ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Vol. 44 N° 4-S, 1994

Characteristics and consequences of interactions of lectins with the intestinal mucosa

Arpad Pusztai

The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland, UK

SUMMARY. Lectins are essential and omnipresent plant (glyco)protein constituents and are ingested daily in appreciable amounts by both humans and animals. As they are biologically highly active, their consumption may have serious consequences for metabolism and health. Lectins, by virtue of their stability and specific recognition and binding by gut brush border epithelial cells, are potent exogenous metabolic growth signals for the gut and the body. As a result of their binding to surface glycans they may affect the turnover and loss of epithelial cells, damage the luminal membranes of the epithelium, interfere with their digestive/absorptive activities, stimulate shifts in the bacterial flora and modulate the immune state of the digestive tract. When eaten in relatively large quantities, these lectins have appreciable antinutritive effects for the consumers. In contrast, lectins which are not bound by the mucosa usually induce little or no harmful effects. From recent studies it is now realized that in addition to the major and sometimes dramatic effects of lectins on the gut which are mediated through their binding to pre-existing membrane glycosyl groups, lectins as metabolic signals, can also radically alter the state of glycosylation of the gut epithelium and thus further amplify their potent physiological effects. Accordingly, with the judicious use of dietary lectins it is now possible 'to engineer' the digestive tract for improved physiological performance and bacterial ecology.

RESUMEN. Características y consecuencias de la interacción de lectinas con la mucosa intestinal. Las lectinas de plantas son componentes esenciales y onmipresentes de naturaleza (glico)proteica que se ingieren en cantidades apreciables diariamente, tanto por humanos como por otras especies animales. Debido a su intensa actividad biológica intrínseca, el consumo de estas proteínas puede acarrear serias consecuencias, tanto a nivel metabólico, como de la salud en general. En virtud de su estabilidad y de su enlazamiento especifico por células epiteliales del tracto digestivo, las lectinas pueden actuar como potentes señales de crecimiento a nivel intestinal y del organismo en general. A consecuencia de su unión a glicanos de superficie, las lectinas pueden afectar los procesos de recambio y la descamación de las células epiteliales, dañar las membranas luminales del epitelio, interferir con los procesos de digestión y absorción, promover cambios de la flora bacteriana y modular el estado inmune del tracto digestivo. La ingesta de estas lectinas en altas cantidades produce efectos antinutricionales apreciables en los consumidores. En contraste, las lectinas que no se unen a la mucosa usualmente causan poco o ningún efecto deletéreo. Estudios recientes nos han llevado al convencimiento de que, además del efecto principal y a veces dramático de las lectinas sobre el intestino y el cual es mediado por su unión a grupos glicosilo pre-existentes en la membrana, las lectinas como señales metabólicas pueden alterar radicalmente el estado de glicosilación del epitelio intestinal y, por ende, amplificar su efecto fisiológico potencial. En consecuencia, con el uso ponderado de las lectinas de origen dietario sería posible «modificar a voluntad» el tracto digestivo para potenciar su respuesta fisiológica y optimizar la ecología bacteriana.

INTRODUCTION

Lectins, or more generally, carbohydrate-binding (glyco)proteins, are essential and omnipresent constituents of plants. As a major part of our food is of plant origin, appreciable amounts of lectins are ingested every day with potentially important consequences. Although some lectins are justly regarded as antinutrients because of their damaging effects on the gut and body metabolism, not all lectins are nutritionally toxic. During evolution man must have eliminated toxic plants from the diet, including those with highly toxic lectin constituents and/or learnt how to de-toxify others. Selection by agricultural means was also extensively exercised in favour of plant varieties which had low residual toxic components; for example the lectin content of some cultivated species of Alliaceae is much lower than in wild varieties. Moreover, plants have occasionally been selected because some of their constituents, including the lectins, possess properties beneficial for health (e.g. garlic).

It is clear that the process of selection by man of nutritionally high-quality crop plants has in the past been based on empirical observations. Although this can now be approached by rational and objective experimentation, direct experimental evidence for the involvement of individual food components in the regulation of gut metabolism is generally scanty. Thus, despite the commonly accepted view that the diet has a major effect on health, rigorous criteria for selection are still not readily available. However, because of the pioneering nutritional studies of Werner Jaffé and Irvin E. Liener, plant lectins may provide one of the few exceptions in this respect. Hopefully, from the sometimes dramatic interactions between ingested lectins and the intestinal mucosa and their consequences for gut and body metabolism, it may be possible in future to select and/or genetically engineer plants for their positive contribution to human and animal health.

Many lectins, regardless of whether they are from plants or bacteria, are potent exogenous growth signals and some can also mimic the action of metabolic hormones (1). Their biological activity is a direct consequence of lectin function: through recognition and binding to specific carbohydrates of receptors on surface membranes, they send signals and deliver messages to cells. Since interaction between lectins and cells depends on their specific recognition of membrane glycans, differences in the potency to bind to cells are mainly due to differences in the state of glycosylation of the cell membrane.

One of the main reasons for the extraordinary effectiveness of ingested lectins on the digestive system is that all lectins studied to date are highly resistant to proteolytic breakdown during gut passage (2). This, coupled with their specific and high reactivity with and binding to surface glycosyl residues of the gut epithelium, can induce major changes in the metabolism, turnover and loss of epithelial cells, may damage their luminal membrane, influence their absorptive capacity for both small and large molecules, stimulate shifts in the bacterial flora and interfere with the immune state of the digestive tract. It is now clear that lectins which avidly bind to the brush border are powerful metabolic signals and growth factors for the entire gut, particularly the small intestine, and induce fully reversible, dose- and polyamine-dependent hyperplastic growth. Other lectins for which no suitable glycosyl receptors exist on the villus surface are usually without any effect (2). These pass through the gut and are excreted in the faeces. However, some of these lectins may also reach the luminal surface of crypt cells whose glycosylation is different from that of fully mature enterocytes. If this is compatible with the sugar specificity of the lectin, binding will occur and anti-mitogenic effects may ensue, leading to a reduction in the rate of crypt cell proliferation and a general slowing down of gut metabolism. Accordingly, strongly mitogenic lectins are usually regarded as antinutrients because these can damage the gut at high doses and on continuous exposure, these lectins may possibly act as adjuvants of chemical carcinogens. Despite these negative attributes, mitogenic lectins can also have some beneficial effects because, at low, non-toxic doses, they can be used to stimulate growth in intestinal hypoplasia caused by parenteral feeding, gut resection and other gut lesions. However, anti-mitogenic lectins probably will have more numerous applications because, by reducing the rate of intestinal cell proliferation, they may also slow down the development of tumours of the large bowel. As most of these lectins are specific for mannose, they can serve as excellent and safe blockers of infections by type-1, mannose-sensitive, fimbriated pathogenic bacteria (3).

One of the best-known and most intensively studied dietary lectin is phytohaemagglutinin, PHA, from kidney bean (*Phaseolus vulgaris*). This is a powerful exogenous metabolic signal and growth factor for the gut (for references see 1). Because of its stability and biological activity of avid binding to receptors of endogenous growth factors, hormones and bacteria expressed on the luminal brush border membrane, PHA delivers potent messages to the epithelial cells leading to changes in gene expression and cellular metabolism. Although it is not clear whether these are direct lectin effects or mediated through endogenous growth factors, there is definite evidence for the binding of lectins to cells of jejunal crypts (2). Therefore it is possible that the PHA signal may directly stimulate crypt cell proliferation and that all its dramatic effects on the gut are a consequence of this. As the state of glycosylation in the small intestine is intricately linked to the development and metabolism of its epithelial cells, major changes in the glycosylation of brush border membrane and cytoplasmic glycoconjugates can be expected as a consequence of the powerful physiological growth factor activity of PHA.

In contrast to the dramatic effects of strongly mitogenic lectins on gut metabolism and receptor glycosylation, the effects of non-mitogenic or poorly mitogenic lectins appear to be more subtle. Thus, the lectin of snowdrop (*Galanthus nivalis*) bulbs, GNA a strictly mannose-specific lectin is a particularly interesting example because although it binds to the crypt (2), its effect appears to be the opposite of PHA. By slowing down the rate of crypt cell proliferation, GNA induced a significant decrease in the length and cell numbers of the crypts and therefore its effects on brush border glycosylation were expected to be particularly revealing in comparison with that of PHA or other similar mitogenic lectins.

There is also convincing experimental evidence that, after binding, some lectins are endocytosed by the gut epithelial cells and there they may interact with internal glycan receptors. As a consequence of this additional metabolic signalling, the powerful physiological effects of PHA and other strongly mitogenic lectins are further amplified and extended, leading to increased rates of crypt cell proliferation, speeding up of epithelial turnover and major changes in the glycosylation of membrane glycans (1).

The most important conclusion emerging from recent studies of the effects of dietary lectins on the alimentary tract of higher animals is that the state of glycosylation of membrane

receptors of gut epithelial cells is highly influenced and in some instances dramatically changed by the presence of a number of biologically active components, particularly lectins, occurring in food/feeds (4). As glycosylation is of paramount importance in deciding how effectively growth factors, hormones, lectins and bacteria can bind to these receptors, the entire metabolic state, digestive/absorptive functions, bacterial ecology and health of the gut can be modulated by the diet through its lectin content.

In a recent histochemical study, by using digoxigenin-labelled lectins as histochemical reagents, comprehensive information on the carbohydrate structure of gut surface receptors was obtained, enabling us to define a low resolution membrane glycosylation map of the small intestine of rats exposed to different dietary lectins in the presence or absence gut flora (4). Although the resolution of digoxigenin-lectin staining at the light microscope level was low and fine structural features of epithelial cells could not be unequivocally located, the results gave an overall view of the state of glycosylation in the lumen of the small intestine. The staining clearly illustrated the presence of saccharide structures on the gut wall or in the cytoplasm of the epithelial cells which were available for possible interactions with dietary factors or resident or infecting bacteria.

In general the results confirmed and extended some of the commonly held views on the state of glycosylation of small intestinal brush border membranes and cytoplasmic glycoconjugates of both villi and crypts in weaned rats possessing a conventional microflora and well-fed on high-quality control diets (5-7). Thus, the villous brush border membrane contained mainly complex glycosyl structures and the expression of terminal mannosyl residues in it, characteristic for immature cells, was vanishingly small. Some of the membrane glycoconjugates were also fucosylated and/or α-2,3 sialylated (7). Although α-2,6 sialylation was absent on the brush border membrane or in goblet cells, these saccharide structures were abundant in the cytoplasm.

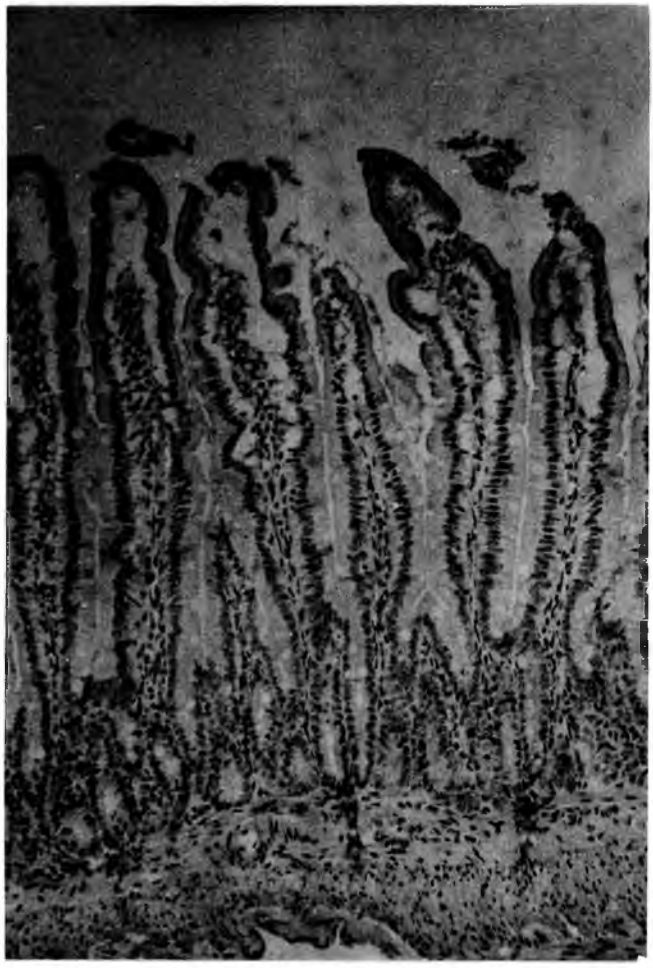
The main and most important finding of this recent study corroborated previous observations and showed conclusively that the state of glycosylation of both membrane and cytoplasmic glycoconjugates was modified by feeding rats on diets containing lectins. Some of these changes were also dependent on the presence or absence of bacteria in the lumen of the gut and, indeed, bacteria themselves could modify the glycosylation of luminal receptors (4). Although the differences between germ-free rats and those possessing a conventional microflora were mainly quantitative, they were obvious in some instances even with the qualitative histological techniques used and in general, the changes in glycosylation caused by lectins were more substantial than those by bacteria.

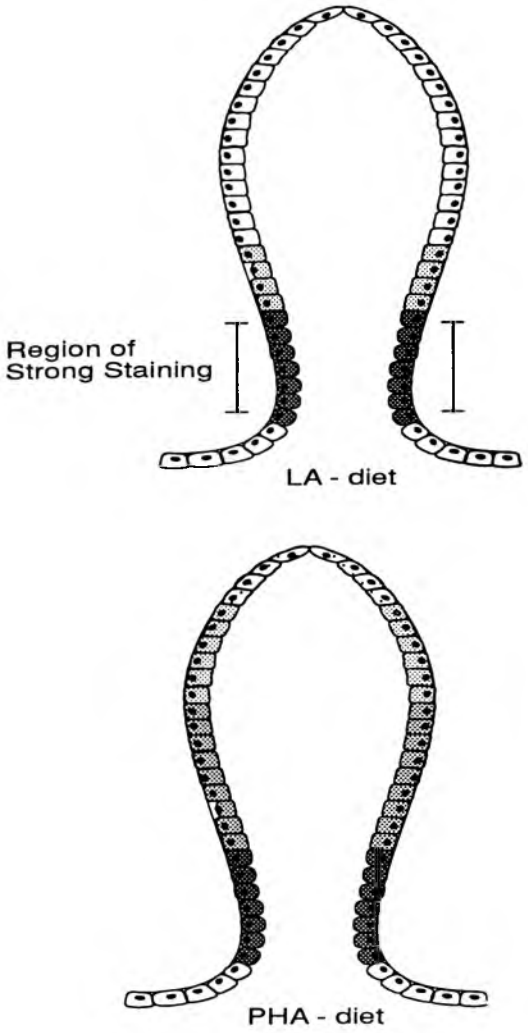
In conclusion, continuous oral exposure of rats to lectins induced major changes in the expression of both membrane and cytoplasmic glycoconjugates altering the structure of terminal saccharides. Some of these were appreciably influenced by the presence or absence of bacteria in the gut lumen. Moreover, some changes in glycosylation also led to major shifts in the bacterial flora of the small intestine such as were found with the dramatic overgrowth of *E. coli* in its lumen after feeding rats on PHA-diets and the successful blocking of this by the dietary administration of GNA (3). However, some of the physiological consequences of the changes induced by lectin exposure are not apparent at present. In general the shifts in glycosylation fall into one of the following categories:

a. *Changes in glycosylation by displacing endogenous ligands.*

Although in healthy rats the brush border membrane of the small intestinal epithelium contains mostly complex glycosyl residues and only few mannosyl terminals, most of these are not shown with the specific histochemical staining for mannose because they are covered by Type-1, mannose-sensitive fimbriated bacteria such as *E. coli* or other endogenous ligands. However, these could be displaced by long-term exposure to diets containing mannose-specific lectins such as GNA, thus revealing the presence of mannosyl terminals whose absence in normal rats is therefore only apparent and not real (Figure 1a, b).

FIGURE 1



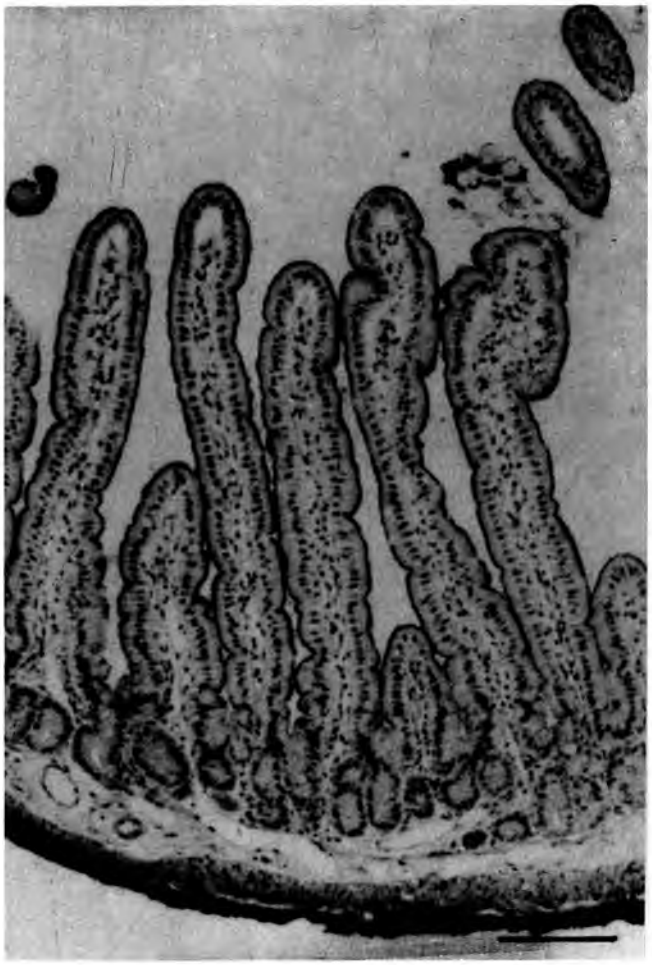


(a) The binding of the *Galanthus nivalis* agglutinin (GNA) is slight on acute exposure but (b) strong binding develops after dietary exposure to this lectin for 10 d indicating the uncovering of mannosyl terminals in epithelial cell membranes. Small intestinal sections were first reacted with monospecific anti-GNA rabbit antibodies, followed by peroxidase-labelled secondary antibodies and development of the colour reaction with 3,3'-diaminobenzidine. Bar = 100 μm.

b. *Changes in glycosylation resulting from increased rate of crypt cell proliferation.*

By speeding up cellular turnover, lectins with powerful growth factor activity can increase the proportion of less differentiated, immature epithelial cells on the villi, with the consequent increase in the concentration of terminally mannosylated cytoplasmic glycoconjugates. Changes in glycosylation can also result from other lectin-stimulated metabolic effects which either increase or decrease the cellular concentration of cytoplasmic and/or membrane glycoconjugates with different terminal glycosyl groups (Figure 2a, b).

FIGURE 2

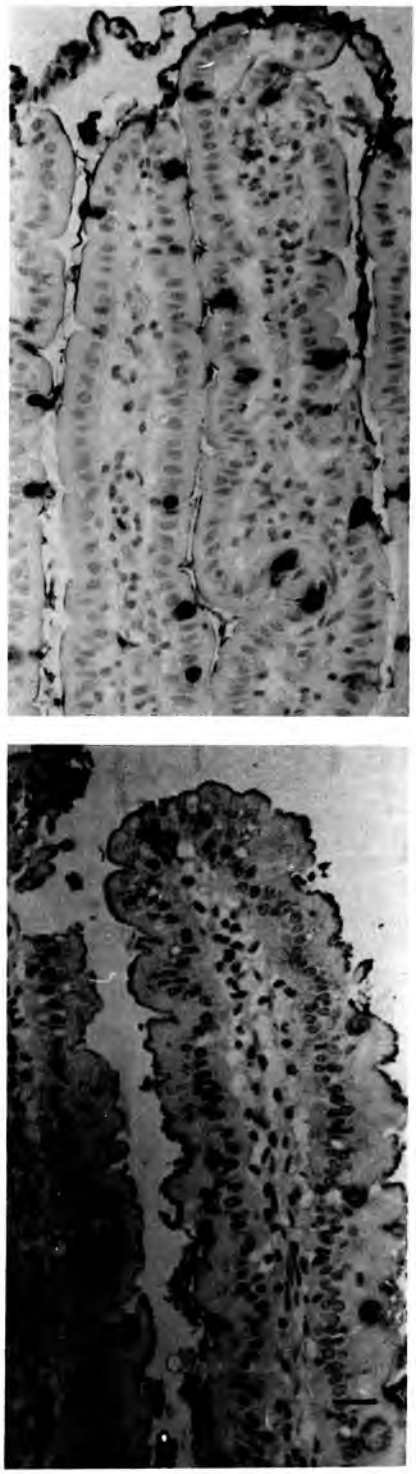


Cartoon of the position of cells on small intestinal villi containing polymannose-type cytoplasmic glycoproteins in rats fed (a) control and (b) PHA-diets. The darker the colour, the more polymannose residues are shown by digoxygenin-labelled GNA.

c. *Changes in glycosylation resulting from the effects of lectins on goblet cells.*

The contents of the goblet cells of both germ-free rats and those with a conventional microflora can nearly be emptied by PHA exposure (Figure 3a, b). In contrast, GNA has the opposite effect. Moreover, exposure to sialic acid-binding lectins can also change the glycosylation of the gut wall by overstimulating and exhausting the mucin synthesizing capacity of goblet cells (2).

FIGURE 3



Staining with digoxigenin-labelled *Aleuria aurentia* agglutinin (specific for α-1,6 fucose) of a section of small intestinal villi of (a) control and (b) PHA-fed rats. There is strong staining of goblet cells in (a) whereas the mucin content of these cells is much depleted in (b). Bar = 25 μm.

Future perspective and practical implications

As dietary lectins induce changes in the glycosylation of brush border membranes or cytoplasmic glycans with the appearance of new or the removal of existing glycosyl structures on the surface of the gut, the binding potential of the small intestinal epithelium for dietary or endogenous factors and bacteria can be radically altered. Since the most critical step in the infection of the gut by bacteria is their attachment to its surface glycosylated receptors through fimbrial- and/or surface adhesins, considerable shifts in the bacterial population of the intestinal tract may be induced by changing the expression of the sugar structures on the luminal surface. Furthermore, as sugar-specificity determines which lectin can bind to the brush border endocrine cells, lectins can also modulate the secretion of gut peptide hormones such as cholecystokinin, gastrin, glucagon and others because, by changing the state of glycosylation of brush border cells, the secretion of these hormones is stimulated or depressed (1,8). By inducing the growth of the gut or modulating the content and/or specific activity of the brush border enzymes in the small intestine or by stimulating the synthesis and secretion of pancreatic enzymes (1,4), lectins can be used to change the physiology and the digestive/absorptive functions of the gut. Some lectins may also be used to stimulate the secretion of mucinous glycoproteins from small intestinal goblet cells. Alternatively, anti-mitotic lectins or incomplete mitogens can be used to slow done or stop unwanted cell proliferation in the gastrointestinal tract. Thus, using dietary lectin(s) it is now possible 'to engineer' the digestive tract for improved performance and bacterial ecology (8). Moreover, as interactions between lectins and gut mucosa are on a solid chemical foundation and predictable, their dietary and medical uses are expected to grow in future.

ACKNOWLEDGEMENTS

The author is a Senior Research Fellow of The Rowett Research Institute and is indebted to The Scottish Office Agriculture and Fisheries Department for financial support.

REFERENCES

1. Pusztai A. Plant Lectins. Cambridge, Cambridge University Press, 1991, p. 1-263.
2. Pusztai A, SWB Ewen, G Grant, WJ Peumans, EJM Van Damme, L Rubio & S Bardocz. 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.
3. Pusztai A, G Grant, RJ Spencer, TJ Duguid, DS Brown, SWB Ewen, WJ Peumans, EJM Van Damme & S Bardocz. Kidney bean lectin-induced *Escherichia coli* overgrowth in the small intestine is blocked by GNA, a mannose-specific lectin. J Appl Bacteriol 75:360-368, 1993.

4. Pusztai A, SWB Ewen, G Grant, WJ Peumans, EJM Van Damme, ME Coates & S Bardocz. Lectins and also bacteria modify the glycosylation of gut surface receptors in the rat. Glycoconjugate J 1995 (In press).
5. Roth J. Subcellular organization of glycosylation in mammalian cells. Biochim Biophys Acta 906:405-436, 1987.
6. Taatjes DJ & J Roth. Glycosylation in intestinal epithelium. Int Rev Cytol 126:135-193, 1991.
7. Roth J. Cellular sialoglycoconjugates: histochemical perspective. Histochem J 25:687-710, 1993.
8. Pusztai A. Dietary lectins are metabolic signals for the gut and modulate immune and hormone functions. Eur J Clin Nutr 47:691-699, 1993.