 Staining and the PAS reaction to allow acid and neutral glycoconjugates to be differentiated (Spicer *et al.*, 1967)

5. The Peroxidase-labelled peanut agglutinin-diaminobenzidine (PO-PNA-DAB) for galactose-(1-3) N-acetylgalactosamine disaccharide residues (Stǫward *et al.*, 1980).

6. The Peroxidase-labelled wheat germ agglutinin for N-acetyl-D-glucosamine residues (Goldstein and Hayes, 1978)

7. The Peroxidase-labelled *Lotus tetragonolobous* agglutinin for ∝-L-fucose residues (Goldstein and Hayes, 1978)

8. The Peroxidase-labelled *Dolichos biflorus* agglutinin for N-acetyl-D-galactosamine residues (Goldstein and Hayes, 1978)

Digestion procedures

Neuraminidase (from Arthrobacter ureafaciens) 1 unit/ml in acetate buffer pH 5.3 containing $CaCl_2$ at 39 - 41°C for 12 - 16 hr (Spicer *et al.*, 1967) prior to staining with AB pH 2.5 or PNA. The neuraminidase was obtained from Marukinshoyu Co.Ltd., Japan. For the enzyme digestion experiments, two types of control procedures were performed : (a) some sections were incubated in the respective buffer solutions without enzyme and (b) other sections were kept intact without any incubation procedures.

RESULTS

The mucous membrane of the chicken coprodeum is thrown into flattened villi and short crypts of Lieberkuhn (Figure 1, 3 and 5). In urodeum, the mucosa lies in a series of irregular folds but true villi do not seem to be present (Figure and 10). The surface of mucosa of both coprodeum and urodeum is covered by simple columnar epithelium in which 2 types of cells can be distinguished-columnar and goblet cells. The columnar cells are uninucleate and provided with a cytoplasm in which the nucleus was basally compressed. In the spaces between the columnar cells, the goblet cells are interposed. In contrast, mucosal surface of proctodeum is found to be a simple columnar epithelium in which 1 layer of only columnar cells are existed (Figure 15 and 16). The villi and goblet cells are not encountered in such epithelium. The mucosa of the vent, the most caudal part of proctodeum, gradually changes to be stratisfied squamous epithelium (Figure 16 and 18). Large amounts of tubular gland together with lymphoid cells occur in tunica

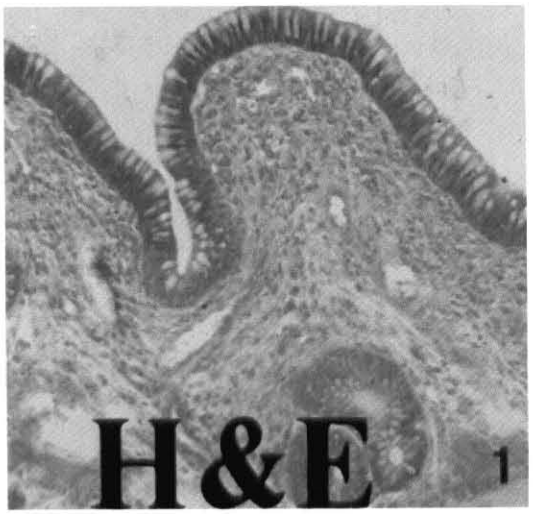

Figure 1. The mucosa of chicken coprodeum consists of a single layer of high columnar and goblet cells, which contain a flattened nucleus in the basal cytoplasm. The cytoplasm of columnar cells stain strongly with eosin. In contrast, the mucous granules of goblet cell stain negatively with eosin. Hematoxylin & eosin x 260

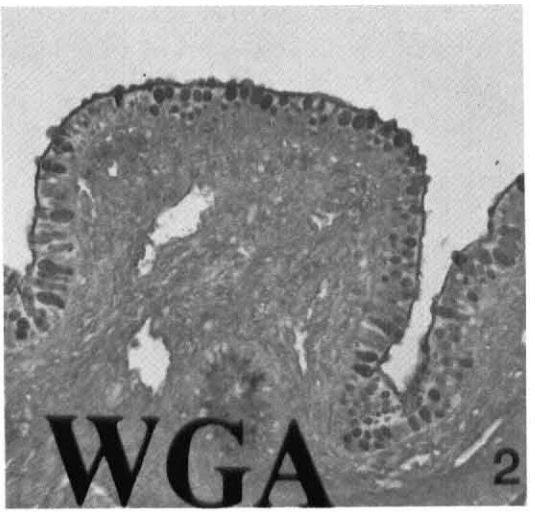

Figure 2. The mucous granules of goblet cells and striated border of columnar cells exhibit strong positive reaction. WGA x 260

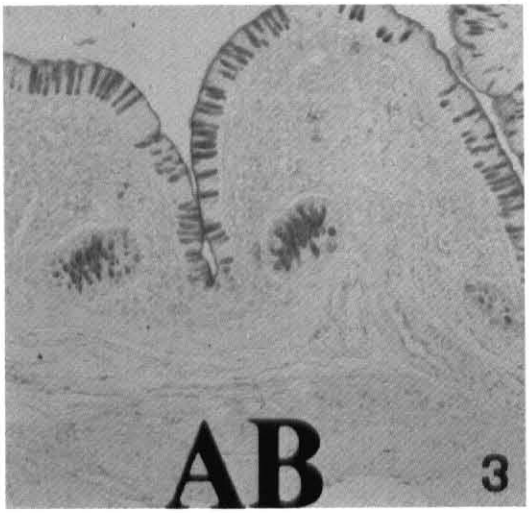

Figure 3. AB pH 2.5. As in Figure 2 x 260

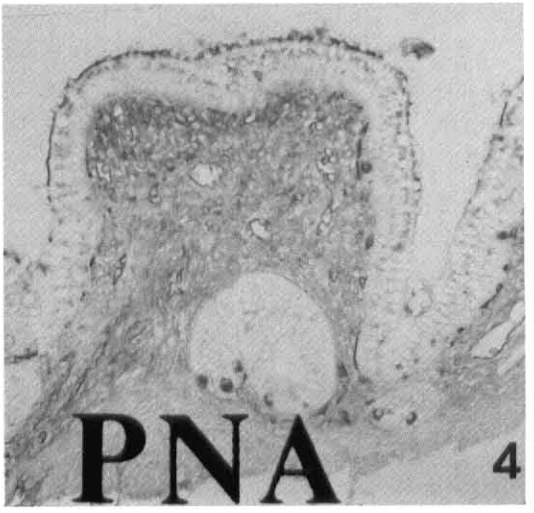

Figure 4. The mucous granules of goblet cells stained negatively with PNA.

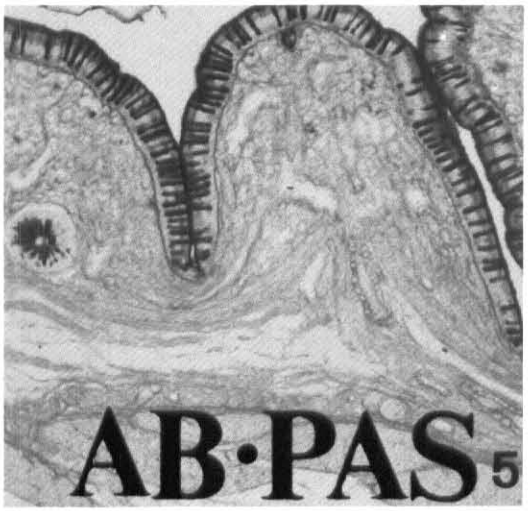

Figure 5. AB pH 2.5-PAS. As in Figure 2 x 260.

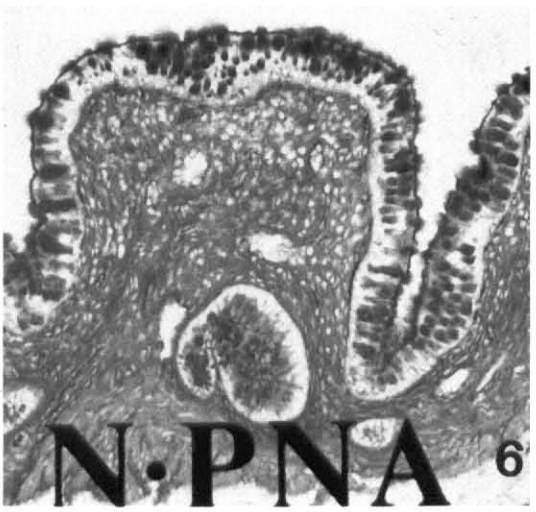

Figure 6. The mucous granules of goblet cells and striated border of columnar cells exhibit strong positive reaction with PNA after enzyme digestion with neuraminidase. x 260

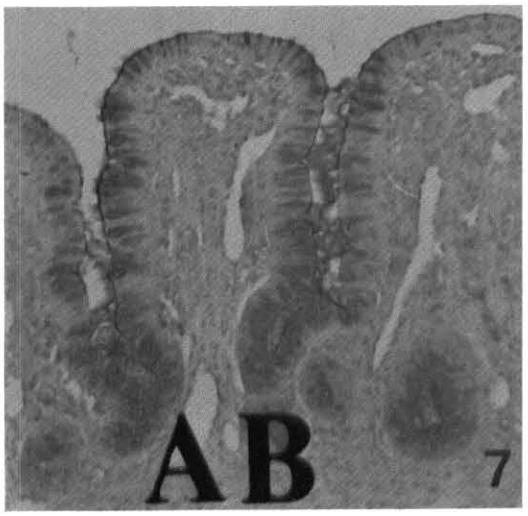

Figure 7. AB pH 2.5. As in Figure 2 x 260.

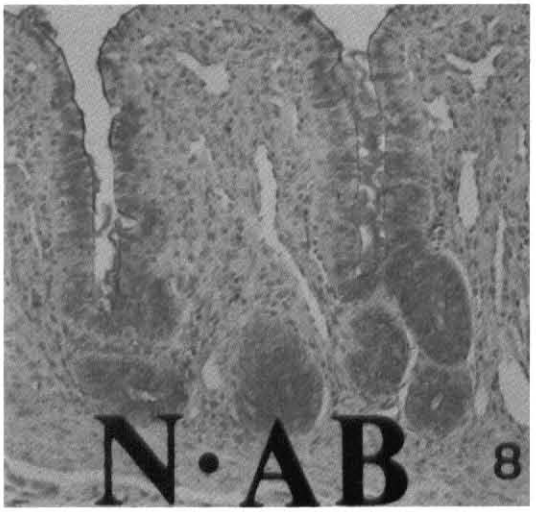

Figure 8. The alcianophilia of mucous granules of goblet cells and striated border of columnar cells is markedly weaker, as compared with that illustrated in Figure 7. AB pH 2.5 following digestion with neuraminidase. x 260.

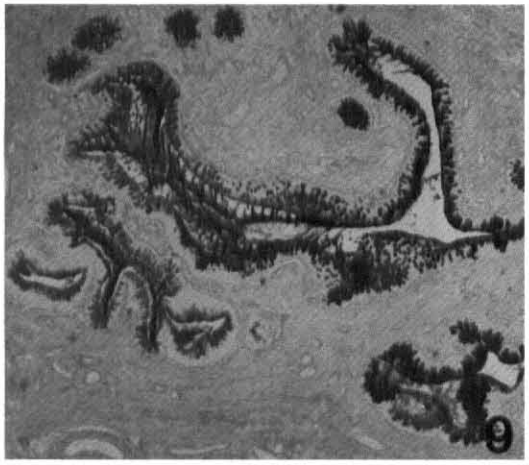

Figure 9. Mucous granules of goblet cells and striated border of columnar cells are colored in shade of deep blue. AB pH 2.5-PAS. x 260.

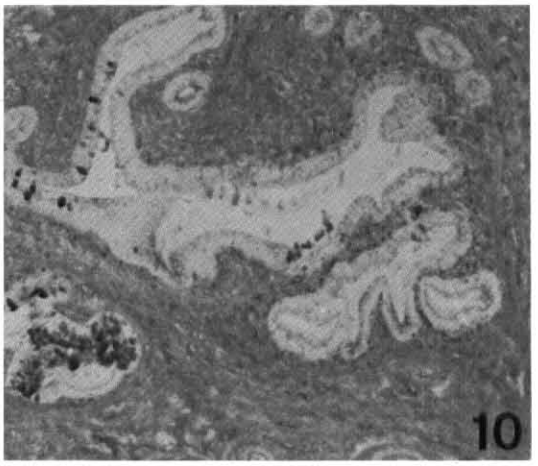

Figure 10. Some of mucous granules of goblet cells stained strongly positive with PNA. x 260.

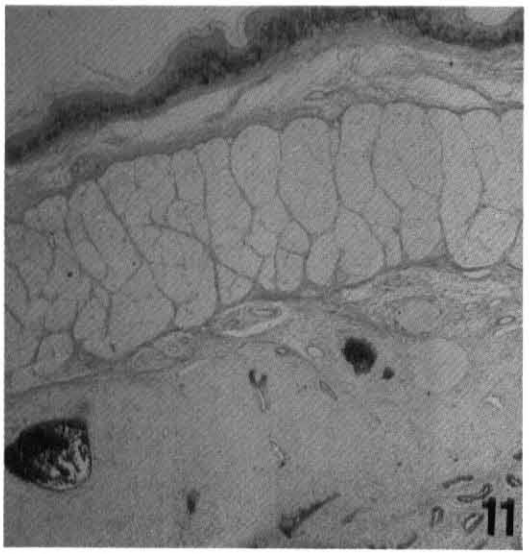

Figure 11. Tubular gland and the epithelial cells lining chicken proctodeum exhibit positive reaction. PAS. × 100.

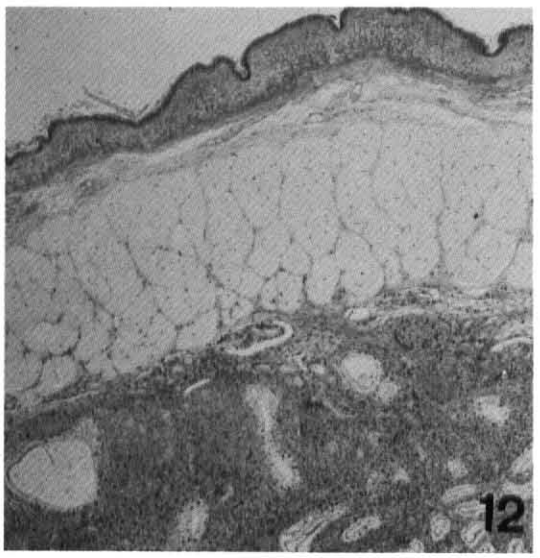

Figure 12. PNA. x 100.

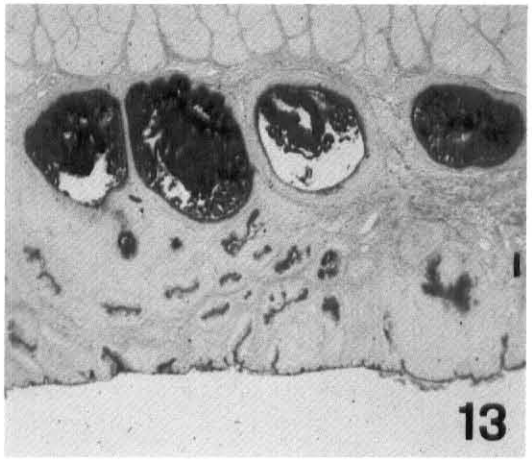

Figure 13. AB pH 2.5-PAS x 260

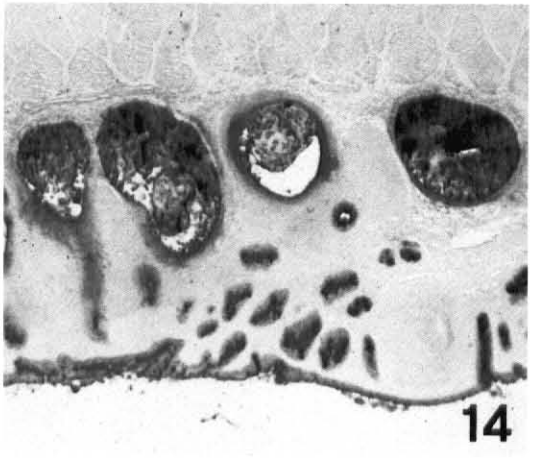

Figure 14. LTA x 260.

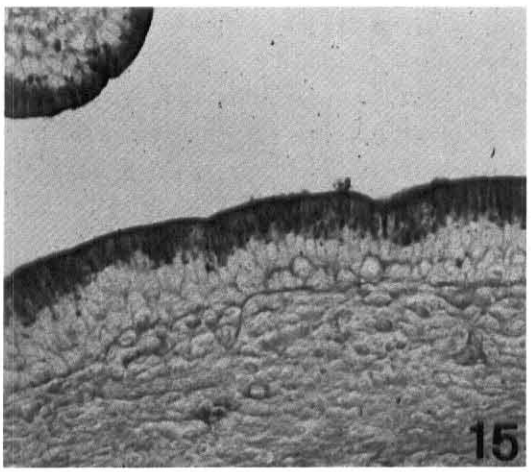

Figure 15. Supranuclear region of columnar cells stained strongly positive with AB pH 2.5-PAS. x 520

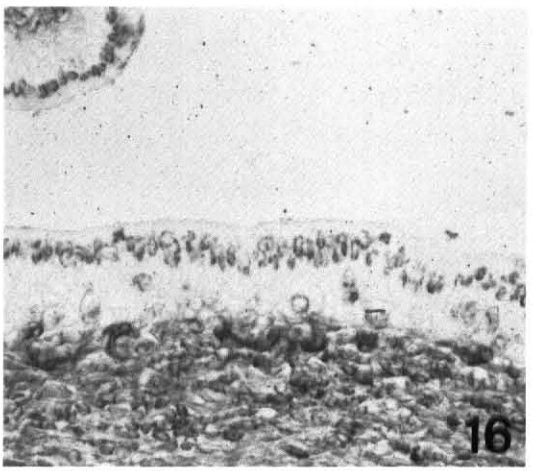

Figure 16. PNA. As in Figure 15 x 520

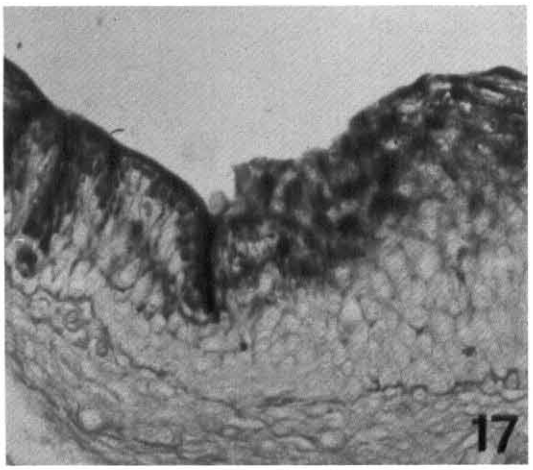

Figure 17. The epithelium at caudal part of proctodeum changed abruptly to be stratisfied squamous epithelium. AB pH 2.5-PAS x 520

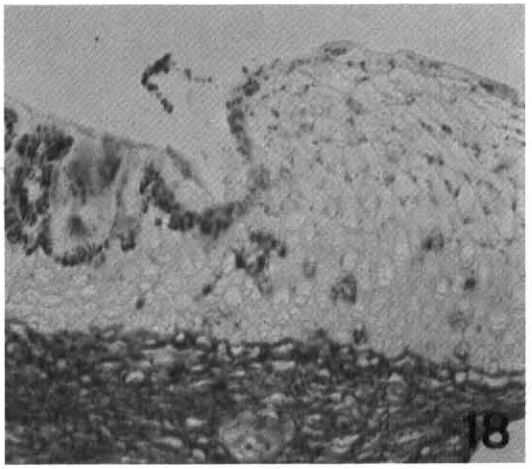

Figure 18. The stratisfied squamous epithelium exhibit negative reaction with PNA. x 520.

propria of the proctodeum. The histochemical staining pattern of glycoconjugates observed in cloaca sample is summerized in Table 1 and Figure 1 - 18.

DISCUSSION

The coprodeum and urodeum were histochemically very similar to that of the avian colon (Suprasert *et al.*, 1987). In view of the mechnism underlying the chemical staining procedures applied to our control samples, the present results would seem to indicate that cloacal epithelium is contained a mixture of salfated acidic and neutral glycoconjugates as presummed from strong positive reaction with (PAS, AB pH 1.0, AB pH 2.5 and AB pH 2.5-PAS.) The mucous granules of goblets cells in coprodeum and urodeum are furthermore involved sialic acid as terminal molecules and galactose residues as penultimate group since they stained strongly positive with PNA after neuraminidase digestion (Spicer *et al.*, 1983), (Figure 4 and 6). In addition, the weak positive reaction with AB pH 2.5 after neuraminidase digestion (Figure 7 and 8) confirmes the presence of acidic glycoconjugates with terminal sialic acid residues (Spicer *et al.*, 1967). The present of terminal or internal N-acetyl-D-glucosamine residues in mucous granules of goblet cells of the coprodeum and urodeum was indicated by the staining results with WGA. (Goldstein and Hayes, 1978).

In the present study, the proctodeum is contained simple columnar epithelium. The villi are diminished and the goblet cells are absent at the proctodeum. The different of epithelial cell lining coprodeum and proctodeum may be due to different in functions of the both part. The absorption capacity may be end at the coprodeum since there were no villi at the proctodeum. The main function of the proctodeum is to resorption of some chemical since the columnar cells were increase at this part. However, the priciples function of lubrication and protection of the both part are the same since they still produce the same glycoconjugates in the both part. In addition, the presence of lymphatic tissues in variable degree throughout the submucosa of the proctodeum is observed in all cases and might be due to an immunological response to viral or bacterial infection to which the animal is very susceptible.

Table 1 Histochemical staining of mucous epithelium of chicken cloaca for the characterization of glycoconjugates.

	Coprodeum		Urodeum		Proctodeum
	Goblet cell	Columnar cell	Goblet cell	Columnar cell	Columnar cells and Tubular gland
AB pH 1.0	+ + +	−	+ + +	−	+ + +
AB pH 2.5	+ + +	−	+ + +	−	+ + +
N.AB pH 2.5	+	−	+	−	+
PAS	+ + +	+	+ +	+	+ + + +
WGA	+ +	−	(+ −)	−	+
DBA	−	+	−	+	+
LTA	−	−	−	(+ −)	+ +
PNA	−	+	(+ −)	+	+
N.PNA	+ + +	+	+ + +	+	+ + +

Symbols indicate the relative intensity of the staining :

− = faint or negligible + + + = intense

\+ = weak + + + + = very strong

\+ + moderate

In the proctodeum, staining profiles of serial sections with different staining procedures might provide information as the whether a single columnar cell contains glycoconjugates with more than one type of terminal sugar. The glycoconjugates stored by mucous granules in the columnar cells contain large amount of terminal sialic acid ∝-L-fucose residues together with terminal galactose-(1-3)N-galactosamine disaccharide residues as evidenced by its affinity for moderate PNA and LTA reaction and strong PNA reaction after neuraminidase digestion.

In a similar way to that described in mammalian species (Sheahan and Jervis, 1976), the epithelial cells of the chicken cloaca contained both sialo--and sulpho- mucins. The precised physiological activities of acidic glycoconjugates remain to be elucidated fully. However, acidic glycoconjugates in the mucus may play an important role in the water resorption in the cloaca as well as resisting the invasion of potentials pathogens (Schauer, 1982). The presence of negative charge groups of carboxyl and sulfate in the three cloacal divisions points to this assumption. Sialic acid and sulfate groups are also believed to play an essential role of the lubrication and protection in the digestive tract, respiratory tract and eleswhere (Werner *et al.*, 1982). Furthermore, sialic acid, which is positioned at the terminal of carbohydrate chain of glycoconjugates, gives ionic changes to the cell surface, being engaged in metabolic process such as transport of materials and plays an important role in cellular specific recognition mechanism (Schauer, 1982).

LITERATURE CITED

Dahm, H.H., U. Schramm and W. Lange. 1980. Scanning and transmission electron microscopic observations of the cloacal epithelia of the domestic fowl. Cell Tissue Research 211 : 83 - 95.

Freeman, H.J., R. Lotan and Y.S. Kim. 1980. Application of lectins for detection of goblet cell glycoconjugate differences in proximal and distal colon of the rat. Lab. Invest. 42 : 405-412.

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

Leppi, T.J. 1968. Morphochemical analysis of mucous cell in the skin and slime gland 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. In : Histochemistry, Theoretical and applied, Vol. 1, 3rd edn, pp. 659-660. Edinburgh, London, New York : J & A Churchill.

Poddar, S., and S. Jacob. 1981. Mucosubstances in the colonic goblet cells of the ferret. Acta histochem. 68 : 279 - 289.

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

Schulte, B.A., K.C. Poon, K.P.P. Rao. and S.S. Spicer. 1985. Lectin histochemistry of complex carbohydrates in human cervix. Histochem. J. 17 : 627 - 654.

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

Spicer, S.S., R.G. Horn, T.J. Leppi. 1967. Histochemistry of connective tissue mucopolysaccharides. In : The Connective Tissue (Edited by Wagner, B.M. and Smith, D.E.) pp. 251 - 303. Baltimore : William & Wilkins.

Spicer, S.S., B.A. Schulte, G.N. Thomopolous, R.T. Parmley and M. Takagi. 1983. Cytochemistry of complex carbohydrates by light and electron microscopy : Available methods and their application. In : Wagner, B.M., R. Fleischmajer and N. Kaufman (eds.) Connective Tissue Diseases. Wilkins, Baltimore, London. pp. 163 - 211.

Stoward, P.J., S.S. Spicer and R.T. Miller. 1980. Histochemical reactivity of peanut lectin-horseradish peroxidase conjugate. J. Histochem. Cytochem. 28 : 979 - 990.

Suprasert, A. and T. Fujioka. 1988. Lectin and ultrastuctural cytochemistry of glycoconjugates in the cecal epithelium of the chicken. Acta. histochem. 83 : 141 - 151.

Suprasert, A., T. Fujioka and K. Yamada. 1987. The histochemistry of glycoconjugates in the colonic epithelium of the chicken. Histochemistry. 86 : 491 - 497.

Thomopolous, G.N., B.A. Schulte and S.S. Spicer. 1983. Light and electron microscopic cytochemistry of glycoconjugates in the rectosigmoid colonic epithelium of the mouse and rat. Am. J. Anat. 168 : 239 - 256.

Turk, D.E. 1982. Symposium : The avian gastrointestinal tract and digestion. The anatomy of the avian digestive tract as related to feed utilization. Poultry Science 61 : 1225 - 1244.

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