Reprinted from
TOXICANTS OCCURRING NATURALLY IN FOODS
ISBN 0-309-02117-0
NATIONAL ACADEMY OF SCIENCES
Washington, D.C. 1973

5 Werner G. Jaffé

TOXIC PROTEINS AND PEPTIDES*

INTRODUCTION

If one considers the varied biological activities of many proteins and peptides such as enzymes, hormones, transport proteins, and antigens, it is not surprising that some members of this large group of substances should exhibit toxic actions in organisms other than those in which they had been produced. Indeed, several proteins—bacterial toxins, snake venoms, and plant toxins (ricin)—are among the most toxic agents known.

The proteins are essential constituents of nearly all foods. Therefore, a discrimination among the nutritional effects caused by amino acid deficiency or imbalance, poor digestibility, and toxic effects may be difficult. Moreover, additional factors, such as protein inhibition of digestive enzymes, may play an important if still unknown role in determining whether or not a certain protein can exert a harmful action.

Most animals have evolved detoxification mechanisms against the action of foreign proteins. In a certain sense the immunological defense mechanisms can be regarded as such, although they may lead to the opposite effect, i.e., hypersensitivity and allergic reactions. Another defense mechanism is gastrointestinal digestion. Although it serves the

*Literature reviewed to March 1970.

purpose of primarily transforming foods into components that can be absorbed through the intestinal wall, it simultaneously destroys the native structure of ingested proteins and peptides, abolishing their biochemical activities and possible toxicities.

Toxicity may be detected, therefore, in certain proteins or peptides existing in foods if they enter the body by other than the oral route or if they resist digestive destruction. They might possibly contain toxic constituents not destroyed by digestion, like selenium, or the proteinaceous substances might act in some way on the food product before it is ingested, as in the destruction of vitamins by certain enzymes.

The specific biological activity of practically all proteins is destroyed by heat, although considerable differences exist between proteins with respect to their rate of heat inactivation. Consequently, most food products, if properly prepared, can be consumed without harmful effect. Notable exceptions are some of the allergy-producing foods, which will be dealt with later on, and the selenoproteins.

The mode of action of most of the toxic proteins is still obscure. In some cases specific *in vitro* activities can be detected, and considerable efforts have been made to relate these to *in vivo* effects. Some of the better known groups of proteins with recognized toxic or antinutritional action are bacterial toxins, animal toxins, hemagglutinins, enzyme inhibitors, vitamin-binding proteins (avidin), vitamin-destroying enzymes, enzymes releasing toxic compounds, and selenium-containing proteins. Not all are discussed in this chapter. For further information on the other toxins the reader should refer to other chapters in this volume.

HEMAGGLUTININS

Hemagglutinins are proteins that have the interesting ability to clump or agglutinate red blood cells (RBC) in a fashion similar to antibodies. Moreover, like antibodies, they show a remarkable specificity in that they may act in high dilution on one kind of erythrocyte and not at all or only weakly on another. Each specific hemagglutinin is therefore clearly different from all others and must be studied individually; the results obtained with one kind cannot be applied to another without verification. Also, the chemical composition of different hemagglutinins may be quite different from one another, as discussed later in this chapter.

Hemagglutinins have been found mostly in plants. Because of the specificity of their action on RBC from different animals, Boyd and Shapleigh[1] have proposed the term *lectins* from the latin word *legere*,

to choose. Plant hemagglutinins are sometimes referred to as phytohemagglutinins, frequently abbreviated simply as PHA.

A small number of similarly acting substances of animal origin have been studied from snails and fish eggs,[2] slugs,[3] sponges,[4] and others. The term *protectins* has been proposed to designate these compounds[5] because they are believed to confer protections against bacterial infection. Nothing is known about their possible toxic action in humans, and they will therefore not be treated further in this chapter.

Occurrence and Nature of Plant Hemagglutinins

Hemagglutinins have been found in a great number of plants and in different plant organs or parts.[6] The first to be recognized was the very toxic ricin from castor beans, which was described as early as 1889 by Stillmark in a paper that led to all later work in this field.[7] The most interesting group of lectins from the standpoint of nutrition is that found in legumes; this group was discovered in 1908 by Landsteiner and Raubitschek.[8]

Between 1890 and 1910 a remarkable number of outstanding research papers was published relating to phytohemagglutinins, including studies of their toxicity and specificity. This early work has been reviewed by Ford.[9] Later, interest in this group of substances declined until Boyd and Reguera[10] and Renkonen[11] discovered the existence of specific hemagglutinins for different human blood groups. In 1960 Nowell described their stimulating effect on mitosis of cultured human leucocytes.[12] Since then the number of papers published on different *in vitro* and *in vivo* effects of phytohemagglutinins has increased enormously and is still rising rapidly. The effects studied include stimulation of DNA, RNA, and protein synthesis in cell cultures[13]; effect on immunorepression[14]; protein–carbohydrate interaction[15]; their use in the investigation of the structure of specific receptor sites in erythrocytes and other cells[16]; and specific interaction with cancer cells.[17] Nevertheless, our knowledge of the toxicity of phytohemagglutinins and of the cause of their toxic action has advanced relatively little in the last 50 yr. Some review articles on the nutritional aspects of phytohemagglutinins have appeared recently.[18,19,20]

A number of edible fruits, seeds, or tubers contain agglutinins. Examples include potatoes,[21] bananas,[22] mangoes,[22] and wheat germ,[23] but nothing is known about their possible toxic action.

By far, however, the most studied group in this respect are the legumes. Many leguminous seeds contain other toxic factors, especially enzyme inhibitors, besides lectins. These different protein factors are

not easily separated from each other, and the extraction and precipitation procedures used for fractionation of toxic seed extracts often will not yield pure products. It is important, therefore, that the different biochemical activities be simultaneously measured in such fractions in order to be able to draw valid conclusions on the relative importance of the hemagglutinins and any other toxicants that might be present. Unfortunately, interpretation of numerous published experiments is difficult, because this precaution has been disregarded.

As proteins, the phytohemagglutinins are heat labile. Normal cooking destroys their specific action. If the nutritional value of a plant product is not enhanced by cooking when tested in a nutritionally complete experimental diet, and if the presence of an agglutinin can be demonstrated in this product, it is safe to conclude that this specific agglutinin is nontoxic for the animal species used, at least at the concentration present in the test diet. Thus, the growth-promoting value of peas, lentils, and peanuts is not significantly improved by heat treatment. Indeed, the isolated hemagglutinin from peas did not exert a significant action on the growth of rats when 1% was included in a test diet.[24]

On the other hand, if the food value of a hemagglutinin-containing plant product is improved by heating, it cannot be concluded that this improvement is due to the destruction of the hemagglutinin. Other factors may be involved: destruction of enzyme inhibitors or heat improvement of the digestibility of proteins may enhance food value.

Still another problem exists in experimental work on toxic phytohemagglutinins. Because of the specificity of their action, standard tests with human or rabbit blood may fail to detect the hemagglutinating activity of some extracts or protein fractions. Therefore, before claims on the separation of agglutinating and nonagglutinating toxic factors are made, it is advisable that a thorough study on the possible hemagglutinating action of the fractions be undertaken with different blood types.

Legume Hemagglutinins

Soybean Hemagglutinin Heated soybeans cause better growth than raw soybeans when fed in an experimental diet to rats or chicks. This fact, known for over 50 yr, points to the existence of heat-labile, toxic factors in these beans. Indeed, it is well established that several enzyme inhibitors and hemagglutinins can be found in soybeans, but their respective effects on the nutritional value is still an object of discussion.

A phytohemagglutinin of the soybean has been studied extensively by Liener and his coworkers.[19] In 1953, Liener and Pallansch[25] iso-

lated a protein from soybeans that agglutinated rabbit blood cells at high dilution. This particular hemagglutinin, although shown to be homogeneous by several criteria, is apparently only one of at least four hemagglutinins subsequently shown by chromatographic separation to be present in soybeans.[26] All of these proteins seem to be quite similar in composition and physicochemical properties. The amount of hemagglutinin present in soybean protein has been estimated by immunochemical methods to be about 3% by Liener and Rose.[27]

When isolated soybean agglutinin was injected into rats, the LD_{50} was found to be in the order of 50 mg/kg,[25] indicating a relatively high toxicity compared to that of the orally ingested agglutinin. Given in an experimental diet to rats at the level of 1%, the hemagglutinin depressed growth to about 75% of normal, but no lethal effect was observed.[28] The oral ingestion of raw soybeans or protein fractions prepared from them has never been demonstrated to have a lethal effect on any animal species tested. The isolated agglutinin was given by stomach tube to rats in the amount of 500 mg/kg without producing death of the animals.[27]

The low toxicity of the soybean agglutinin when given orally, as compared to its rather high toxicity when injected, is noteworthy, because the observations with other bean agglutinins are quite different. The LD_{50} of hemagglutinin of black bean (*Phaseolus vulgaris*)[29] when injected is similar to that of the soybean agglutinin. Diets containing raw beans or orally administered bean agglutinin cause loss of weight and death of the rats.[30] Different susceptibility to digestion could possibly account for the difference in oral toxicity of these hemagglutinins, since the soybean agglutinin is inactived *in vitro* by pepsin[31]; the bean agglutinin is relatively resistant to this enzyme,[32] and hemagglutinating action can be detected in the feces of rats after the ingestion of raw beans.[33] A portion of the soybean agglutinin may nevertheless withstand gastric digestion, which may thus account for its moderate oral toxicity. This aspect of the problem awaits further investigation.

Unheated soybean meal increases the animals' requirements for certain vitamins, minerals, and other nutrients, compared to heated soy protein. Although nothing is known about the exact nature of the responsible factors, their heat lability points to their proteinaceous nature. The hemagglutinin of kidney beans (*Phaseolus vulgaris*) reduces intestinal absorption and will be discussed later in this chapter. The soybean hemagglutinin may exert a similar effect when raw soybeans are fed to animals, but this point has not been investigated in any detail.

Rats fed a diet containing raw soybeans develop enlarged thyroid glands.[34] This effect may be destroyed by steaming the soybeans. The

goitrogenic effect of soybeans is readily overcome by adding supplemental iodine to the diet.[35] Several workers have reported cases of goiter in human infants fed soy milk.[36,37] Fecal loss of thyroxine was observed in rats fed raw soybeans, which may be attributed to the fact that reabsorption of thyroxine from the gut, where it has been excreted via the bile, is inhibited.[38]

Reduced amino acid absorption through the intestinal wall can be observed in rats fed raw soybeans.[39] Low fat absorption due to the presence of raw soybean fractions in an experimental diet has likewise been described.[40] This effect may be related to the increased fecal excretion of bile acids in chicks fed a raw soybean diet.[41] Evidence for increased requirement for the fat-soluble vitamins A,[42] D,[43] and K,[44] in animals subsisting on a raw soybean diet has also been presented. For example, turkey poults suffered severe rickets when fed a soy-protein glucose ration, even though the diet contained normally adequate levels of vitamin D_3, calcium, and phosphorus. Growth and tibia ash were improved by an increase of vitamin D_3 or by the replacement of the unheated soybean protein by heated soybean meal.[43] Likewise, unheated soybean protein increased the vitamin D_2 requirement of baby pigs.[45] The enhanced requirement for vitamin K in chicks receiving unheated soybean meal[44] has been attributed to the use of trichlorethylene in the extraction procedure.[46] Vitamin B_{12} in amounts that support normal growth in animals on a control diet was insufficient when a diet containing unheated soymeal was fed.[47] Recent results seem to exclude the explanation that an impairment in absorption plays a role in producing a deficiency of this vitamin in rats fed unheated soybean flour, but the real cause is not clear.[48]

The reasons for the increased requirements for some nutrients in animals fed diets containing unheated soy protein remain to be elucidated. Although in some cases crude preparations of trypsin inhibitor have been found to decrease intestinal absorption,[39] the possibility exists that these inhibitor fractions may have also contained hemagglutinins and other proteins[49] that complicate the issue.

The existence of other unindentified, heat-labile, toxic factors in soybeans was suggested by Stead *et al.*[50] because they found that two of the protein fractions separated by chromatography on DEAE-cellulose were toxic when injected into rats. Only one of them, however, had significant hemagglutinating activity when tested with rabbit blood. After extraction of the soluble proteins from soy flour, the residue was still not capable of supporting normal growth if not submitted to heat treatment.[51] An unidentified growth-depressing factor can be released by papain digestion from extracted soybean meal.[52] The possible pres-

ence of a toxic peptide fraction from soybeans will be discussed later in this chapter.

No other foodstuff has been reported to produce as many antinutritional effects as the soybean.[53] Is this because this legume is exceptionally rich in different heat-labile toxicants or because no other foodstuff has been tested so thoroughly? If the latter alternative is indeed true, one might expect many more unrecognized toxicants in foods that have not received as much detailed study.

Other Bean Hemagglutinins Hemagglutinins have been purified from extracts of black beans,[32] red beans,[30] yellow wax beans,[54] and other varieties of *Phaseolus vulgaris*.[55,56] In most cases, at least two different hemagglutinating factors were present and could be separated by electrophoresis or chromatography. Two hemagglutinins differing in molecular weight and amino acid composition were isolated from a particular variety of *P. vulgaris* grown in Sweden.[56] In general, most of the hemagglutinins isolated from different varieties of *P. vulgaris* have molecular weights ranging from 91,000 to 130,000; differences in isoelectric points, however, have been observed.[56]

The toxic properties of these purified preparations have been studied in only a few cases. Thus, Honavar *et al.* observed a marked inhibitory effect on the growth of rats fed a diet containing purified kidney-bean agglutinin at a level of 0.5%.[30] All animals died within 2 weeks of ingestion of this diet. An agglutinin isolated from black beans was likewise toxic when fed to rats or when injected into mice.[29] In the latter case, the LD_{50} was about 50 mg/kg. The black-bean agglutinin was found to be somewhat less toxic than that obtained from red kidney beans.[30]

Diets containing unheated, ground kidney beans cause loss of weight, and their ingestion usually results in death of the experimental animals. Food consumption is very low, and most of these animals have diarrhea. Balance studies have shown that overall digestibility and nitrogen retention are low in rats ingesting raw bean diets, compared with animals kept on a diet prepared with properly heated beans.[29] The extracted residue of the bean meal from which the hemagglutinin had been prepared retained part of the toxicity of the original material, since it reduced the growth of rats when added to a casein diet.[29]

The supplementation of a raw bean diet with predigested casein had no beneficial effect on growth and survival of rats, an observation that was interpreted to mean that the trypsin inhibitors present in the beans were not responsible for the toxic manifestations.[57] In similar experiments, diets prepared with bean varieties with strong hemagglutinating activity produced the effects already described (i.e., rapid weight loss

and death of the rats), whereas diets containing nonhemagglutinating bean meals had no lethal action and permitted slow growth. The addition of predigested casein resulted in normal growth in the latter case but was without effect when used in a diet prepared with hemagglutinin-containing bean meal.[33]

Notwithstanding the evidence cited for the toxicity of the bean hemagglutinins, there are several claims for a partial separation of the toxic and hemagglutinating activities of beans[50,58,59] and for lack of correlation between toxic and hemagglutinating activities measured simultaneously in a number of different bean species and varieties.[60] These observations do not allow the conclusion that bean hemagglutinins are nontoxic, but they are consistent with the assumption that there may be present other unidentified heat-labile and toxic factors in raw beans.

Recent observations of differences between hemagglutinins from various bean varieties in specificity, heat resistance, and toxicity may help to explain some of these contradictory observations.[61] By the use of rabbit red blood cells (RBC) and trypsin-activated cow RBC, it is possible to distinguish four types of bean hemagglutinins, because the extracts of some bean samples agglutinate only one of these blood preparations, both of them, or none at all. Toxicity tested by IP injection of extracts into mice and by addition of the ground beans to experimental diets of rats was detected only in the bean varieties containing the hemagglutinins active with trypsin-activated cow RBC. Heating to 85 °C for several hours did not destroy the toxicity and the action on cow blood cells, but the action on rabbit blood cells disappeared. Cooking the soaked beans 1 h at 100 °C destroyed toxicity and hemagglutinating activity completely.

Treatment with proteolytic enzymes has been recommended for the reduction of the cooking time required for the preparation of beans.[62] Mixtures of ground beans with ground cereals are being used in infant feeding programs in order to raise the protein intake and to achieve an optimal proportion of these ingredients.[63] These mixtures are quite palatable after a relatively short cooking time at 90 °C. Too-short cooking of partially precooked bean flakes produced a serious outbreak of poisoning in Berlin.[64]

The toxicity of bean products will depend on the heat treatment they have received. Some of the intermediary stages of toxicity may not be easily detected. Before new bean products are used in industrial procedures or feeding programs, it is advisable that the heat treatment required for complete destruction of toxic factors be established by careful investigations.

Other Legume Hemagglutinins Field beans or hyacinth beans (*Dolichos lablab*) are consumed in India, Africa, and some parts of South America. They show a degree of toxicity similar to that of kidney beans when fed to rats. A hemagglutinin obtained from these seeds produced loss of weight and death of rats when given in amounts of 1.5 or 2.5% in a casein diet. Necropsy revealed focal liver necrosis in these animals.[65]

Lima beans or double beans (*Phaseolus lunatus*) contain two blood-group-A-specific hemagglutinins.[66] A partly purified preparation of a lima-bean agglutinin reduced growth of rats when fed with a casein diet but did not produce death under these conditions. When injected into rats or mice, this preparation was lethal at a dose of 65 and 140 mg/kg, respectively, but a highly purified preparation was less toxic.[67]

Another anti-A-group-specific hemagglutinin has been isolated from the horse gram (*Dolichos biflorus*).[68] Rats fed with a diet containing horse gram showed greatly retarded growth and did not survive for more than 3 weeks,[69] but these effects were not observed when autoclaved material was used. A crude hemagglutinin preparation had a growth-retarding effect, but the purified hemagglutinin was innocuous when parenterally administered to rats or mice.[70]

Properties of Hemagglutinins

Degree of Toxicity The experimental animal species used for testing, the route of administration, and even the age of the test animals may be responsible for different toxic effects. Hemagglutinin purified from kidney beans and added to a rat diet caused loss of weight and death,[30] but in similar experiments with chicks no lethal action was detected.[71] Rats or chicks fed diets prepared with unheated soybean meal as the only source of protein grow fairly well, although less than animals given heated soy meal; mice kept on a raw soy diet may lose weight,[72] as do calves fed a lightly cooked soy-flour milk replacer.[73] It has not been proved, however, that the factor causing body-weight loss in these cases is the soybean hemagglutinin.

Among the legume seeds that contain measurable amounts of hemagglutinins,[74] and that will show no or only a minimum improvement of food value after heating, are peas (*Pisum sativum*), red gram (*Cajanus cajan*), and lentils (*Lens culinaris*).[18] The different degrees of oral toxicity of soybean and kidney-bean agglutinins have been mentioned already. The most toxic hemagglutinins of the edible legumes are those of kidney beans and the field beans. In human diets prepared with well-cooked beans of either kind, no toxic effects have been observed. The uncooked beans are highly unpalatable.

Mode of Toxic Action of Hemagglutinins It seems likely that both the hemagglutinating and the toxic actions are related to the ability of these plant proteins to react with certain receptor groups of the membrane of the target cells.[17] Only those plant agglutinins capable of interaction with receptors of cells of certain animal species would be expected to be toxic for the corresponding animals. The cancerostatic action of several hemagglutinins (described below) may be an example of such a specific cell toxicity.

Interaction of hemagglutinins from kidney beans with the cells lining the gut has been postulated as an explanation for their oral toxicity.[29] The adsorption of bean agglutinin on the mucosal cells of rat intestine can be shown in *in vitro* experiments.[29] Their resistance to digestion in the animal is easily demonstrated by the hemagglutinating activity of extracts from feces of rats after they ingested a raw bean diet.[33] As mentioned, reduced intestinal nitrogen absorption can be observed in rats fed a raw bean diet.[29] Glucose absorption from a ligated intestinal loop is low, compared to the control animals previously given black-bean agglutinin by stomach tube.[75] Rats fed a bean diet may experience severe hypoglycemia, which could be caused by poor absorption of glucose.[77] Evidence for interference of raw beans with aminoacid absorption and for the reduced utilization of vitamin E in chicks[76] has been presented.[57] It has been mentioned above that raw soybeans may also interfere with the absorption of various nutrients.

The food intake of animals fed raw beans, soybeans, or field beans is impaired. This may be related to slow stomach emptying.

The fact that the different phytohemagglutinins react in a specific manner with different receptor groups existing on the surface of cells makes them valuable tools for the investigation of those groups and, at the same time, may offer a clue to their toxicity. The observation of receptor groups for lectins on cancer cells, their appearance during cancerous transformation in cell cultures,[78] and the anticancerous effect of various phytohemagglutinins merit special interest. Thus, Nungester and Van Halsema[79] found that bean extracts may interact with Flexner–Jabling carcinoma cells of rats; Steck and Wallach[17] found purified bean PHA to react with Ehrlich ascites carcinoma of mice. Concanavalin A, the agglutinin from the jack bean (*Canavalia ensiformis*), has a definite cancerostatic action in mice inoculated with a cancer-producing virus,[78] and the trypsin-treated lectin inhibits *in vivo* growth of cancer cells.[80] Ricin and abrin, the agglutinins from the castor bean and from the seeds of *Abrus precatorius,* respectively, suppress Ehrlich ascites tumor growth in mice[81]; the wheat-germ hemagglutinin reacts with some kinds of cancerous cells as well as with some normal cells.[82] The

receptor groups of the cancer-cell surface may also exist on normal cells. It is, therefore, likely that some of the hemagglutinins may interact with some normal cells, resulting in a toxic effect, but this effect would be more difficult to prove experimentally than is the case with the cancer cells.

Chemical Properties Until now most plant hemagglutinins obtained in pure form have been carbohydrate-containing proteins; the only exception is concanavalin A from the jack bean, which is free of sugars.[83] Only two plant hemagglutinins are known to contain sulfhydryl groups: the lima-bean agglutinin[84] and that of the meadow mushroom *Agarius campestris.*[85] Among the hemagglutinins obtained in pure form from edible legumes are soybean,[26] black beans,[32] wax bean,[54] lentil,[86] pea,[24] lima bean,[66] horse gram,[68] *Vicia cracca,*[87] and peanut.[88] In most of these seeds several hemagglutinins with very similar characteristics are present. The agglutinins from kidney beans[89] and from the jack bean[90] can be dissociated into subunits by urea.

The kidney-bean agglutinin is probably a lipid-containing protein, as is shown by the staining of the corresponding immunoprecipitates.[91] In two plant hemagglutinins, concanavalin A from the jack bean[92] and the agglutinin from lentils,[93] significant amounts of calcium and manganese exist as constituents essential for the activity. Many plant hemagglutinins are remarkably stable against the action of a great number of proteolytic enzymes.

Detection

Hemagglutinins are detected in the extracts of ground seeds prepared with physiological saline solution by their action in serial dilution on washed RBC. A microdilution test has been used in many of the recent papers on phytohemagglutinins.[87] A photometric method was proposed by Liener.[94] As all phytohemagglutinins are more or less specific, the choice of the right kind of blood cells is important. Treatment of the red cells with proteolytic enzymes often enhances the sensitivity of the assay. Pronase has been found to be well suited for this purpose in most cases.[95] For the soybean agglutinin, rabbit blood is the most satisfactory, but for the kidney-bean agglutinins, trypsin-activated cow blood and rabbit blood should be used, because some types of these agglutinins are detected by only one of these blood types and not by the other.[61] The agglutinins from horse gram (*Dolichos biflorus*) and from lima beans will agglutinate only human-group-A blood. Some agglutinins

are active at low temperature but will not give a positive reaction at 37 °C.[74]

It should be remembered that a positive agglutination test does not necessarily distinguish between toxic and nontoxic hemagglutinins. The test is most useful for the detection of agglutinins in seed varieties or fractions suspected to be toxic. In special cases a distinction between toxic and nontoxic agglutinins may be possible, as has been mentioned for the kidney bean.

ALLERGENIC FOODS

Many people show more or less violent local or generalized reactions after ingestion of certain foods or contact with certain types of proteinaceous material, i.e., pollen, which does not produce harm in other persons. The nature of these allergic reactions is not yet fully understood, notwithstanding the tremendous amount of research devoted to the subject. Unlike effects from other food toxins, the intensity of this reaction will depend not so much on the quantity of the food ingested as on the sensitivity of the affected person. The allergens are, therefore, not toxic food constituents in the general sense, and the reason for the toxic action rests rather with the individual who has an altered reactivity (allergy). For this reason, allergens will be treated only very briefly here; the interested reader is referred to an extensive review by Mansmann in the first edition of this book[96] and to other recent review articles.[97,98,99]

Allergy is a rather common affliction. It has been estimated that about 20% of American schoolchildren suffer some kind of allergy.[100] Most foods have been reported to induce allergy in some people; exceptions are fats, pure sugar, and salt. Common causes of food allergy are grains, milk, eggs, fish, crustaceans, tomatoes, strawberries, nuts, chocolate, and many others. Milk allergy has been studied mostly in children. Diarrhea, abdominal pain, and vomiting are the most common symptoms.[101] Although the exact nature of the allergens in foods is generally unknown, they are probably mostly proteins.[102] In some cases, but not in all, heating to 120 °C for 30 min will abolish allergenicity.[103] The best way to avoid food allergies is the complete elimination of the offending product from the diet. Desensitization and the use of various specially developed drugs are other therapeutic measures sometimes used to alleviate the suffering of the allergic patient.

The allergic reaction is brought about by a certain type of antibody

called reagins, but the reagin titer is not always correlated with the patient's sensitivity. There is considerable evidence for a hereditary factor in human allergy, although little is known about specific genetic factors that cause allergy in humans. While most organs can be involved in allergic reactions, the most commonly affected are the skin and the respiratory tract. The predominant characteristic of reagins is their ability to become fixed to the skin or to other tissues. A recently discovered class of immunoglobulin, IgE, appears to be the skin-sensitizing antibody in man.[104] By far the most commonly employed procedure for the detection of food allergens is the skin test, performed by superficial scarification or by intradermal injection of extracts of the suspected products, which will result in a wheal or flare reaction. Such other methods as the dietary history and elimination diets are also used for diagnostic purposes.

CELIAC DISEASE

Celiac disease is a malabsorption syndrome mostly seen in children but that can also be observed in adults. Although the exact cause is unknown, it is clearly related to the ingestion of wheat products and specifically of gluten.[105] Indeed, the complete elimination of gluten-containing foods from the patient's diet is the most important therapeutic measure, and relapse after ingestion of a test dose of gluten is an important diagnostic sign. In the celiac patient a normal food protein has a toxic effect, a situation similar to that for food allergies. In adults a gluten-sensitive enteropathy is often referred to as nontropical sprue, idiopathic steatorrhea, or adult celiac disease.

The mucosa of biopsy samples of the jejunum taken from celiac patients is flat, and the villi are small or absent.[106] Abnormally large amounts of fat are present in the stool, and the absorption of many other nutrients, such as amino acids, glucose, vitamins K, B_{12}, and others is impaired. Celiac disease may therefore lead to a state of advanced malnutrition. Not only wheat but also rye, barley, and oats may be harmful to patients with celiac disease, and milk also is often not well tolerated. A gluten-free diet must be observed, often for many years.[107] A secondary deficiency of the intestinal enzymes, disaccharidase and peptidase, has often been observed.[108] Gluten is not well digested,[109] and the oligopeptides derived from gluten may cause intestinal lesions.[110] When different peptides obtained through *in vitro* digestion of gluten are tested in celiac patients, important differences are observed in their ability to elicit the pathological signs characteristic of celiac disease.[111]

From immunological studies the absorption of antigenic derivatives of gluten into intestinal epithelium has been postulated.[112] A hereditary factor may be involved in the susceptibility to this syndrome.[113]

SELENIUM-CONTAINING PROTEINS

Selenium is abundant in some types of soil and is absorbed by most plants more or less efficiently. Thus it can accumulate in diverse foods. (See Chapters 3 and 7.) It may be incorporated into amino acids, replacing sulfur atoms, and these seleno–amino acids may then be incorporated into proteins. If the selenium concentration is high enough, these products may produce toxic effects. From dialysis experiments with extracts of wheat and other cereals, it was concluded many years ago that selenium may be present in protein-bound form.[114] Olson *et al.* showed that wheat with a high selenium content contains seleno-methionine in the gluten, but it contains no seleno-cystine.[115] The selenium in defatted sesame seeds is also present in the seed proteins,[116] and the major part of selenium in fish can be recovered in the protein fraction.[117]

The occurrence of selenium-containing proteins in edible plant products is of special significance when these materials are used for the preparation of protein isolates, because in this way the toxic selenium may inadvertently be concentrated. This possibility should be kept in mind when oilseed products are processed for high-protein foods, particularly since the seleno-proteins cannot be rendered harmless by heat processing.

ENZYMES

Because of their specific activity on biological compounds, some enzymes can produce toxic effects, but only very few are important in foods. They may act through the decomposition of such essential constituents as the vitamins, or they may liberate toxic compounds.

Among the first group, the thiamine-destroying enzymes, called thiaminases, may be mentioned. They have been found in bracken fern, *Pteridum aqualinum,*[118] which is sometimes eaten by horses or cattle, in fish, especially the carp,[119] and the sardine,[120] and in some crustaceans.[118]

A lipoxygenase that oxidizes and destroys carotene has been observed in soybeans.[121] Low blood values of vitamin A and carotene have been found in the liver and blood of calves fed a diet containing raw

soybeans, but the importance of the lipoxygenase in causing these low vitamin blood levels has not been ascertained.[42]

Great amounts of urease are found in some legume seeds, notably in the jack bean (*Canavalia ensiformis*) and to a lesser degree in the soybean. This enzyme may produce toxic effects when ingested by animals[122] and is highly toxic when injected, because it will liberate ammonia from the blood urea. Its harmful action may be of importance only when urea is used in feed for cattle or sheep.

Several foods contain cyanogenetic glucosides. (See Chapter 14, p. 300.) Specific glucosidases that are frequently present in the same products release the highly toxic hydrocyanic acid from these compounds. This release is enhanced when the plant products are crushed and soaked in water.

Intestinal β-glucosidase may release the toxic methylazoxymethanol from the glucoside cycasin that exists in plants of the cycad family.[123] In these cases, the enzyme acts in releasing a toxic factor, and it is a matter of semantics whether or not one should call it toxic.

TOXIC PEPTIDES

Peptides are often not recognized in complex biological materials because they are lost in many of the fractionation procedures, especially if these include prolonged dialysis. This may be the reason only few toxic peptides have been identified in food products. In a recent study of the toxic principles of raw soybeans, Schingoethe *et al.*[72] observe that a fraction separated from a crude preparation of the soybean trypsin inhibitor had a growth-inhibitory activity when fed to mice. By its retardation on a Sephadex G-25 column and its passage through dialysis membranes the peptide nature of this product was indicated. The presence of at least eight positively charged peptides, one of which was a glycopeptide, could be shown by high-voltage electrophoresis.

A highly toxic cyclic peptide called islanditoxin has been isolated from so-called yellow rice, that is, rice infected with the mold *Penicillium islandicum*.[124] It contains β-aminophenylalanine, two molecules of serine, aminobutyric acid, and a dichlorinated proline. Islanditoxin is a highly toxic carcinogenic hepatoxin, but removal of the chlorine atoms gives a nontoxic product. Feeding of low amounts of rice infected with *P. islandicum* induces liver cirrhosis in experimental animals.

Collection of wild mushrooms for the purpose of eating them is a popular hobby not free of danger. Knowledge of the edible and inedible varieties should be an indispensable prerequisite, because mistakes may

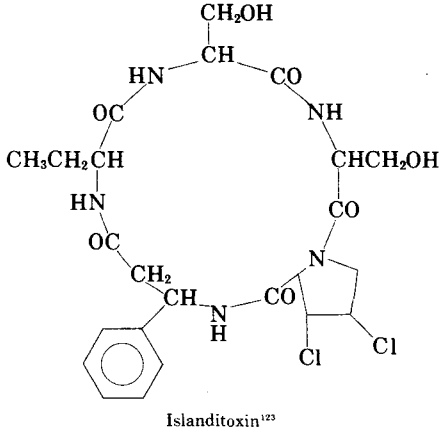
have a fatal outcome. By far the most common cause of mushroom poisoning is the ingestion of species belonging to the genus *Amanita,* which might be confused with the edible champignon. They contain a variety of very toxic peptides, the structure of which has been elucidated mostly by the work of Wieland and his collaborators.[125,126,127]

Two groups of peptides called phallotoxins and amatoxins can be distinguished. Phallotoxins are cyclic heptapeptides, and amatoxins are comprised of closely related cyclic octapeptides. Both have several unusual characteristics. There is a thioether formed between a L-cysteine and L-tryptophan in the phallotoxins, while in the amatoxins these two amino acids are united in a sulfoxide structure. The phallotoxins contain hydroxyamino acids derived from L-leucine, while the amatoxins contain derivatives of γ-hydroxyisoleucine.

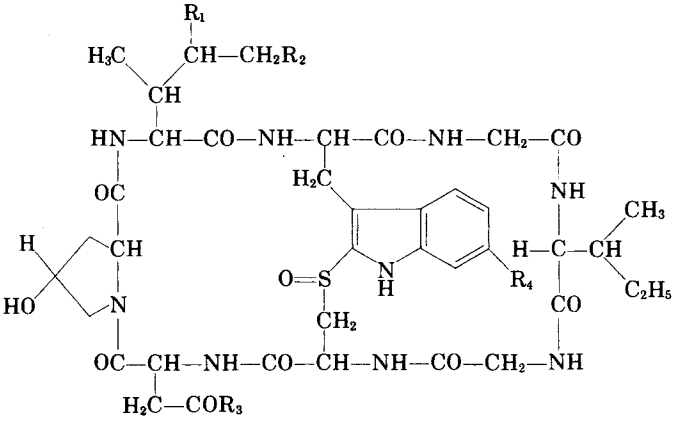
The two groups of toxins show striking differences in their respective biological actions; amatoxins are much more toxic than the phallotoxins. Since both groups of peptides are present at approximately the same concentration in poisonous species of *Amanita,* the poisonous nature of these mushrooms must be attributed mainly to the amatoxins.

The organ affected by both groups of peptide toxins from mushrooms is the liver.[128] The toxins have been identified in liver extracts from fatal human cases of intoxication.[129] Phalloidin has a marked affinity for the microsomal fraction of the liver, as has been shown with labeled toxins.[130] It is interesting that phalloidin acts only if administered to the intact animal. It seems likely, therefore, that it is converted into an unknown toxic substance by some metabolic process.

The toxic action of amatoxins appears considerably later after ingestion than that of phallotoxins, and the nuclei are the part of the liver cell mostly affected.

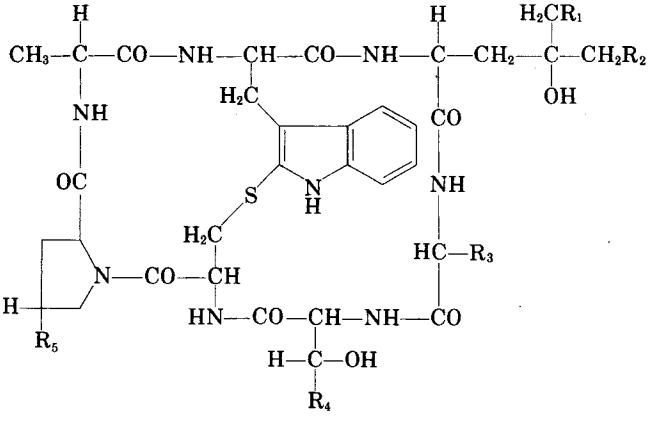


Islanditoxin[123]



Amatoxins[125]

	R_1	R_2	R_3	R_4
α-Amanitin	OH	OH	NH_2	OH
β-Amanitin	OH	OH	OH	OH
γ-Amanitin	H	OH	NH_2	OH
Amanin	OH	OH	OH	H
Amanullin	H	H	NH_2	OH



Phallotoxins[125]

	R_1	R_2	R_3	R_4	R_5
Phalloidin	OH	H	CH_3	CH_3	OH
Phalloin	H	H	CH_3	CH_3	OH
Phallisin	OH	OH	CH_3	CH_3	OH
Phallicidin	OH	H	$CH(CH_3)_2$	COOH	OH
Phallin B (tentatively)	H	H	$CH_2C_6H_5$	CH_3	H

Several other biologically active principles exist in mushrooms of the *Amanita* family, but they are not peptides and seem to be of little importance relative to toxicity. The poisonous *Amanita* mushrooms contain about 10–15 mg of both toxins per 100 g fresh weight. A single mushroom weighing 50 g may contain enough toxins to kill a man.

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NOTE

After this chapter was completed, two extensive review articles were published on the biochemistry of plant agglutinins by Nathan Sharon and Halina Lis: *Science 177*(4053):949 (1972) and *The Annual Review of Biochemistry* (1973).