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In vitro binding of FITC-labelled plant lectins to gastrointestinal cells in the hen *(Gallus domesticus)*

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ABSTRACT

Binding of a panel of FITC-labelled lectins to deparaffinized histological sections from the crop, glandular stomach and duodenum of hens was studied. Succinyl-ConA produced a diffuse staining in the cytoplasm of epithelial cells in the three organs examined. In the epithelium of the crop, staining of the intercellular matrix by WGA, PHA, PNA and LCA displayed a net-like pattern. In the deep gastric glands, oxyntopeptic cells did not react with lectins except succinyl-ConA. Duodenal and gastric mucous cells were stained by WGA and PNA, but not by UEA-I or SNA-I. Binding of WGA and RPA-I to duodenal brush border was demonstrated. PHA did not attach to the brush border, which explains why this lectin is harmless if fed to chicken. The subepithelial connective tissue was strongly stained by LCA. The findings are discussed in relation to carbohydrate receptors and compared to the reactivity of mammalian tissues.
(Keywords: lectin, hen, avian, crop, stomach)

ÖSSZEFOGLALÁS

FITC-jelzett növényi lektinek in vitro kötődése a tyúk (*Gallus domesticus*) gyomor-bélcsatornájához

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Szövettani metszeteket egy sorozat, különböző kötési specificitású lektinnel kezeltünk a szénhidrát-receptorok kimutatására a tyúk gyomor-béltraktusában. A begy epitheliumban a hámsejtek közötti anyag számos lektint megkötött, ami komplex szénhidrátok jelenlétére utal. A gyomor és a bél nyálkasejtjei WGA-val és PNA-val festődtek, de UEA-I és SNA-I lektinekkel nem, jóllehet az emlősökben a nyálkasejtek általában reagálnak az utóbbiakkal. A gyomor mély mirigyeiben az oxyntopepticus sejtek (amelyek pepszinogént és sósavat is szekretálnak), nem festődtek PNA-val és PHA-val, hasonlóan a patkány-gyomor fősejtjeihez, de kifejezetten eltértek ennek az emlős fajnak a fedősejtjeitől. A hámsejtek mindegyik szervben diffúzan festődtek succinyl-ConA-val, ami arra utal, hogy polimannóz-típusú éretlen glikozidok vannak jelen a citoplazmában. A PHA, amely a patkány bélhámján megkötődik és biológiai

hatásokat vált ki, a tyúk duodenumban nem reagált a kefeszegély glikoproteinjeivel. Ez megmagyarázza azt a megfigyelést, hogy a PHA ártalmatlan a tyúk számára.
(Kulcsszavak: lektin, tyúk, madár, begy, gyomor)

INTRODUCTION

Lectins constitute a large family of proteins; these are carbohydrate-binding proteins with the exception of carbohydrate-binding immunoglobulins and enzymes. Lectins are widely distributed in nature, from viral haemagglutinins to several human proteins. In nature they are used for aggression (bacterial adherence), protection (antinutritive plant lectins) or communication between cells.

Lectins occur in seeds and other avian feeds, and recently several lectin genes were introduced into transgenic plants as a form of plant protection. Several lectins resist heat treatment and digestion. In the rat most of the lectins bind to carbohydrate receptors (glycoprotein side chains) on the cell surface in the gut, resulting in various biological effects (*Pusztai,* 1991*; van Damme et al.,* 1998). This binding is a prerequisite for biological effects. Recent experiments (*Pusztai* et al.) Indicate that birds respond differently to lectins from the rat. In the present work, therefore, we examined the direct binding of a panel of FITC-labelled lectins to the avian gastrointestinal cells *in vitro*.

MATERIALS AND METHODS

Animals and preparation of samples

Adult hens were kept in a small farm on mixed diet. They were anaesthesized by Nembutal (pentobarbitone) and killed by bleeding from the jugular vein. After evisceration a 0.5 cm wide strip was cut out of the middle of the crop and the glandular stomach, and a 0.5 cm wide ring from the duodenum. The samples were fixed in phosphate-buffered saline (PBS) containing 8% formaldehyde, for three days and embedded in paraffin. Five μm thick sections were cut, deparaffinized in xylene, rehydrated in a descending series of ethanol solutions and stored in PBS (pH 7.4) until use.

Isolation and labelling of lectins

Robinia bark lectin (RPA-I) (*van Damme et al.,* 1995) and *Sambucus* bark lectin (SNA-I) was isolated on fetuin-Sepharose column (*Broekaert et al*., 1984) dialyzed against distilled water, lyophilized and checked by SDS-PAG electrophoresis and agglutination of rabbit red cells.

For labelling, 2 mg SNA-I or RPA-I was dissolved in 1 ml carbonate-bicarbonate buffer (0.05 M, pH 9.5). Fluorescein isothiocyanate (FITC) adsorbed to Celite (Sigma) was added to a final concentration of 50 μg/ml and incubated at 4oC overnight. Unbound FITC was removed on a Sephadex G-25 column calibrated with Dextran blue (Pharmacia, Uppsala). The other FITC-labelled lectins (*Table 1*) were purchased from Sigma.

Binding experiments

A 0.75 mg/ml stock solution was prepared from each of the FITC-labelled lectins in pH 7.5 PBS and filtered through a Millipore membrane (22 μm). Ten-fold dilution was made before use, except WGA, that was applied in a 300-fold dilution. The rehydrated tissue was first incubated with 1% BSA in PBS at room temperature for 1 hour in a humid chamber, then with the labelled lectin solution for another 60 minutes, rinsed in PBS three times and mounted in glycerol. The preparations were examined under a Nikon-104 or Alpha 2001

YL fluorescence microscope and photographed onto high sensitivity Fujichrom 1600D film. Tissue autofluorescence was checked with PBS-treated control slides and also using rhodamine filter. Specificity of binding was examined in control experiments using haptenic sugars in 1% solution before adding the labelled lectin. No such control was performed with PHA and RPA-I, due to the lack of haptenic sugar.

Table 1

FITC-labelled lectins used in binding experiments

Abbreviation(1)	Source(2)	Latin name(3)	Specificity(4)
Succ.-ConA	jack bean(5)	*Canavalia ensiformis*	Man/Glc
LCA	lentil(6)	*Lens culinaris*	Man/Glc
WGA	wheat germ(7)	*Triticum aestivum*	GlcNAc
PNA	peanut(8)	*Arachis hypogaea*	Gal-GalNAc
UEA-I	gorse seed(9)	*Ulex europaeus*	fucose
SNA-I	black elder bark(10)	*Sambucus nigra*	Gal-sialic acid
PHA	kidney bean(11)	*Phaseolus vulgaris*	complex
RPA-I	black locust bark(12)	*Robinia pseudoacacia*	complex

Succ.=succinyl, ConA=concanavalin A, Gal=galactose, Glc=glucose, Man=mannose, GalNAc=N-acetyl-galactose, GlcNAc=N-acetyl-glucose

1. táblázat: A kötési kísérletekben használt FITC-jelzett lektinek

Rövidítés(1), Forrás(2), Latin név(3), Specificitás(4), jack bean (trópusi pillangós növény magja)(5), lencse(6), búzacsíra(7), földidió(8), zanót mag(9), bodzakéreg(10), kerti bab(11), akáckéreg(12)

RESULTS

Crop

All the layers of crop mucosa were stained by LCA (*Fig. 1*) and succinyl-ConA. LCA showed an especially strong binding with the connective tissue. In the epithelium, binding of lectins to the intercellular matrix resulted in a characteristic net-like staining pattern. LCA (*Fig. 1*), succinyl-ConA and WGA stained both the *stratum spinosum* and *stratum granulosum*, while PNA did bind to the *stratum spinosum* only (*Fig. 2*). Less intense staining was found in the basal layer. PHA reacted like WGA. Cytoplasmic staining was observed with succinyl-ConA only. No binding could be demonstrated with UEA-I, SNA-I and RPA-I in the crop mucosa.

Gastric deep glands

The cytoplasm of the oxyntopeptic cells was stained by succinyl-ConA (*Fig. 3*), but none of the other lectins was able to bind. However, PNA (*Fig. 4*) and LCA markedly stained the connective tissue.

Duodenum and gastric superficial glands

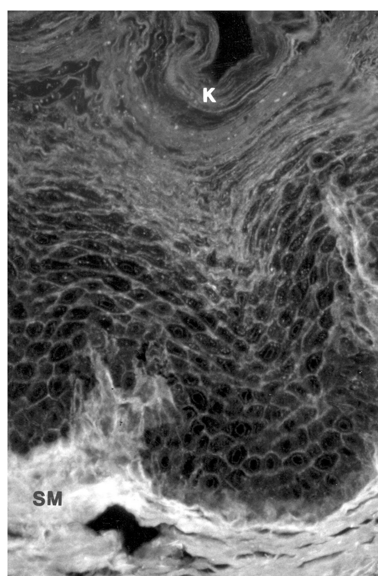
Cytoplasmic staining by succinyl-ConA and less markedly by LCA, was observed in the duodenal epithelium.

WGA (*Fig. 5-6*) and less markedly, PNA stained the mucous cells both in stomach and small intestine, but none of the other lectins reacted with them.

Binding of lectin to the luminal surface of epithelial cells could be demonstrated with WGA (*Fig. 6*), RPA-I (*Fig. 7*) and in some regions with PNA, but never with PHA.

Fig. 1

Crop tissue stained with FITC-LCA

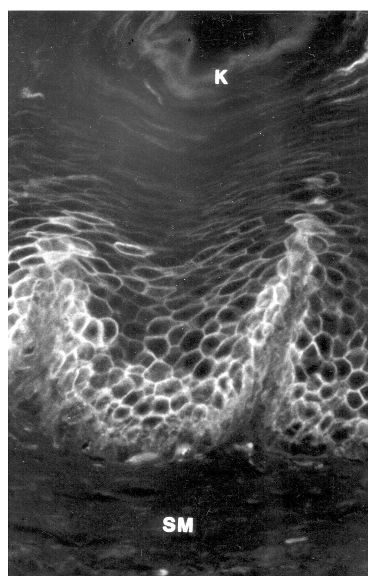


K=keratinized layer (*keratinizált réteg*), SM=submucosa

1. ábra: FITC-LCA-val festett begyszövet

Fig. 2

Crop tissue stained with FITC-PNA

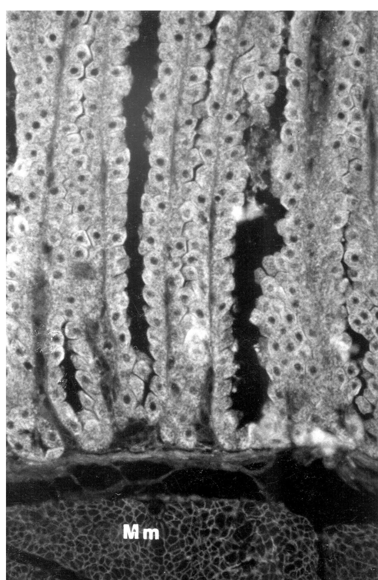


K=keratinized layer (*keratinizált réteg*), SM=submucosa

2. ábra: FITC-PNA-val festett begyszövet

Fig. 3

Deep gastric gland, cytoplasmic staining of oxyntopeptic cells with FITC-labelled succinyl-ConA

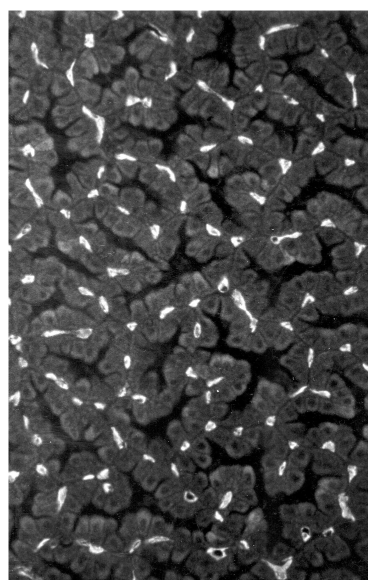


Mm=*muscularis mucosae*

3. ábra: Az oxyntopepticus sejtek citoplazmás festődése FITC-jelzett succinyl-ConA-val a gyomor mély mirigyeiben

Fig. 4

Cross section of processes in the deep gastric gland, stained with FITC-PNA*

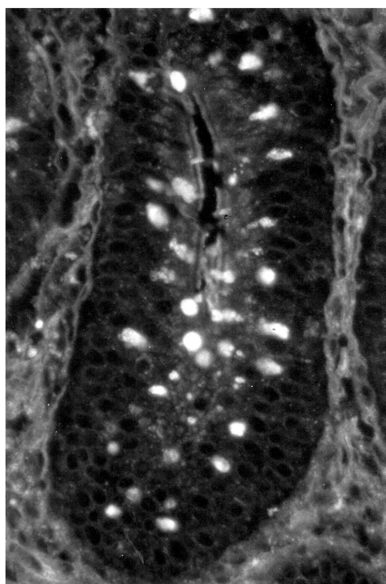


*The lectin bound only to the connective tissue (*A lektin kizárólag a kötőszövethez tapadt*)

4. ábra: FITC-PNA-val festett nyúlványok keresztmetszete a gyomor mély mirigyeiben

Fig. 5

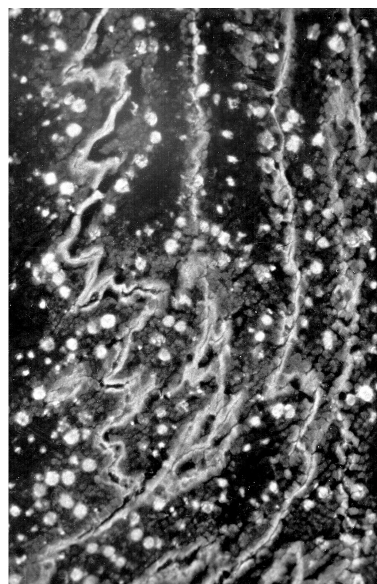
Staining of mucous cells with FITC-WGA in a superficial gastric gland



5. ábra: A nyálkasejtek festődése FITC-WGA-val a gyomor felületes mirigyeiben

Fig. 6

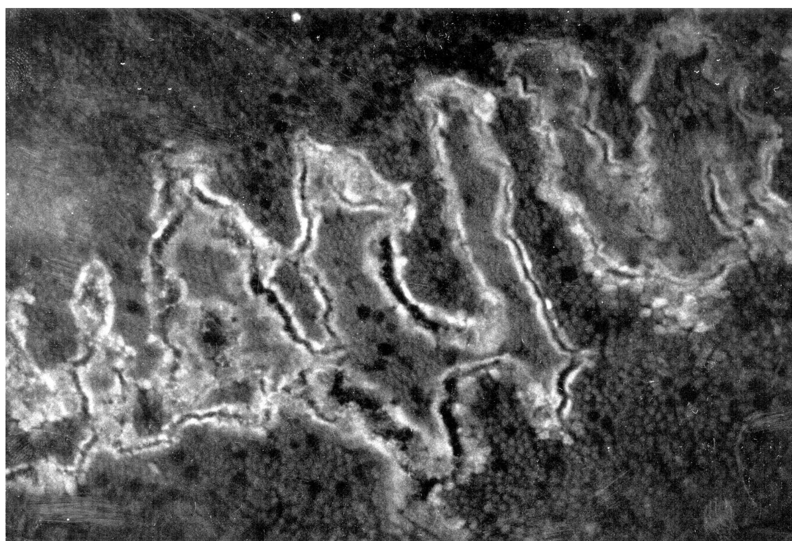
Staining of duodenal mucous cells and brush border with FITC-WGA.



6. ábra: A kehelysejtek és a kefeszegély festődése FITC-WGA-val a duodenumban

Fig. 7

Staining of the duodenal brush border with FITC-RPA-I between mucosal folds



7. ábra: A kefeszegély festődése FITC-RPA-I-gyel a duodenum nyálkahártyaredői között

DISCUSSION

A panel of lectins with different binding specificities (*Table 1*) was applied to histological sections to visualize glycoside or glycoprotein receptors on the cell surface and in the extracellular material in the avian gastrointestinal tract. A single monovalent lectin, succinyl-ConA was also included.

In the crop epithelium the intercellular material bound several lectins, indicating the presence of complex carbohydrates. Binding of PNA shows that galactose is localized in terminal position, in other words, the PNA-reactive carbohydrate is not sialylated. Although the the human skin, another multi-layered keratinized epithelium (*Reano et al*.,19882) gave a similar picture, the lectin binding pattern clearly differed from that of the avian crop.

The mammalian gastric gland secretes both mucus and digestive secretions. The gland of the avian glandular stomach is divided to a mucous superficial gland and a digestive deep gland. In the latter the oxyntopeptic cells secrete both pepsinogen and HCl. These cells did not react with lectins (except succinyl-ConA) in the present study, similarly to the gastric chief cells of the rat, but clearly differed from the rat parietal (oxyntic) cells (*Baintner et al*., 2000) which bound both PNA and PHA.

The rat goblet cell mucus appears to be of a more complex nature than that of the hen, because the latter did not bind UEA-I and SNA-I (*Baintner et al*., 2000) lectins with fucose and sialic acid-galactose specificity (the latter in an α[2,6] configuration), respectively.

PHA, a gut-binding and biologically potent lectin for the rat (*Bardocz et al*., 1995; *Pusztai et al*., 1975) did not react with the brush border glycoproteins in the avian duodenum. This explains the observation that oral PHA is harmless for the chicken (*Pusztai et al*.).

In all the organs the epithelial cells were stained diffusely by succinyl-ConA, indicating the presence of immature glycosides of polymannose type in the cytoplasm.

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