10.1053.paor.2000.0240 available online at http://www.idealibrary.com on IDEAL

ARTICLE

Binding of FITC-Labelled Lectins to the Gastrointestinal Epithelium of the Rat

Károly BAINTNER,[1] Gábor JAKAB,[2] Zsuzsa GYÔRI,[2] Péter KISS[3]

[1]Department of Physiology, Faculty of Animal Science, Pannon Agricultural University, Kaposvár, Hungary; [2]Department of Neurology, Uzsoki Hospital, Budapest, Hungary; [3]Department of Agricultural Chemistry, University of Agriculture, Gödöllô, Hungary

Biotechnology uses lectin genes to transfect into crop plants for protection against insects and nematodes. On the other hand, the information is limited on lectin-binding properties of cells in the gastrointestinal tract. Therefore, binding of a panel of FITC-labelled plant lectins to gastrointestinal cells of the rat was studied. In the stomach, cytoplasmic staining of parietal cells by PHA appeared to be due to glycoproteins attached to the tubulovesicles. PNA also stained the parietal cells, but only in the isthmus and neck regions, reacting with desialylated glycoproteins. WGA bound to the mucous neck cells with higher affinity than to the surface and foveolar mucous cells. The mucous cells were also stained by SNA-I, UEA-I and, less intensively, by LCA. Chief cells did not show detectable reaction with any of the applied lectins. Binding of PHA to gastric cells showed differences when compared with the results of *in vivo* studies. Small intestinal brush border was stained with UEA-I and SNA-I, the latter lectin also strongly stained the surface of small intestinal crypts. Both lectins reacted with the mucus of goblet cells. In the large intestine UEA-I and SNA-I stained the goblet cells at the base and upper part of the crypts, respectively. Accordingly, we provided evidences for the unique lectin-binding phenotype of the various segments of the gastrointestinal tract. (Pathology Oncology Research Vol 6, No 3, 179–183, 2000)

Keywords: rat, gut, stomach, lectin, ConA, LCA, PHA, RPA, PNA, WGA, SNA, UEA

Introduction

The gastrointestinal epithelium is exposed to various proteinase-resistant lectins of food origin.[8,13] Peanut lectin even survives heat treatment in substantial quantities and a much smaller part is absorbed to the circulation.[14] Recently biotechnology raised renewed interest towards lectins: their occurrence, properties and fate in the GI tract. Lectin genes from non-food organisms were transferred into crop plants to exert biological protection against insects and nematodes, e.g. the gene of snowdrop bulb lectin (GNA) into potato[4] and of *Bacillus thuringiensis* delta-endotoxin into soybean and corn. The product of the latter gene consists both lectin and cytolytic domains. Genetically modified soybean and corn is produced on millions of acres on the Western hemisphere. Recently safety concerns were raised for the GNA-transgenic potato, although the problems may originate from the gene construct applied, rather than from the transgene itself.[4] Accordingly, more information is required about the binding potential of lectins to the gastrointestinal tract.

Lectin binding of gastric cells was studied by several workers with *in vitro* methods in various mammalian species. In spite of studies with a few lectins in the rat,[3,9,10,11] to our knowledge this is the first systematic study of lectin binding pattern of the stomach, jejunum and colon of rat (which is the frequent test system for bioengineered food products).

Received: Dec 9, 1999; *revised:* April 20, 2000; *accepted:* May 20, 2000;

Correspondence: Károly BAINTNER, PATE, 7401 Kaposvár, Pf. 16. Fax: 36 82 313562, E-mail: baintner@atk.kaposvar.pate.hu

 1219-4956/00/030179+05 $ 12.00/0

Material and Methods

Animals and preparation of samples

Female Wistar rats (140 g) were fed on commercial chow. In the morning, without previous fast, the animals were anaesthesized by Nembutal and killed by decapitation. After evisceration an 0.5 cm wide strip was cut along the *curvatura major* from the esophageal part of the stomach to the antral region. Other samples were taken from the middle of jejunum and colon. The tissue was fixed in PBS-buffered 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 placed in phosphate-buffered saline (PBS, pH 7.4) for use.

Isolation and labelling of lectins

Robinia bark lectin (RPA-I)[12] and *Sambucus* bark lectin (SNA-I) was isolated on fetuin-Sepharose column[2], dialyzed against distilled water, freeze-dried and checked by SDS-PAG electrophoresis and for agglutination of rabbit red cells.

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

Binding experiments

An 0.75 mg/ml stock solution was prepared from each of the FITC-labelled lectins in pH 7.5 phosphate buffered saline (PBS) and filtered through a Millipore membrane (0.22 μm). Ten-fold dilution was made before use, except for WGA, where 300-fold dilution was applied. The rehydrated tissue was first incubated with 1% BSA in PBS at room temperature in a humid chamber for 1 hour, then with the FITC-lectin solution for 60 minutes, rinsed in PBS three times and mounted with glycerol. The preparations were examined under a Nikon-104 or Alpha 2001 YL fluorescence microscope and photographed to high sensitivity Fujichrom 1600D film.

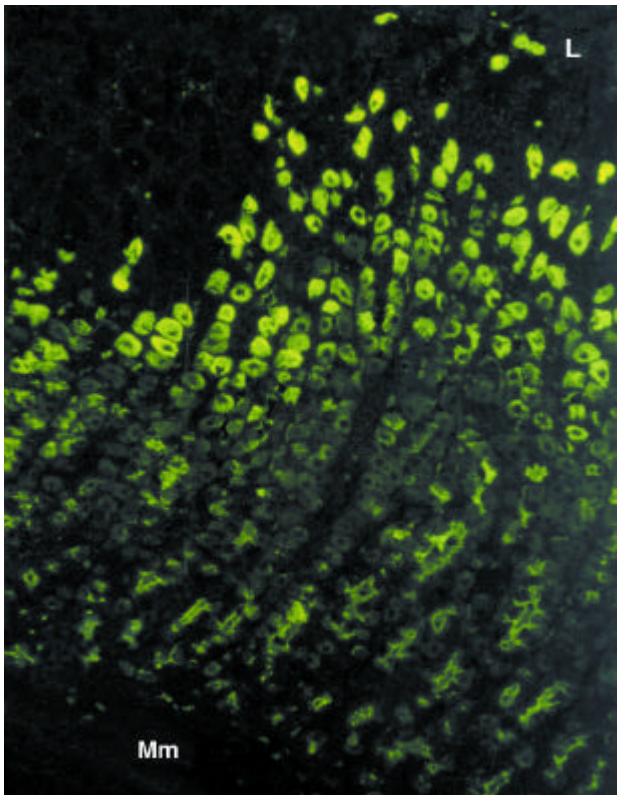

Figure 1. FITC-PNA staining of parietal cells in the isthmus region of gastric glands. L = lumen, Mm = muscularis mucosae.

Specificity of lectin binding was checked with sugars that are able to inhibit the lectin-carbohydrate interaction (so-called haptenic sugars) as shown in *Table 1*. In the control experiments the labelled lectin was mixed with the respective sugar (2% solution in PBS) before it was applied onto the tissue. Chitotriose was used in 1% solution. No such control was done for SNA-I and lectins with „complex specificity", i.e. those not inhibited by any simple sugar. Tissue autoflu-

Table 1. **FITC-labelled lectins used in binding experiments**

Abbreviation	*Latin name*	*Source*	*Inhibition by sugar (specificity)*
Succ.-ConA	*Canavalia ensiformis*	jack bean	glucose
LCA	*Lens culinaris*	lentil	glucose
WGA	*Triticum aestivum*	wheat germ	chitotriose
PNA	*Arachis hypogaea*	peanut	galactose
UEA-I	*Ulex europaeus*	gorse seed	fucose
SNA-I	*Sambucus nigra*	black elder bark	Gal-sialic acid*
PHA	*Phaseolus vulgaris*	kidney bean	complex spec.
RPA-I	Robina Pseudoacacia	black locust bark	complex spec

Abbreviations: Succ. = succinyl; Gal = galactose; GalNAc = N-acetyl-galactosamine.
* sialyl-α(2,6)Gal / GalNAc specificity

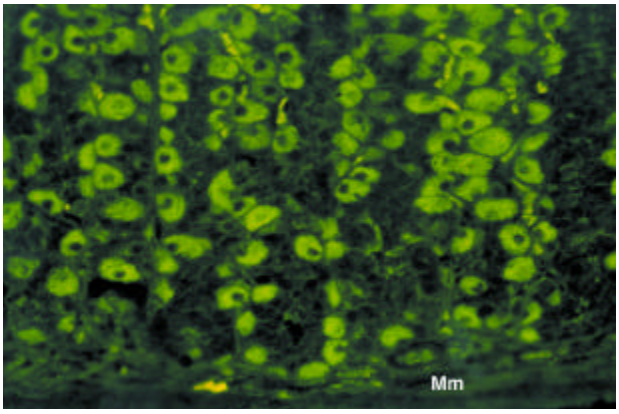

Figure 2. FITC-PHA staining of parietal cells in the base of gastric glands. Mm = muscularis mucosae.

orescence was checked with PBS-treated control slides and using rhodamine filter. Some sections were stained with haematoxylin-eosin.

Results and Discussion

Stomach

The whole cytoplasm of the parietal cells appeared to react both with PNA *(Figure. 1)* and PHA *(Figure. 2).* In fact, this appearance may be due to glycoproteins situated on the numerous invaginations of the plasma membrane,

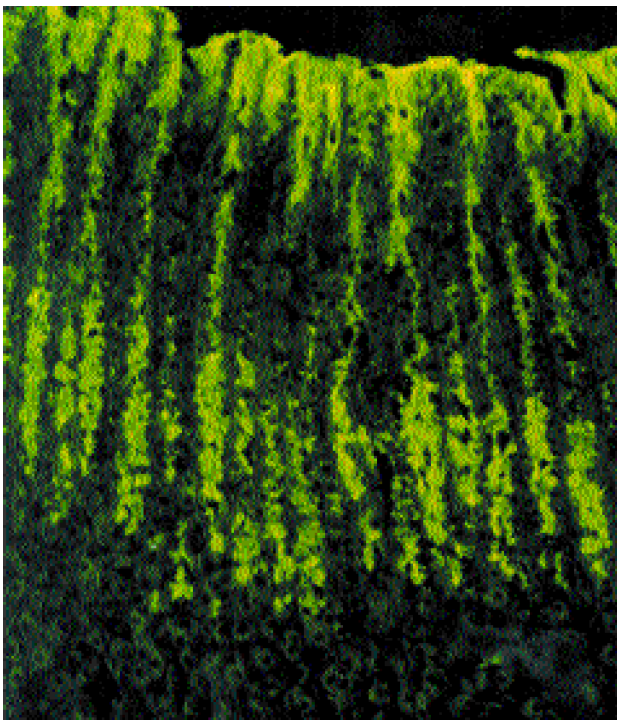

Figure 3. FITC-WGA staining of surface mucous cells and mucous neck cells in absence of haptenic sugars.

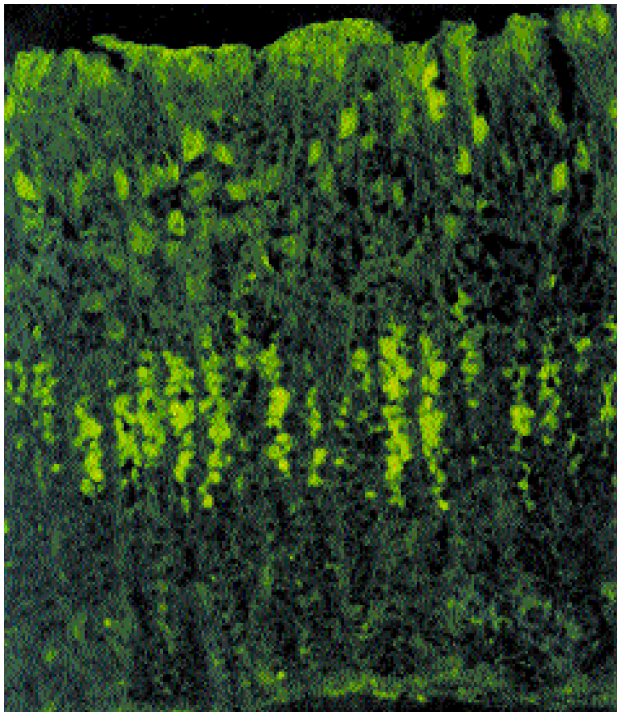

Figure 4. FITC-UEA-I staining of mucous neck cells.

also known as tubulovesicles. The proton pump ATPase constitutes the principal membrane glycoprotein as shown in mice by electron microscope[5] and with biochemical studies.[6] The glycoproteins of the parietal cells are desialylated,[6,15] exposing galactosyl units at terminal positions and, therefore, allowing them to react with PNA.

The round parietal cells reacted with PHA in all regions of the gastric gland. A different pattern was shown by PNA, which stained the parietal cells in the isthmus region very strongly, but the staining faded away towards the basal region. Some reactive substance could also be observed in the lumen of the glands and on the luminal surface of some unidentified cells.

WGA stained two types of mucous cells strongly and in very high dilution: the surface and foveolar mucous cells and the mucous neck cell *(Figure 3).* Between the two regions (isthmus), some WGA-reactive material was observed in the lumen of the glands and on the luminal surface of the cells. Binding of WGA was not affected by 2% N-acetyl-glucosamine. When the concentration of this sugar was raised to 16%, binding of WGA to surface and foveolar mucous cells was inhibited without affecting the strong staining of the mucous neck cells. This high affinity binding could be inhibited only with chitotriose, a polymeric form of acetylated glucosamine.

UEA-I gave a similar staining to that of WGA, but higher lectin concentration was required. LCA stained the mucous neck cells clearly *(Figure 4),* but more faintly than WGA or UEA-I. Staining by succinyl-ConA, a monova-

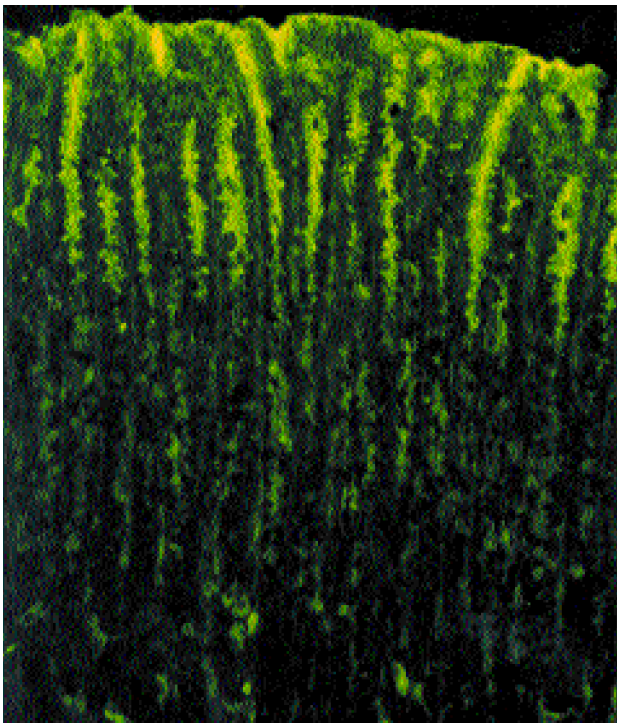

Figure 5. FITC-SNA-1 staining of the surface and foveolar mucus cells.

lent derivative of ConA, was very faint. SNA-I reacted with the mucous cells in the surface, foveolar and isthmus region *(Figure 5),* but little staining could be observed at the neck region. RPA-I did not react with any of the gastric cell types.

In the basal part of the gastric glands a sporadic cell type of pyramidal shape was stained faintly by UEA-I and SNA-I; these cells may be identical to non-functional parietal cells found by Karam et al.[7] in the rabbit. Chief cells did not show detectable reaction with any of the applied lectins.

The present findings are in agreement with the PNA binding studies of Schulte and Spicer[10] and with the WGA binding studies of Suzuki et al.[11] Callaghan et al[3] used tomato lectin (LEA) as an NAcGlc specific lectin, which also stained gastric parietal cells in addition to the mucous cells.

Bardocz et al[1] fed young rats with a diet containing isolated PHA and examined the effect of the lectin on organ growth and metabolism. In their experiments PHA was demonstrated by antibody peroxidase-antiperoxidase (PAP) staining inside clusters of cells in the neck region of gastric glands even at 3 days after the end of PHA feeding. Although the authors considered them parietal cells, we reidentified them as mucous neck cells. This cell type could not be stained with PHA in the present

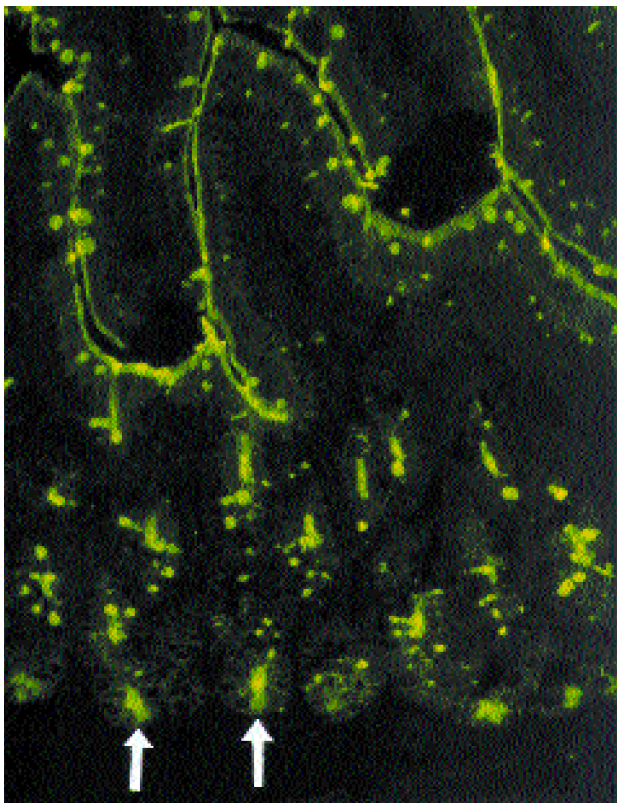

Figure 6. FITC-UEA-I staining of villus (V) and crypt surface in the jejunum. Arrows show the base of crypts.

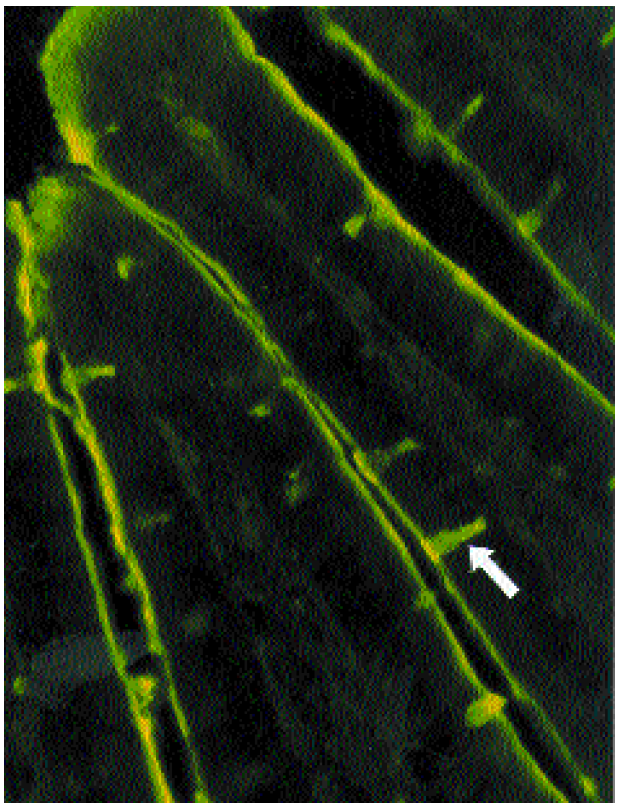

Figure 7. FITC-SNA-I staining of brush border and goblet cells (arrow) on intestinal villi.

Table 2. **FITC-Lectin binding pattern of rat gastrointestinal epithelial cells in vivo**

Lectin	*Stomach*	*Jejunum*	*Colon*
PNA	Parietal cells	NS	NS
PHA	Parietal cells	NS	NS
WGA	Mucus cells	NS	NS
LCA	Mucus cells	NS	NS
UEA-	Mucus cells (neck)	Enterocytes, villi, crypt surface	Cryptal mucus cells
SNA-	Mucus cells, pyramidal cells	Brush border, goblet cells	Crypt surface, goblet cells
RPA-	NS	Enterocytes, villi, crypt surface	NS

NS = not studied

work. Further work is required to clarify the cause of discrepancy between the *in vivo* and *in vitro* study and the function of these cells.

Intestine

Binding of six lectins with different specificities to the small intestinal epithelium was studied by Pusztai et al.[9] using PAP staining. Highest reaction was shown with PHA. We extended this work using three other lectins. The brush border was stained faintly with RPA-I and more strongly with UEA-I *(Figure 6)* and SNA-I *(Figure 7)*. The latter lectin also strongly stained the surface of the small intestinal crypts. UEA-I and SNA-I also reacted with the mucus of goblet cells both in small and large intestine. In the latter organ UEA-I and SNA-I stained the goblet cells at the base and the upper part of the crypts, respectively. Our data is summarized on *Table 2*.

Our studies indicated a clear cell-type specific expression of glycoconjugates on the surface of the cells of the gastrointestinal tract which bind selectively authetic lectins such as the parietal of the stomach (PNA), UEA-binding of mucus cells of the stomach, jejunum and colon, RPA-binding to the villi of the enterocytes and SNA-binding of goblet cells in the colon. Therefore, we suggest that the biotechnological introduction of lectins into crops and vegetables needs special consideration of these lectin-binding properties of the modified product which may specifically modulate the function of certain gastrointestinal cells. The effect of lectin genes introduced into food plants on gastrointestinal histology will be published later.

Acknowledgements

The authors express their thanks to Dr Susan Neogrády and Dr Peter Gálfi (Dept. of Physiology, Univ. of Veterinary Medicine, Budapest) for FITC-labelled lectins; Dr Stanley W.B. Ewen (Dept. of Pathology, Univ. of Aberdeen Medical School) for the high sensitivity film, Ida Rózsavölgyi (Dept. of Pathology, County Hospital, Kaposvár) for the histological preparations and the late Dr János Fischer (Dept. of Biochemistry, SZOTE, Szeged) for valuable advices.

The work was supported by OTKA T6400, OTKA M 27218 and FKFP 503 grants.

References

1. *Bardocz S, Grant G, Ewen SWB, et al:* Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. Gut 37:353-360,1995.
2. *Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ*: A lectin from elder (*Sambucus nigra* L.) bark. Biochem J 221:163-169,1984.
3. *Callaghan JM, Toh B-H, Pettitt JM, et al:* Poly-N-acetyllactosamine-specific tomato lectin interacts with gastric parietal cells. J Cell Science 95:563-576,1990.
4. *Ewen SWB, Pusztai A:* Effects of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. Lancet 354:1353-4,1999.
5. *Fischer J*: Alternative ultrastructural localization of *Dolichos biflorus* lectin binding sites in proton secreting parietal cells of mice. Biochemistry 87:1-4,1989.
6. *Goldkorn I, Gleeson PA and Toh B-H*: Gastric parietal cell antigens of 60-90 kDa, and 100-120 kDa associated with autoimmune gastritis and pernicious anaemia. Role of N-glycans in the structure and antigenicity of the 60-90 kDa component. J Biol Chem 264:18768-74, 1989.
7. *Karam SM, Yao X, Forte JG*: Functional heterogeneity of parietal cells along the pit-gland axis. Am J Physiol 272: G161-G171, 1997.
8. *Pusztai, A*: Plant Lectins. Cambridge University Press.1991.
9. *Pusztai A, Ewen SWB, Grant G et al*: The relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. Digestion 46: (suppl. 2) 308-316,1990.
10. *Schulte B and Spicer SS*: Light microscopic histochemical detection of terminal galactose and N-acetylgalactosamine residues in rodent complex carbohydrates using a galactose oxidase-Schiff sequence and peanut lectin-horseradish peroxidase conjugate. J Histochem Cytochem 31:19-24,1983.
11. *Suzuki S, Tsuyama S and Murata F*: Post-embedding staining of rat gastric mucous cells with lectins. Histochemistry 73:563-575,1982.
12. *Van Damme EJM, Barre A, Smeets K, et al:* The bark of *Robinia pseudoacacia* contains a complex mixture of lectins. Plant Physiol 107:833-843,1995.
13. *Van Damme EJM, Peumans WJ, Pusztai A, et al*: Handbook of Plant Lectins. John Wiley & Sons, Chichester, 1998.
14. *Wang Q, Lu-Gang Y, Campbell BJ, et al*: Identification of intact lectin in peripheral venous blood. Lancet 352:1831-1832,1998.
15. *Zolotarev AS, Townsend RR, Stuart-Tilley A, et al*: HCO_3^--dependent conformational change in gastric parietal cell AE2, a glycoprotein naturally lacking sialic acid. Am J Physiol 271:G311-G321,1996.