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Mark R. Ackermann, United States Department of Agriculture
N. F. Cheville, United States Department of Agriculture
P. G. Detilleux, United States Department of Agriculture

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What is This?
Lectin Histochemistry of Trachea and Lung of Healthy Turkeys (Meleagris gallopavo) and Turkeys with Pneumonia

M. R. Ackermann, N. F. Cheville, and P. G. Detilleux

US Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA

Abstract. Thirteen lectins were used to characterize lectin-binding specificity of glycoconjugates on sections of formalin-fixed lung and trachea from seven normal turkeys, two turkeys with acute pneumonia, and two turkeys with chronic pneumonia. Neuraminidase was used to digest sialic acid residues. One N-acetylgalactosamine-binding lectin and two N-acetylgalactosamine/galactose-binding lectins stained the apical membrane and cytoplasm of multifocal cells that lined air atria and hyperplastic granular cells. Other lectins in these groups stained ciliated cells of the trachea and bronchi and air capillary epithelial cells. Sialic acid residues were on apical surfaces of ciliated and nonciliated tracheal and bronchial lining cells, air capillary epithelial cells, and vascular endothelial cells. Mannose/glucose-binding lectins stained reticular and elastic fibers in the lamina propria of trachea, primary and secondary bronchi, and the tunica adventitia of arteries and veins. By transmission electron microscopy, colloidal gold-Arachis hypogaea (peanut agglutinin) labeled microvilli on the apical surface of mature granular cells. The L-fucose-binding lectin, in addition to several other lectins, stained nonspecifically in both trachea and lung. These studies show that granular cells that line air atria can be identified with lectins of N-acetylgalactosamine and N-acetylgalactosamine/galactose groups, and that apical surfaces of epithelial cells and endothelial cells in the trachea and lung express terminal sialic acid residues.

Key words: Avian species; granular cells; lectins; peanut agglutinin; type II pneumocytes.

Glycoconjugates comprising the glycocalyx of the respiratory epithelium are essential to normal mucociliary and respiratory function. Sugar residues of glycoconjugates can also serve as receptor sites for respiratory pathogens. Experimentially, fimbrial-mediated attachment of Escherichia coli, a respiratory pathogen of poultry, to the tracheal mucosa of chickens can be blocked by D-mannose residues. In turkeys, fimbria-mediated adherence of E. coli to the respiratory tract epithelium has not been demonstrated.

Biochemical characterization of epithelial cell glycoconjugates is generally difficult, since samples are often contaminated with sloughed cells, bacteria, and mucus. Biotinylated lectins have been used histochemically, therefore, to determine binding affinities to respiratory epithelial cells lining the trachea or lung of the rat, mouse, hamster, human fetus, and the human adult. Studies of human fetal lung have demonstrated sialidation of alveolar lining cells during maturation. Normal and hyperplastic type II pneumocytes isolated from lungs of adult human beings can be identified with a lectin, Macula pomifera agglutinin, which binds a type II cell-specific glycoprotein. Granular cells of turkeys that are capable of hyperplasia and secrete surfactant may be analogous to type II cells of mammals.

This study was designed to 1) identify adhesion sites for type 1 (D-mannose-binding) fimbriae of E. coli, 2) characterize lectin-binding affinities to specific cell types, and 3) identify sialic acid residues. A panel of lectins was used to stain trachea and lung from healthy 3-week-old turkeys, 18-day-old turkeys with acute pneumonia, and 10-week-old turkeys with chronic pneumonia and granular cell hyperplasia. Sialic acid residues were identified using neuraminidase enzyme digestion.

Materials and Methods

Distal trachea and lung sections from seven (Nos. 1–7), Broad-Breasted White, 3-week-old turkeys were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin, Verhoeff-van Gieson stain, Gomori's reticulum stain, Masson's Trichrome stain, and 13 different biotinylated lectins (Vector Laboratories, Burlingame, CA) using a direct peroxidase technique (Table 1). Briefly, serial (3 μm) sections were deparaffinized with xylene. Endogenous peroxidase was quenched with absolute methanol containing 0.6% H2O2 and 0.074% HCl. Sections were rinsed with distilled water, a Tris solution containing 0.1 mM Ca++ and Mg++, then 1% bovine serum albumin in the Tris solution, followed by a 1-hour incubation with a lectin-Tris solution. Diaminobenzic acid was used as a chromogen, and sections were counterstained with Harris' hematoxylin. The lowest concentration of each lectin that resulted in specific, definitive staining was used for both trachea and lung sections.
Table 1. The concentrations of the lectins used in this study (Golstein and Porez).∗

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Abbreviation</th>
<th>Lectin Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose/mannose group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conavalia ensiformis (Jack bean)</td>
<td>Con A</td>
<td>5.0</td>
</tr>
<tr>
<td>Lens culinaris</td>
<td>LCA</td>
<td>2.5</td>
</tr>
<tr>
<td>Pisum sativum</td>
<td>PEA</td>
<td>2.5</td>
</tr>
<tr>
<td>N-acetylglucosamine group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triticum vulgare (Wheat germ)</td>
<td>WGA</td>
<td>2.5</td>
</tr>
<tr>
<td>Succinylated Triticum vulgare</td>
<td>sWGA</td>
<td>2.5</td>
</tr>
<tr>
<td>N-acetylgalactosamine/galactose group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycin maximus (Soybean agglutinin)</td>
<td>SBA</td>
<td>2.5</td>
</tr>
<tr>
<td>Sophora japonicum</td>
<td>SJA</td>
<td>5.0</td>
</tr>
<tr>
<td>Arachis hypogaea</td>
<td>PNA</td>
<td>2.5; 5.0</td>
</tr>
<tr>
<td>Phaseolus vulgaris (leukoagglutinin)</td>
<td>PHA-L</td>
<td>2.5</td>
</tr>
<tr>
<td>Ricinus communis I</td>
<td>RCA-I</td>
<td>1.25</td>
</tr>
<tr>
<td>Griffithia simplicifolia I</td>
<td>BSL-I</td>
<td>50.0</td>
</tr>
<tr>
<td>Phaseolus vulgaris (erythraagglutinin)</td>
<td>PHA-E</td>
<td>50.0</td>
</tr>
<tr>
<td>L-fucose group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulex europaeus I</td>
<td>UEA-I</td>
<td>50.0</td>
</tr>
</tbody>
</table>

∗ Concentration used on tissue sections from 10-week-old pouls.

Lectin-binding specificity was tested by mixing each lectin with a 0.1 M solution of its inhibitory sugar for 20 minutes before application, or by treating sections with 1% sodium periodate, for 10 minutes, prior to labeling; both techniques prevented staining. Several sections were incubated with Vibrio cholerae neuraminidase (NA, 1 U/ml [Sigma, St. Louis, MO]) for 18 hours at 37 C and then stained with Arachis hypogaea (PNA) or Triticum vulgare (WGA) for the detection of sialic acid residues. Several sections were incubated with galactose oxidase (5 U/ml) for 18 hours at 25 C and stained with PNA. Galactose oxidase oxidizes galactose residues into aldehydes that are not bound by PNA.

To determine if acute inflammation altered lectin affinities, the battery of lectins was also used on similarly prepared sections of trachea and lung from two (Nos. 8, 9) 3-week-old Broad-Breasted White turkeys experimentally inoculated intratracheally with E. coli 0143:K*:H27. The battery of lectins was also used on trachea and lung sections of two (Nos. 10, 11) 10-week-old Broad-Breasted White turkeys with chronic pneumonia, caused by experimental infection with Chlamydia psittaci, to determine if hyperplastic granular cells stained similarly to non-hyperplastic granular cells. Normal areas of lung from turkeys with acute and chronic pneumonia served as internal controls to compare staining patterns to 3-week-old healthy pouls (Nos. 1–7).

Arachis hypogaea (PNA) conjugated with 15-nm colloidal gold (Polysciences, Inc., Warrington, PA) was directly labeled to lung tissue for transmission electron microscopy. Lung samples were fixed in 2.5% glutaraldehyde (pH 7.4) for 4 hours and stored in 0.1 M cacodylate buffer (pH 7.4). The samples were rinsed in microtiter plate wells containing 0.2 M phosphate buffered saline with 1% bovine serum albumin for 5 minutes and transferred to a solution of gold-labeled PNA (34 µg/ml) for 1 hour, then washed three times for 5 minutes with bovine serum albumin. Control tissues were either processed without lectin or incubated with the gold-lectin previously mixed with either 0.1 M N-acetylglucosamine or 0.1 M D-galactose. Tissues were post-fixed with 1% osmium tetroxide (pH 7.4), embedded in Epon, and thin sections were stained with lead citrate and uranyl acetate. Thin sections were examined on a Philips electron microscope (Mahwah, NJ).

Results

Each lectin-binding group (mannose-glucose, N-acetylglucosamine, N-acetylgalactosamine/galactose, and L-fucose) stained different areas of tissue, but most lectins within a group stained similarly (Tables 2, 3). No staining was present in lung or trachea when the

<table>
<thead>
<tr>
<th>Groups*</th>
<th>D-mannose</th>
<th>nAcGlc</th>
<th>nAcGal</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con A</td>
<td>LCA</td>
<td>PEA</td>
<td>WGA</td>
<td>PHA-L</td>
</tr>
<tr>
<td>Lamina propria</td>
<td>7/7†</td>
<td>7/7</td>
<td>7/7</td>
<td>0‡</td>
</tr>
<tr>
<td>Adventitia of blood vessels</td>
<td>7/7</td>
<td>7/7</td>
<td>7/7</td>
<td>0</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5/7</td>
</tr>
<tr>
<td>Nonciliated cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Group abbreviations indicate: D-mannose group; nAcGlc = N-acetylglucosamine group; nAcGal = N-acetylgalactosamine group; NA = neuraminidase (NA) treatment followed by staining with Arachis hypogaea (PNA).

† Indicates number of pouls with specific staining/number tested.

‡ 0 = no specific staining.

Table 2. Lectin histochemical staining of components of distal tracheas from seven, healthy, 3-week-old turkeys (Nos. 1–7).
appropriate blocking sugar was preincubated with lectin or in periodate-treated sections.

In healthy turkeys, the mannose/glucose-binding lectins, Conavalia ensiformis (Con A), Lens culinaris (LCA), and Pisum sativum (PEA) consistently (7/7 turkeys) stained the lamina propria of the trachea and pulmonary bronchi and the adventitia of lung and trachea blood vessels (Fig. 1). These lectins also stained small vacuoles in epithelial cells that lined trachea, but failed to stain the apical membrane. Triticum vulgare (WGA) inconsistently stained the apical membranes of ciliated cells of the trachea (5/7), primary and secondary bronchi (6/7), and capillary endothelial cells (5/7), and consistently stained cells that lined air atria and air capillary epithelial cells. In contrast, succinylated Triticum vulgare (sWGA) stained only the apical cytoplasm and surface of cells that line air atria and did not stain tracheal cells.

Glycin maximus (SBA), Arachis hypogaea (PNA), Phaeosolus vulgaris (PHA-L), and Ricinus communis I (RCA-I) stained the apical surface of cells that line air atria; in addition, PHA-L and RCA-I inconsistently stained other cell types, i.e., PHA-L stained apical surfaces of nonciliated cells of the trachea, and ciliated and nonciliated cells of the primary and secondary bronchi, while RCA-I stained apical surfaces of nonciliated cells of the trachea, primary and secondary bronchi, and air capillary epithelial cells. Staining of air atria lining cells by both N-acetylglucosamine (WGA and sWGA) and N-acetylgalactosamine/galactose (SBA, PNA, PHA-L, and RCA-I)-binding lectins was discontinuous, i.e., multifocal, individual cells were stained (Figs. 2, 3).

Following incubation with neuraminidase, PNA consistently stained additional cell types. The apical surfaces of ciliated and nonciliated tracheal and bronchial cells, air capillary epithelial cells, and vascular endothelial cells were all stained by PNA following neuraminidase treatment (Figs. 4–6). No differences in staining were seen in the cells that line air atria between neuraminidase-PNA-stained sections and PNA-stained sections. The WGA staining following neuraminidase digestion was restricted to air atria lining cells. Galactose oxidase-treated sections inhibited PNA staining both before and after neuraminidase.

Lungs from turkeys (Nos. 8, 9) experimentally infected with E. coli had acute, marked focally-extensive suppurative pneumonia. Lectin staining of degenerate epithelial and adventitial fibers in lungs with acute pneumonia was decreased in intensity and often pale in areas of acute fibrinosuppurative inflammation. In degenerate or necrotic cells, lectin staining was often irregular, pale, or absent, and the cytoplasm of adjacent heterophils and macrophages often stained (Figs. 7, 8). Areas of lung unassociated with acute inflammation stained similarly in both turkeys and were also similar to lungs of healthy pouls. In normal areas of lung, there was consistent staining (2/2) of bronchial lamina.

### Table 3. Lectin histochemical staining of components of lungs from seven, healthy, 3-week-old turkeys.

<table>
<thead>
<tr>
<th>Groups*</th>
<th>D-mannose</th>
<th>nAcGlc</th>
<th>nAcGal</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con A</td>
<td>LCA</td>
<td>PEA</td>
<td>WGA</td>
</tr>
<tr>
<td>Lamina propria of bronchi</td>
<td>7/7†</td>
<td>7/7</td>
<td>7/7</td>
<td>0‡</td>
</tr>
<tr>
<td>Adventitia of blood vessels</td>
<td>7/7</td>
<td>7/7</td>
<td>7/7</td>
<td>0</td>
</tr>
<tr>
<td>Ciliated bronchial cells§</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6/7</td>
</tr>
<tr>
<td>Nonciliated bronchial cells§</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5/7</td>
</tr>
<tr>
<td>Air capillary epithelial cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7/7</td>
</tr>
<tr>
<td>Air atria lining cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7/7</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5/7</td>
</tr>
</tbody>
</table>

* Group abbreviation indicate: D-mannose group; nAcGlc = N-acetylglucosamine group; nAcGal = N-acetylgalactosamine group; NA = neuraminidase (NA) treatment followed by staining with Arachis hypogaea (PNA).

† Indicates number of pouls with specific staining/number of pouls tested.
‡ 0 = no specific staining.
§ Primary and secondary bronchi only.

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**Fig. 1.** Trachea; healthy turkey. Peroxidase staining of Pisum sativum (PEA) binding sites in the lamina propria. Harris’ hematoxylin counterstain.

**Fig. 2.** Lung; healthy turkey. Peroxidase staining of Arachis hypogaea (PNA) binding sites of the apical surface and cytoplasm of cells that line air atria trabecula. Note multifocal staining of individual cells. Harris’ hematoxylin counterstain.

**Fig. 3.** Lung; normal area, 10-week-old turkey. Peroxidase staining of Arachis hypogaea (PNA) binding sites of multiple foci of cells lining air atria. Harris’ hematoxylin counterstain.
Fig. 4. Lung; healthy turkey. Peroxidase staining of *Arachis hypogaea* (PNA) binding sites following neuraminidase digestion. There is diffuse staining of the cilia of cells lining the trachea, the apical portion of nonciliated cells, and the surface of capillary endothelial cells. Harris' hematoxylin counterstain.

Fig. 5. Lung; healthy turkey. *Arachis hypogaea* (PNA) staining following neuraminidase digestion. There is diffuse staining of the apical membrane of arterial endothelial cells. Harris' hematoxylin counterstain.

Fig. 6. Lung; healthy turkey. Peroxidase staining of *Arachis hypogaea* (PNA) binding sites following neuraminidase digestion. The apical membrane of lymphoepithelial cells that cover the bronchus-associated lymphoid tissue are stained. Note the thin follicular-associated epithelium at the dome apex. Harris' hematoxylin counterstain.
propria and vascular adventitia by Con A, PEA, and LCA; ciliated bronchial cells, air capillary epithelial cells, and endothelial cells with WGA; air atria cells (2/2) with PNA, SBA, sWGA, and neuraminidase-PNA. Inconsistent staining (1/2) was seen in nonciliated bronchial cells with WGA, RCA-I, and in air capillary epithelial cells with RCA-I. No differences in staining pattern were seen in stained sections of trachea. Consistent staining (2/2) of the lamina propria was seen with Con A, PEA, and LCA. Ciliated and nonciliated cells stained consistently with neuraminidase-PNA, but inconsistently (1/2) with WGA, PHA-L, and RCA-I.

*Chlamydia*-infected lungs (turkey Nos. 10, 11) were characterized by a chronic, marked multifocal lymphoplasmacytic pneumonia with marked multifocal hyperplasia of granular cells. In lungs of 10-week-old turkeys with chronic pneumonia, lectin staining patterns and neuraminidase-PNA staining patterns were similar in normal areas of lungs (non-hyperplastic) to the normal 3-week-old turkeys; however, consistent (2/2) staining of nonciliated bronchial cells was seen with WGA. There was multifocal staining of cells that line air atria using lectins sWGA, SBA, and PNA. The apical cytoplasm and surface of hyperplastic granular cells that lined air atria also stained with PNA, SBA, and sWGA (Fig. 9). Tracheal sections of those turkeys also stained similarly to 3-week-old poults; however, there was consistent staining of ciliated cells with WGA.

Verhoeff-van Gieson, Gomori’s, and Masson Trichrome stains highlighted areas with interstitial fibers, e.g., lamina propria of the trachea and pulmonary bronchi, the pleura, and the tunica adventitia of tracheal and pulmonary arteries and veins were stained. *Ulex europeas* (UEA-I), *Phaesolus vulgaris* (erythrophagglutinin), *Sophorajaponicum* (SJA), and *Griffonia simplicifolia-I* (BSL-I) either did not stain or stained sec-

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**Fig. 7.** Lung; turkey with acute pneumonia. Peroxidase staining of *Lens culinaris* (LCA) binding sites. The individual fibers tunica adventitia of a medium-sized pulmonary artery are separated by edema. *Lens culinaris* staining of fibers in the outer tunica adventitia is pale when compared with the inner portions of the adventitial layer. Some of the heterophils and macrophages in the peripheral infiltrates have stained cytoplasm. Harris’ hematoxylin counterstain.

**Fig. 8.** Lung; turkey with acute pneumonia. Peroxidase staining of *Glycin maximus* (SBA) binding sites. Air atria are distended by numerous, closely-packed bacteria, along with sloughed cells and several heterophils. The cells lining air atria are degenerate and some contain bacteria. Note the pale and haphazard staining of degenerate cells with *Glycin maximus* (SBA). Harris’ hematoxylin counterstain.

**Fig. 9.** Lung; turkey with chronic pneumonia. Peroxidase staining of *Glycin maximus* (SBA) binding sites. Hyperplastic cells, which are closely packed and cuboidal, line a distended air atrium. The apical cytoplasm and surface of many of these cells are stained. The air atrium is filled with seroproteinaceous fluid and low numbers of macrophages, heterophils, and lymphocytes. Harris’ hematoxylin counterstain.
sections diffusely and nonspecifically at lectin concentrations of 0.5, 2.5, 5.0, 25, and 50 μg/ml. The blocking sugars and periodate treatment inhibited all staining in control sections.

With transmission electron microscopy, colloidal gold-PNA conjugate labeled the plasma membrane of microvilli extending from the apical surface of granular cells. These cells had abundant cytoplasm and contained several osmiophilic inclusion bodies (Fig. 10). Cells that were not labeled included immature granular cells with scant amounts of cytoplasm and few osmiophilic inclusion bodies, nongranular cells, and air capillary epithelial cells (Fig. 11). In addition, the conjugate did not label surfactant and trilaminar substance that was within the cytoplasm. Bar = 1 μm.

**Discussion**

In this study, we were unable to identify epithelial cells in the lung or trachea to which there was an affinity for the D-mannose binding lectins *Conavalis ensiformis* (Con A), *Lens culinaris* (LCA), or *Pisum sativum* (PEA). Lack of staining by Con A was especially surprising, since this lectin can bind internal sugar residues of glycoproteins. Lack of epithelial cell staining by these lectins may reflect the following: low numbers of D-mannose residues present in the apical surface of these cells in turkeys; blocking of D-mannose or glucose residues by a complex, three dimensional conformation of the glycoconjugates; and loss of these residues during processing. Most glycolipids, for example, are lost following routine processing, and decreased lectin-binding affinities can be seen in paraffin-embedded sections when compared with frozen sections. Low numbers of available mannose residues may explain why adherence by *Escherichia coli* strains expressing mannose-binding type I fimbria has not been demonstrated in the turkey.

Staining of interstitial and lamina propria fibers by lectins may indicate the presence of glycosylated collagen or elastin. Elastic fibers and reticular fibers were prominent with traditional histochemical stains and were in identical locations to areas stained by these lectins. Sugar residues on these structures may facilitate interstitial invasion and lead to serosal spread of *E. coli* that express type 1, D-mannose-binding fimbriae.

There is evidence in this study that granular cells, but not the nongranular cells, were stained by N-acetylgalactosamine/galactose and N-acetylglucosamine-binding lectins. The affinity of several lectins to hyperplastic atrial cells supports a granular cell-lectin affinity. Since staining by three of the lectins (PNA, sWGA, and SBA) was restricted to cells that line air atria and hyperplastic atrial cells, these lectins may be

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**Fig. 10.** Electron micrograph. Lung; turkey. Colloidal gold particles (15 nm in diameter) conjugated to gold-*Arachis hypogaea* (PNA) are adhered to the apical plasma membrane and microvilli of a granular cell. Note that the gold particles do not adhere to the whorled lamella of surfactant (S) present in the airway lumen. Note osmiophilic inclusion bodies within the granular cell cytoplasm (arrows). Bar = 1 μm.

**Fig. 11.** Electron micrograph. Lung; turkey. The apical portion of a nongranular cell. The cell is a short distance from the granular cell shown in Fig. 10, and it lacks colloidal gold particles on its apical plasma membrane. Trilaminar substance (TLS) is within the cytoplasm. Bar = 1 μm.
useful markers for avian granular cells. This is similar to the use of Maclura pomifera agglutinin (MPA) marker used for rat and human type II cells. Maclura pomifera agglutinin (MPA) is in the N-acetylgalactosamine/galactose group, as is Arachis hypogaea (PNA) and Glycin max (SBA).

Lectins staining the apical surface of granular cells probably bind the plasma membrane rather than the overlying surfactant. This was demonstrated for PNA with the colloidal-gold-PNA conjugate. In addition, the apical surface of air capillary epithelial cells are also often covered by a thin layer of surfactant,12,13 but of the six lectins (WGA, sWGA, SBA, PNA, PHA-L, and RCA-I) that stained atrial cells (cells that secrete surfactant), only two (WGA and RCA-I) stained the apical surface of air capillary epithelial cells. Of these two lectins, Triticum vulgare (WGA) may adhere to sialic acid residues of the plasma membrane, while Ricinus communis I (RCA-I) staining was not seen in all turkeys (5/7). Although much of the surfactant may be lost with tissue processing, surfactant-like membranes are retained in tissues processed for electron microscopy and were seen in this study. Lectins may also bind trilaminar substance, but this material was not seen on transmission electron microscopy sections of 10-week-old turkeys, and was limited to occasional air atria of 3-week-old turkeys (unpublished observations).

The PNA staining of neuraminidase-digested sections indicates that respiratory lining cells and endothelial cells express sialic acid residues. Neuraminidase cleaves sialic acid residues and exposes penultimate galactose residues that can be stained by PNA. Sialic acid residues can then be determined by comparing PNA staining before and after neuraminidase treatment. Sialic acid residues may be an important receptor for certain pathogens of turkeys. For example, virulent E. coli strains may adhere to sialic residues to enter pneumocytes that line air capillaries, and Bordetella avium binds sialic acid residues of ciliated tracheal cells.1,3 Respiratory epithelia of other species also express sialic acid. These include tracheal mucous cells in the rat and mouse, and alveolar cells of human fetal lung.8,22,23 The presence of sialic acid residues was confirmed by similarity of staining patterns with WGA and neuraminidase/PNA. The WGA can bind sialic acid, but succinate residues on sWGA prevent this lectin from binding sialic acid. Cells that stained with WGA, but not sWGA, therefore, indicate the presence of sialic acid. In addition, WGA following neuraminidase treatment resulted in a staining pattern similar to sWGA (sucnylation inhibits binding of sWGA to sialic acid). One exception to this staining pattern was the lack of staining of nonciliated tracheal cells by WGA and the consistent staining of these cells by PNA following neuraminidase.

Interference of lectin staining by inflammation was demonstrated in lungs with acute pneumonia. This was likely due to the action of glycosidases and hydrolases present in acute inflammatory exudates. Altered lectin staining patterns with neoplasia and inflammation have also been reported in other studies.5,7,14,26

In this study, the lowest concentration of lectin that resulted in specific, definitive staining was used. For several lectins (UEA-I, PHA-L, SJA, and BSL-I), staining specificity was poor regardless of the lectin concentration used. Although Ulex europeae I (UEA-I) stains human endothelial cells and can be used as a marker to identify some of these cells in hemangiomias and hemangiosarcomas of human beings and dogs, it did not stain turkey endothelial cells. This lectin does not stain the normal endothelial cells of rodents, chickens, or endothelial cells of canine skin, kidney, and liver, and renal endothelial cells of chickens, quail, and other animal species.2,6,11,20

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Request reprints from Dr. M. R. Ackermann, US Department of Agriculture, Agricultural Research Service, PO Box 70, Ames, IA 50010 (USA).