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Histochemical Detection of Glycoconjuagates in Chicken Colonic Epithelium with a Specific Horseradish Peroxidase Conjuagated Lectin

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ABSTRACT

Chicken colons were stained with a battery of seven horseradish peroxidase-conjugated lectins : *Ricinus communis* agglutinin-I (RCA-I) peanut agglutinin, wheat germ agglutinin, *Ulex europaeus* agglutinin, *Limax flavus* agglutinin, *Dolichos biflorus* agglutinin, and *Pisum sativum* agglutinin. Lectin staining revealed striking differences in the structure of oligosaccharides between intracellular secretory glycoconjugates in goblet cells and in columnar cells. The mucous granules of goblet cells were found to contain glycoconjugates with sialic acid-galactose dimer, α-D-mannose, α-D-glucose, N-acetyl-D-glucosamine and ß-D-galactose residues. In contrast, terminal or internal N-acetyl-D-glucosamine and terminal galactose-N (1-3)-acetyl-D-galactosamine disaccharides were detected in supranuclear region of the columnar cells. The striated border of the colonic columnar cells were also found to contain glycoconjugates similar in reactivity to those of the goblet cells. The existence of glycoconjugates with various saccharide residues in colonic epithelium of the chicken is reported for the first time in this study.

INTRODUCTION

It is known that lectins bind to specific sugar residues (Goldstein and Hayes, 1978). They have been widely used as a powerful probe for cytochemical demonstration of carbohydrate moieties in cells and tissues (Roth, 1978). Using lectins, histochemically and cytochemically, important roles of the complex carbohydrate have been shown on differentiation (Lis and Sharon, 1973; Kawai *et al.*, 1979), fertilization (Ahuja, 1980), cell adhesion and cell interaction (Rauvala *et al.*, 1981), and aging (Aubery *et al.*, 1980). Of particular interest is the possibility that glycoconjugates could play a crucial role in many pathological processes, including malignancy (Boland *et al.*, 1982).

In colonic epithelium of different mammals, numerous histochemical studies have been made on glycoconjugate elaborated by their secretory cells (Sheahan and Jervis, 1976; Freeman *et al.*, 1980; Thomopolous *et al.*, 1983). As a result, a wealth of information is available on the histophysiology of such carbohydrates in the mammalian colonic epithelium. Little is known, however, about the cytochemical aspects of glycoconjugates of the chicken colonic epithelium. Furthermore, our recent histochemical study has been shown by only conventional and a few kinds of lectin staining method (Suprasert *et al.*, 1987).

The aim of this study is to specify the cytochemical nature of secretory substance being elaborated by colonic epithelium of the chicken by using the light microscopic methods of peroxidase-conjugated lectins. A large number of lectins was also employed to gather as much information as possible about the characteristics of lectin labelling under carefully controlled expedrimental conditions.

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MATERIALS AND METHODS

A total of 15 adult male Brown Leghorn Chickens were killed by pentobarbital anesthesia followed by exsanguination. The proximal part of colon was removed and fixed immediately either in Rossman's fluid for 12 h at 4°C, or Carnoy's fluid for 6 h at room temperature. They were then processed for 3 μm paraplast sections by routine method. To characterize the saccharide residues, a variety of lectin staining procedures were applied to tissue sections.

Ricinus communis agglutinin-I (RCA-I), peanut agglutinin (PNA), wheat germ agglutinin (WGA), *Ulex europaeus* agglutinin-I (UEA-I), *Limax flavus* agglutinin (LFA), *Dolichos biflorus*-agglutinin (DBA), and *Pisum sativum* agglutinin (PSA) were employed as lectins. All these lectin preparations labelled with horseradish peroxidase were purchased from E.Y. Laboratory (San Mateo, California, USA.)

Cytochemical control Lectin-peroxidase conjugates containing a particular sugar were used in the control procedures. In order to detect the activity of endogenous peroxidase in the tissues, some control tissue sections were reacted for DAB only.

The lectins used in this study, along with their reported carbohydrate binding specificities, are listed in Table 1.

Digestion Procedures

Neuraminidase (from *Arthrobacter ureafaciens)* 1 unit/ml in acetate buffer pH. 5.3 containing 0.04 M $CaCl_2$ at 39-41°C for 12-16 h. (Spicer *et al.*, 1967) prior to staining with LFA 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.

Table 1. Lectins used in histochemical studies

Agglutinin : lectin name (common name) abbreviation	Carbohydrate binding specificity	Reference
Ricinus communis (castor bean) RCA-I	ß-D-Gal a-D-Gal	Yamada and Shimuzu 1977
Arachis hypogaea (peanut) PNA	ß-D-Gal-(1-3)-D-GalNAc	Stoward *et al.* 1980
Triticum vulgaris (wheat germ) WGA	GlcNAc	Goldstein and Hayes 1978
Ulex europaeus (Gorse seed) UEA-I	α-L-Fuc	Goldstein and Hayes 1978
Limax flavus (slug bean) LFA	Neu 5 Ac	Schulte *et al.* 1984
Dolichos biflorus (horse gram) DBA	α-D-Gal NAc	Goldstein and Hayes 1978
Pisum sativum (pea) PSA	α-D-glucose, α-D-mannose	Goldstein and Hayes 1978

Table 2. Lectin staining of mucous epithelium of chicken colon for the characterization of glycoconjugates

Histochemical technique	Goblet cells	Columnar cell		Connective tissue tunica propria
		supranuclear region	striated border	
Ricinus communis agglutinin-I (RCA-I)	1 - 2 Br	1 Br	1 - 3 Br	0 - 1 Br
Peanut agglutinin (PNA)	0	2 Br	3 Br	1 - 2 Br
Wheat germ agglutinin (WGA)	3 Br	1 - 2 Br	3 - 4 Br	1 - 2 Br
Ulex europaeus agglutinin-I (UEA-I)	0	0	0	0
Limax flavus agglutinin (LFA)	4 Br	0	4 Br	1 Br
Dolichos biflorus agglutinin (DBA)	0	0 - 1 Br	0 - 1 Br	0
Pisum sativum agglutinin (PSA)	3 Br	1 Br	3 - 4 Br	1 - 2 Br
N-PNA	3- 4 Br	2 Br	4 Br	1 Br
N-LFA	0	0	0 - 1 Br	1 Br

Abbreviation
Br = Brown, 0 = Negative reaction, 1 - n = Number indicates intensity of staining reaction, N = Neuraminidase.

RESULTS

Specific staining patterns observed for each lectin are summarized in Talbe 2. There was no significant difference in staining patterns between the two kinds of the fixative. Lectin staining of mucous epithelium of chicken alone for the characterization of glycoconjugates, obtained by light microscopic methods of peroxidase-conjugated lectins, are also illustrated in Figure 1-6.

Goblet cells The goblet cells did not stain with PNA (Figure 1), UEA-I (Figure 4) and DBA.- However, they stained strongly with WGA (Figure 3), LFA (Figure 5) and PSA, and stained weakly with RCA-I.

Columnar cells The supranuclear region of the coloumnar cells exhibited positive reaction with PNA (Figure 1) and WGA (Figure 3). However, it failed to stain with UEA-I (Figure 4), LFA (Figure 5) and DBA. In contrast, the striated border of columnar cells reacted strongly with PNA (Figure 1), WGA (Figure 3) and LFA (Figure 5) but reacted negatively with DBA and UEA-I (Figure 4)

Connective tissue The connective tissue in tunica propria of each villi stained mildly with PNA, LFA and WGA. However, they exhibited negative reaction with DBA and UEA-I.

Cytochemical controls All positive reactions completely disappeared in the colon when sections were incubated with lectins in the presence of

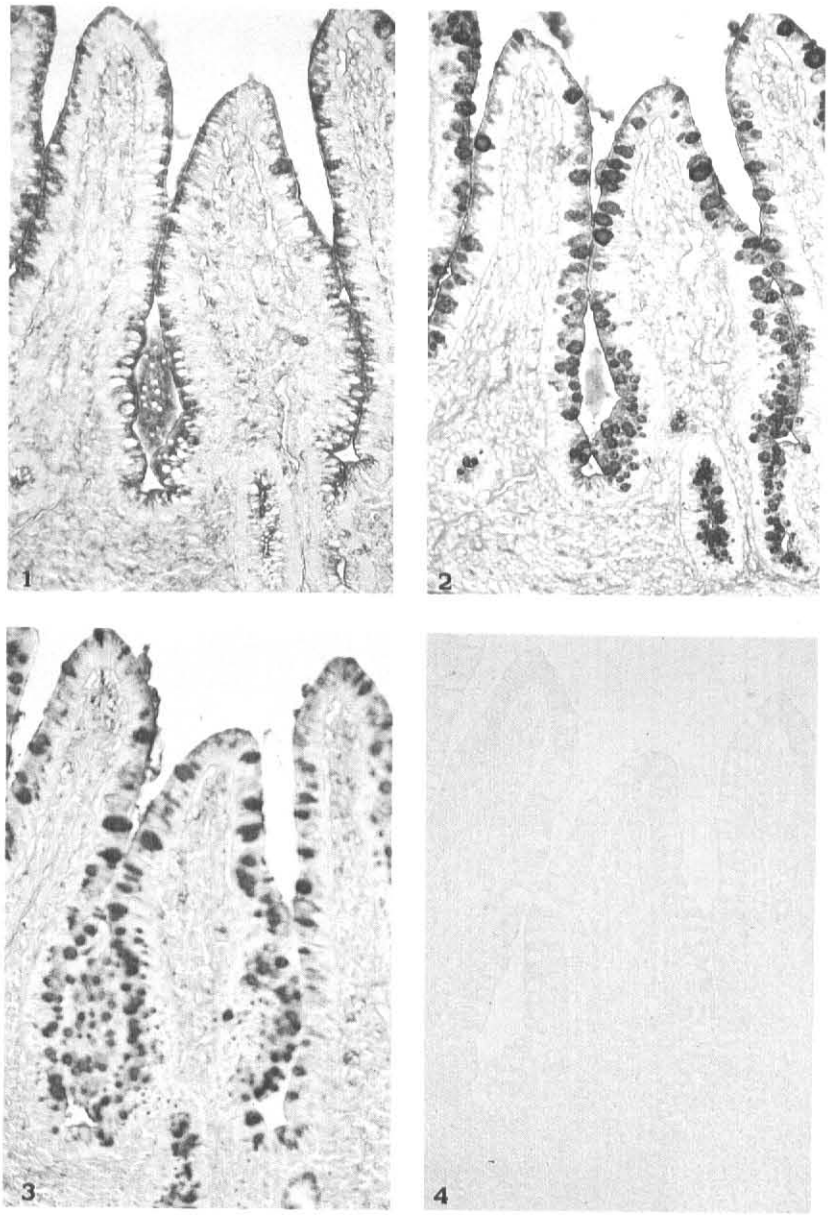
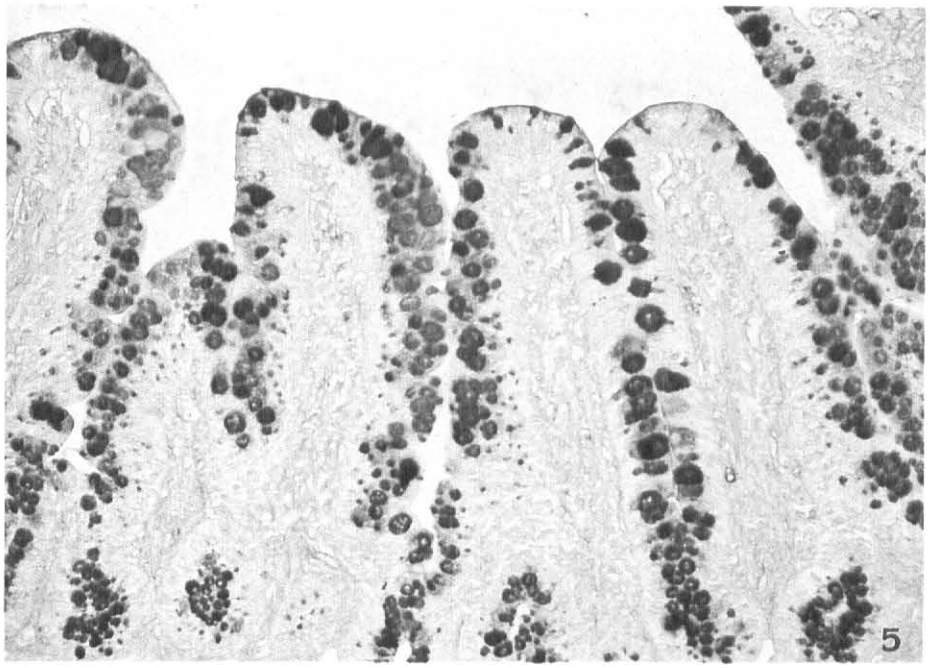


Figure 1. Supranuclear region and striated border of columnar cell are strongly reactive. In contrast, the mucous granules of goblet cells exhibit negative reaction. PNA. (x260).

Figure 2. The mucous granules of goblet cells and striated border of columnar cells exhibit strong positive reaction with PNA after enzyme digestion with neuraminidase, (x260).

Figure 3. The positive reaction of mucous granules of goblet cells and striated border of columnar cells is strongly intensity. However, supranuclear region of columnar cells are mildly reactive. WGA. (x260).

Figure 4. Colonic epithelium stained negatively with UEA-I. (x260).



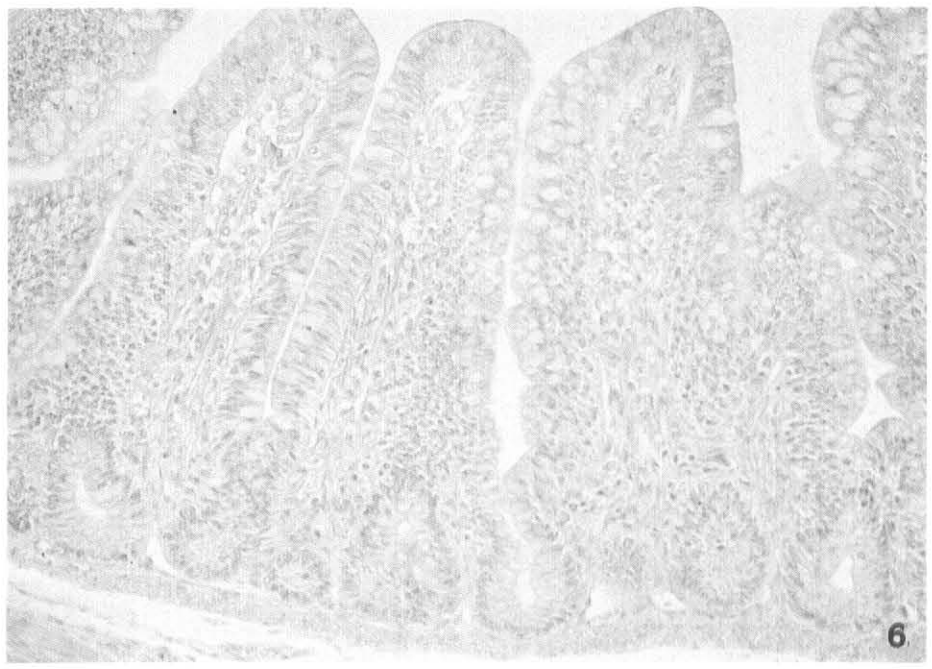


Figure 5. Mucous granules of goblet cells and striated border of columnar cells are colored in shade of black. LFA. (x260).

Figure 6. Mucous granules of goblet cells and striated border of columnar cells stained negatively with LFA after enzyme digestion with neuraminidase. (x260).

the appropriate sugar. Neither endogenous peroxidase activity nor nonspecific binding of lectin-HRP was observed in the colon in the present study.

Lectin staining following neuraminidase treatment Neuraminidase treatment resulted in significant changes in the labelling patterns (Table 2). After neuraminidase treatment, PNA stained strongly the mucous granules of goblet cells (Figure 2). In contrast, LFA did not stain mucous granules of goblet cells after treatment (Figure 6).

DISCUSSION

Glycoconjugate histochemistry, in general, allows demonstration of hexoses with perio - date reactive vicinal diol groups, and of carboxyl and sulfate groups with selective affinities for various cationic reagents. Recently, a new approach in the study of secretory glycoconjugates can be acheived by the use of lectins as histochemical markers of specific saccharide groups. This technique has already been applied to the study of guinea pig, rat, and human small and large intestinal epithelium (Etzler and Branstrater, 1974; Boland *et al.*, 1982; Fischer *et al.*, 1984). A specific labelling pattern for each lectin studied was observed. This method allowed a further differentiation of cell glycoconjugates as compared to routine studies with alcian blue and periodic acid-Schiff.

In agreement with some previous studies on animal and human colonic epithelium (Sheahan and Jervis, 1976; Freeman et al., 1980; Boland *et al.*, 1982), our investigations revealed characteristic differences in the binding pattern of some lectins in different cell types of normal chicken colonic epithelium. In the present study, the lectin histochemical reactions showed that the mucous granules of goblet cells and the striated border of columnar cells contained acidic glycoconjugates with terminal sialic, residues, in view of the positive reaction with LFA (Spicer *et al.*, 1983). The strong positive reaction of mucous granules of goblet cells with PNA after neuraminidase digestion further confirmed the presence of acidic glycoconjugates with terminal sialic acid residues with galactose residues as subterminal molecules (Stoward *et al.*, 1980).

However, the mucous granules of goblet cells stained negatively with PNA (without previous digestion with neuraminidase) thereby suggested that the mucous granules of goblet cells lack terminal galactose-N (1-3) acetylgalactosamine disaccharides (Stoward *et al.*, 1980). The goblet cells are furthermore provided with α-D-mannose, α -D-glucose, ß-D-galactose and N-acetyl-D-glucosamine as all the mucous granules of goblet cells stained moderately with PSA, RCA-I and WGA. The negative reaction with DBA and UEA-I will most probably indicate the absence of N-acetylgalactosamine and α - L-fucose residues as terminal molecules of glycoconjugates or sterically hindered sialic acid residues in their carbohydrate portions. The presence of various saccharide residues in the chicken colonic epithelium is reported for the first time in this investigation.

We suggest that the glycoconjugates synthesised by the chicken colonic epithelial cells have different physiological functions such as lubrication and protection. Such biological roles of these substances are determined by their high carbohydrate content, of which sialic acid is the most prominent component. The net negative charges of sialic acid on the luminal surface are thought to be involved in ion transport, secretion and membrane stability (Suprasert and Fujioka, 1988). The repulsive, electrostatic forces of sialic acids contribute to the rigidity of the cell surface (Schauer, 1982). Moreover, the cell-surface sialoglycoconjugates appear to play a role in cellular recognition phenomena (Gesner and Ginsburg, 1964) and may also represent components of receptor sites of cell membrane. Functional activities of other sugar residues of glycoconjugates are still unknown.

Finally, we are still a interpretative phase in our investigation. Availability and application of further lectins with other particular specificities will enhance, in the near future, the interpretation of normal and diseased states of intestinal glycoconjugate synthesis. Histological lectin

bindings studies will give more information on the structural composition of cellular oligosaccharide moieties in healthy and diseased animals.

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