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MINIREVIEW

Biological Effects of Plant Lectins on the Gastrointestinal Tract:Metabolic Consequences and Applications

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Abstract

Lectins are one of the most important physiologically active ingredients and potent exogeneous biological signals in the diet. Although the amounts of lectins in foodstuffs can vary considerably, they can dramatically affect the entire digestive tract and its bacterial population, body metabolism and health. Their extraordinary effectiveness stems from resistance to gut proteolysis and a high and specific chemical reactivity with endogenous surface receptors of the epithelial cells of the gut of both higher animals and lower organisms. Lectins are powerful oral and parenteral immunogens and some of their physiological effects are intricately linked to interference with immune function. However, the primary effects and the potency of lectins as biological signals are the direct result of their specific chemical reactivity with saccharides. As these reactions are predictable, the use of lectins as blockers of pathogens, immune stimulants, hormone modulators and metabolic agents in clinical-medical applications and as natural insecticides in trans-genic plants, offers great promise.

A. Introduction

Lectins are carbohydrate-binding (glyco)proteins of non-immune origin capable of specific recognition of, and reversible binding to, carbohydrates without altering their covalent structure (1). Ligand-binding which is the first step in the reaction between lectins and carbohydrates is similar for both lectins and carbohydrate-reactive enzymes. However, the subsequent steps are different because, in contrast to the action of enzymes, formation of the lectin-sugar complex is not followed by splitting of chemical bonds in the covalent structure of the carbohydrate ligands nor the dissociation of the complex.

Lectins occur widely in plants (for refs.see 2). Consequently, most plant-based food/feed stuffs contain appreciable amounts of lectins, some with striking biological activities on gut function. Although in general lectins are more resistant to heat-denaturation than other plant proteins, legume lectins can be inactivated by moderately prolonged cooking. However, as heat-processing is expensive and potentially damaging, it is usually kept to a minimum even with legumes, particularly when the product is to be used in animal nutrition. Furthermore, as many non-legume lectins are not denatured by normal heating practices and green vegetables and fruits are usually not cooked, the digestive tract is regularly exposed to biologically active lectins. Moreover, cooking and other heat-treatments are recent inventions of the human civilisation, therefore exposure to dietary lectins must have played an important part in the evolutionary development of alimentary tract of both humans and higher animals.

As lectins react with the surface epithelium of the digestive tract, they can cause antinutritional, mild allergic or other subclinical effects in higher animals and humans, particularly when consumed in large quantities (2). The importance of understanding of the precise mechanism of these diet-gut interactions and the ensuing effects on metabolism is self-evident. However, studies of the effects of lectins on the gut have revealed that oral administration of low doses of lectins can also have many beneficial effects on the digestive/absorptive efficiency of the gut, its immune system and

bacterial ecology and that, by modulating the secretion of gut hormones, some lectins can influence the body's endocrine system with beneficial consequences for general metabolism (3). It is becoming clear that with these potentially wide range of applications, lectins may provide one of the practical and most natural means for improving both the nutritional value and safety of the diet.

The realisation that lectins may have many potential uses has been given an added impetus by the recent discovery that some lectins are far more toxic for insects than for higher animals(4). It is therefore possible that some crops are naturally resistant to pests because their constituent lectin interacts strongly with the surface glycosyl groups of the less highly differentiated cells of the insect digestive tract. This may then lead to potent inhibition of the process of food assimilation in the insect gut with severe consequences for their survival and reproduction. Thus, some lectins are regarded as natural insecticides. Moreover, with the advent of genetic engineering it is now possible to transfer lectin genes into plants which do not normally possess these and therefore protect important and sensitive crop plants against harmful pests without using chemical insecticides and pesticides (4).

B. Lectin - Gut Interactions

B-1. Resistance of Plant Lectins to Proteolytic Degradation

Dietary proteins are rapidly degraded during passage through the gut by digestive enzymes in the small bowel. Any residual undigested matter is then degraded by bacteria in the large intestine and used as sources of energy. In contrast to dietary proteins, lectins resist degradation in the small intestine (5) and are also resistant to breakdown by most gut bacteria. Thus, as most lectins survive at least in part the passage through the digestive tract in an immunologically and functionally intact form (Table I), they can exert their potent biological activities in vivo. Similar to the enhanced stability of hormones and growth factors bound to receptors, lectins are also protected by receptor-binding. However, this is not necessary as shown by the almost complete survival of the agglutinin from *Galanthus nivalis*, snowdrop bulbs (GNA) which does not bind to the gut wall on acute oral exposure suggesting that resistance may also result from particular features of the molecular structure (6).

Table I. Survival and binding of pure lectins to the small intestinal mucosa*.

Lectins	Specificity	Binding	Recovery (%)
PHA (*Phaseolus vulgaris*)	Complex	+++	>90
Con A (*Canavalia ensiformis*)	Man/Glc	+	>90
GNA (*Galanthus nivalis*)	Man	-	>90
SNA-I (*Sambucus nigra*)	α-2,6-neuraminyl-Gal	+	50–60
SNA-II " "	GalNAc	+++	>60
SBA (*Glycine max*)	GalNAc/Gal	++	40–50
LEL (*Lycopersicon esculentum*)	GlcNAc	+	40–50
WGA (*Triticum vulgare*)	GlcNAc	++	50–60
PSL (*Pisum sativum*)	Man/Glc	±	30–40
VFL (*Vicia faba*)	Man/Glc	±	20–30
DGL (*Dioclea grandiflora*)	Man/Glc	±	18–20

Rats were intragastrically intubated with 10 mg of individual lectins. The amounts of lectin surviving in the stomach and small intestine were estimated from luminal washings and supernatants of the tissues homogenized with 0.1M solution of the appropriate specific carbohydrate in phosphate-buffered saline, pH 7.6. The strength of binding is marked on an arbitrary scale: +++ strong binding and - represents no binding at all.
*From ref. no. 5.

As part of the normal turnover of the gut epithelium, cells are shed from the villus tips into the lumen and most cellular material is then digested and recycled. The presence of lectins attached to these cells does not interfere with the breakdown of cell contents but the liberated lectin can move further down the gut and bind to the next receptor with an appropriate carbohydrate moiety. Although lectin-binding is most frequently studied in the small intestine, similar binding can occur throughout the entire digestive tract, from the stomach to the distal colon. However, as surface glycosylation varies in the different functional parts of the gut, lectin-binding is not uniform in the digestive tract. Thus, when a particular glycosyl group is absent from the surface of the small bowel but present on the large intestinal epithelium, specific lectin-binding will almost exclusively be to the wall of the colon and vice versa. Accordingly, with the appropriate choice of lectins, it is possible to selectively affect the metabolism of different parts

of the gut.

As some lectins are partially heat-stable and most remain active during the passage through the gut, interactions between lectins in the diet and the gut can occur, occasionally with dramatic consequences. Although it is known from animal studies that lectins can damage the gut and this may lead to various nutritional disorders (for refs., see 2), it is not generally appreciated that this only occurs at relatively high lectin concentrations in the diet. However, lectins can also have beneficial effects particularly at low dietary inclusions, and these may find uses in medical/clinical practice (3).

B-2. Carbohydrate Structures on the Surface of the Gut

The surface epithelium of the gut is extensively glycosylated (7-10) mainly because most membrane proteins including hormone and growth factor receptors, transport proteins and brush-border enzymes are glycosylated before being embedded in the brush-border membrane.

Additionally, membrane lipids and gangliosides are also glycosylated, and all secreted mucins are carbohydrate-rich glycoproteins. Accordingly, the scope of potential lectin-gut interactions is wide. However, for these reactions to occur it is necessary that the correct carbohydrate structures should be present on the surface of the gut mucosa, i.e. the one which the lectin is specific for. Thus, despite extensive glycosylation of the surface of the alimentary tract, not all lectins react with the epithelium and even those which react vary in their ability to recognise and bind to specific types of carbohydrate receptors. Moreover, the structure of glycosyl side-chains depends on many factors even within one species, such as the age of the animal, its blood group specificity, genetics, the mucosal cell types and their state of differentiation/maturation and position on the crypt/villus axis and along the gastrointestinal tract (Table II). For example, it is believed that the glycosyl side-chains of membrane glycoproteins of the less differentiated crypt cells are usually of the polymannose type, whereas the fully mature cells on the villi express complex glycosyl side-chains.

Table II. Factors influencing membrane glycosylation in the intestines.

* Animal species
* Blood group specificity
* Age
* Site in the intestines
* Position along crypt - villus axis
* Stem cell genetics
* State of differentiation and maturation
* Diet
* Bacterial status
* Pathology

The epithelium of the small bowel is composed of a monolayer of epithelial enterocytes whose main function is to digest and absorb the nutrients. These cells are interspersed with minor cell types: mainly goblet cells producing mucins, and enteroendocrine cells, which are responsible for the synthesis of gut peptide hormones. The small intestinal epithelium is organized into two functionally and morphologically distinct compartments: the crypts, where stem cells proliferate and differentiate, and the villi, where these differentiated cells mature while migrating toward the tip of the villi. During migration along the crypt-villus axis, there is a continuous change in the cellular membrane; its protein composition, the pattern and activity of the enzymes expressed in it and the state of glycosyl-ation of its components go through distinct phases of development. Finally, after the mature cells have reached the apical area of the villus, they are extruded into the lumen.

As all cell-surface proteins have to be transported from the site of synthesis to the plasma membrane, they have to go through several steps of glycosylation. However, the pattern of glycosylation of the membrane glycoconjugates varies in different species. Indeed, this great variability in membrane glycosylation may help to explain why lectins differ in their ability to interact with the gut surface (6). Unfortunately, at present, quantitative information on the precise carbohydrate structure of the gut surface receptors, their location along the crypt-villus axis and the changes occurring during normal turnover or under different conditions of age and dietary status, is limited. However, the epithelium of the intestinal tract has one of the highest cell turnover rates in the body which enables it to react rapidly to dietary changes. Moreover, surface morphology of the gut and changes in its receptors follow and can truly reflect the causes which initiated these changes. Indeed, mapping the lectin receptor sites by lectin-histochemistry and/or determination of their lectin-binding capacity can give valuable information on the functional state of the gut.

B-3. Lectin-induced Changes in Cell Metabolism

As surface membrane receptors of cells are glycosy-lated, lectins are good mimics of the effects of endogenous growth factors, hormones and cytokines in all types of cells (for ref., see 2). Receptor proteins are usually composed of more than one subunit. The signal molecules bind to the subunits exposed on the external side of the membrane and these are glycosylated. The other subunit(s) span the membrane, and these are responsible for the transmission of the signal message and the activation of the second messenger system(s). Depending on their position, the sugar-structures can be present on or near to the active centre of the receptor. Although the lectin binding site (the glycosyl side chains) is clearly not the normal functional binding site of the receptor, the resulting conformational change in the receptor subunits embedded in the membrane and the ensuing signal transduction may be similar irrespective of whether the activation was by the physiological ligand or the lectin. In this case the lectin can mimic the effect of the natural ligand and induce similar physiological reactions. However, it is also possible that the bound lectin may not induce a conformational change but, by physically blocking the active site of the receptor, attenuate or completely abolish the physiological effect of the natural ligand. Some of the so-called non- or anti-mitogenic lectins probably fall into this category. Finally, lectin-binding to the external receptor subunits may also additively or synergistically reinforce the effects of the natural ligand by an allosteric mechanism.

B-4. Interaction with the Brush Border Membrane

For a lectin to exert its biological activity, the carbohydrate structure which it is specific for, must be present and free on the gut surface. The interaction between them can be best described by a key-lock analogy. If both the lectin (key) and receptor (lock) are present, the binding is immediate and the lectin can open the lock and send messages into the cell via second messengers or by the lectin itself entering the cell. Either way, the lectin can influence the metabolic activity of gut epithelial cells or, for that matter, any other cell.

Recognition between lectins and receptors is instantaneous. However, the strength of the binding is dependent on the association constant between the lectin and the glycosyl group and the number of unoccupied receptor sites. If there are many carbohydrate side-chains with the `right' sugar structure, the lectin will bind extensively and cross-link them. With only few sites, particularly when they are well-separated, only weak or no binding occurs. Moreover, lectins which avidly bind to epithelial cells are also readily endocytosed and transcytosed although the signals necessary for these processes are not well understood (6).

Lectins which bind avidly to the brush border membrane are potent hyperplastic growth factors for the gut (2, 5). Indeed, lectin-binding to the epithelium is obligatory for growth stimulation and their growth factor activity is determined mainly by the strength and intensity of their binding (6). Thus, the kidney bean (*Phaseolus vulgaris*) agglutinin, PHA, is one of the most avidly binding lectins and also one of the most potent intestinal growth factors (5, 6, 11). Although during this growth the length of the villus is rarely affected significantly, the size of the crypts, the number of cells they contain and the crypt cell production rate (CCPR) are substantially increased and with continuous exposure to PHA, the cell turnover time can decrease from 72 to 12h. However, while during the hyperplastic growth cell migration on the villi speeds up, the time taken for cellular differentiation remains unchanged. Consequently, there is an increase in the proportion of immature cells on the villi whose protein and enzyme patterns are typical of the immature cell type. This is clearly shown by the reduction in the amounts/activity of maturation marker enzymes, such as diamine oxidase (12) or sucrase-isomaltase and alkaline phosphatase in gut tissues (unpublished). As a result, the

capacity of cells to digest/absorb nutrients is reduced.

With other lectins similar changes may occur. However, these are dependent on the strength of lectin-binding which correlates well with their effectiveness as growth factors. Even when binding is relatively weak, lectins can disturb the organization of the epithelial membrane by cross-linking surface receptors, and induce slight gut growth.

In adult rats there are few free terminal mannose residues on the brush-border membranes (7), therefore, on acute exposure the binding of the mannose-specific GNA to the jejunal epithelium is slight and no growth occurs. Interestingly, Concanavalin A (Con A) from jack bean (*Canavalia ensiformis*), which has a similar specificity reacts somewhat more strongly than GNA, but due to patchy binding there is only slight growth (7). In contrast, as on the luminal surface mainly complex glycosyl groups are expressed, PHA-binding is extensive. Due to the presence of N-acetylglucosamine in hybrid glycosyl moieties in brush border enterocytes, wheat (*Triticum vulgare*) germ agglutinin (WGA) also binds well and is endocytosed (7, 13), particularly at mid villus, though this also occurs lower down on the villi.

B-5. Binding and Endocytosis by the Stomach and the Intestines

Although studies in model systems have indicated that reaction between most lectins and their specific ligands is abolished at acid pH (pH 3 or less), lectins can apparently bind to the stomach epithelium *in vivo* even when the luminal pH is 3 or less (14, 15). This may, in part, explain why the presence of lectins in the diet slows down stomach-emptying in rats.

Binding of lectins and their endocytosis by enterocytes occurs throughout the gut although endocytosis in the small intestine becomes appreciable only in the presence of large numbers of commensal bacteria. As expected, endocytosis of lectins is more extensive in the colon where bacterial counts are high (14). Thus, lectins may be particularly suited to serve as site-directed targeting agents for delivering drugs to the colonic epithelium (16).

B-6. Effects of Plant Lectins on the Gut Immune System

Type-1, immediate hypersensitivity reactions by the gut immune system occur even to highly degradable food proteins. Allergic reactions to the more stable lectins which persistently bind to brush border cells can be more extensive (for refs., see 2). Thus, the anaphylactic response of the gut to PHA, as assessed from the leakage of ^{125}I-labelled serum proteins into the gut of rats given a single intragastric dose of lectin, is appreciable even on first exposure. This increased vascular permeability is not IgE-mediated but due to the direct degranulation of submucosal mast cells by PHA (17) and cross-linking of membrane glycans by the multivalent PHA. However, the allergic response was elevated in rats pre-fed on PHA-diets, suggesting that it was amplified by the formation of anti-PHA IgE (17).

C. Modification of Epithelial Cell Glycosylation by Lectins

Depending on the specificity of the lectin and the strength of its binding, three main types of lectin-induced changes were observed in the glycosylation of membrane and/or cytoplasmic glycoproteins of epithelial cells of the small intestine.

C-1. Effect of Increased Crypt Cell Production Rate (CCPR) on Glycosylation

After exposure to lectins which bind avidly to and are endocytosed extensively in the small intestine, the pattern of glycosylation of brush-border cells becomes distinctly different from that of fully mature apical cells in control rats. In particular large amounts of polymannosyl groups are exposed on both membrane and cytoplasmic glycoconjugates, readily detectable by histological staining with GNA-digoxigenin (7). It is possible that with the lectin-induced increase in CCPR and the correspondingly shortened transit time, the less differentiated crypt cells, which are highly polymannosylated, penetrate further up the villus than they would under normal physiological conditions. The more reactive the lectins are, the shorter the transit time becomes, with the result that there are more immature cells on the epithelial surface which contain more poly-mannosyl side-chains in the cellular glycoproteins. Accordingly, the most effective growth-stimulating lectins are also the most powerful agents for inducing this type of change in carbohydrate

receptor expression of the gut epithelium.

C-2. Reaction of Lectins with Secreted Glycoproteins

Lectins which react with sialic acid in secreted glycoproteins, such as those from the bark of the elder tree (*Sambuccus nigra*), SNA-I, and *Maackia amurensis*, MAA, can induce changes in receptor glycosylation by overstimulating and exhausting the capacity of goblet cells to synthesise mucin (7). The action of these lectins results in the partial disappearance of mucin `receptor sites' containing neuraminyl-alpha2,6-lactose and neuraminyl-alpha2,3-lactose glycosyl structures respectively, and this may leave the luminal surface partially uncovered. Thus, the reactivity of the intestinal epithelium with SNA-I or MAA disappears almost completely after extended dietary exposure to these lectins. As the binding of soya bean (*Glycine max*) agglutinin, SBA, to glycans with terminal sialic acid is relatively weak, the extent of its binding is increased after the removal of mucins containing terminal sialic acid.

C-3. Displacement of Endogenous Ligands by Dietary Lectins

By displacing endogenous ligands bound to glycosyl moieties of luminal receptors, lectin can change the availability of glycosyl groups on the membrane. Thus, GNA-binding by the small intestinal brush-border is essentially nil in rats with a conventional microflora but is significant in germ-free animals (7). This may reflect the fact that *Escherichia coli* or other mannose-sensitive fimbriated bacteria can block the limited number of surface mannosyl groups but these groups are free in the absence of bacteria in the lumen (6, 7). However, when lectin concentration in the lumen is high during feeding rats diets containing GNA, the newly emerging epithelial cells may bind the GNA in preference to the bacteria.

C-4. Nutritional Penalty of Growth Stimulation

The hyperplastic growth of the gut has a nutritional penalty for the animal, since the need to renew the gut surface more quickly than normal means that more of the dietary protein and energy are used up to maintain the faster turnover (18). However, this may be offset by the gain in absorption efficiency of the fresh gut surface after the removal of the lectin from the diet. With lectins which are powerful growth factors for the small bowel, the cost in nutritional terms can be appreciable. At very high dietary intakes of these lectins, most or all of the diet is used by the gut alone with the result that other organs are starved of nutrients. However, as in practice the dietary intake of lectins is low, their growth stimulating activity has no measurably negative effect on nutritional performance.

D. Effect of Lectins on Gut Bacteria

It has recently been demonstrated that the orally administered lectins which are generally regarded as toxic are not deleterious per se but that most of their harmful consequences are due to their interaction with gut bacteria (19-24). Although PHA and other so-called "toxic" lectins bind just as extensively to the epithelium and stimulate the growth of the small intestine of germ-free rats as they do in rats carrying a conventional microflora, the consequences of the binding are essentially harmless for the germ-free rats (2, 19, 20) . The deleterious effects of the consumption of large amounts of PHA by animals with a normal microbial flora are probably due to the stimulation of a selective bacterial overgrowth in the small intestine by the lectin. This may be exacerbated by the increased endocytosis by the epithelial cells of PHA and possibly the bacteria and/or their toxic metabolites or toxins. After transcytosis, these toxic substances may enter the blood circulation and exert their deleterious effects by stopping vital body functions. They may also interfere with the body's hormone balance and disturb normal metabolic reactions.

D-1. Direct Interaction of Lectins with Gut Bacteria

Lectins can directly interact with and agglutinate some resident bacteria of the digestive tract and then selectively remove these from the lumen. Therefore, diets which contain these lectins can change the bacterial ecology of the gut. However, lectins can also interfere with the attachment of bacteria to the brush border by speeding up the turnover of epithelial cells and, by changing receptor glycosylation, effectively remove potential attachment sites for particular bacteria.

D-2. Lectin-induced Coliform Overgrowth in the Small Intestine

The stability of the bacterial flora in the small intestine of healthy, well-fed rats was clearly demonstrated by our inability to orally infect them with Type-1, mannose-sensitive fimbriated E. coli isolated from their own gut without first changing their diet (24). Even after six days of oral exposure of the rats to broth containing 10^8 and 10^9 viable *E. coli* ml^{-1}, coliform counts remained at control levels, 10^3 to 10^4, in the whole small intestine. In contrast, a highly significant, dose-dependent and damaging but fully reversible overgrowth of *E. coli* occurred in the small bowel of rats given PHA in the diet (Table III). The source of E. coli for this selective overgrowth was the commensal flora. Although at high doses of PHA there was also a slight increase in some non-lactose-fermenting coliforms (mainly *Proteus spp*.), counts of *Lactobacillus spp*. and Bacteroides were essentially unchanged even at the highest PHA concentrations. The proliferating E. coli was avidly bound to the small intestinal glycocalix and could not be removed by simple washing of small intestinal sections unless mannose was used in the washing buffer. This provides convincing experimental evidence for the involvement of bacterial lectins in the adhesion of bacteria to the small intestinal epithelium.

Similar overgrowth of the small intestine by commensal, Type-1, mannose-sensitive fimbriated *E. coli* can be induced by other avidly binding lectins, such as SBA, WGA or Robinia lectin, although to achieve the same effect their amounts have to be increased to compensate for the lower avidity of their binding.

Table III. PHA Dose-dependence of *E. coli* overgrowth in the rat small intestine.

Dose of PHA mg/rat/day	Log_{10} counts of *E. coli*	
	Washed	Unwashed
24	-	4.8×10^{4}
28	-	5.9×10^{5}
44	-	5.5×10^{5}
50	-	6.9×10^{6}
54	-	6.8×10^{6}
58	1.0×10^{7}	6.6×10^{6}
66	0.9×10^{8}	1.0×10^{9}
72	9.0×10^{8}	9.0×10^{8}
78	-	2.0×10^{8}
80	-	1.0×10^{8}
98	-	3.2×10^{7}

Morphological studies have confirmed that *E. coli* overgrowth in the small intestine occurs only with lectins which are potent growth factors. As explained above, in healthy rats the villus epithelium is populated mainly by highly differentiated, mature enterocytes expressing complex saccharide groups and only few terminal a-linked mannose residues on membrane- and cytoplasmic glycoconjugates. Thus, in the normal rat small intestine the residual population of Type-1, mannose-sensitive fimbriated *E. coli* is low. This is confirmed by the near absence of binding of GNA but a high reactivity with PHA. However, after PHA-induced overgrowth of *E. coli*, alpha-linked terminal mannosyl groups become numerous as shown by strong reaction with GNA (7, 24) and, as the brush-border can now provide receptors for the bacteria, selective proliferation ensues. The overgrowth is reversible because the mannosyl residues disappear within three days of the removal of the PHA from the diet.

Lectin-induced coliform overgrowth is not confined to laboratory conditions. Injuries to the small intestinal epithelium automatically stimulate its hyperplastic growth to make good the damage regardless of whether it is caused by dietary or bacterial lectins or other erosive factors or diseases. Under these conditions the proportion of immature cells expressing polymannosylated membrane and cytoplasmic glycans increase inevitably leading to coliform overgrowth. The resulting diarrhoea and other digestive/absorptive problems usually exacerbate the initial gut damage and, when combined with poor nutritional status such as in children from Third World countries, may lead to serious and wide-ranging health problems.

D-3. Prevention of Colonization of the Gut by Pathogens Using Dietary Lectins (Chemical Probiosis)

Bacterial overgrowth in the small intestine is a major cause of ill-health and of production loss in farm animals. Control by antibiotics is not always possible because this may give rise to the development of antibiotic-resistance. Moreover, the methods proposed for the reduction of harmful bacteria in the small intestine by administration of sugars or complex carbohydrates are somewhat ineffective. In contrast, lectins such as GNA and possibly other non-toxic lectins are highly effective in inhibiting the overgrowth of Type-1 *E. coli* in the rat small intestine (Table IV). However, this can only happen if the sugar- specificity of the blocker lectin is complementary to that of the bacterial

Table IV. Microbiological analysis of the small intestine (jejunum) of rats treated with PHA (kidney bean lectin) in the presence or absence of GNA (snowdrop bulb lectin) and appropriate controls*.

	Treatment groups					
	LA (control)	GNA+PHA	PHA	GNA	PHA→LA	SED
Log_{10} Bacterial counts g^{-1} wet tissue						
Lactose fermentor coliforms	3.3^a	6.5^b	8.7^c	3.1^a	3.3^a	0.9
Non lactose-fermentor coliforms	2.8^a	4.8^b	5.8^c	2.8^a	2.7^a	0.6
Lactobacilli	4.2^a	6.4^b	7.4^c	4.7^a	2.8^d	1.0

Groups (n = 12) of rats were fed on different diets: LA, control lactalbumin for 6 d; GNA+PHA, GNA incorporated into control diet for 6 d and intubated with PHA for the last 3 d; PHA, control diet for 6 d and intubated with PHA for the last 3 d; GNA, GNA incorporated into control diet for 6 d and PHA→LA, control diet for 6 d and intubated with PHA for the first 3 d. Groups with different superscripts in horizontal rows are significantly different (at least $P<0.05$). *From ref. no 24.

adhesin (24).

Non-toxic lectins are good candidates to replace antibiotics because they can be administered with the diet and therefore may provide the most natural means for the control of pathogenic infection. Thus, lectin research and exploration of their ability to block bacterial adherence in the gut (Chemical probiosis) may ultimately lead to the discovery of strategies for the prevention of intestinal disease and improvements in food safety.

E. Systemic Effect of Lectins

Lectins may influence systemic metabolism by two different but possibly simultaneous mechanisms (for refs., see 2). Lectins can indirectly influence the endocrine system of the body by binding to the neuroendocrine cells of the gut and

stimulating the secretion of gut peptide hormones into the systemic circulation. Alternatively, lectins can be transmitted through the gut wall into the blood circulation and thus may directly influence peripheral tissues and body metabolism by mimicking the effects of endocrine hormones. The organs most often affected are the pancreas, skeletal muscle, liver, kidneys and thymus (25).

E-1. Oral Immunization

Although most food proteins are rapidly degraded in the small intestine, nutritionally insignificant amounts are absorbed systemically through M cells of the gut-associated lymphoid tissue and presented by macrophages to competent lymphocytes of the immune system (for refs., see 2). Allergic responses to these absorbed proteins in adults are minimized by T suppressor cells and therefore harmful effects seldom occur and only in susceptible individuals. In contrast, as luminal levels of the more stable lectins are high, their transport through the gut wall is appreciable. Thus, PHA is a powerful oral immunogen and produces a high titre of monospecific anti-PHA antibody of IgG-type in animals, including ruminants (26, 27). There is, in fact, some evidence that most lectins given orally are immunogenic (28). The recent finding of high titre anti-banana lectin (BanLec-1) IgG_4 antibodies in pooled human blood further supported this suggestion (29).

The time-course of antibody development follows the normal course of immunization with a primary response by ten days after the first oral dose. Further oral exposure results in booster effects. Thus, the putative gut anti-lectin-s-IgA system must be ineffective since it cannot prevent the absorption of PHA (2). This abrogation of s-IgA response to PHA may remove one of the major obstacles to the repeated use of lectin-targeted oral drug conjugates.

Lectins can modulate IgE responses to other antigens. Thus, although both Con-A and PHA affect the synthesis of anti-ovalbumin IgE in mouse, Con-A enhances it under most conditions(30), while the effect of PHA depends on the time of injection (31). In DBA/2 mice parenterally immunised with ovalbumin, oral sensitisation to jacalin (jackfruit, *Artocarpus heterophyllus*, lectin) increased IgE responses to both jacalin and ovalbumin in a time- and dose-dependent way. This suggested that jacalin is a mitogenic immunomodulator for the production of IgE but not IgG_1 antibodies against these two antigens (32). Thus, the use of lectins to reduce or abolish IgE responses to allergens without affecting the synthesis of IgG- type humoral antibodies, promises well for the future treatment of allergies.

E-2. Effects of Dietary Lectins on the Pancreas

Most dietary lectins produce trophic changes within the exocrine pancreas which parallel the growth of the small intestine (Table V). The best known example is PHA, which is a growth factor for both the gut and pancreas. The trophic effect of lectins on the pancreas is mainly mediated through cholecystokinin (CCK), a gut peptide hormone which is released by PHA action from duodenal enteroendocrine cells. The growth of the pancreas is intimately involved with polyamine metabolism in the tissue (Table V). However, the mechanism of lectin-induced, polyamine-dependent pancreatic growth appears to involve a route of hormonal mediation which is, at least in part, different from that caused by soya trypsin inhibitors despite that both are mediated by cholecystokinin (33).

Table V. Effect of lectins and trypsin inhibitor on the pancreas*.

	Weight	Acinar area	Polyamines
PHA	145	156	140
RPA; Robina	143	n.d.	126
SNA-II; Elder	136	150	140
SBA; Soya	132	145	153
WGA; Wheat germ	118	n.d.	114
DSA; Datura	111	n.d.	101
UDA; Nettle	98	n.d.	94
SBA-I; Elder	132	132	133
MAA; Maackia	132	132	106
STI; Trypsin inhibitor	135	151	160

The weight, acinar area of true tranverse pancreatic sections (stained with haematoxylin and eosin, perimeter traced by computer-linked pixel planimetry and measured by a Joyce-Loebl 'Magiscan' image analyser) and polyamine content of pancreas of rats fed with diets containing 0.7% lectins or trypsin inhibitor are expressed relative to those of the controls (taken as 100%).*From ref. no 33.

As secretion of digestive enzymes by the pancreas is severely reduced in chronic pancreatitis, it is possible that the administration of low doses of non-toxic lectins which stimulate pancreatic hypertrophy may find use in clinical practice. This is because lectins in contrast to trypsin inhibitors, do not inhibit pancreatic proteases and, therefore, the secreted enzymes remain active and contribute to the digestion of nutrients in the small intestine.

E-3. Effects on Hormone Balance

PHA affects the endocrine pancreas and interferes with insulin secretion and thus changes the hormone balance of the body (33). SBA is also a growth factor for pancreas, but as it does not affect circulating insulin levels, its action is confined to the exocrine pancreas.

Most of the effects of lectins on systemic metabolism are mediated through changes in insulin levels. Thus, insulin concentration is depressed in rats fed on kidney bean diets or intubated with pure PHA but the absence of changes in blood glucose indicates that the animals are not diabetic (34). As a single acute oral dose of PHA also leads to a significant but transient decrease in blood insulin, this reduction is not due to inadequate nutrition but probably is a direct effect of the lectin on insulin synthesis and/or secretion by the pancreas.

To compensate for the depression of blood insulin levels, various homeostatic processes are activated by PHA, including changes in other hormone levels such as changes in glucagon (2) and glucocorticoid levels. It is therefore clear that PHA does not only modulate insulin levels but can also induce complex changes in hormone balance of the body.

It is generally believed that eating legume proteins is beneficial for human health because they appear to reduce the concentration of plasma and body lipids. Although it is not clear whether lectins are involved in this process, it is known that PHA and other lectins (2, 35) are potent lipolytic agents.

E-4. Effects on Peripheral Organs and Tissues

Skeletal muscle: PHA and several other lectins have long been known to mimic most of the *in vitro* biological effects of insulin (36, 37). PHA also binds to the insulin receptor of muscle cells (2), but unlike insulin, it does not stimulate protein synthesis in this tissue. At very high oral intakes of PHA (over 0.5 g kg-1 body weight) this results in a loss of about 30% of skeletal muscle in rats fed kidney bean diets for ten days (25). One of the main causes of muscle catabolism is the reduction of the fractional rate of protein synthesis which occurs without a similar decrease in protein degradation rate, thus leading to a net loss of muscle protein (38). These results suggest that the muscle atrophy is the consequence of the abrogation of the stimulatory effect of insulin on muscle protein synthesis because the receptor sites for insulin are blocked by PHA. However, muscle wastage is not as extensive as could be expected from the low blood insulin concentration, because a parallel compensatory upregulation of the mRNA of the insulin-receptor and insulin-sensitive glucose transporter by the dietary PHA increases the efficiency of insulin-receptor interaction (39). Despite these compensatory changes, large amounts of nutrients and polyamines are released from the atrophying muscles and are then used to support the growth of the gut (40).

Thymus, spleen and other organs: At high intake of antinutrient lectins (2,25) the thymus and spleen undergo atrophic changes. Some of these are irreversible with potentially serious consequences for the immune system, especially T cell-mediated immunity.

Similar to skeletal muscle, lectins may also affect the heart by reducing its weight and the rate of protein synthesis (38). Some lectins cause a slight reduction in liver weight which is mainly due to losses in lipids and glycogen after ten days of oral exposure to PHA (2). The kidneys are also slightly enlarged in these rats. However, this effect is non-specific as other forms of protein-deprivation lead to similar enlargement. Although these lectin-effects have been observed with animals, it is expected that some of them may also apply to humans.

F. Perspectives of the Use Lectins (Genes) as Natural Insecticides in Transgenic Plants

Lectins appear to have a dual role in the plant; they can serve as convenient forms of nitrogen storage and may also fulfil a defensive-protective function. It is realised that their main function may be to protect plants against pests and, indeed, convincing evidence exists that lectin function and plant protection are correlated.

It is obvious that an individual lectin of defined carbohydrate specificity cannot defend plants against all potential predators because the glycosyl structures expressed on the external surfaces of the different bacteria, fungi, insects and mammals are usually different. Fortunately, as most pests are host-specific to some extent, it is possible, at least theoretically, for a plant during evolution to acquire lectins with the "right" carbohydrate specificities. However, to be effective, the plant will also need different strategies against each predator and this will depend on both the pest and the plant. To be successful against bacteria and fungi, the lectin will have to be able to stop their attack at an early stage of infection before they can penetrate deeply into plant tissues. This is usually achieved by binding the bacterial

or fungal cell wall to the lectin on the surface of the plant. The best examples of these are the chitin-binding proteins of plant tissues, including chitin-binding lectins which, by inhibiting spore germination and hyphal growth, can protect the plants. However, the nature of the interactions between plants and insect and/or higher animal predators is more complex and requires a thorough understanding of the structure and function of the digestive tract of the predator. Animals will first have to eat the plant and it is the consequent harmful effects of the diet which will discourage the predator to go on with the eating (4). However, this type of passive protection has serious consequences for the entire food chain as lectins can also be harmful for the human and higher animal consumers. Lectins protect the plant against insects because, by interacting with surface saccharides of cell membrane glycoconjugates in the insect gut, they interfere with food assimilation and metabolism in the pest. However, if the lectin reacts in a similar fashion with the mammalian gut epithelium, it is likely that similarly antinutritive or occasionally toxic effects will follow. Thus, there is an inextricable link between lectin-mediated protection of plants against predators and the potential nutritional problems of prospective mammalian consumers of the plants.

In conclusion, transgenic research is now focussing on the identification of lectin genes which are suitable for transfer into plants to enhance their resistance towards insect and nematode pests, but have minimum impact on non-target, beneficial organisms, the environment and livestock feeding on these plants, and which present no health risks for humans either directly or indirectly the food chain.

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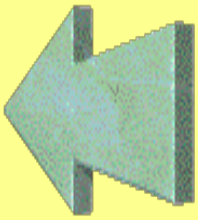

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