Phytohemagglutinins: Their Nutritional Significance

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Substances which have the ability to agglutinate red blood cells, the so-called phytohemagglutinins or lectins, are widely distributed in the plant kingdom. The fact that the lectins are found in those legumes which constitute an important source of dietary protein for many segments of the world's population raises the question as to their possible nutritional significance. Phytohemagglutinins isolated from a number of legumes, including the soybean and several varieties of *Phaseolus vulgaris,* have been shown to be toxic when injected into animals and growth inhibitory when incorporated into the diet. The detection of hemagglutinating activity *in vitro* in crude extracts of the seed depends on the type of red blood cells employed as well as their pretreatment. A number of lectins have been isolated in pure form and have been well characterized with respect to their chemical and physical properties. The practical importance of suitable processing conditions for the destruction of the phytohemagglutinins in plant materials intended for animal and human consumption will be stressed.

That the seeds of certain plants are highly toxic to man and animals has been known for a long time. During the latter part of the nineteenth century, when the science of bacteriology was coming of age and having a marked influence on the scientific thinking of the times, it was widely believed that the toxicity of such seeds was due to a bacterial toxin. This theory was disproved, however, when, in 1884, Warden and Waddell (1884) observed that the toxicity of the jequirity bean, *Abrus precatorius,* resided in a fraction which could be precipitated by alcohol from an aqueous extract of the bean. Several years later Dixson (1886–1887) obtained a highly toxic concentrate from extracts of the castor bean, *Ricinus communis.* Stillmark (1889), however, appears to have been the first to observe that a protein fraction of the castor bean, which he called *ricin,* was capable of agglutinating red blood cells, a property which led to the term *phytohemagglutinins* which is still used today in referring to this class of substances. The work of Stillmark attracted the attention of Ehrlich, who chose to work with ricin rather than the bacterial toxins which were then so popular among the bacteriologists of that time. The use of these substances led Ehrlich to the discovery of the most fundamental principles of immunology (Ehrlich, 1891, 1897).

Landsteiner and Raubitschek (1908) showed for the first time that even the seeds of edible species of some common legumes such as navy beans, lentils, and garden peas contained these so-called phytohemagglutinins. Landsteiner (1936) subsequently pointed out that the relative hemagglutinating activities of various seed extracts were quite different when tested with erythrocytes from different animals and compared this specificity with that of antibodies of animal blood serum. It was in fact this specificity toward specific types of blood cells that later led Boyd and Shapleigh (1954) to coin the word *lectin* (Latin, *legere,* to choose), a term which is today used interchangably with phytohemagglutinin.

Although the phytohemagglutinin from the jack bean, concanavalin A, was crystallized as early as 1919 by Sumner (1919), the phytohemagglutinins attracted little attention and remained little more than a laboratory curiosity until 1948. In that year Renkonen (1948) in Finland and, 1 year later, Boyd and Reguera (1949) in this country reported that extracts of various seeds displayed a high degree of specificity toward human red blood cells of various blood groups. This discovery aroused the interest of immunologists and thus began an intense systematic investigation of the distribution and specificity of the phytohemagglutinins in the plant kingdom. In a study involving 2663 plant species, about 800 showed hemagglutinating activity (Allen and Brilliantine, 1969). Other significant developments which led to a revival of interest in the lectins were the observations that they could initiate mitosis in cultures of human leukocytes (Nowell, 1960) and that some of the lectins reacted specifically with tumor cells (Aub *et al.,* 1963; Burger and Goldberg, 1967). Interesting and significant as these observations are, my concern today is with another part of the story of the phytohemagglutinins about which we hear very little, and that relates to their role in determining the nutritive properties of plant protein.

It is a well known fact that many of the legumes which play an important part in the diet of large segments of the world's population possess little nutritive value and in fact may be toxic, unless subjected to some form of heat treatment (Liener, 1969). Since these same legumes are the very plants in which the phytohemagglutinins can be found (Table I), it is indeed curious that one finds so little information in the literature regarding what would seem like an obvious relationship between the phytohemagglutinins and the poor nutritive value of raw legumes. Perhaps one explanation for this vacuum lies in the fact that the discovery of a trypsin inhibitor in soybeans (Ham and Sandstedt, 1944; Kunitz, 1945) and other legumes (Borchers *et al.,* 1947) served to divert attention from the phytohemagglutinins, and nutritionists seem to have become preoccupied with studying the role of the trypsin inhibitors, particularly in the soybean. It is my intent, therefore, to try to redirect your attention to the role that the phytohemagglutinins might play in affecting the nutritional properties of plant protein.

SOYBEANS (*Glycine max*)

The beneficial effect of heat on the nutritional value of soybean protein was first observed by Osborne and Mendel (1917) and, with the demonstration of a heat-labile trypsin inhibitor in raw soybeans (Ham and Sandstedt, 1944), it was generally assumed that this was the factor responsible for the poor nutritive quality of the unheated bean. Liener *et al.* (1949), however, presented evidence to show that the trypsin inhibitor could not account for all of the growth inhibition observed with rats on a raw soybean diet and succeeded in isolating from raw soybeans a protein fraction which had hemagglutinating activity and was toxic when injected into rats (Liener 1951; Liener and Pallansch, 1952). Of greater significance, however, was the observation that when the soybean hemagglutinin was incorporated into the diet at a level equivalent to the ac-

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Table I. Some Edible Plants in Which Hemagglutinating Activity Has Been Detected[a]

Latin name	Common name	Toxicity[b]		Effect of heat on nutritive value[c]
		Peritoneal	Oral	
Arachis hypogaea	Peanut, ground nut	?	?	+
Canavalia ensiformis	Jack bean	+	+	+
Dolichos biflorus	Horse gram	−	+	+
Dolichos lablab	Field bean Hyacinth bean	+	+	+
Glycine max	Soybean	+	+	+
Lathyrus odoratus	Sweet pea	?	?	+
Lens esculenta	Lentil	?	?	+
Phaseolus acutifolius	White tipary bean	+	?	?
Phaseolus aureus	Mung bean	−	?	−
Phaseolus lunatus	Lima bean, double bean	−	+	+
Phaseolus multiflorus	Scarlet runner bean	+	?	?
Phaseolus vulgaris	Kidney bean, navy bean, pinto bean, wax bean	+	+	+
Pisum arvense	Black pea	?	?	?
Pisum sativum	Field pea	−	−	−
Ricinus communis	Castor bean	+	+	+
Vicia faba	Horse bean Broad bean	?	?	+
Vicia sativa	Common vetch	?	?	+

[a] This table is based on information taken from the following sources: de Muelenaere (1965); Huprikar and Sohonie (1961); Jaffé (1969); Liener (1962); and Manage *et al.* (1972). [b] Peritoneal toxicity refers to the observation that the intraperitoneal injection of the seed extract or the purified phytohemagglutinin into a test animal produces death. Oral toxicity refers to death or marked inhibition of growth of animals when purified phytohemagglutinins are incorporated into the diet. + and − denote positive or negative effects respectively; ? denotes that no information is available. [c] + indicates that the nutritive value of the plant foodstuff is improved by heat treatment; − indicates that heat does not have any beneficial effect; ? indicates that no information is available.

tivity found in raw soybean meal, a significant depression of the growth of rats was obtained (Table II). It can be estimated from these data that approximately one-half of the growth inhibition that is obtained with unheated soybeans can be attributed to the soybean hemagglutinin. Substances other than the hemagglutinin, including trypsin inhibitors (Liener and Kakade, 1969) or other growth inhibitors (Schingoethe *et al.*, 1970), may be assumed to account for the remainder of the growth inhibition.

Liener and Hill (1953) suggested that the hemagglutinating activity of soybean meal could be used as an index of their nutritive value since the decrease in hemagglutinating activity effected by heat treatment paralleled the improvement in nutritive value as measured with chicks. In their experiments, exposure to steam at atmospheric pressure for at least 60 min or autoclaving at 15 lb of pressure for at least 20 min was sufficient to inactive the hemagglutinin.

Concurrent with studies on the nutritional effects of the soybean hemagglutinin were studies dealing with the physicochemical characterization of this protein both in this laboratory (Pallansch and Liener, 1953; Wada *et al.*, 1958) and later by Sharon's group in Israel (Lis *et al.*, 1964, 1966b, 1969, 1970). These properties are summarized in Table III. The soybean hemagglutinin is a glycoprotein with a molecular weight of about 110,000; the carbohydrate moiety comprises about 5% of the molecule and consists largely of mannose and *N*-acetyl-D-glucosamine. Unpublished data indicate that it is probably composed of four identical subunits (Lotan *et al.*, 1973). At least three

Table II. Growth Inhibitor Effect of the Soybean Hemagglutinin, SBH (Liener, 1953)

Protein component of diet	Wt gain in 2 weeks, g	% growth inhibition
25% heated soybean meal	60.0	0
25% raw soybean meal	28.0	43.2
25% heated soybean meal + 0.8% SBH	45.0	25.6

other minor components possessing hemagglutinating activity have been reported to be present in soybean oil meal (Catsimpoolas and Meyer, 1969; Lis *et al.*, 1966a).

BEANS BELONGING TO THE GENUS PHASEOLUS

The common bean, *Phaseolus vulgaris*, constitutes an important source of dietary protein for large segments of the world's population, and numerous reports may be found in the literature concerning the toxic effects which have sometimes accompanied the ingestion of raw or inadequately cooked beans (Liener, 1969). Although the presence of phytohemagglutinin had been reported earlier by Landsteiner and Raubitschek (1908), the toxicity of partially purified preparations of the phytohemagglutinin from *P. vulgaris* was first reported by Jaffé and his coworkers (Jaffé, 1960; Jaffé and Gaede, 1959; Jaffé *et al.* 1955). In order to evaluate the possible nutritional significance of this phytohemagglutinin we undertook the task of isolating sufficient quantities of this material from *P. vulgaris* in order to feed it to rats at approximately the same level of activity as is found in the raw bean (Honavar *et al.*, 1962). This was preceded by a study to ascertain the effect of heat on the nutritive value of several legumes which enjoy popular consumption in some of the underdeveloped countries. It is apparent from Table IV that of the five legumes tested, only the black bean and kidney bean, both classified as *P. vulgaris*, were markedly improved by heat treatment. Table V shows that these same two beans were the only ones to display high levels of hemagglutinating activity. When the phytohemagglutinins from these two beans were purified to the point where the trypsin inhibitor activity was eliminated, their growth inhibitory effect and toxicity to rats became readily apparent (Table VI). Levels as low as 0.5% of the diet caused a definite inhibition of growth, and higher levels of the phytohemagglutinin hastened the onset of death. Similar effects were subsequently reported with chicks as the experimental animal (Wagh *et al.*, 1965).

Preliminary soaking prior to autoclaving seems to be required for the complete elimination of the toxicity of the kidney bean (Jaffé, 1949), although Kakade and Evans (1965b) found that autoclaving alone for 5 min was sufficient to eliminate the toxicity of finely ground navy bean meal. de Muelenaere (1964) noted, however, that autoclaving for 30 min was necessary to destroy the hemagglutinating activity of certain African varieties of *Phaseolus vulgaris*. Of particular significance was the observation that hemagglutinating activity was still detectable after 18 hr of *dry* heat.

Although the toxicity of most phytohemagglutinins can be readily demonstrated by intraperitoneal injection, there is some evidence to indicate that the toxic component may not be identical to the component responsible for the hemagglutinating activity exhibited by preparations from *P. vulgaris*. Kakade and Evans (1965a,b) working with navy beans and Stead *et al.* (1966) with the Natal round yellow bean isolated fractions of low hemagglutinating activity which were more toxic than fractions with higher hemagglutinating activity. Similar observations were reported by Jaffé (1962) and de Muelenaere (1965) with other strains of *P. vulgaris*. The question of the nonidentity of a phytohemagglutinin with a toxic

Table III. Properties of Some Phytohemagglutinins Which Have Been Purified from Edible Plants[a]

Plant source	Molecular weight	Carbohydrate %	Carbohydrate Major sugars[b]	No. of subunits	Sugar specificity
Canavalia ensiformis					
Jack bean	112,000	0		4	α-D-Man
Glycine max					
Soybean	110,000	5.0	D-Man D-GlcNAc	4	D-GalNAc
Lens esculenta					
Lentil	42,000–69,000	2.0	GlcN, Glc	2	α-D-Man
Phaseolus lunatus					
Lima bean	269,000	4.0	Glc, Man, Fuc	8	D-GalNAc
Phaseolus vulgaris					
Wax bean	132,000	10.4	D-Man	4	D-GalNAc
Kidney bean	98,000–138,000	4.1	GlcN, Man	4	D-GalNAc
Black bean	128,000	5.7	Hexosamine, Man, Xyl		
Ricinus communis					
Castor bean	46,000[c]	3.7	Man, D-Gal		D-Ga
	88,000[d]	3.7	Man, D-Gal		D-Gal
Solanum tuberosum					
Potato	20,000	5.2	Ara		D-GlcNAc
Triticum vulgaris					
Wheat	26,000	4.5	Glc, Xyl, hexosamine		D-G cNAc

[a] Based on information taken from Sharon and Lis (1972) and Lis and Sharon (1973). [b] The following abbreviations are used: Glc, glucose; D-Gal, D-galactose; D-Man, D-mannose; D-GlcN, D-glucosamine; D-GalNAc, *N*-acetyl-D-galactosamine; D-GlcNAc, *N*-acetyl-D-glucosamine; Fuc, fucose; Ara, arabinose; Xyl, xylose. [c] Toxic but nonagglutinating (Waldschmidt-Leitz and Keller, 1970). [d] Agglutinating but nontoxic (Waldschmidt-Leitz and Keller, 1970).

Table IV. Effect of Heat on Nutritive Value of Some Legumes (Honavar *et al.*, 1962)[b]

Source of protein	Gain in weight, g/day Raw	Heated
Phaseolus vulgaris	−1.94 (4–5)	+1.61
Black bean	−1.04 (11–13)	+1.48
Kidney bean		
Cicer arietinum		
Bengal gram	+1.25	+1.16
Cajanus cajan		
Red gram	+1.33	+1.74
Phaseolus aureus		
Mung bean	+1.05	+1.07

[a] 100% mortality observed during period (in days) shown in parentheses. [b] Reprinted with permission of American Institute of Nutrition: Honavar, P. M., Shih, C. V., Liener, I. E., *J. Nutr.* **77,** 109 (1962).

Table V. Hemagglutinating and Antitryptic Activities of Crude Extracts of Raw Legumes (Honavar *et al.*, 1962)[b]

Legume	Hemagglutinating activity,[a] HU/ml	Antitryptic activity,[a] TIU/ml
Phaseolus vulgaris		
Black bean	2450	2050
Kidney bean	3560	1552
Cicer arietinum	0	220
Cajanus cajan	0	418
Phaseolus aureus	0	260

[a] HU = hemagglutinating units; TIU = trypsin inhibitor units. [b] Reprinted with permission of American Institute of Nutrition: Honavar, P. M., Shih, C. V., Liener, I. E., *J. Nutr.* **77,** 109 (1962).

factor will only be resolved when and if it becomes possible to separate these two activities, as has been done in the case of ricin (see below).

One of the complicating factors involved in relating hemagglutinating activity to toxicity is the fact that there are hundreds of different strains and cultivars of *P. vulgaris*. The hemagglutinins present in their seeds are known to exhibit different degrees of specificity, depending on the species of animal from which the red blood cells have been derived and whether or not the cells have been pretreated with proteolytic enzymes such as trypsin. Jaffé and his colleagues (Brücher *et al.*, 1969; Jaffé and Brücher, 1972; Jaffé *et al.*, 1972) have made a systematic study of the hemagglutinating activity of a large number of different varieties and cultivars of *P. vulgaris* with respect to their action on the blood from different animals, with and without trypsinization, and the toxicity of their extracts when injected into rats. They made the significant observation that only those extracts which agglutinated trypsinated cow cells were toxic when injected into rats (Table VII). Feeding tests confirmed the fact that those varieties which exhibited agglutinating activity toward trypsinated cow cells were also toxic and supported very poor growth when fed to rats (Jaffé and Brücher, 1972; Jaffé and Vega Lette, 1968). Those varieties which were nonagglutinating or agglutinated only rabbit cells were nontoxic when fed. These results serve to emphasize the importance of testing the hemagglutinating activity of seed extracts against several species of blood cells before one is justified in concluding that a particular bean is toxic or not. The use of trypsinated cow cells would appear to be the most useful system for detecting potentially toxic beans.

Other species of *Phaseolus* which have demonstrated hemagglutinating activity are the lima or double bean (*Phaseolus lunatus*), mung bean (*P. aureus*), white tipary bean (*P. acutifolius*), and the scarlet runner bean (*P. multiflorus*) (see Table I). The phytohemagglutinins from the lima bean and mung bean have been reported to be nontoxic, however (Manage *et al.*, 1972; de Muelenaere, 1965). Despite its lack of toxicity, the oral administration of the lima bean hemagglutinin severely restricted the growth of rats (Manage *et al.*, 1972). Similar studies with the mung bean hemagglutinin have not been reported. Extracts of the white tipary bean and the scarlet runner bean do display some degree of toxicity when injected into rats (de Muelenaere, 1965), but the extent to which they

Table VI. Effect of Purified Hemagglutinin Fractions from the Black Bean and Kidney Bean on Growth of Rats (Honavar *et al.*, 1962)[c]

Source of of hemag-glutinin	Purified hemag-glutinin 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 period recorded. Blank space indicates no deaths observed. [b] Solution of hemagglutinin boiled for 30 min and dried coagulum fed at level indicated. Hemagglutinating activity was completely destroyed by this treatment. [c] Reprinted with permission of American Institute of Nutrition: Honavar, P. M., Shih, C. V., Liener, I. E., *J. Nutr.* **77,** 109 (1962).

influence the nutritive properties of these beans is not known.

Of the many varieties and strains of *Phaseolus* which contain phytohemagglutinins, several have been purified from different varieties of *P. vulgaris* and from *P. lunatus*. It is evident from a comparison of their properties (Table III) that the lectins isolated from different varieties of *P. vulgaris* are very similar and resemble the soybean hemagglutinin with respect to size, composition, and subunit structure. The lima bean lectin, on the other hand, consists of a greater number of subunits, each of which appears to contain a free sulfhydryl group which seems to be located at the saccharide-binding site (Gould and Scheinberg, 1970).

CASTOR BEAN (*Ricinus communis*)

Ricin, the phytohemagglutinin of the castor bean, was one of the first lectins to attract the attention of investigators, presumably because of its extreme toxicity; its MLD is about 0.001 μg/g (mice), which makes it about 1000 times more toxic than most of the other bean lectins (Jaffé, 1969). This toxicity persists even after oral ingestion and, for this reason, detoxification of castor pomace is essential for its safe handling and its utilization for animal feeding. Steam heating, as used for the recovery of solvents employed for the extraction of the castor oil, has been found to produce a thousandfold reduction in toxicity and to render the pomace harmless for sheep, rabbits, and rats when used in the respective diets in a proportion of not more than 10% (Clemens, 1963). Jenkins (1963) found that 1-hr steam heating at 15 lb of pressure reduced the toxicity of castor bean meal to 1/2000 of its original value. Rats fed 23.9% of the autoclaved meal were in good health after 4 weeks, although growth and food conversion were lower than in casein controls. Effective detoxification can also be achieved by extraction with hot water (Vilkjalmsdottir and Fisher, 1971) and hot water treatment with dilute alkali or formaldehyde (Fuller *et al.*, 1971; Gardner *et al.*, 1960).

Not to be confused with the toxic effects of ricin is the presence of the castor bean allergen and the alkaloid ricinine (Jenkins, 1963). The latter is generally considered harmless and is growth inhibitory to chicks only when fed in large amounts (Murase *et al.*, 1966). Individuals handling castor bean pomance which has not been properly processed are known to develop severe symptoms of irritation of eyes, nose, and throat, asthma, nausea, vomiting, weakness, and pain (Perlman, 1969). Although most of these symptoms are most likely due to the castor bean allergen, Cooper *et al.* (1964) attribute at least some of these reactions to ricin.

Table VII. Correlation of Specific Hemagglutinating Activity with the Intraperitoneal Toxicity in Rats of Extracts of Different Varieties and Cultivars of *P. vulgaris* (Jaffé and Brücher, 1972)

Variety	Rabbit blood	Trypsinated cow blood	Toxicity, no. of injected rats/no. of dead rats
Valin de Albenga	+	+	5/4
Merida	+	+	9/9
Negro Nicoya	+	+	5/4
Saxa	+	+	5/5
Peruvita	+	−	5/0
Palleritos	+	−	6/0
Juli	+	−	5/0
Cubagua	+	−	5/0
Porillo	−	+	5/5
Negra No. 584	−	+	5/3
Varnica Saavegra	−	+	10/6
Hallado	−	−	5/0
Madrileno	−	−	5/0
Alabaster	−	−	5/0
Triguito	−	−	6/0

Although highly toxic preparations of ricin had been prepared by a number of early investigators (Kabat *et al.*, 1947; Karrer *et al.*, 1924; Osborne *et al.*, 1905), it was first obtained in crystalline form by Kunitz and McDonald (1948). More recent work in Japan by Funatsu and his group (Ishiguro *et al.*, 1964; Takahashi *et al.*, 1962a,b) has shown that a toxic protein devoid of hemagglutinating activity, designated as ricin D, may be separated from other protein fractions which display hemagglutinating activity. A separation of the toxin from the agglutinin has also been reported by Waldschmidt-Leitz and Keller (1970); the toxin had a molecular weight of 46,000, whereas the molecular weight of the agglutinin was 87,000. Other workers have similarly reported the isolation of fractions from the castor bean which differ in the relative ratios of toxic and hemagglutinating activities (Nicolson and Blaustein, 1972; Tomita *et al.*, 1972). These observations, of course, raise the question as to whether the toxicity observed with other phytohemagglutinins may not be due to a protein which does not depend on a hemagglutinin, with which it must be strongly associated, in order for it to manifest its toxicity.

JACK BEAN (*Canavalia ensiformis*)

Concanavalin A is the name given to the phytohemagglutinin first isolated from the jack bean by Sumner (1919). Because of the ease with which it may be purified in good yield, especially by affinity chromatography (Agrawal and Goldstein, 1965; Olson and Liener, 1967), this particular protein has been the object of more study than any other lectin. It has, for example, provided a most useful technique for probing alterations in the surface of cell membranes, particularly those changes associated with malignancy or transformation (Lis and Sharon, 1973).

Despite the attention which concanavalin A has received, very little is known regarding its toxicity. It was noted many years ago that the direct injection of this protein into animals caused the agglutination of red blood cells, followed by hemolysis, and finally death (Dameshak and Miller, 1943; Ham and Castle, 1940; Lee *et al.*, 1944). Jack bean meal is of poor nutritive value unless heated (Borchers and Ackerson, 1950), and consumption of the

raw bean has been reported to cause a variety of pathological lesions in rats (Orru and Demel, 1941) and cattle (Shone, 1961). Jayne-Williams (1973) has recently reported that quail raised under germ-free conditions were able to tolerate the toxic effects of raw jack bean meal or the concanavalin A isolated therefrom much better than conventional birds. It was postulated that one of the effects of concanavalin A was to interfere with the ability of the animal to prevent the translocation of bacteria from the lumen of the gut into the blood system and other organs. There appears to be some doubt, however, as to whether the harmful effects accompanying the ingestion of raw jack bean meal are entirely due to concanavalin A. Dennison *et al.* (1971) removed the agglutinating activity of a crude extract of jack bean meal by selective absorption on Sephadex and observed that the unabsorbed fraction still retained some toxicity, albeit less than the original extract, when injected into rats. This would indicate that a portion of the toxicity of the jack bean meal may very well reside in a fraction devoid of hemagglutinating activity.

Concanavalin A is the most thoroughly characterized lectin, and some of its more important properties are summarized in Table III. The most distinguishing characteristic of concanavalin A is that, unlike most of the other lectins, it is not a glycoprotein. Chemical and physical evidence (Abe *et al.*, 1971; Edmundson *et al.*, 1971; Kalb and Lustig, 1968; McKenzie *et al.*, 1972; Wang *et al.*, 1971) support the view that concanavalin A consists of an aggregation of identical subunits, each of which has a molecular weight of 25,500. The exact number of subunits which associate is pH-dependent; thus, at pH values below 5.6, concanavalin A exists in solution as a single molecular species made up of two subunits, whereas above pH 5.6, the protein forms tetramers with a molecular weight of about 112,000. X-Ray crystallographic studies have extended our knowledge to the three-dimensional structure of concanavalin A. These studies have confirmed its tetrameric structure (Greer *et al.*, 1970; Hardman *et al.*, 1971; Quiocho *et al.*, 1971) and have revealed the location of the saccharide (Becker *et al.*, 1971) and metal (Mn^{2+} and Ca^{2+}) binding sites (Weinzierl and Kalb, 1971). Each subunit appears to have one sugar-binding site and two separate binding sites for Mn^{2+} and Ca^{2+}. With the recent elucidation of its amino acid sequence (Edelman *et al.*, 1972), concerted efforts are being made to relate the structure of concanavalin A to its mode of action as a phytohemagglutinin.

OTHER PLANTS

Although the extracts of various kinds of peas (*Pisum sativum*) exhibit hemagglutinating activity (see Table I), the isolated hemagglutinin does not produce any toxic effects when injected into rats (Manage *et al.*, 1972; de Muelenaere, 1965) or when incorporated into their diet at a level of 1% (Huprikar and Sohonie, 1965). This perhaps may explain why the nutritive value of peas is not affected by heat treatment. The pea hemagglutinin has been purified by affinity chromatography (Entlicher *et al.*, 1969) and shown to require Ca^{2+} and Mn^{2+} for activity (Paulova *et al.*, 1971).

The phytohemagglutinin from the field bean (*Dolichos lablab*) is toxic when injected into rats (Manage *et al.*, 1972) and, when fed at a level of 2.5% in the diet, causes an inhibition of the growth of rats accompanied by zonal necrosis of the liver (Salgarkar and Sohonie, 1965). The phytohemagglutinin presumably accounts for only part of the growth depression and toxicity of the raw field bean since the effects obtained with the purified hemagglutinin are less than that obtained with raw field bean meal containing an equivalent level of hemagglutinating activity. Although the hemagglutinin of the horse gram (*Dolichos biflorus*) is nontoxic, it retarded the growth of rats when administered orally (Manage *et al.*, 1972).

Although the nutritive value of the lentil (*Lens esculenta*) can be improved by heat treatment, there do not appear to be any studies relating to the possible toxic effects of the isolated lectin. The latter has now been obtained in a high state of purity, and its properties are somewhat similar to that of concanavalin A, particularly with regard to the specificity of its binding to mannose (Table III).

The broad bean (*Vicia faba*) is known to contain hemagglutinins but this activity would appear to bear little relationship to the disease known as "favism," which sometimes accompanies the ingestion of this bean (Mager *et al.*, 1969).

Other edible plants known to contain hemagglutinins are the potato (*Solanum tuberosum*), wheat germ (*Triticum vulgaris*), and the peanut (*Arachis hypogaea*), but there is very little evidence to indicate that these lectins play any significant role in the nutritive properties of these foods.

MECHANISM OF TOXICITY

If one accepts the premise that the toxicity of the phytohemagglutinins is related to their ability to interact with specific receptor sites on the surface of the cell, a reasonable explanation for the deleterious effects of orally ingested hemagglutinins is the one proposed by Jaffé (Jaffé, 1960; Jaffé and Camejo, 1961; Jaffé *et al.*, 1955). One of the *in vivo* effects observed when the phytohemagglutinin from the black bean (*P. vulgaris*) was given orally to rats was a sharp diminution in the absorption of all nutrients. *In vitro* experiments with intestinal loops taken from rats fed this lectin revealed a 50% decrease in the rate of absorption of glucose across the intestinal wall compared to controls. Jaffé postulated that the action of the hemagglutinin is to combine with the cells lining the intestinal wall, thus causing a nonspecific interference with the absorption of nutrients.

If, on the other hand, the toxicity of the lectins is due to a nonhemagglutinating factor intimately associated with it, as seems to be the case with ricin, then some other explanation must be sought. In this connection, it is of interest to note that Olsnes and Pihl (1972) have recently shown that the nonagglutinating toxic fraction of ricin markedly inhibits protein synthesis in a cell-free system by inactivating some component essential for the elongation of peptides. Whether a similar mechanism is responsible for the toxic effects of other lectins remains to be established.

CONCLUSION

Although the toxic effects of the hemagglutinins present in plant foodstuffs can be generally eliminated by proper heat treatment, it should be recognized that conditions may prevail whereby complete destruction of the phytohemagglutinins may not always be achieved. For example, Korte (1973) has recently observed that in mixtures of ground beans and ground cereal prepared under the field conditions prevailing in Africa, the hemagglutinin was not always destroyed, and the cooked product produced diarrhea and other signs of toxicity. A reduction in the boiling point of water in mountainous regions could also result in incomplete destruction of toxicity. An outbreak of massive poisoning after the consumption of partially cooked bean flakes has been reported by Griebel (1950). The marked resistance of phytohemagglutinins to inactivation by dry heat (de Muelenaere, 1964) deserves special emphasis. Thus, the addition of kidney bean flour to wheat flour for the manufacture of bread (*Chem. Ind. Eng. News*, 1948) and the use of bean flour for making baked goods (Marcos and Boctor, 1959) should be viewed with caution.

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