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Toxicology of Plant Lectins

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I. INTRODUCTION

The presence of heat-labile toxic factors in plant products, mainly legume seeds, makes them unsuitable for human consumption unless they are properly cooked. Among the antinutritional factors that are inactivated by cooking are the lectins or phytohemagglutinins (PHA), which are subject of this chapter.

Lectins were discovered in the last century when Stillmark (1889) presented evidence that the extreme toxicity of castor beans (*Ricinus communis*) could be attributed to a protein fraction which agglutinated red blood cells and which he called "ricin." Ehrlich (1891) compared it with the toxic abrin from the jeriquinty seeds (*Abrus precatorus*) and thus initiated his immunological research on the specificity of antisera obtained by the use of these lectins. This research meant the initiation of many fundamental advances in the knowledge of immunological reactions (Ford, 1913; Lis and Sharon, 1977; Jaffé, 1977; Sharon, 1989).

Landsteiner and Raubitscheck (1908) observed that extracts of many edible crude legume seeds would likewise agglutinate red blood cells, but no toxic action was detected at that time. Probably this was due to the relative low toxicity as compared to the very high activity of the above-mentioned lectins.

Today the widespread occurrence of lectins in plant and animal tissues is well known (Gold and Balding, 1975; Goldstein and Hayes, 1978). Lectins have been reported in more than 800 plant species (Lis and Sharon, 1973).

II. GENERAL PROPERTIES

The term "lectin," derived from the Latin *legere* to choose, was proposed by Boyd and Reguera (1949) to indicate the selectivity of their interaction with specific blood cells. Etzler (1985) discusses the various definitions of lectins. Besides the polyvalent, agglutinating protein molecules, the definition should also include the monovalent ones, which bind reversibly to complex carbohydrates in cell surfaces without clumping and are structurally and genetically related to agglutinating lectins.

Lectins possess specific affinity for certain carbohydrate residues. Since sugars are structural features of most animal cell membranes, lectins may attach to these receptor groups, if the specific structure of the latter fits the

former. As this name indicates, hemagglutinins can be characterized and detected by their activities on red blood cells, while other cells can be agglutinated as well. In many cases the binding may occur without visible clumping. The receptor site of the cell surface must exist in an exposed position in order to be able to react with a specific lectin. For detecting hemagglutination in some cells like cow red blood cells (RBC), this may be achieved by partial proteolysis. Trypsin and pronase are often used for this purpose.

Hemagglutination is only one of the numerous detectable consequences of the lectin-receptor interaction. They play a key role in the control of various normal and pathological processes in living organisms. In addition to plants, lectins are commonly present in animal and bacterial cells (Sharon and Lis, 1989).

III. WHAT RENDERS FOOD TOXIC?

The definition of toxins is especially critical when it is applied to food. A snake may be toxic or nontoxic—the bite is a single definite event. Food is ingested daily, and with time harmful effects may accumulate and produce undesirable consequences for the health of the consumer.

Cultures and tradition have given way to habits that are strongly influenced by etiological factors—the food available in a certain environment. The availability of limited choices of food products may have obscured hidden health damage.

The definition of toxicity for a food ingredient is therefore a rather subtle problem. Many normal food ingredients can cause harm when consumed in high amounts. Saturated fats, salt, and cholesterol are well-known examples. Practically any food if consumed in excess may be harmful, but it will not be considered toxic.

Toxicology is concerned with biological effects characterized by distinct dose-response relationships. A substance may be lethal beyond a certain level of intake. The toxicity may be assessed in terms of an LD_{50}, which is the dose that causes the death of 50% of the tested animals. Tests of toxicity are normally performed with laboratory or farm animals. Unfortunately, animals and humans do not react equally to many stress situations like an intoxication.

Lack of exact toxicological evidence of natural foodstuffs or ingredients is generally taken as evidence for safety. The term "generally recognized as safe" (GRAS) expresses this belief and is applied to food legislation (WHO, 1967). It is accepted that there must exist some finite level below which no food compound is toxic. The definition of what this level may be has great practical importance. It may vary according to the physiological condition of the consumer, age, sex, etc. Some of the many plant lectins are known to be sources of hazard; the implications of their prolonged consumption are, however, unknown. Some lectins may kill experimental animals when ingested in sufficient amounts or may interfere with the bioavailability of nutrients. The term "antinutritional" is usually used for this situation (Liener, 1983).

Antinutritional activity of a compound or food may remain unrecognized under conditions of a normal and varied diet, but may exert harmful effects

under stress situations, malabsorption syndromes, malnutrition, etc. It is therefore surprising that very little research has been done on "toxicological insignificance" (Goldberg, 1970). The fact that a food ingredient has been consumed for generations without apparent harm is certainly no proof that it is safe under any condition. Control groups are never available for comparison, like populations with identical consumption habits with the only difference, for example, that one consumes tomatoes and the other does not.

No animal species consumes foods as varied as humans do. The great variability probably has resulted in a certain adaptation to the digestion and absorption of different food components (Waterlow, 1990). It is only a guess whether such a capacity for adaptation may also exist for the ingestion of antinutritional factors.

In this chapter, we shall therefore mention some lectins of common foods for which no toxic activity has been demonstrated.

IV. LECTINS IN FOODS

Plant lectins have been found in a great variety of constituents of human diets. A thorough survey on the presence of lectins in commonly consumed foods in the United States was reported by Nachbar and Oppenheim (1980). They tested 88 food items with respect to their ability to agglutinate human erythrocytes of three blood groups, natural and enzyme treated, and also to clump several bacterial strains. Positive results were found in 29 food extracts. Moreover, they list literature on 53 edible plants in which phytohemagglutinins have been identified. Great variations in agglutinating activity were observed in the same food items purchased from different stores or on different days, a fact that may obscure the detection of positive samples. It can be concluded that human exposure to lectins is a widespread event notwithstanding the fact that cooking and contact with digestive enzymes may blunt or abrogate activity. Andersen and Ebbesen (1986) detected lectin activity in 40 of the 75 foods tested. Several papers review the nutritional significance of lectins (Liener, 1976, 1978, 1983, 1986; Jaffé, 1980, 1983; McPherson, 1989).

Liener (1989) in his latest comprehensive review points out the fact that legumes and some cereal grains of primary concern in human nutrition are rich in phytoagglutinins. He also includes a list of edible plants in which lectins have been detected. A slightly modified version is presented in Table 1.

Legumes are an important ingredient of the diets of most developing countries (Aykroyd and Doughty, 1964). In the future an increased consumption can be anticipated as a cheap and nutritious source of protein. Already, cereal–bean mixtures, which can be prepared locally in rural areas, are recommended as weaning food (King et al., 1966).

Following the observation that some legume lectins distinguish between different human and animal blood groups, a great number of extracts from this vegetable family were tested for agglutination (Krüpe, 1956; Makela, 1957; Tobiska, 1964). Toms and Western (1971) list nearly 500 legume species and varieties, the seeds of which show some blood agglutinating activity. It must be

Table 1 Edible Plants That Contain Lectins

CEREAL GRAINS			
Avena sativa	Oats	*Secale cereale*	Rye
Hordeum vulgare	Barley	*Triticale* spp.	Triticale
Oryza sativum	Rice	*Triticum vulgare*	Wheat
Sorghum bicolor	Sorghum	*Amaranthus caudatus*	Amaranth
		Zea mays	Corn
VEGETABLES			
Abelmoschus esculentus	Okra	*Cucurbita maxima*	Pumpkin
Apium graveolens	Celery	*Cucurbita sativus*	Cucumber
Asparagus officinalis	Asparagus	*Ipomea batatas*	Sweet potato
Beta vulgaris	Swiss chard	*Lactuca scariole*	Prickly lettuce
Brassica camapestris rapa	Turnip/Beet	*Lycopersicon esculentum*	Tomato
Brassica napobrassica	Rutabaga	*Medicago sativum*	Alfalfa
Capsicum annum	Sweet pepper	*Petrosecinum hortense*	Parsley
Solanum tuberosum	Potato	*Rheum rhapontium*	Rhubarb
FRUITS			
Carica papaya	Papaya	*Malus species*	Apple
Citrus aurantium	Orange	*Musa paradisiaca*	Banana
Citrullus vulgaris	Watermelon	*Prunus americana*	Plum
Citrus medica	Grapefruit	*Prunus avium bigarreaus*	Cherry
Cucumis melo cantalupensis	Cantaloupe	*Ribees rubrum*	Currant
Cydonia oblonja	Quince	*Rubus idacus*	Raspberry
Fragaria versa	Strawberry	*Rubus fructicosus*	Blackberry
SPICES			
Allium sativum	Garlic	*Myristica fragrans*	Nutmeg
Labiacea origanum	Marjoram	*Menta piperita*	Peppermint
Pimienta officialis	Allspice		
OTHERS			
Agaricus bisporus	Mushroom	*Juglans regia*	Walnut
Carum carvi	Caraway seeds	*Helianthus annus*	Sunflower seeds
Cocos nucifera	Coconut	*Sesamum indicum*	Sesame seeds
Coffee arabica	Coffee	*Theobroma cacao*	Cocoa
Corylus avellania	Hazelnut		

remembered that negative results may be due to the fact that the right blood type was not used.

In agglutination studies of 54 species of legumes on 18 different blood types, only 6 samples gave positive results with trypsin-treated cow erythrocytes. However, these RBC are the best for detection of the toxic bean varieties (Casotto et al., 1984).

V. ANIMAL GROWTH ASSAYS

Lectin toxicity is usually evaluated by injection or by oral ingestion. The latter is obviously of primary concern in relation to the corresponding plant products as food. Most toxic lectins are more or less heat labile. Therefore, cooking of lectin-containing food products will have a beneficial effect on the nutritive value. Despite this apparent association, one cannot assume that this effect can be attributed solely to the inactivation of toxic lectins. Enzyme inhibitors, other thermolabile compounds, and increase of digestibility may also be responsible.

In order to distinguish the effect of trypsin inhibitors, amino acid deficiency, and toxic lectins in beans, the addition of predigested casein (casitone) and methionine to the experimental diets for growing rats has been useful (Jaffé and Vega-Lette, 1968). Poor growth performance of rats consuming beans with low lectin activity could be restored to normal with this procedure. In contrast, animals given a diet of toxic beans did not respond with growth improvement. They grew well, however, on a diet of autoclaved beans supplemented with methionine. The oral toxicity of common beans (*Phaseolus vulgaris*) was recognized by Everson and Heckert (1944), Jaffé (1949), Jaffé et al. (1955), Jaffé and Gaede (1959), Honavar et al. (1962), Wagh et al. (1963), DeMuelenaere (1964, 1965), Evans et al. (1973), and confirmed by Pusztai et al. (1975), Bender and Reaidi (1982), Lojolo and Mancini (1988), and others.

The fact that purified bean lectin is highly toxic for rats was demonstrated by adding it to an experimental diet. It produces growth inhibition and will kill the animals within a few days, depending on the level and origin of the lectin as shown in Table 2 (Honavar et al., 1962). The opposite experiment with delectinized bean diet showed disappearance of toxicity as measured by normal growth of animals (Pusztai and Palmer, 1977).

VI. DIGESTIBILITY OF LECTINS

In order to exert toxic or antinutritional actions, lectins must resist digestion, in cooked foods be thermo-stable, and interact with the brush-border membrane of the intestinal mucosa. In the case of raw toxic beans given to experimental animals, the hemagglutinating activity and the specific precipitation of thyroglobulin can easily be detected in extracts of feces. These results indicate that at least part of the ingested lectins have resisted digestion (Jaffé and Vega-Lette, 1968). Similar observations have been reported for winged bean agglutinin (Higuchi et al., 1989).

In vitro experiments with pepsin and trypsin corroborate the resistance toward digestion of these proteins from beans (Jaffé and Hannig, 1965). Soybean lectins, on the other hand, have been found to be digested by gastrointestinal enzymes (Liener, 1958). Very little is known about many other lectins that may be present in our daily diet, as in cucumbers, melons, and other fruits that are eaten raw.

Tomato lectin resists digestion by rats. It can be detected bound to intestinal villi and in the feces of the experimental animals after consumption of a lectin-containing diet. ^{125}I-Labeled tomato lectin was found in human blood,

Table 2 Effect of Purified Hemagglutinin Fractions from the Black Bean and Kidney Bean on Growth of Rats

Source of hemagglutinin	Purified hemagglutinin in diet (%)	Gain in weight (g/day)	Mortality[a] (days)
Black bean	0	+2.51	
	0.5	+1.04	
	0.5[b]	+2.37	
	0.75	+0.20	
	1.2	–0.91	15–19
	2.3	–1.61	12–17
	4.6	–1.72	5–7
Kidney bean	0	+2.31	
	0.5	–0.60	13–16
	0.5[b]	+2.29	
	1.0	–0.87	11–13
	1.5	–1.22	4–7

[a]100% mortality observed during the period recorded. Blank space indicates no deaths observed.
[b]Solution of hemagglutinin boiled for 30 min and dried coagulum was fed at level indicated. Hemagglutinating activity was completely destroyed by this treatment.
Source: Honavar et al., 1962.

indicating that it also resists digestive breakdown in man (Kilpatrick et al., 1985). Nevertheless, tomatoes are often consumed raw, and no antinutritional effects have been reported, as far as we are aware.

VII. EFFECT OF HEAT TREATMENT

Cooking of beans does not necessarily destroy their toxic properties. Autoclaving at 118°C for 30 min of beans previously soaked in water for 2–3 h abolished toxicity completely (Jaffé and Vega-Lette, 1968). Contradictory opinions have been reported in the literature on whether soaking prior to cooking is essential for the improvement of the nutritional quality of beans. Pack et al. (1978) concluded that soaking was not necessary for detoxification of bean meal and whole beans. Overnight soaking was applied by Lowgren and Liener (1986) in their bean-cooking tests. Soaking alone caused a 10–15% loss of hemagglutinating activity. No significant advantage was observed by Kakade and Evans (1966) or by El-Nahry et al. (1977) for attaining complete elimination of agglutinating and toxic properties. These differences may be due to the so-called "hard-to-cook" phenomenon (Jackson and Varriano-Marston, 1981), a consequence of poor drying and storage conditions of seeds used in the assays.

The effect of cooking at relatively low temperatures, as in so-called "slow cookers" or "crockpots" on the hemagglutinating action of kidney beans, was studied by Lowgren and Liener (1986). As most previous works had been conducted at fixed temperatures, these authors determined the disappearance

of the agglutinating activity, taking into account the lag of temperature rise and the rate of heat penetration into the bulk of the beans in the slow cooking vessel. After 5 h of heating, when the temperature had almost reached 80°C, over 90% inactivation was achieved, while heating to 100°C for 20 min abolished hemagglutinin activity completely.

Coffey et al. (1985) applied a sensitive electronic cell counter for measuring the hemagglutinating activity in slowly cooked beans. They observed some activity after 12 h of heating at 82°C. (This is the boiling temperature of water in Mexico City.) Moreover, energy shortage is an economic reason for applying slow cooking. Addition of sodium bicarbonate to cooking water shortens cooking time.

Dry heating had little effect on hemagglutinating activity of certain varieties of *P. vulgaris*, and some activity was still detectable after several hours of heating (DeMuelenaere, 1964). Heintze (1950) found dry heating or high frequency treatment ineffective for the cooking of beans. However, the heating of soaked beans was fully effective. Too much heating reduces the growth induced by bean diets, probably due to the impairment of the nutritive value of the proteins (Kakade and Evans, 1965).

Another procedure to prepare edible pulses is sprouting or germination, which may lower, but does not eliminate, lectin activity (Chen et al., 1977; Nielsen and Liener, 1988).

VIII. DIFFERENCES IN LECTIN CONTENT, TYPE, AND TOXICITY IN COMMON BEANS

Large differences in toxicity of lectins have been observed. Compared with the extremely toxic ricin, the LD_{50} of the toxic bean lectin is about 1/1000 times smaller. Some bean cultivars and lectins are known to be nontoxic (Jaffé and Gomez, 1975; Pusztai et al., 1979). This may be due to the lectin concentration in the seed or to the nature or type of lectins present.

In relation to specificity, there are at least four clearly distinguishable groups of bean lectins (Jaffé et al., 1972). The most abundant type A will agglutinate both rabbit and trypsinized cow blood cells. Type B is more active on rabbit than on cow blood. Type C is specific for cow blood, and type D does not agglutinate either but will agglutinate pronase-treated hamster blood (Table 3).

When extracts of the different bean types were tested by injection into mice (Jaffé et al., 1972) or by feeding the ground seeds to rats (Jaffé and Vega-Lette, 1968), it was established that only the A- and C-type beans were toxic, whereas types B and D were of low toxicity. Only the toxic beans showed mitogenic activity on human leucocytes (Jaffé et al., 1972; Jaffé, 1973) (Table 4).

Some samples of cow blood are inactive, probably due to different blood group specificities. Pronase-activated hamster blood is very sensitive to all types of bean lectins and several others, too.

Lectins of the kidney bean seed groups show remarkable differences when submitted to heat treatment. The toxic lectins which agglutinate trypsinized cow blood cells (A and C) will not lose either this ability or its toxicity after 90

Table 3 Hemagglutinating Activity of Extracts of Beans on Different Blood Samples[a]

	Bean cultivar							
	Saxa (A)		Cubagua (B)		Porillo (C)		Mountan. half runner (D)	
Type of blood	nat.	pron.[b]	nat.	pron.[b]	nat.	pron.[b]	nat.	pron.[b]
Rabbit	8	11	9	11	0	2	0	2
Cow[c]	0	12	0	2	0	12	0	1
Swine	11	12	9	12	0	0	0	0
Mouse	6	12	3	8	3	7	1	5
Rat	6	11	2	8	0	9	0	4
Hamster	8	12	7	11	6	12	3	7
Cat	12	12	5	12	0	5	0	4
Cock	6	12	6	11	0	9	0	3
Sheep	4	12	3	6	2	7	0	1
Human blood group AB	6	12	6	12	0	4	0	3
Human blood group O	6	12	6	12	0	3	0	3

[a]The highest dilution step of the crude seed extracts (1 g of ground seeds + 10 ml physiological saline) producing visible agglutination within 1 h is indicated. Only 12 dilution steps were tested.
[b]Pronase-treated RBC.
[c]Cow RBC were treated with trypsin, instead of pronase.
Source: Jaffé et al., 1972.
(A), (B), (C), (D) refer to the four lectin specificity groups.

min heating at 80°C. Under these conditions the activity toward rabbit blood is strongly diminished or completely abolished (Brucher et al., 1969; Jaffé et al., 1972). This observation should be an alert that the results of an in vitro agglutinin test must be interpreted with caution when used as evidence of the persistence for toxicity (Table 5).

The existence of these different lectin types has been confirmed by several authors. Lima et al. (1980) studied 16 Brazilian bean varieties. They found that those active as agglutinins of rabbit and trypsinized cow RBC and as mitogens were toxic, although some nontoxic samples had mitogenic activity. Mancini and Lajolo (1981) established the existence of four bean lectin types similar to those described by Jaffé et al. (1972). Koehler et al. (1986) screened 24 samples of American beans and found three of the four lectin types. The toxic lectins were detected by hemagglutination of trypsinized cow blood and also by SDS electrophoresis. Pusztai et al. (1979) found 11 of 13 cultivars of kidney beans to be active in hemagglutination and toxicity, while 2 low-lectin bean varieties had no appreciable effect on the intestine and were nontoxic in rats. Extensive variation in bean lectin types has also been observed by two-dimensional electrophoretic patterns (Brown et al., 1982). Lectin-free bean cultivars were described by Osborn et al., 1985).

Table 4 Correlation of Specific Hemagglutinating Activity with Intraperitoneal Toxicity in Mice of Extracts of Different Varieties and Cultivars of *P. vulgaris* and Their Mitogenic Action

Variety	Rabbit blood	Trypsinated cows' blood	Toxicity (no. of injected mice/ no. dead mice)	Mitogenic activity
Balin de albenga (A)	+	+	5/4	+
Merida (A)	+	+	9/9	+
Negro Nicoya (A)	+	+	5/4	+
Saxa (A)	+	+	5/5	+
Peruvita (B)	+	–	5/0	–
Palleritos (B)	+	–	6/0	–
Juli (B)	+	–	5/0	–
Cubagua (B)	+	–	5/0	–
Porillo (C)	–	+	5/5	+
Negra No. 584 (C)	–	+	5/3	+
Vainica Saavega (C)	–	+	10/6	+
Hallado (D)	–	–	5/0	–
Madrileno (D)	–	–	5/0	–
Alabaster (D)	–	–	5/0	–
Triguito (D)	–	–	6/0	–

Source: Jaffé et al., 1974.

Table 5 Hemagglutinating Activity of Extracts of Different Bean Cultivars to Rabbit and Trypsin-Treated Cows' Red Blood Cells Before and After Heating at 80°C for 90 min

	Agglutination titer			
	before heating		after heating	
Bean cultivar	rabbit	cow	rabbit	cow
Saxa (A)	9	11	4	11
Balin de albenga (A)	9	10	5	10
Guateian (A)	11	9	2	9
Juli (B)	8	3	1	0
Cubagua subline (B)	9	2	0	0
San Fernando (B)	8	3	0	0
Porillo (C)	0	9	0	9
Ecuador 4A (C)	0	11	0	11
Rabuda (C)	0	9	0	9
Mountaneer half runner (D)	0	0	0	0
Great northern (D)	0	0	0	0
Kaiser Wilhelm (D)	0	0	0	0

The highest dilution step producing visible agglutination in 1 h is indicated. The extracts were heated in presence of the ground beans used for extraction.
Source: Jaffé, 1972.

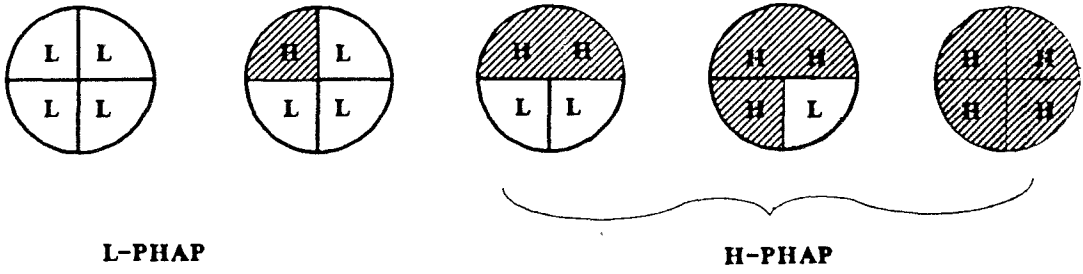

Figure 1 Tetrameric structures of bean agglutinins. L = leukoagglutinating mitogenic unit. H = hemagglutinating unit.

Field fertilization can effect the lectin content of beans (Tobiska and Lhotecka-Brazdova, 1960). It was also shown that the seeds of one bean variety grown and harvested under various environmental conditions differed in lectin activity (Fernandez et al., 1981).

The hemagglutinating and mitogenic activities have been explained by the tetrameric structure of bean lectins. They have been separated by dissociation in 8.0 M urea (Rigas and Head, 1969). Two different subunits, which can produce five combinations, have been recognized. One of the subunits has high affinity for lymphocyte membrane receptors but low or no affinity for rabbit and human RBC (Allen et al., 1969; Miller et al., 1973, 1975). This so-called leukoagglutinin has been isolated from commercial phytohemagglutinin by Weber et al. (1972). It will agglutinate trypsin-treated cow RBC and pronase-treated hamster cells (Jaffé et al., 1974) (Fig. 1).

IX. SOYBEANS

Soybeans and garden beans aroused the most interest in relation to the toxicity of the lectins. Osborne and Mendel (1917) observed the growth-promoting effect of heating on soybean meal, which pointed to the presence of heat-labile antinutritional compounds in these seeds. Among these, protease inhibitors and lectins have been studied most. Liener (1953) presented evidence that the growth inhibition observed in rats on a raw soybean diet could not fully be explained by the trypsin inhibitors. When purified soya lectin was added to the heated soybean meal diet, some growth depression was apparent. This could be attributed to decreased food intake of the experimental animals.

Soybean proteins may be freed of lectin by affinity chromatography. This delectinized material did not support better growth than the original lectin-containing extract (Turner and Liener, 1975a). Soybean lectin is digested by pepsin (Liener, 1958). This may account for the little, if any, direct effect on the nutritional properties of soybean proteins.

When injected into rats, the isolated lectins resulted toxic (Liener and Pallansch, 1952). Grant et al. (1987a) reported toxic manifestation in rats when 0.75% of soybean lectin were added to the diet, an amount higher than

would be provided by a diet with 10% protein derived from soy, which is commonly used.

Loss of brush-border enterocytes, atrophy of intestinal villi, and other toxic effects in rats fed soybean lectin were described by Jindal et al. (1984). Growth was, however, not significantly impaired in the 28-day assay. In our laboratory it was observed that mice are more susceptible than rats to the ingestion of raw soybean meal and that hemagglutination can be detected in the feces of the corresponding animals.

X. WINGED BEANS

Winged beans (*Psophocarpus tetragonolobus*) are legumes of nutritional importance in Asian-tropical countries, where seeds, roots, and immature pods are commonly consumed. Diets prepared with 10% protein derived from winged beans are toxic for rats. The experimental animals will not survive for more than 10 days. Autoclaved beans are nontoxic. Their extracts will agglutinate rabbit erythrocytes (Jaffé and Korte, 1976).

Higuchi and Iwai (1985) purified winged-bean lectin by affinity chromatography. Kortt and Caldwell (1987) observed two distinct lectins in the roots of winged beans. They were essentially identical to the ones found in the seeds.

XI. FIELD BEANS

Ground field beans (*Dolichos lablab*) as part of rat diet reduced growth significantly and caused the death of the experimental animals (Jaffé, 1950). Autoclaving, but not germination, abolished toxicity (Salgarkar and Sohonie, 1965).

The isolated lectin from field beans is toxic when injected into rats (Manage et al., 1972). When fed at a level of 2.5% in the diet, it inhibits normal growth of rats (Salgarkar and Sohonie, 1965).

XII. JACK BEAN SEEDS

The nutritional value of jack bean (*Canavalia ensiformis*) meal improves markedly with heat treatment. It contains several antinutritional factors, such as trypsin and amylase inhibitors, canavanin—an arginine analog—canalin, the lectin concanavalin A (ConA), and canatoxin.

Although ConA can easily be isolated from the meal extract by affinity chromatography, much more is known about its structure than about its toxicity. Similar to other legume lectins, ConA can interact and damage cells lining the gastrointestinal tract (Weaver and Bailey, 1987).

A toxic protein named canatoxin has been isolated from jack bean seeds and separated from the hemagglutinating ConA. It is considered to be a monovalent or hemi-lectin. The presence of similar proteins has been demonstrated in several other legume seeds by immunological methods (Carlini et al., 1988).

XIII. OTHER LEGUME LECTINS

Salgarkar and Sohonie (1965) also isolated lectins from horse gram (*Dolichos biflorus*). Manage et al. (1972) observed retarded growth in rats fed this lectin, which was nontoxic when injected into rats and mice. These results contrast those obtained by the above authors with lima bean (*Phaseolus lunatus*) lectins, which were growth inhibiting when fed and toxic when injected. Levy et al. (1985) fed diets prepared with raw or autoclaved lima beans and garden beans. The diet of raw garden beans was lethal within 1 week; the raw lima bean diet did not support any growth in 28 days but did not cause death. Both permitted normal growth performance after heat treatment.

The nutritive value of peas (*Pisum sativum*) measured in rats is not enhanced by heat treatment (Schneider and Miller, 1954). The isolated lectin is slightly toxic when injected into rats (Manage et al., 1972).

Peanut lectin has been used for fractionation of thymocyte subpopulations into mature and immature cells (Reisner et al., 1976). No toxic action of this phytoagglutinin has been reported.

XIV. CEREAL LECTINS

In cereal grains such as oats, barley, rice, rye, sorghum, and wheat, lectins are present mainly in the germ (Stinissen et al., 1983; Peumans, 1985; Liener, 1989). Wheat germ agglutinin survives the digestive process of the human body and can reach the colon in biologically intact form (Brady et al., 1978). Lectin activity is also present in endosperm and can be traced to albumin, globulin, zein, and glutelin fractions. Some of the endosperm lectins are heat stable (Newburg and Concon, 1985). It has been proposed that the pathogenesis of celiac disease, an intolerance toward wheat products, is related to lectinlike activity of wheat gliadin (Kottgen et al., 1983). Nevertheless Colyer et al. (1986) did not observe any agglutination of normal and celiac human enterocytes or mammalian erythrocytes. The authors suggest, therefore, that in celiac disease there is probably no interaction of gliadin with intestinal cells in polyvalent lectinlike manner.

Lectin activity in rice is concentrated in the germ fraction and is heat labile (Barber and Barber, 1978).

XV. ANTINUTRITIONAL AND TOXIC ACTIVITIES

Based on evidence available in the 1950s, Jaffé (1960) proposed that a possible explanation for the toxic action of lectins, resistant to gastric and intestinal digestion, is that they combine with cells lining the intestinal wall, causing lesions and nonspecific interference with the absorption of nutrients. Since then several groups of researchers have produced direct evidence to substantiate the fact that bean lectins interact specifically with intestinal epithelium cells (Etzler and Brandstator, 1974), damage, and even kill them both in vivo and in vitro (King et al., 1980; Sotello et al., 1980; Pusztai et al., 1981). Etzler

(1984) described the specific lectin receptors (carbohydrate residues) on the small intestinal cell surface for several plant agglutinins.

Since surface-bound lectins are able to produce profound physiological effects on the cells, with which they interact, their capacity to absorb nutrients from the gut may be impaired, causing growth inhibition and even death. Rats switched from a toxic bean diet to a commercial diet recovered weight quickly, but histological studies showed severe ulceration and necrosis of intestinal villi in the surviving animals (Oliveira et al., 1989). The rapid loss of body weight may be explained by the observed doubling of the mucosal protein synthesis rate, which uses up large amounts of N and thus starves the body of its amino acid supply (Pusztai, 1987).

In addition, dietary lectins are specifically taken up into the systemic circulation of rats, either through the disrupted intestinal wall or by breaching the gut absorptive barrier. The concentration of toxic bean PHA in blood is higher than that of nontoxic tomato agglutinin. Up to 10% of bean lectin was detected in blood vs. 0.1% of the tomato lectin (Pusztai, 1989).

The latter, taken up at a lower level of the intestinal villi by endocytosis than PHA, is retained in the liver and detoxified. The former can act on various organs of the body, carried by the blood circulation (A. Pusztai and S. Bardocz, personal communication, 1991).

Dietary exposure to lectins or toxic lectin-containing beans produces polyspecific antibodies against the major bean proteins in rats (Grant et al., 1985), pigs (Begbie and King, 1985), and steers (Williams et al., 1984/85). The binding of PHA to the membrane receptors and its endocytosis by epithelial cells of the small intestine stimulates cellular metabolism, protein, and glycoprotein synthesis and increases DNA and RNA content. This hyperplasia is accompanied by an elevated polyamine concentration. PHA may utilize similar polyamine-dependent pathways as used by mucosal growth-stimulating signals during adaptive growth (Pusztai et al., 1988).

Consumption of kidney beans induces depletion of lipid and glycogen reserves, loss of protein, and reduction of circulating insulin. The interaction of the absorbed lectins on the pancreas could have suppressed insulin secretion and caused some of the catabolic changes observed. Several hormonal and metabolic changes were also detected in soybean-fed rats (Grant et al., 1987b).

Grant et al. (1985) conclude that the toxicity of *Phaseolus vulgaris* lectins are independent of age or maturity of the experimental animals after pair feeding rats aged between 30 and 123 days with bean diets. No significant differences between age groups could be detected in protein utilization, food intake, disruption of intestinal lining, or circulating antilectin antibodies.

In vivo studies using ConA in neonatal guinea pigs show mucosal damage in stomach, crypt cells, and villi. A substantial increase in urinary lactulose: mannitol excretion ratio, indicative of a loss of mucosal integrity, was detected. According to the authors, these findings may "have implications for the pathogenesis of childhood celiac disease" (Weaver and Bailey, 1987).

ConA binding disturbs the functional formation of brush-border response to dietary alterations (Nakata and Kimura, 1986). Similar effects of the brush-border cells were observed for the wing bean lectin (Kimura et al., 1986).

ConA given to rats at a level of 0.3 and 0.5% in the diet prevented normal adaptive changes of sucrase, alkaline phosphatase, and amino-peptidase (Nakata and Kimura, 1985).

According to Rouanet et al. (1982), of several intestinal hydrolases only alkaline phosphatase is not inhibited by bean lectin in vitro. However, in pair-fed rats receiving diets with and without PHA, the total activity of intestinal hydrolases decreases in similar proportion (Rouanet et al., 1985). Jindal et al. (1984) found loss of brush-border cells and atrophy of the villi in albino rats fed soybean lectins. They also observed a decrease in both disaccharidases and proteases, but report an increase in alkaline and acid phosphatases and Ca^{2+}-ATPase.

Lectins were shown to interact with human intestinal hydrolases (Triadou and Audran, 1983). Purified brush-border aminopeptidase and dipetidylpeptidase recognize sepharose-bound bean lectins (Erickson and Kim, 1983).

The in vivo binding of navy bean lectins to the brush-border cells decreases the rate of glucose absorption to one half of its normal value (Donatucci et al., 1987). These results definitely confirm the observations reported by Jaffé et al. (1955) and Jaffé and Camejo (1961) on the effect of kidney bean lectin on intestinal absorption.

In vitro studies revealed noncompetitive inhibition of pancreatic and salivary amylases by red kidney bean lectins. Thompson and Gabon (1987) therefore suggest that lectins may play a role in reducing the rate of starch digestion.

The oral toxicity of raw kidney beans in rats involves, in addition to hypertrophy of the pancreas, due in part to trypsin inhibitors, atrophy of the thymus and doubling in weight of the small intestine. Changes in tissue composition include protein, carbohydrate, and DNA increases. Mineral content, urea concentration, and some enzyme activities in serum and urine were also changed (Greer et al., 1985).

On the mammary gland level, ConA was shown to specifically inhibit milk secretion in minced tissue (Patton et al., 1980). The cobalamine transport to liver and kidney in vivo was inhibited by ConA. It affected the binding of the transcobalamin II-cobalamin to its receptor, interfering with the tissue uptake of the vitamin (Ramasamy et al., 1990).

Despite the considerable body of evidence on the harmful effects of the ingestion of active lectins, it has nevertheless been suggested that they may serve to protect against colon cancer by causing hypersecretion of intestinal mucus or by exerting direct toxic effect on tumor cells (Freed and Green, 1975). Indeed many lectins agglutinate and damage neoplastic cells in preference to their normal equivalents (Raedler and Raedler, 1985).

Another toxic effect in animals, observed upon ingestion of lectin-containing diets, is the decrease of food intake. Donatucci et al. (1987) and Rouanet et al. (1985) describe the phenomenon in rats, Leon et al. (1991) in chicks, Williams et al. (1984/85) in fast-growing steers, and Begbie and King (1985) in pigs. The mechanism of the above effect is still unknown. Its remarkable feature is its velocity, for food intake decreases within 30 min after ingestion of lectin diet (Leon et al., 1991).

The real importance of these findings is still obscure. It is obvious, though, that lectins from legumes may damage the intestinal structures and interfere with their normal functions.

XVI. BACTERIOLOGICAL CONSEQUENCES OF LECTIN INGESTION

Germ-free animals are better able to tolerate lectin-containing diets than conventional ones. An overgrowth or colonization of coliform bacteria has been observed in the small intestines of experimental animals. Banwell et al. (1985) found that, in addition to structural damage, the mucous layer of the jejunal, ileal, and cecal surfaces were heavily colonized by bacteria and protozoa in weanling rats fed 0.5% PHA in the diet.

There is a clear increase in lectin toxicity in conventional quail (Jayne-Williams and Burgess, 1974), chicks (Hewitt et al., 1973), and rats (Rattray et al., 1974) as compared to germ-free ones. The binding of lectins to the cells lining the intestine may interfere with their defense mechanism, which prevents the normally innocuous bacteria from passing from the lumen to lymph, blood, and other animal tissues. The ulcerative disruption of the gut may also have allowed bacteria to invade tissues.

Several lectins clump some bacteria specifically. They may act on adherence of bacteria to the intestinal wall. *Salmonella typhimurium* adherence is promoted by ConA but not by the lectins from beans, groundnut, and wheat germ. This activity may enhance bacterial colonization and infection (Abud et al., 1989).

XVII. HUMAN INTOXICATION

Several cases of intoxication in humans by the consumption of partially cooked beans have been reported. A paper by Faschingbauer and Kofler (1929) was probably the first to describe this occurrence. During the blockade of Berlin in 1948, preheated bean flakes were air-lifted to the city. They caused gastrointestinal distress among many consumers (Griebel, 1950). Acute gastroenteritis, nausea, and diarrhea following the consumption of soaked beans as part of a salad or of slow-cooked beans have been reported from England (Noah et al., 1980; Bender and Reaidi, 1982).

Korte (1972) assayed agglutination of trypsin-treated cow erythrocytes by extracts from weaning food used in Tanzania. Locally produced corn and beans were ground and heated in open vessels on three stone fireplaces to palatability. Of 91 samples tested, 20 showed positive agglutinin reaction, indicating remaining active lectins.

The possible role of bean lectins in the ethyology of protein-energy malnutrition has been analyzed in a comprehensive paper by McPherson (1989).

XVIII. INHERITANCE OF LECTINS

The presence or absence of the toxic lectin was shown to be inherited as a single gene in wild beans (*Phaseolus aborigeneus*) (Brücher et al., 1969) and also in kidney beans (Jaffé et al., 1972) (Table 6). Osborn et al. (1985) studied 46 bean

Table 6 Hemagglutinating Activity in the F_2 Generation of Types A and D Bean Plant

No.	Types of cross	Agglutination of rabbit and trypsin-treated cow RBC	
		Number of seeds	
		+	−
1	A/D	23	13
2	A/D	76	28
3	A/D	22	14
4	D/A	30	6
5	D/A	55	22
6	D/A	241	81
7	D/A	231	77
Total		678	241
		73.7%	26.3%

Source: Jaffé et al., 1972.

cultivars and observed significant differences with respect to lectin type and quantity. The F_2 plants of crosses segregated in a Mendelian fashion for two paternal lectin types, indicating single gene inheritance. It is, therefore, possible to remove seed lectins genetically by backcross-breeding (Osborn and Bliss, 1985).

The occurrence of a certain soybean lectin has been attributed to a single dominant gene (Orf et al., 1978). Later Tsien et al. (1983) were able to show the presence of very small amounts of this lectin in soybean cultivars, which had previously been considered inactive. The quantities present were 1000 to 10,000 times less than those found in a reference cultivar.

XIX. ASSAY METHODS

The detection of seed lectins is most commonly performed by serial dilution. In a microtiter dish, constant amounts of normal or protease-treated RBC are added and clumping is visually determined. The acceptance of this method is widespread, among other considerations, because of the small amounts of lectin samples it requires. Screening of single seeds or seed parts is feasible with the above technique.

RBC of different species, animal and human, should be used, since lectins could be overlooked due to inactivity toward some erythrocytes. Burger (1974) has critically evaluated this procedure. In addition to blood agglutination, clumping of bacterial strains have been used (Nachbar and Oppenheim, 1980).

To overcome the semiquantitative nature of the serial dilution test, Liener (1955) devised a photometric measurement of the RBC that are left unagglutinated by the lectin. Stabilization of RBC with glutaraldehyde rendered the

above method more sensitive and reproducible for the detection of nanograms of soybean agglutinin (Turner and Liener, 1975b). More recently, an electronic particle counter was applied to count the nonagglutinated cells in the microtiter wells, to give a quantitative measure of lectin activity (Koehle and Kauss, 1980; Coffey et al., 1985).

Screening of lectins in foods has been realized by a combination of line-dive immunoelectrophoresis and hemadsorption lectin test. Both methods could detect about 0.2 μg purified lectin (Andersen and Ebbesen, 1986). Osborn and Bliss (1985) prepared antibodies against isolated bean lectins, incorporated them into gels, and applied rocket immunoelectrophoresis for the quantitative determination of agglutinins in inbred backcross lines.

Nanogram quantities of castor bean lectins were detected by a technique based on the interaction of the lectin with sugar immobilized to lysozyme and the measurement of the remanent lytic effect of *Micrococcus luteus* cells. In this case the preparation of antibodies was unnecessary (Ghosh et al., 1979). Jaffé and Gómez (1975) used an intradermic test in mice to compare partially purified lectins from different bean cultivars.

XX. CHEMICAL CHARACTERISTICS

The structures of some legume lectins have been studied in detail. Many, but not all, are glycoproteins that contain different *N*-linked glycosyl residues. These are probably not essential for sugar binding. Bivalent metal ions—Ca^{2+} and Mn^{2+}—are often found as ligands in specific sites. Their removal abolishes activity. Lectins frequently contain hydrophobic regions, which may be related to cell-binding properties. They are a family of homologous proteins and quite different in composition and structure from ricin and abrin. Complete amino acid sequence has been established for some 15. The homologies are particularly striking between lectins of the same tribe. About 10% of the amino acids are invariant in all legume lectins (Sharon and Lis, 1990).

Oligomeric structures of two or four equal or different subunits are common, as mentioned in Chapter 8. This feature explains the existence of isolectins in plant tissues (Fig. 1). They may possess varying degrees of toxicity. Each subunit has one carbohydrate-binding site. Monomeric or hemi-lectins are, therefore, nonagglutinating molecules (Etzler, 1985).

ConA was the first lectin to be crystallized by Sumner. The three-dimensional structure of its carbohydrate complex was studied by high-resolution X-ray crystallography by Derewenda et al. (1989). The configuration of a few other lectins and lectin–carbohydrate complexes has been solved by this method (see Sharon and Lis, 1990).

XXI. TOXIC LECTINS IN NONEDIBLE PLANTS

Besides being found in the legume plant family, toxic lectins have been detected mostly in *Euphorbiaceae* since the last century: in *Ricinus communis* (Stillmark, 1889), *Croton triglium* (Elfstrand, 1897), *Jatropha curcas* (Siegel, 1893), and *Hura crepitans* (Richet, 1909). The body of evidence that relates

edible-plant lectins to antinutritional effects observed in experimental animals is large. However, the mechanism by which they influence or damage the organisms and the cells has not been as thoroughly studied.

On the other hand, interesting details on the action of the lectins, ricin, abrin, modeccin from *Adenia digitata*, and viscumin from the mistletoe (*Viscum album*) derived from nonedible plants, and which possess different molecular structures than most legume agglutinins, are known. They exert cytotoxicity by a mechanism similar to bacterial diphtheria toxin (see Olsnes and Sandvig, 1988).

Ricin is synthesized as a single polypeptide in maturing castor beans, where it accumulates in the storage granules of the seeds (Youle and Huang, 1976). Unlike most agglutinins, ricin and abrin are cleaved afterwards into two disulfide-linked glyco-polypeptides, termed the A- and B- chains.

Chain B acts as a galactose-specific lectin that interacts with cell membrane glycoproteins and glycolipids and thus permits the translocation of chain A into the cytosol. The A-chain has enzyme activity that is basically responsible for killing the infected cell. It is a highly specific *N*-glycosidase that removes adenine from a single adenosine residue located in a conserved region near the 3'-end of 28-S RNA. This cleavage inactivates the 60S ribosomal unit required for protein synthesis (see van Deurs et al., 1990) (Fig. 2).

The inhibition of in vitro synthesis in a rabbit reticulocyte cell-free system by toxic trypsic peptides from ricin has been described by Lugnier et al. (1974).

Lethal doses of abrin and ricin are about 1 μg toxin/kg body weight in mouse, rat, and dog. Rabbits are 10 times more sensitive to these toxins, while goats are more resistant (Field, 1910). However, due to a selective effect of ricin and abrin on malignant cells, they have been assayed in treatment of human cancer (Hsu et al., 1974). The cytostatic phenomenon may be explained by the fact that malignant cells are more efficient in internalizing the toxins (Nicolson et al., 1975). The inhibitory effect on the growth of human cancer in nude mice has been demonstrated by Fodstad et al. (1977).

Cases of intoxication of operators handling press cake from the castor oil industry (Cooper et al., 1964) and of children who swallowed ricinus seeds (Alstalf, 1963) have been reported.

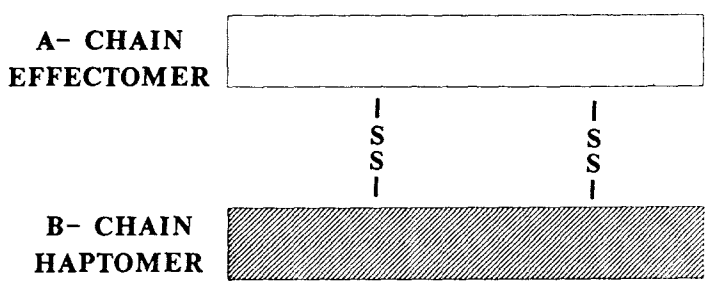

Figure 2 Schematic structure of ricin and abrin.

XXII. FINAL COMMENTS

Given the significant exposure of the consumers to dietary lectins and the diversity of their biological activities, it is rather surprising that the weight of scientific research directed to these interesting compounds has been focused mostly on chemical and biochemical aspects with little attention given to their possible antinutritional and toxicological role. It is evident that complete destruction of lectins is not always achieved by all the possible conditions of culinary preparations. What does it mean when a lectin is labeled nontoxic? Nontoxic for whom? Rats, chicks, humans?

Much more research is needed to evaluate any nutritional or physiological implications of the long-term consumption of low levels of toxic lectins and also of those until now considered nontoxic, or even those not yet detected.

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