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Lectin Histochemistry on Esophageal Mucosa of Pigs

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Summary

Lectin binding sites in normal esophageal mucosa of three pigs were studied using seven biotinylated lectins and the avidin-biotin peroxidase complex method. Esophageal squamous epithelium revealed regional distribution of lectin binding in their respective individual layers. Con A, WGA and RCA-I moderately or weakly stained the cell membrane of epithelial cells. PNA staining was observed in the spinous and inner cornified layers in two animals, but in a third animal PNA stained basal and suprabasal cells. SBA bound to spinous and cornified layers. DBA stained only the partial inner cornified layer. UEA-I stained preferentially, weak to strong, the upper spinous and lower cornified cells of two animals, but it was negative in all the layers of another. Subepithelial connective tissues showed positive stainings with Con A, WGA and RCA-I.

Lectins isolated from various plants and animals specifically bind regular carbohydrate residues of cellular glycoconjugates. They are useful tools for the study of stable features in cell surface carbohydrates which change with cell differentiation, development or ageing, and malignancy (1-5). Some reviews have summarized histochemically detectable lectin binding sites in the epithelial tissues of the digestive tract (1, 5, 6). It has clearly been shown with lectin histo- and cytochemistry, that the degrees and localizations of glycoconjugates change with the cell differentiation and the cell migration of the epithelial cells in the digestive mucosa (7-9). However, there was some information concerning the lectin binding pattern in esophageal epithelium of humans (3, 10) and mice (11). In this current study, the regional distributions of lectin binding sites in esophageal mucosa of pigs were investigated, using seven biotinylated lectins and the avidin-biotin peroxidase complex (ABC) method.

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Materials and Methods

Tissue Sections

Young adult crossbred pigs (three females) were used. The animals were Duroc × (Landrace × Large White). They were killed at an abattoir in Sendai. Tissue samples were taken from the proximal and distal esophagus. Tissues were fixed in 10% buffered formalin, embedded in Paraplast and cut into 4 μm sections. For histological observation, sections were stained by hematoxylin and eosin, and periodic acid-Schiff (PAS).

Lectins

Seven biotinylated lectins were used : concanavalin A (Con A), for detecting mannose (Man) and glucose (Glc) ; wheat germ agglutinin (WGA), for N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-neuraminic acid (NeuNAc) ; *Ricinus communis* agglutinin-I (RCA-I), for galactose (Gal) ; peanut agglutinin (PNA) and soybean agglutinin (SBA), for galactose (Gal) and N-acetyl-D-galactosamine (GalNAc) ; *Dolichos biflorus* agglutinin (DBA), for N-acetyl-D-galactosamine (GalNAc) ; *Ulex europaeus* agglutinin-I (UEA-I), for α-L-fucose (Fuc). The lectins used were purchased from E.Y. Laboratory Inc. in the USA.

Lectin Staining

The sections were treated in 0.3% H_2O_2/methanol for 30 min in order to inactivate the endogenous peroxidase. They were rinsed in 10 mM phosphate buffered saline (PBS, pH 7.2) and then covered with 1% bovine serum albumin in PBS (BSA-PBS) for 10 min. The excess BSA-PBS was shaken off, and then the sections were incubated with biotinylated lectins (25 μg/ml) in 0.1% BSA-PBS in a hymidity chamber, for 30 min at room temperature. The sections were rinsed with PBS, and then incubated with the avidin-biotin horseradish peroxidase complex (ABC ; Vectastain ABC kit, Vector Lab. Inc., USA) in 0.1 M Tris-HCl buffer (pH 7.6) for 30 min at room temperature. After washing with PBS, the sections were immersed in 3, 3′-diaminobenzidine-4HCl (DAB, 0.2 mg/ml)-H_2O_2 (0.005%) in 0.05 M Tris-HCl buffer (pH 7.6) for 10 min. The sections were rinsed with distilled water, dehydrated and mounted.

Controls

The specificity of lectin binding was confirmed by incubating the sections with biotinylated lectins in the presence of hapten sugars. The sugars were 0.2 M α-methyl-D-mannoside for Con A, 0.2 M GlcNAc for WGA, 0.2 M lactose for RCA-I, 0.1 M Gal for PNA, 0.1 M GalNAc for SBA and DBA, and 0.1 M Fuc for UEA-I. To confirm the absence of a nonspecific binding of ABC, the sections were treated with ABC and DAB-H_2O_2. The endogeneous peroxidase activity

was also checked by incubating the sections with DAB-H_2O_2 alone.

Results

The structure of esophageal mucosa of pigs showed very thick epithelium and subepithelial connective tissue. Numerous large connective tissue papillae extended into the upper spinous layer (Figs. 1-10). The epithelial cell nuclei did not disappear in the surface cornified layer. The cornified layer did not show higher cornification or tightly stacked structure (Fig. 1) as in the epidermis. Keratohyalin granules stained with hematoxylin were not clearly observed in pig esophageal epithelium, and consequently it was difficult to distinguish the granular layer from the spinous and cornified layer. Some lectin bindings showed different degrees in the luminal and basal sides of the cornified layer. For that reason, in this study, the epithelium were classified into four layers as follows; outer and inner cornified, spinous and basal layers (Table 1).

Lectin Binding in Esophageal Epithelium

Each lectin showed particular regional distribution in esophageal mucosa as summarized in Table 1. Regional distribution of each lectin binding was the same in proximal and distal esophageal mucosa of each animal. In the epithelium, binding degrees and regional distributions of three lectins, PNA, DBA and UEA-I, were different among three animals. Surface exfoliating epithelium and surface mucin were weakly or moderately stained with all the lectins studied (Figs. 2-10). The basement membrane showed no binding with any of the lectins used in this study (Figs. 2-10). The each lectin staining pattern in the epithelium was as follows.

i) Con A moderately stained the cell membrane of cells in the spinous and inner cornified layers of the epithelium. Basal cells showed a weak staining, and the upper cornified layer showed a slight or negative one (Fig. 2).

ii) WGA staining, although stronger than Con A, was moderate in the outer spinous and inner cornified layers. Basal and inner spinous cells showed weak or negative staining (Fig. 3).

iii) RCA-I showed a slight staining in the outer cornified layer and moderate in other layers (Fig. 4).

iv) Distribution of PNA binding was different among animals (Table 1). In animal 1 and 2, PNA staining was weak or moderate in spinous and inner cornified cells, and slight or negative in basal and outer cornified cells (Fig. 5). In animal 3, reversely, it was weakly or moderately positive in basal cell membranes and weak or slight in inner spinous cell membranes (Fig. 6).

v) The SBA binding degree was different among animals. It was trace or weak in the inner cornified layer in one animal (Fig. 7) and weak to strong in other two animals (Fig. 8). The cell membrane of basal and inner spinous cells showed

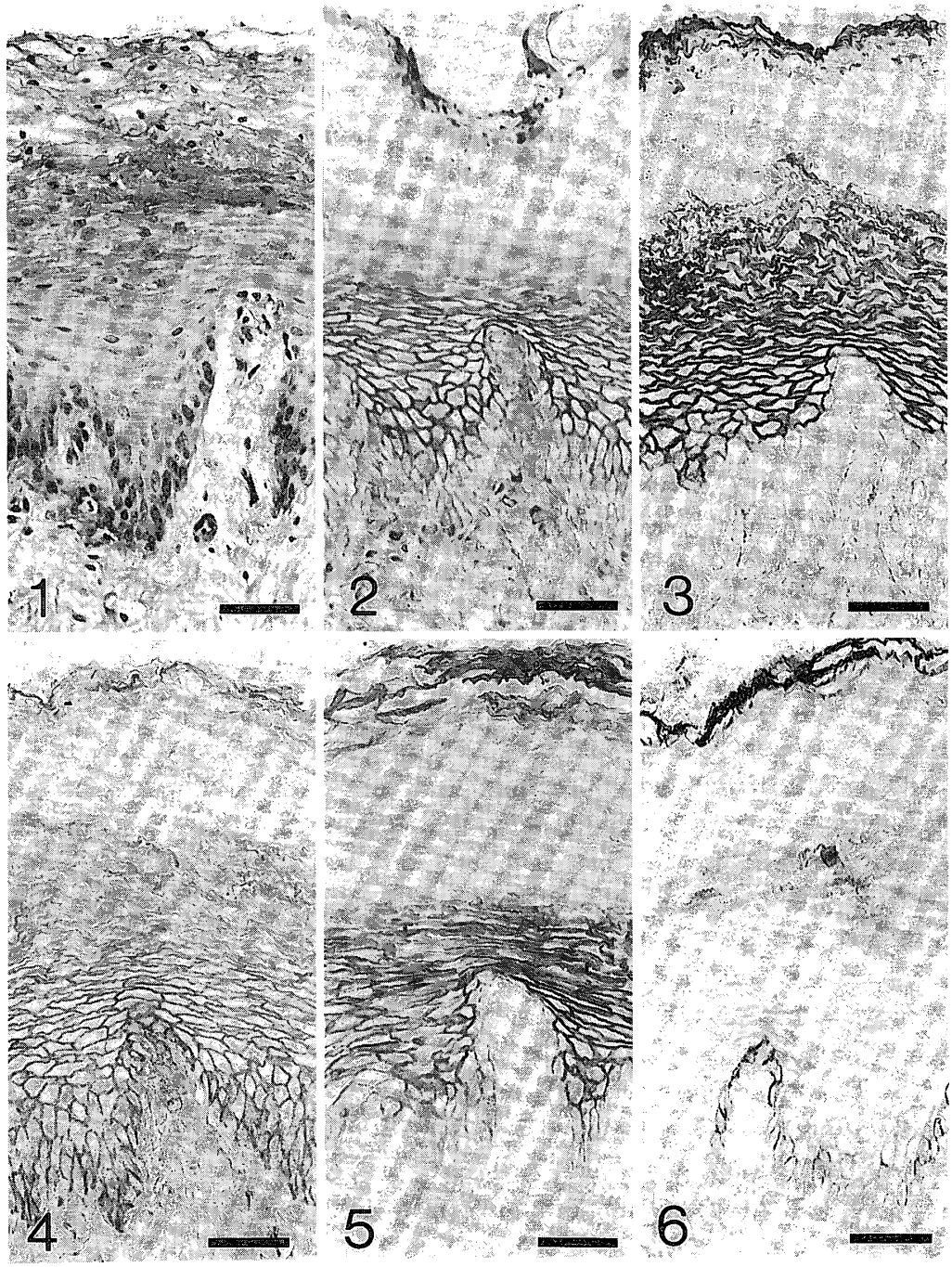
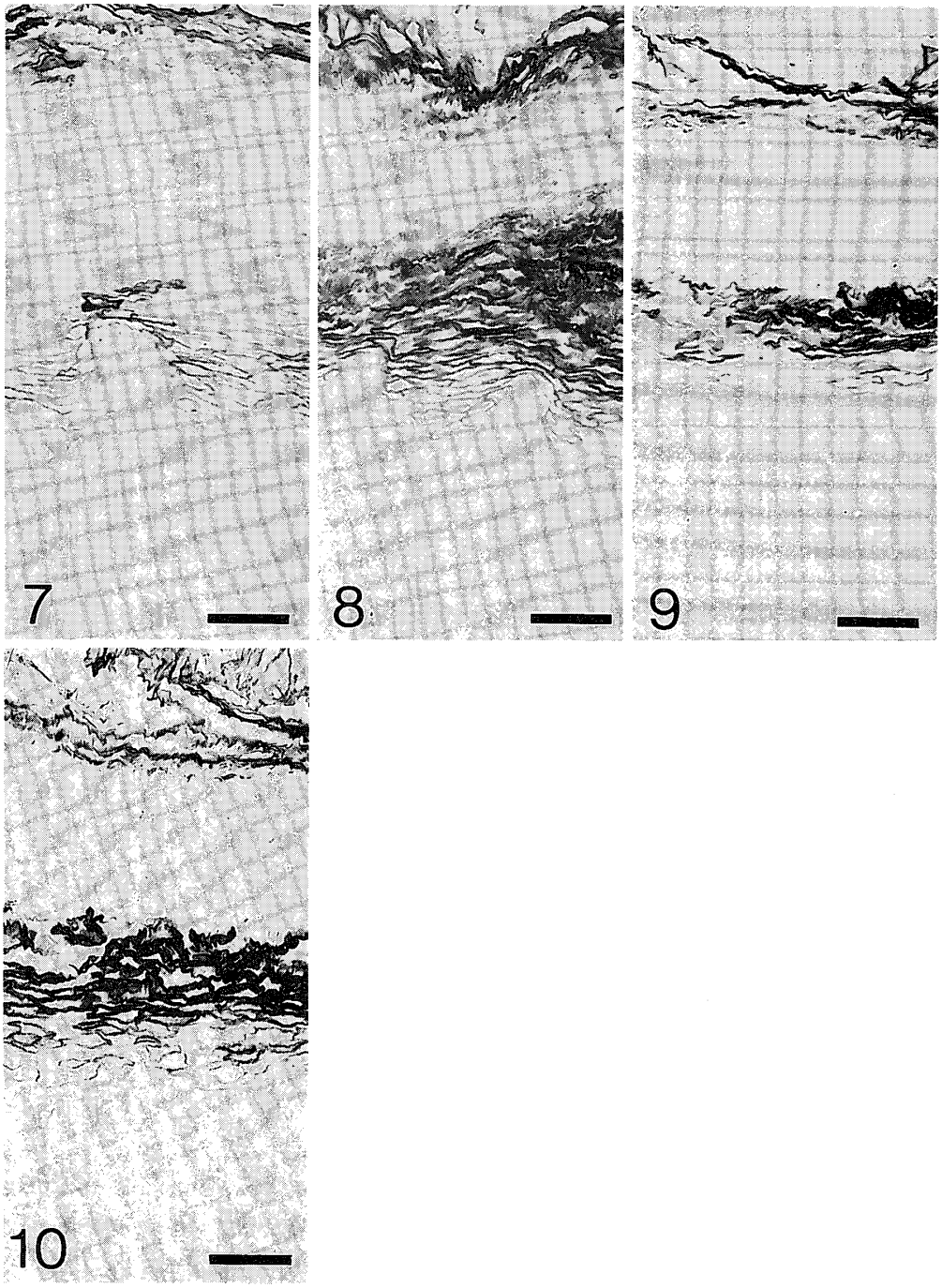


FIG. 1. PAS-hematoxylin staining. Figs. 2-6. Lectin staining. Con A (Fig. 2), WGA (Fig. 3), RCA-I (Fig. 4), PNA in animal 1 (Fig. 5) and animal 3 (Fig. 6). For details of each figure, see text. Bars = 50 μm.



FIGS. 7–10. Lectin staining. SBA in animal 1 (Fig. 7) and 3 (Fig. 8), DBA in animal 3 (Fig. 9), and UEA-I in animal 3 (Fig. 10). For details of each figure, see text. Bars = 50 μm.

TABLE 1. *Regional Distribution of Lectin Binding in Esophageal Mucosa of Pigs*

Lectin	Con A	WGA	RCA-I	PNA		SBA	DBA		UEA-I	
Animal	1, 2, 3	1, 2, 3	1, 2, 3	1, 2	3	1, 2, 3	1, 3	2	1	2, 3
Epithelium Cornified										
outer*	±	±/+	−/+	+	−	+	−	−	−	+
inner	+	+	+	+/#	−/±	±/#	−/+	−	−	+/#
Spinous	+	+/#	+	±/+	−/±	±/+	−	−	−	−/+
Basal	±/+	−/±	±/+	−/±	±/+	±	−	−	−	−
Subepithelial tissues	+	+	±/+	−	−	−	−	−	−/±	−/±

Staining intensities: − = negative, ± = slight or very weak, + = weak, # = moderate, ## = strong

* : Surface exfoliating epithelium and surface mucin were weakly or moderately stained with all lectins used.

a weak or trace reaction and some basal cells negative.

vi) DBA showed a weak staining in the partial inner cornified layer of animal 1 and 3 (Fig. 9). In animal 2, it was negative in all layers.

vii) UEA-I stained preferentially, weak to strong, in outer spinous and inner cornified cells of animal 2 and 3 (Fig. 10). Basal cells and some spinous cells in the epithelial pegs were negative in these two animals. In animal 1, UEA-I was negative in all layers of the epithelium.

Lectin Binding in Subepithelial Tissue

Subepithelial connective tissue showed a moderate or weak staining to Con A, WGA and RCA-I (Figs. 2-4), and a slight one to UEA-I, as in Table 1. The other three lectins were negative.

Controls

When any lectin solution contained hapten sugars, it gave a negative staining or a decreased slight staining as compared with the originals. The nonspecific binding of ABC and the endogenous peroxidase activity, which was not inactivated by H_2O_2/methanol, was not observed in the esophageal mucosa.

Discussion

The cell membrane of esophageal squamous epithelium of pigs revealed the regional distribution of lectin binding in their respective individual layers as summarized in Table 1. Con A, WGA and RCA-I evenly stained the epithelial cell membrane and subepithelial connective tissues of pig esophagus, and therefore there were Man, Glc, GlcNAc, Gal and NeuNAc residues in cell membranes of the epithelium and connective tissue. The staining patterns of these three lectins in

the pig esophageal epithelium were almost the same as that in the epithelium of previous reports (10, 14-17), but the negative Con A staining in the cytoplasm of epithelial cells in pig esophagus was different from the positive staining of the epithelial cell cytoplasm in the previous reports (10, 14-17).

PNA and SBA showed different staining patterns. The lectin stainings were comparatively stronger in the spinous layer more than in other layers. There were two patterns of PNA binding in the cornified squamous epithelium: one type was negative or very weak in basal cells and positive in differentiated cells (10, 13, 14, 16, 17), and the another one was positive in basal cells and disappeared with cell differentiation (11). In this study, the epithelium of the pig esophagus showed these two patterns of PNA binding (Table 1, Figs. 5 and 6). SBA binding sites in the esophageal mucosa of pigs were almost similar to that of the human esophagus (10), human skin (14) and oral mucosa (15), except for the negative staining in the highly keratinized cornified layers of skin and oral mucosa. The DBA binding pattern in the pig esophageal epithelium was the same as in the pig snout skin (15) but different from the results of negative DBA staining in the human esophagus (10, 11) and in the human epidermis (14). These results show that GalNAc and Gal residues were added on the cell membrane in the process of cell migration from basal to spinous layer and disappeared in the process of cornification.

UEA-I staining in the epithelium of the pig esophagus was similar to that of the skin epidermis (2, 13, 17), the esophageal (3, 10) and oral mucosal epithelium (15). UEA-I binding was negative in basal cells and positive in spinous and granular cells in these epithelium. In these epithelium, therefore, Fuc residues were added to the glycoconjugates of cell membranes with the cell differentiation (18).

Lectin binding sites in esophageal mucosa have been reported in humans (3, 10), mice (11) and the pigs of this study. The degree of keratinization in the stratified squamous epithelium of esophagus varies with species (12). It is generally nonkeratinized in humans and carnivores, slightly keratinized in pigs and ruminants, and highly keratinized in rodents. Positive staining was not seen with any of the lectins studied in the highly keratinized layer of the epidermis (12, 13), oral mucosa (15) and mouse esophageal epithelium (11). But in the epithelium of pig esophagus, the lectin staining in the cornified layer was weaker than in the spinous layer as shown in Table 1. Moreover, in the nonkeratinized human esophagus, the superficial layer showed positive reaction at almost the same degree in the middle spinous layer (10). It is concluded, therefore, the lectin binding degree relates to the degree of keratinization in cornified or surface layer of the squamous epithelium.

Cell surface sugar residues change during the course of the cellular differentiation or ageing from the basal to the surface cornified layers in the

esophageal epithelium of pigs as reported in the squamous epithelium. In this study, the epithelium of the pig esophagus showed individual differences in PNA, DBA and UEA-1 binding (Table 1). Previous reports have described no difference of lectin binding pattern related with sex (10, 11, 15) or animal strains (19). We used only three animals in this study, and we need to investigate a larger number of animals in the future.

The basement membrane did not show binding with all lectins studied, as reported by Rittman and MacKenzie (20) that lectin binding in the basement membrane is usually lost by formalin fixation and wax processing at 60°C.

Lectin staining in the subepithelial connective tissues of the esophageal mucosa of pigs was comparably similar to the snout skin of pigs except for the positive DBA staining in the snout skin (16).

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