Significance for Humans of Biologically Active Factors in Soybeans and Other Food Legumes

I. LIENER, Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul, MN USA

ABSTRACT

Among the many biologically active factors present in the soybean, only protease inhibitors (PI) have been shown to exert significant adverse effects on animals consuming diets containing soybean protein. Evidence is presented to suggest that (a) PI are only partially responsible for the poor nutritive value of inadequately processed soybeans, (b) low levels of PI are relatively harmless to animals, (c) human trypsin is only weakly inhibited by PI, and (d) the human pancreas is probably insensitive to the hypertrophic effects of PI. Parelleling the wide spread distribution of PI in the plant kingdom are the so called phytohemagglutinins or lectins. Unlike the lectin present in soybeans which appears to have only a marginal effect on the nutritional quality of the protein, the lectin of the common bean (*Phaseolus vulgaris*) is quite toxic. Moreover, the major storage protein of such beans is quite resistant to digestion unless denatured by heat, thus emphasizing the importance of adequate processing of those legumes when used in the human diet. Although goiter-inducing compounds are present in most cruciferous plants and cyanide-producing substances may be found in cassava and lima beans, traditional methods of preparation and present technology have served to minimize any harmful effects that may accompany the ingestion of these foods by man. Brief mention will also be made of two human diseases, lathyrism and favism, associated with the consumption of *Lathyrus sativus* and *Vicia faba,* respectively, their causative agents and mechanism of action. Although there are numerous examples of so called toxic constituents in legumes, they nevertheless have provided a valuable source of protein to man over the centuries. This can be attributed, in part, to the fact that man has learned how to detoxify them by suitable preparative measures. The varied nature of our diet also minimizes the contribution of a toxicant from any one foodstuff. Nevertheless, there is the ever present possibility that the prolonged consumption of a particular legume that may be improperly processed could bring to the surface toxic effects that otherwise would not be apparent. As the shortage of protein becomes more acute, it is not unlikely that much of the population of the world will be faced, in the future, with a more limited selection of protein-foods, most of which will be of plant origin and, hence, potential carriers of toxic constituents. The food scientist should at least be cognizant of such a possibility and be prepared to apply his knowledge and skill to meeting this challenge.

INTRODUCTION

It is well recognized that, for whatever reason, Nature has seen fit to endow plants with the capacity to synthesize biologically active substances which may have an adverse effect on animals and man. The very fact that we are having this conference bears testimony to the importance that is being attached to use of plants as a source of protein in the human diet, so it is understandable that we should give some consideration to the question as to whether these biologically active constituents of plants are of any significance with respect to man. In general one can say that there are two categories of these biologically active substances. There are those whose effects have been studied in a wide variety of animals, but whose effects in humans must remain, at least for the moment, largely a matter of conjecture. Then there are those foods of plant origin which are known to produce adverse reactions in humans, but in which the causative factor has been difficult to identify because the same disease is difficult and often impossible to reproduce in animals.

Since time does not permit a coverage of all of the many biologically active substances known to be present in plant foodstuffs (1), this paper will be confined to a consideration of some selected examples of each of these two categories. In the first category, an attempt will be made to evaluate the significance of protease inhibitors and lectins in the diet of man, since these substances are the principle antinutritional factors associated with the legumes most commonly consumed by man. Brief consideration will also be given to a group of chronical substances known as glycosides which, although innocuous in themselves, can be converted to either goiter-inducing agents (goitrogens) or can release toxic levels of cyanide (cyanogens). In the second category, our current knowledge concerning two diseases in man associated with the consumption of specific legumes, namely lathyrism and favism, will be briefly summarized.

PROTEASE INHIBITORS

Historical Background

It was not long after soybeans were introduced into the United States, primarily as a source of oil, that Osborne and Mendel (2) made the significant observation that soybeans had to be heated in order to support the growth of rats. With the demonstration of a heat-labile trypsin inhibitor in soybeans and its subsequent crystallization (3), it was generally assumed that the beneficial effect of heat treatment could be ascribed to the destruction of this inhibitor. The inactivation of the trypsin inhibitor does in fact appear to parallel the improvement in nutritive value effected by heat as demonstrated with rats (Fig. 1). Further evidence came from experiments in which it was shown that the addition of purified preparations of the trypsin inhibitor to heated soybeans, so as to provide the same inhibitory activity as raw soybeans, caused a significant reduction in growth (Table I). It is important to note, however, that adding the trypsin inhibitor did not reduce the PER to the same level of growth as was observed on raw soybeans, indicating that heat treatment was doing something more than just inactivating the trypsin inhibitor. This is a point to which will be referred to later.

With the recognition of the presence of a trypsin

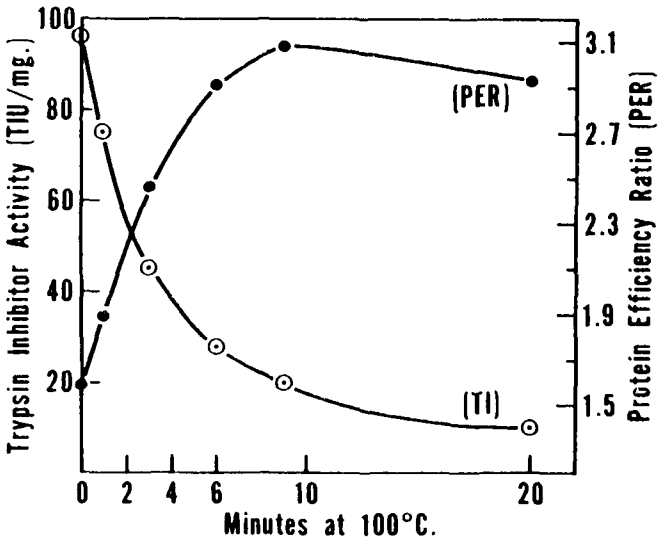

FIG. 1. Effect of heat treatment on trypsin inhibitory activity and protein efficiency ratio of soybean protein (4).

TABLE I

The Effect of Adding Partially Purified Soybean Trypsin Inhibitor (STI) to Diets Containing Heated Soybean Meal in the Presence and Absence of Methionine (5)

Diet	PER - met	PER + met[a]
Raw soybeans	1.40	2.42
Heated soybeans[b]	2.63	2.99
Heated soybeans + 1.8% STI	1.95	2.63

[a]Diets were supplemented with 0.6% methionine.
[b]Autoclaved at 15 lb pressure (115) for 20 min.

inhibitor in soybeans, it was tempting to conclude that the growth inhibition which it evoked in animals was simply due to an inhibition of digestion of dietary protein by proteolytic enzymes present in the intestinal tract. The most destructive blow to this theory was the observation that preparations of trypsin inhibitor were capable of inhibiting growth even when incorporated into diets containing predigested protein or free amino acids (5-7). Such experiments obviously rule out an inhibition of proteolysis as the sole factor responsible for growth inhibition and thus served to focus attention on some alternative mode of action of the trypsin inhibitor.

Mode of Action

Perhaps the most significant observation which has ultimately led to a better understanding of the mode of action of the soybean inhibitor was the finding that raw soybeans and the trypsin inhibitor itself could cause hypertrophy of the pancreas, an effect which is accompanied by an increase in the secretory activity of the pancreas (8). This led to the suggestion that the growth depression caused by the trypsin inhibitor might be the consequence of an endogenous loss of essential amino acids being secreted by a hyperactive pancreas (9,10). Since pancreatic enzymes such as trypsin and chymotrypsin are particularly rich in the sulfur-containing amino acids, pancreatic hypertrophy causes a drain on the body tissue of these particular amino acids in order to meet an increased need for the synthesis of these enzymes. This loss in sulfur-containing amino acids serves to accentuate an already critical situation with respect to soybean protein, which is inherently deficient in these amino acids.

The mechanism whereby the trypsin inhibitor induces pancreatic enlargement is still not fully understood. Lyman

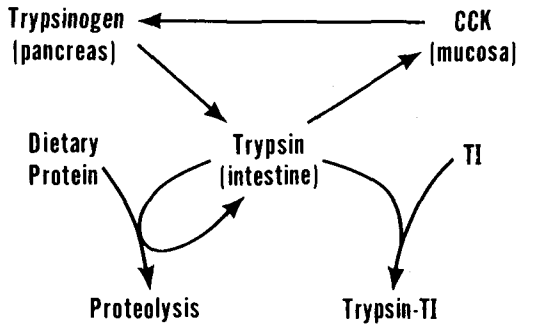

FIG. 2. Regulation of secretion of trypsin by the pancreas. CCK, cholecystokinin; TI, trypsin inhibitor.

and coworkers (11-13) have shown that pancreatic secretion is controlled by a mechanism of feedback inhibition which depends upon the level of trypsin and chymotrypsin present at any given time in the small intestine. When the level of these enzymes falls below a certain critical threshold value, the pancreas is induced to produce more enzyme. The suppression of negative feedback inhibition can occur if the trypsin is complexed with the inhibitor or by dietary protein itself (see below). It is believed that the mediating agent between trypsin and the pancreas is the hormone cholecystokinin (CCK), which is released from the intestinal mucosa when the level of trypsin in the intestine falls below its threshold level. These relationships are illustrated in Fig. 2.

The Role of Protein Digestibility

It was mentioned earlier that the trypsin inhibitor itself did not appear to account fully for the growth inhibition observed on raw soybeans (Table I). A further indication that this might be true came from an investigation of a large number of different varieties of soybeans in which the PER of such beans were compared with their trypsin inhibitor activity. As shown in Fig. 3, there is no correlation whatsoever between these two parameters. Furthermore, if the trypsin inhibitor activity of a crude extract of soybeans is removed by affinity chromatography on Sepharose-bound trypsin, the resulting extract is still capable of causing growth inhibition and pancreatic hypertrophy (Table II). It may be estimated from these data that the trypsin inhibitor accounts for about 40% of the growth inhibition observed with raw soybeans. It is also significant to note that only about 40% of the enlargement of the pancreas produced by the ingestion of raw soybeans is also accounted for by the trypsin inhibitor.

These findings raise the question as to what is responsible for the remaining 60% of the growth-retarding and pancreatic-inducing effects of raw soybeans. A possible clue comes from experiments in which the crude soybean extract from which the inhibitor had been removed was subjected to digestion with trypsin in vitro (Fig. 4). Heat treatment of this soybean protein produces an increase in the digestibility of the protein over and above the digestibility of a similar preparation from which the inhibitor had been removed. This observation suggests that native, undenatured soybean protein is in itself refractory to enzymatic attack unless denatured by heat. If this undenatured protein is in fact capable of binding trypsin by forming an enzyme-substrate complex (16,17), this undigested protein can likewise remove feedback inhibition of pancreatic secretion by trypsin. It would appear, therefore, that the trypsin inhibitor and the refractory nature of the soybean protein act through a common mechanism to inhibit the growth of rats.

Physiological Significance in Man

It should be appreciated that all of the experiments

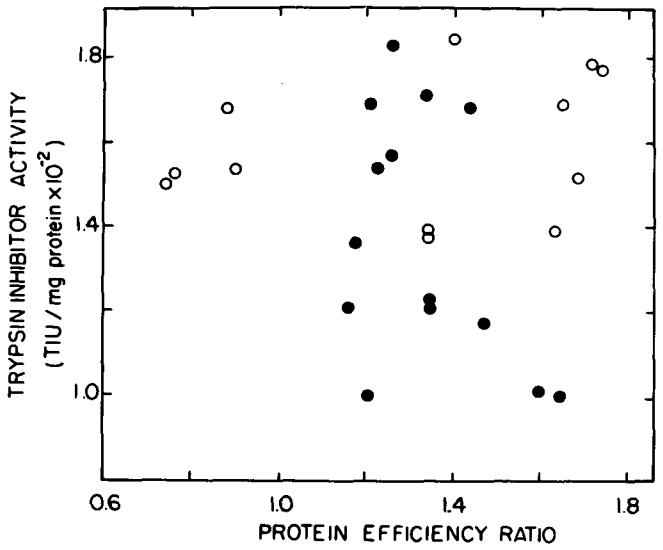

FIG. 3. Relationship of trypsin inhibitor activity of different soybean varieties (14).

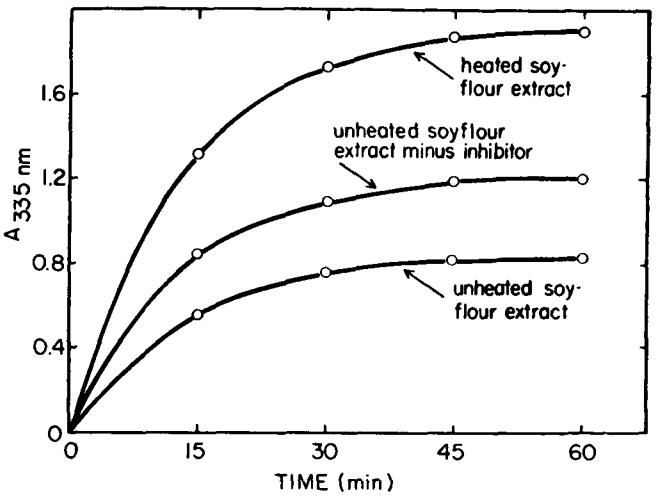

FIG. 4. In vitro digestibility by trypsin of soybean extract with and without trypsin inhibitor removed compared to the heated extract (14).

described thus far were conducted with rats as the experimental model. As a basis for speculation as to the relevance of such experiments to humans, the following lines of evidence will be considered which suggest that the trypsin inhibitors are most likely of little consequence when soybean products are used for human food.

Many of the soybean products on the market today have been made from protein isolates which, depending on their mode of preparation, may contain as much as 30% of the trypsin inhibitor activity of the raw bean. An

TABLE III

Trypsin Inhibitor Activities of Soybean Flour, Isolate, Fiber, and Finished Textured Products (18)

	Antitrypsin activity TIU[a] g dry solids x 10^{-3}	% of Soy flour
Soy flour (unheated)	86.4	100
Soybean isolate	25.5	30
Soybean fiber	12.3	14
Chicken analog	6.9	8
Ham analog	10.2	12
Beef analog	6.5	7

[a]TIU = trypsin inhibitor units.

examination of the trypsin inhibitor activity of several textured meat analogs during the course of their manufacture reveals that, although the protein isolate may be rich in antitryptic activity, the inhibitor activity is reduced to very low levels in the final product (Table III). Household cooking of such products would be expected to reduce these levels even further. Kotter et al. (19) have reported that canned Frankfurter-type sausages containing 1.5% soy isolate were essentially devoid of any trypsin inhibitor activity after the canning process. Furthermore, Nordal and Fossum (20) have reported that the trypsin inhibitor activity provided by soy isolate in meat products was actually more labile to heat inactivation due to some component in the meat ingredients. They postulated that this factor increased the sensitivity of the trypsin inhibitors to heat inactivation by causing the rupture of disulfide bonds in the inhibitor molecule, particularly the Bowman-Birk inhibitor, which is rich in disulfide bonds.

Of particular concern to the pediatrician is the possibility that infants fed soy milk formulated with soy isolate might be more sensitive to the same physiological effects of the inhibitor as observed in young rats. Churella et al. (21), however, have recently demonstrated that the heat treatment involved in the processing and sterilization of infant soy formulas containing soy isolate reduced the trypsin inhibitor activity to less than 10% of the original activity of the isolate. This residual activity did not produce any weight reduction or pancreatic hypertrophy in rats. These observations are consistent with the findings of Rackis et al. (22), who found that no pancreatic hypertrophy occurred in rats fed soy flour in which only 54% of the trypsin inhibitor activity was destroyed, and maximum PER corresponds to a destruction of only 80% of the inhibitor activity of soy flour (Table IV). The further increase in growth and PER may be attributed to an increase in protein digestibility rather than to the further destruction of the trypsin inhibitor.

Assuming for the sake of argument that the processing conditions may have been inadequate to reduce the level of trypsin inhibitory activity below the levels shown to be safe for rats, would the residual activity still pose a risk to human health? Let us first address the more basic ques-

TABLE II

Contribution of Trypsin Inhibitors to the Growth Inhibition and Pancreatic Hypertrophy Induced in Rats by Diets Containing Unheated Soybean Protein (15)

Source of protein	PER	Wgt. of pancreas g/100 g body weight
Soy flour extract, unheated	1.4	0.71
Soy flour extract, heated	2.7	0.57
Soy flour extract minus inhibitor[a]	1.9	0.65
Percent change due to removal of inhibitor	+38	-41

[a]Trypsin inhibitors removed by passage of unheated soy flour extract through a column of Sepharose-trypsin.

TABLE IV

Effect of Soy Flour Containing Various Levels of Trypsin Inhibitor on Growth and Size of Pancreas of Rats (22)

Trypsin inhibitor content mg/100 g diet	% destruction	Body wgt. g.	PER	Pancreas wgt. g/100 g. body wgt.
887	0	79	1.59	0.70
532	40	111	2.37	0.56
282	68	121	2.78	0.50
157	82	134	2.97	0.49
119	87	148	3.08	0.47
71	92	142	3.03	0.45
Casein	---	145	3.35	0.55

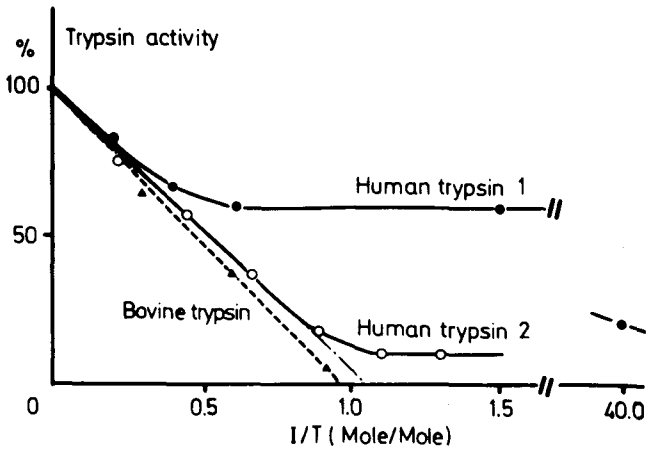

FIG. 5. Inhibition of human trypsins by soybean trypsin inhibitor. Trypsin 1, cationic species; trypsin 2, anionic species. (24).

tion of whether the soybean inhibitors do in fact inhibit human trypsin. Trypsin inhibitor activity is invariably measured in vitro on the basis of the ability of soybean preparations to inhibit *bovine* or *porcine* trypsin since these are readily available commercially in "pure" crystalline form. Human trypsin is known to exist in two forms, a cationic species which constitutes the major component, and an anionic species which accounts for less than one-third of the total trypsin activity of pancreatic juice (23-25). As shown in Fig. 5, while the minor anionic species of trypsin (trypsin 2) is inhibited by the soybean inhibitor in a stoichiometric fashion, the cationic species of trypsin (trypsin 1), which constitutes over two-thirds of the total trypsin activity of the human pancreas, is only very weakly inhibited.

In further support of the probability that the soybean inhibitors are relatively ineffective against human trypsin, there is the rather interesting relationship that seems to exist between the size of the pancreas of various species of animals and the nature of the response of their pancreas to the trypsin inhibitor. As shown in Table V, there appears to be a direct relationship between size of the pancreas and sensitivity of response to raw soybeans or the isolated inhibitor. Pancreases of those species of animals whose weights exceed 0.3% of their body weight become hypertrophic when fed raw soybeans or the inhibitor, whereas those animals whose pancreas fell below this value do not respond to the hypertrophic effects of the trypsin inhibitor. The guinea pig appears to be on the boderline of this relationship in as much as a positive response is noted in the case of the adult animal, but not in the case of the immature animal. One would predict from these data that the pancreas of the human would be insensitive to the effects of soybean inhibitor, although it must be emphasized that there is no direct experimental evidence bearing on this point.

LECTINS

It is well recognized that, in addition to protease inhibitors, most legumes and cereals contain substances, the so called phytohemagglutinins or lectins, which have the unique property of binding to the sugar components of a wide variety of biological components (33). With the red blood cells, the interaction of lectins with glycoproteins present on the cell surface is manifested in vitro by an agglutination of the cells. Ever since the days of Ehrlich, it has been shown that some of these lectins, such as ricin from the castor bean, are extremely toxic to animals. Little is known, even now, concerning the extent to which these substances might be responsible for the well recognized fact that legumes constitute a very poor source of protein unless heated (34).

Soybeans

Soybeans contain several lectins comprising an estimated 1%-3% of the protein of defatted soybean flour (35). A seven-fold variation in hemagglutinating activity among 108 soybean varieties and strains was reported by Kakade et al. (14). Soybean lectin, like the trypsin inhibitors, is readily destroyed by heat treatment, and destruction is accompanied by a marked improvement in the nutritive

TABLE V

Relationship between Size of Pancreas of Various Species of Animals and the Response of the Pancreas to Raw Soybeans or Trypsin Inhibitor

Species	Size of pancreas (% of body wgt)	Pancreatic hypertrophy	Ref.
mouse	0.6 - 0.8	+	(26)
rat	0.5 - 0.6	+	(27)
chick	0.4 - 0.6	+	(27)
guinea pig	0.29	±[a]	(28)
dog	0.21 - 0.24	-	(29)
pig	0.10 - 0.12	-	(30)
human	0.09 - 0.12	(-)[b]	(31,32)
calf	0.06 - 0.08	-	

[a]Observed in young guinea pigs but not in adults.
[b]Predicted response.

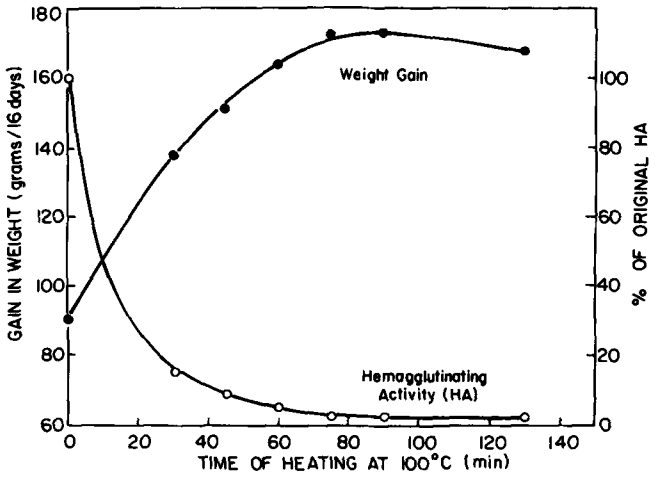

FIG. 6. Effect of heat treatment of soybeans on hemagglutinating activity and growth response of chicks (36).

value of the protein (Fig. 6). As it turns out, however, it appears that the soybean lectin is a relatively minor factor contributing to the poor nutritive value of raw soybeans. This conclusion is based on experiments by Turner and Liener (37), who fed rats raw soybean extract from which the soybean lectin had been selectively removed by affinity chromatography on Sepharose-bound concanavalin A. As shown in Table VI, the PER was essentially unchanged by lectin removal, but the expected improvement in protein quality was achieved by heat treatment.

Other Legumes

Since lectins are present in many other commonly consumed legumes and are quite diverse in their physicochemical and biological properties, it does not necessarily follow that the lectins of other legumes are as innocuous as the soybean lectin. Table VII shows the effect of heat on the growth-promoting property of a number of legumes which enjoy popular consumption by large segments of the world's population. It is evident that the two beans which are botanically classified as *Phaseolus vulgaris* are quite toxic to rats unless subjected to heat treatment. These two beans likewise display extremely high levels of hemagglutinating activity compared to those which do not respond to heat treatment. When purified preparations of the lectins from the black bean and kidney bean were fed to rats, growth depression was noted which became more marked as the level of lectin in the diet increased (Fig. 7). In fact, at the higher levels of lectin, greater than 1% of the diet, a high incidence of mortality was observed. It is important to note that autoclaving of the lectins for 20 min destroyed their toxicity. These results have been confirmed by Pusztai and Palmer (39) for the kidney bean lectin. These authors also found that kidney bean protein from which the lectin had been removed by affinity chromatography was nontoxic.

Mode of Action

Jaffé (40) has proposed that the toxic effect of lectins when ingested orally may be due to their ability to bind to specific receptor sites on the surface of intestinal epithelial cells resulting in an interference with the absorption of nutrients across the intestinal wall. This was reflected in vivo by a decrease in the apparent digestibility of the protein when the purified lectin was added to a diet containing casein (41). In vitro experiments with isolated intestinal loops taken from rats fed the black bean lectin showed a 50% decrease in the rate of absorption of glucose across the intestinal wall compared to control animals not receiving the lectin (40). Support for this hypothesis comes from the studies of Etzler and Branstrator (42), who found that a number of different lectins react with different regions of the intestine depending on the specificity of the lectin. Since surface-bound lectins are known to cause profound physiological changes in the cells with which they interact (43), one of these effects could be a serious impairment

TABLE VI

Effect of Removing Soybean Hemagglutinin (SBH) on the Growth-promoting Activity of Raw Soybean Extracts (37)

Protein component of diet	Hemagglutinating activity	PER
	Units/g protein x 10^{-3}	
Original soybean extract	324	0.91
Original soybean extract - SBH[a]	29	1.13
Original soybean extract, heated	6	2.25
Raw soy flour	330	1.01
Heated soy flour	13	2.30

[a]SBH was removed from an aqueous extract of soybeans by passage through a column of Sepharose-bound concanavalin A.

TABLE VII

Effect of Heat on Nutritive[a] Value and Hemagglutinating Activity of Some Legumes (38)

	Gain in weight (g/day)		Hemagglutinating activity (units/g)	
Legume	Raw	Heated	Raw	Heated
Phaseolus vulgaris				
black bean	-1.94(4-5)[1]	+1.61	2450	0
kidney bean	-1.04(11-13)[1]	+1.48	3560	0
Cicer arietinum				
chick pea	+1.25	+1.16	0	0
Cajanus cajan				
Red gram	+1.33	+1.74	0	0
Phaseolus aureus				
mung bean	+1.05	+1.07	0	0

[a]100% mortality observed during period (in days) shown in parentheses.

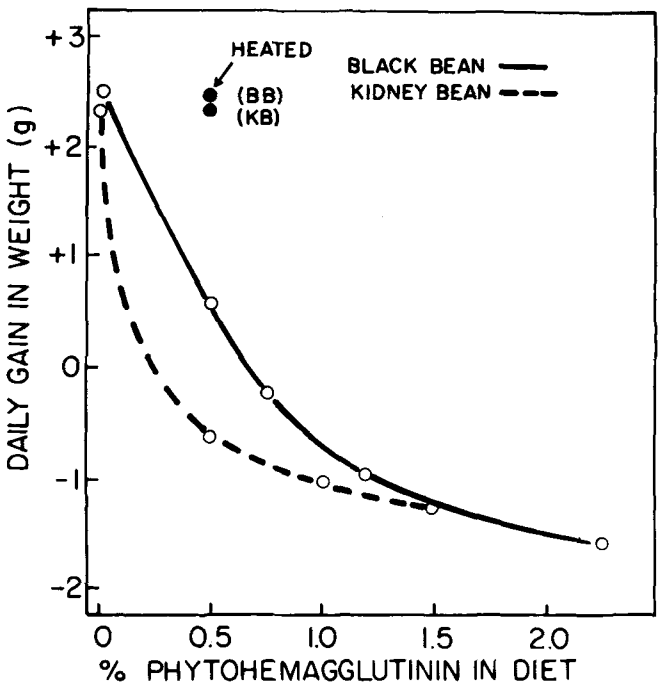

FIG. 7. The effect of black bean and kidney bean lectins on growth of rats (38).

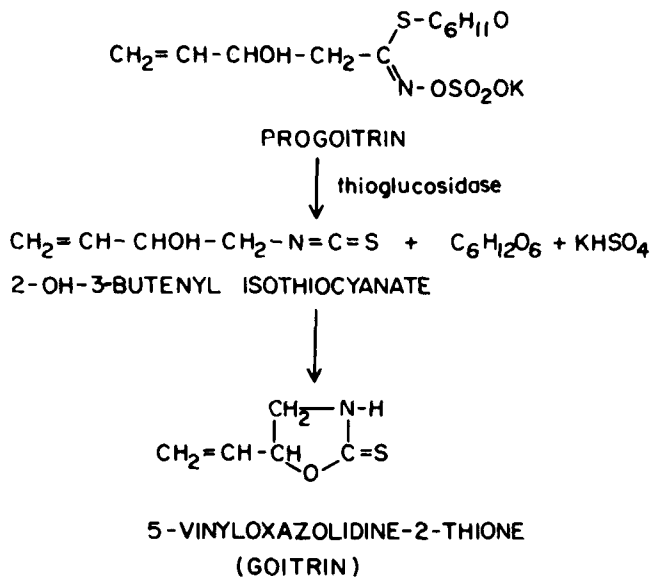

FIG. 8. Reactions depicting the products of the enzymatic hydrolysis of the goiter-producing substance (progoitrin) of rapeseed.

in the ability of these cells to absorb nutrients from the gastrointestinal tract, thus causing an inhibition of growth and, in extreme cases, even death.

An alternative effect on the intestinal wall has been suggested by Jayne-Williams (44,45) who observed that germ-free Japanese quail were much better able to tolerate the toxic effects of concanavalin A and the navy bean lectin than conventional birds. It was theorized that the binding of lectins to the cells lining the intestinal wall interfered with the normal defense mechanism of these cells whereby otherwise innocuous intestinal bacteria are prevented from passing from the leumen of the gut into the lymph, blood, and other tissues of the animal body.

Significance in Human Diet

It is difficult, of course, to assess the significance of lectins in the human diet based on experiments in animals

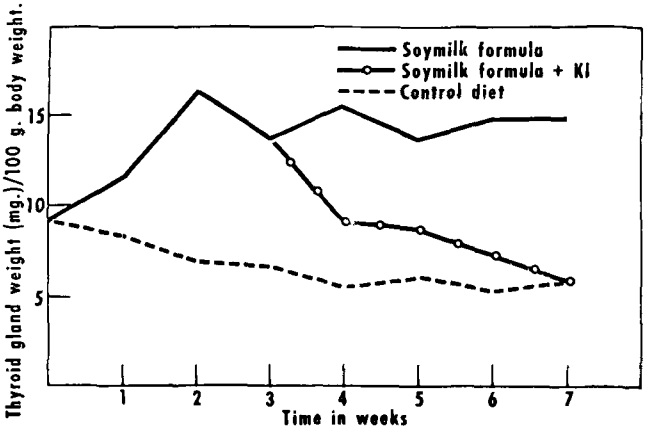

FIG. 9. Effect of a soybean milk diet with and without iodide supplementation on the thyroid gland of the rat (58).

alone. As long as sufficient heat treatment has been applied to insure complete destruction of the lectins, there would appear to be little cause for concern in the human diet. Nevertheless, it should be recognized that conditions may prevail wherein complete destruction of the lectin may not always be achieved. For example, a massive outbreak of poisoning occurred in Berlin in 1948 after the consumption of partially cooked bean flakes (46). Mixtures of ground beans and cereals have been recommended in child-feeding programs in lesser developed countries (47). Such mixtures can be prepared locally from easily available foodstuffs and can be formulated in proportions to give an amino acid pattern comparable to that of milk protein. However, cooking such mixtures requires a relatively short heating time to become palatable so that the lectin may not be completely destroyed (48). Furthermore, primitive cooking is often done in earthen pots on a wood fire, so that with a tough viscous mass like cooked beans, heat transfer may be imperfect, and, in the absence of constant and vigorous stirring, the temperature reached in parts of the preparation may well be inadequate for the destruction of the lectins. A reduction in the boiling point of water such as would be encountered in certain mountainous regions of the world might also result in incomplete destruction of lectins.

The marked resistance of lectins to inactivation by dry heat (49) deserves special emphasis. Thus, the recommendation that raw bean flour be added to wheat flour in bread formulations (50) and in other baked goods (51) should be viewed with caution.

GOITROGENS

Goiter-producing agents in the form of glycosides are found in oil meal residues from such plants as the rapeseed, mustard seed, and crambe. The rapeseed in particular has received a great deal of attention as a potential source of protein because of its high protein content (40-45%) and balanced amino acid composition (52,53). Its use as a potential ingredient of the human diet has been restricted by the presence of thioglycosides or glucosinolates which, although innocuous in themselves, are enzymatically hydrolyzed to yield products which are goitrogenic and growth inhibitory. The predominant glucosinolate of rapeseed is progoitrin which, upon enzymatic hydrolysis, releases an isothiocyanate and 5-vinyloxazolidine-2-thione (Fig. 8). The latter two compounds are antithyroid agents which inhibit the organic binding of iodine by the thyroid gland. Thus, their goitrogenic effect is not prevented by the addition of iodine to the diet. Efforts to breed strains of rapeseed with low glucosinolate content has met with some degree of success (54). One such variety (Bronowski) was found to promote better growth than commercial rapeseed

TABLE VIII

Cyanide Contents of Certain Plants (63)

Plant	HCN yield, mg/100 g
Lima bean (*Phaseolus lunatus*)	
Samples incriminated in fatal human poisoning	210.0-312.0
Normal levels	14.4-16.7
Sorghum	250.0
Cassava	113.0
Linseed meal	53.0
Black-eyed pea (*Vigna sinensis*)	2.1
Garden pea (*Pisum sativum*)	2.3
Kidney bean (*Phaseolus vulgaris*)	2.0
Bengal gram (*Cicer arietinum*)	0.8
Red gram (*Cajanus cajans*)	0.5

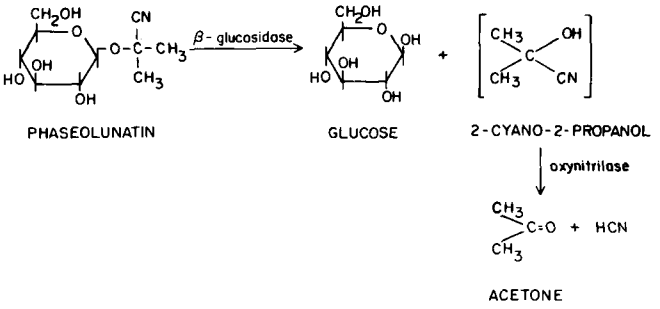

FIG. 10. Enzymatic release of HCN from phaseolunatin, the cyanogenetic glycoside of lima beans (*Phaseolus lunatus*).

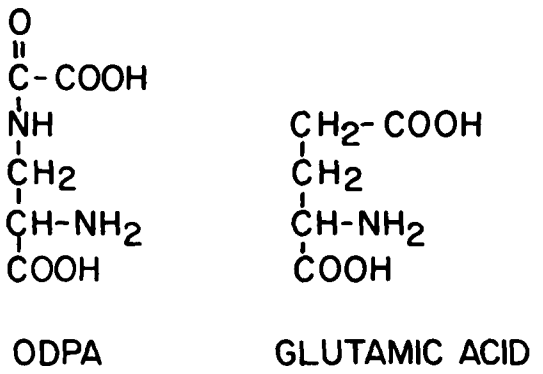

FIG. 11. Structure of β-N-oxalyl-α,β-diaminopropionic acid, the lathyritic principle of *Lathyrus sativus*, compared with glutamic acid.

meal, although there is some indication of a high molecular weight constituent which still inhibits growth and is unrelated to the glucosinolates (55).

Among the legumes, only the soybeans and peanuts have been reported to produce goitrogenic effects in animals. Unheated soybeans, for example, cause a marked enlargement of the thyroid gland of the rat and chick, an effect which can be counteracted by the administration of iodide or partially eliminated by heat (56,57). An example of the therapeutic effectiveness of iodide in overcoming the goitrogenic effect of soymilk is shown in Fig. 9. Several workers (59,60) have reported a number of cases of goiter in human infants fed soybean milk. Apparently the heat treatment employed for sterilizing these particular soybean preparations was not sufficient to destroy the goitrogenic agent. Iodine supplementation, however, alleviated this goiter condition in human infants (59). The goitrogenic principle from soybeans has been partially purified and characterized as a low molecular weight oligopeptide composed of two or three amino acids or a glycopeptide containing one or two amino acids and a sugar residue (61,62).

Rats fed peanuts also develop enlarged thyroids, but in this instance, the goitrogenic principle has been identified as a phenolic glycoside which resides in the skin (63). It was suggested that the phenolic metabolites formed from this glycoside are preferentially iodinated and thereby deprive the thyroid of available iodine. Thus the goitrogenic effect of peanuts is effectively counteracted by iodine supplementation but not by heat treatment.

CYANOGENS

A wide variety of plants are potentially toxic because they contain glycosides from which HCN may be released by hydrolysis (64). It will be noted in Table VIII that the legumes predominate in terms of their cyanide-producing potential. In the years immediately following the turn of the 20th century and again during World War I, lima beans imported into Europe from tropical countries (Java, Puerto Rico, and Burma) were responsible for serious outbreaks of cyanide poisoning, and cases of human intoxication from the consumption of certain varieties of lima beans are not uncommon today in some of the tropical countries. Most of the lima beans consumed in the United States and Europe at the present time are well below the toxic levels implicated in fatal cases of poisoning.

Cyanide in the form of HCN is released from a glycoside (*phaseolunatin* in the case of lima beans) through the action of an enzyme present in the plant tissue (Fig. 10). Hydrolysis occurs quite rapidly when the ground bean meal is cooked in water, and most of the liberated HCN is lost by volatilization. Further cooking also leads to the eventual destruction of the enzyme. Yet many cases of human intoxication have occurred even with cooked lima beans. For example, it has been reported (65) that when lima beans which had been cooked to destroy the enzymes responsible for cyanide formation were fed to human subjects, cyanide could be detected in the urine. This had led to the supposition that perhaps enzymes secreted in the intestinal tract, or by the microflora of the colon, may be responsible for releasing HCN after ingestion of the cooked beans.

LATHYRISM

Lathyrism, as it is known to occur in humans, is a disease associated with the consumption of a legume or peas known scientifically as *Lathyrus sativus*, or by its common name as chicking vetch or kesari dal. This disease is particularly prevalent in India, especially during periods of famine resulting from droughts when the field crops become blighted, and, as an alternate crop, this particular legume is cultivated. We are not dealing here with an occasional case of poisoning but with a disease which can almost reach epidemic proportions. As recently as 1975 over 100,000 cases of lathyrism in men between the ages of 15 and 45 years were reported (66). Lathyrism seems to affect only males, particularly young adults. This disease is characterized by a nervous paralysis of the lower limbs

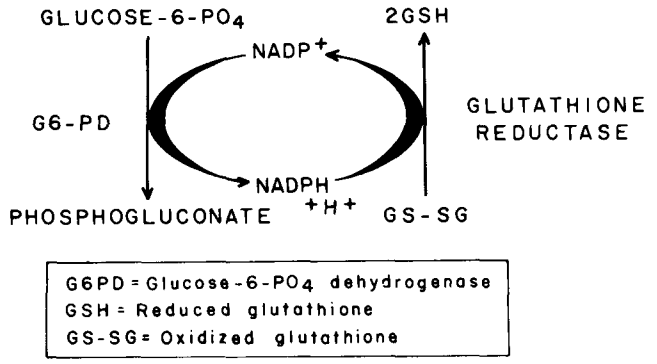

FIG. 12. Metabolic reactions governing the level of reduced glutathione (GSH) in the blood.

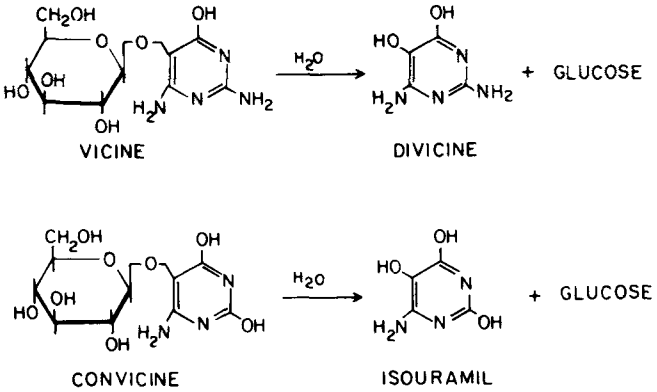

FIG. 13. Structure of vicine and convicine, the favism-producing factors in *Vicia faba*.

which forces the victim to walk on his toes with short, jerky steps; death may result in extreme cases. This form of lathyrism, sometimes referred to as "neurolathyrism," should be clearly differentiated from "osteolathyrism," a disease involving collagen synthesis which is noted in animals consuming the sweet pea (*Lathyrus odoratus*). Progress towards the identification of the causative factor of human lathyrism has been hampered by an inability to reproduce a similar disease in animals. However, a compound, β-N-oxalyl-α,β-diamino-propionic acid (Fig. 11), has been isolated from *L. sativus* which, when injected into young rats, mice, chicks, and monkeys, does produce neurotoxic symptoms closely resembling the symptoms of human lathyrism (67). Because of its structural similarity to glutamic acid, it is not surprising that ODAP has been found to interfere with the role of glutamic acid as an excitory neurotransmitter in brain tissue (68).

Despite the experimental evidence which would appear to implicate ODAP as the causative factor of neurolathyrism, it remains to be demonstrated that the neurological symptoms of lathyrism can be demonstrated by feeding *L. sativus* to animals. Nevertheless, the detoxification of *L. sativus* seeds can be effected by destroying or removing ODAP by rather simple processing procedures (69). Over 90% of the toxin can be removed by cooking the seeds in excess water which is then discarded, or by soaking the seeds overnight followed by steaming, roasting, or sun drying. The dried seeds can then be ground into flour for making chapatis, an unleavened Indian bread. Actually *L. sativus* contains 24-28% protein which is rich in lysine (70). Thus, despite the tarnished reputation which this legume has had over the years, it may yet have potential as a protein supplement in a country which sorely needs it.

FAVISM

The field or broad bean (*Vicia faba*) has been extensively

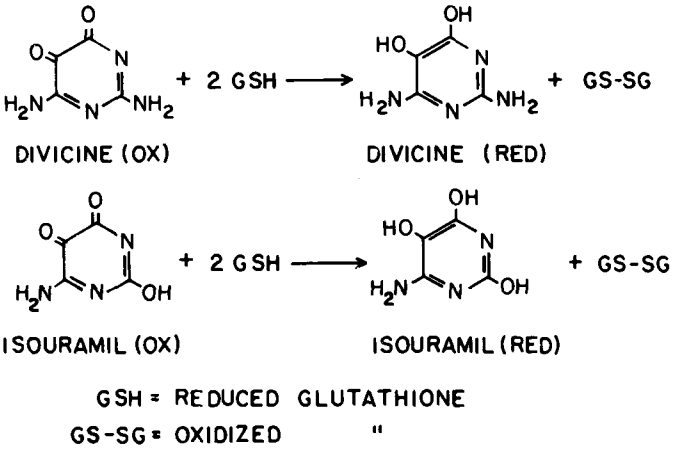

FIG. 14. Reactions showing the manner in which divicine and isouramil can lead to reduced levels of GSH.

used as a source of good quality protein for feeding livestock and poultry (71). However, its use for human consumption has been tempered by the fear that its consumption could lead to a disease known as favism in some susceptible individuals. This would be particularly true in the Middle East, where the field bean is a major good staple and where the genetic defect associated with favism is more prevalent.

Favism is a disease characterized by hemolytic anemia which affects certain individuals following the ingestion of the field bean (72). Symptoms of this disease include weakness or fatigue, pallor, jaundice, and hemoglobinuria. Favism is confined largely to inhabitants of the Mediterranean basin, although individuals of the same ethnic background residing in other countries frequently suffer from favism. Although about two-thirds of the cases of favism are associated with the consumption of the fresh or dried beans, the remainder of the cases are caused by cooked beans.

One of the main difficulties in trying to elucidate the pathogenesis of favism has been the inability to reproduce this disease in an animal model. Although heating definitely improves the nutritive value of the field bean for experimental animals, no symptons resembling human favism have been observed with the raw bean.

Extensive clinical studies with favism-prone individuals has revealed that the blood cells of such individuals are genetically deficient in glucose-6-phosphate dehydrogenase (G6PD) and contain low levels of reduced glutathione (GSH). The latter is necessary for maintaining the structural integrity of the cell membrane, and the role of G6PD is to generate NADPH via the pentose phosphate shunt. NADPH is necessary for the action of glutathione reductase which serves to reduce oxidized glutathione to GSH. These relationships are shown in Fig. 12.

It follows that any factors which lead to a decrease in GSH, particularly in the absence of G6PD, might be expected to cause hemolysis of red blood cells. The field bean is known to contain the glucosides, vicine and convicine (Fig. 13), the aglycone components of which (divicine and isouramil respectively) have been shown to cause a rapid oxidation of GSH in G6PD-deficient erythrocytes but not in normal cells (73) (Fig. 14). If vicine and convicine are in fact the causative agents of favism, it would appear that the only way of diminishing the risk of this disease is to effect their removal by genetic breeding or by some form of processing. With the availability of relatively simple chemical tests for vicine and convicine (74-76), it should be possible to screen cultivars of *V. faba* and to assess various processing techniques for the effectiveness in eliminating these compounds. Although protein concen-

trates and isolates have been prepared from the field bean and have been found to be of good nutritional quality in animal tests (71,77), the extent to which they might still be contaminated with vicine or convicine does not appear to have received the attention this problem deserves if such products are to be used in the human diet. It is evident that in order for divicine and isouramil to function as oxidizing agents in vivo, they must first be released from their parent glycosides and converted to their corresponding ortho-quinones. How this is accomplished is not known; this presumably could take place through hydrolysis by β-glycosidases in the intestinal tract (78).

ACKNOWLEDGMENT

A portion of the work described in this paper was supported by Grant AM-18324 from the National Institutes of Health.

REFERENCES

1. "Toxic Constituents of Plant Foodstuffs," Edited by I.E. Linener, Academic Press, N.Y., 1969.
2. Osborne, T.B., and L.B. Mendel, J.Biol. Chem. 32:369 (1917).
3. Kunitz, M., Science 101:668 (1945).
4. Rackis, J.J., in "Soybeans: Chemistry and Technology," Edited by A.K. Smith and S.J. Circle, Avi Publishing Co., Westport, Connecticut, 1972, pp. 158-202.
5. Liener, I.E., H.J. Deuel, Jr., and H.L. Fevold, J. Nutr. 39:325 (1949).
6. Desikachar, H.S.R., and S.S. De, Science 106:421 (1947).
7. Westfall, R.J., D.K. Bosshardt, and R.H. Barnes, Proc. Soc. Exp. Biol. Med. 68:498 (1948).
8. Chernick, S.S., S.S. Lepkovsky, and I.L. Chaikoff, Am. J. Physiol. 155:33 (1948).
9. Lyman, R.L., and S.S. Lepkovsky, J. Nutr. 62:269 (1957).
10. Booth, A.N., D.J. Robbins, W.E. Ribelin, and F. DeEds, Proc. Soc. Exp. Biol. Med. 104:681 (1960).
11. Green, G.M. and R.L. Lyman, Ibid. 140:291 (1972).
12. Schneeman, B.O., and R.L. Lyman, Ibid. 142:162 (1973).
13. Lyman, R.L., B. Olds, and G.M. Green, J. Nutr. 104:105 (1974).
14. Kakade, M.L., N.R. Simons, I.E. Liener, and J.W. Lambert, J. Agric. Food Chem. 20:87 (1972).
15. Kakade, M.L., D.E. Hoffa, and I.E. Liener, J. Nutr. 103:1772 (1973).
16. Seidl, D., M. Jaffé, and W.G. Jaffé, J. Agric. Food Chem. 17:1318 (1969).
17. Green, G.M., B.A. Olds, G. Mathews, and R.L. Lyman, Proc. Soc. Exp. Biol. Med. 142:1162 (1973).
18. Liener, I.E., unpublished data.
19. Kotter, L., A. Palitzsch, H.-D. Belitz, and K.-H. Fischer, Die Fleischwirtschaft. 8:1063 (1970).
20. Nordal, J., and K. Fossum, Z. Lebensm. Unters. Forsch. 154:144 (1974).
21. Churella, H.R., B.C. Yao, and W.A.B. Thompson, J. Agric. Food Chem. 24:393 (1976).
22. Rackis, J.J., J.E. McGhee, and A.N. Booth, Cereal Chem. 52:85 (1975).
23. Robinson, L.A., W.J. Kim, T.T. White, and B. Hadorn, Scand. J. Gastroenterol. 7:43 (1972).
24. Figarella, C., G.A. Negri, and O. Guy, in "Baeyer Symp. V on Proteinase Inhibitors," Edited by H. Fritz, H. Tschesche, L. J. Greene, and E. Truscheit, Springer-Verlag, Berlin and New York, 1974, pp. 213-222.
25. Figarella, C., G.A. Negri, and O. Guy, Eur. J. Biochem. 53:457 (1975).
26. Schingoethe, D.J., S.D. Aust, and J.W. Thomas, J. Nutr. 100:739 (1970).
27. Liener, I.E. and M.L. Kakade, in "Toxic Constituents of Plant Foodstuffs," Edited by I.E. Liener, Academic Press, New York, 1969, pp. 7-68.
28. Patten, J.R., J.A. Patten, and H. Pope, II Food Cosmet. Toxicol. 11:577 (1973).
29. Patten, J.R., E.A. Richards, and J. Wheeler, Life Sci. 10:145 (1971).
30. Yen, J.T., A.H. Jensen, and J. Simon, J. Nutr. 107:156 (1977).
31. Kakade, M.L., R.M. Thompson, W.E. Engleslad, G.C. Behrens, R.D. Yoder, and F.M. Crane, J. Dairy Sci. 59:1484 (1976).
32. Long, C., in "Biochemists' Handbook," D. Van Nostrand Co., Inc., Princeton, New Jersey, 1961, p. 675.
33. Liener, I.E., Ann. Rev. Plant Physiol. 27:291 (1976).
34. Liener, I.E., J. Agric. Food Chem. 22:17 (1974).
35. Liener, I.E., and J.E. Rose, Proc. Soc. Exp. Biol. Med. 83:539 (1953).
36. Liener, I.E., and E.G. Hill, J. Nutr. 49:609 (1953).
37. Turner, R.H., and I.E. Liener, J. Agric. Food Chem. 23:484 (1975).
38. Honavar, P.M., C.-V. Shih, and I.E. Liener, J. Nutr. 77:109 (1962).
39. Pusztai, A. and R. Palmer, J. Sci. Food Agric. 28:620 (1977).
40. Jaffé, W.G., Arzneim. Forsch. 12:1012 (1960).
41. Jaffé, W.G., and G. Camejo, Acta Cient. Venez. 12:59 (1961).
42. Etzler, M., and M.L. Branstrator, J. Cell Biol. 62:329 (1974).
43. Lis, H., and N. Sharon, Ann. Rev. Biochem. 42:541 (1973).
44. Jayne-Williams, D.J., Nature 243:150 (1973).
45. Jayne-Williams, D.J., and C.D. Burgess, J. Appl. Bacteriol. 37:149 (1974).
46. Griebel, C., Z. Lebensm. Unters. Forsch. 90:191 (1950).
47. King, K., W. Fourgere, J. Foucald, G. Dominique, and I.D. Begkin, Arch. Latinoam. Nutr. 16:53 (1966).
48. Korte, R., Ecol. Food Nutr. 1:303 (1972).
49. DeMuelenaere, H.J.H., Nature 201:1029 (1964).
50. Anonymous, Chem. Ind. Eng. News 26:2516 (1948).
51. Marcos, S.K. and A.M. Bocter, Brit. J. Nutr. 13:163 (1959).
52. Miller, E.R., D.E. Ullrey, C.J. Zutant, S.H. Hoefer, and R.L. Luecke, J. Nutr. 85:347 (1965).
53. Ballester, D., R. Rodrigo, L. Nokorezi, C.O. Chiebester, E. Yanez, and F. Mochenberg, J. Sci. Food Agric. 21:140 (1970).
54. Josefson, E., J. Sci. Food Agric. 21:98 (1970).
55. Josefson, E., and L. Munck, J. Sci. Food Agric. 23:861 (1972).
56. Patton, A.R., H.S. Wilgus, Jr., and G.S. Harshfield, Science 89:162 (1939).
57. Block, R.J., R.H. Mandl, H.W. Howard, C.D. Bauer, and D.W. Anderson, Arch. Biochem. Biophys. 93:15 (1961).
58. Anderson, D.W., Proc. Conf. Soybean Products for Protein in Human Foods, Northern Regional Research Laboratory, Peoria, Illinois, 1961, p. 173.
59. Van Wyk, J.J., M.B. Arnold, J. Wynn, and F. Pepper, Pediatrics 24:752 (1959).
60. Hydowitz, J.D., N.Engl. J. Med. 262:351 (1960).
61. Konijn, A.M., S. Edelstein, and K. Guggenheim, J. Sci. Food Agric. 23:549 (1972).
62. Konijn, A.M., B. Gershon, and K. Guggenheim, J. Nutr. 103:378 (1973).
63. Sreeniwasan, V., N.R. Mougdal, and P.S. Sarma, Ibid. 61:87 (1957).
64. Montgomery, R.D., in "Toxic Constituents of Plant Foodstuffs," Edited by I.E. Liener, Academic Press, New York, 1969, pp. 143-157.
65. Gabel, W. and W. Kruger, Muensch. Med. Wochschr. 67:214 (1920).
66. Natajaran, K.R., Chemistry 49:12 (1976).
67. Sarma, P.S., and G. Padmanaban, in "Toxic Constituents of Plant Foodstuffs," Edited by I.E. Liener, Academic Press, New York, 1969, pp. 267-291.
68. Lakshamanan, J., and G. Padmanaban, Nature 249:469 (1974).
69. Mohan, V.S., V. Nagarajan, and C. Gopalan, Indian J. Med. Res. 54:510 (1966).
70. Sarma, P.S., J. Vitaminol. 14:53 (1968).
71. Bell, J.M., in "Nutritional Aspects of Common Beans and Other Legume Seeds for Animal and Human Foods," Arch. Latinoam. Nutr., Caracas, Venezuela, 1973, p. 165.
72. Belsey, M.A., Bull. WHO 48:1 (1973).
73. Mager, J., A. Razin, and A. Hershko, in "Toxic Constituents of Plant Foodstuffs," Edited by I.E. Liener, Academic Press, New York, 1969, pp. 294-318.
74. Brown, E.G., and F.M. Roberts, Phytochemistry 11:3203 (1972).
75. Higazi, M.I., and W.W.C. Reed, J. Agric. Food Chem. 22:570 (1974).
76. Hamalian, J., F. Aylward, and B.J.F. Hudson, Ind. Plant Foods Human Nutr. 26:331 (1976).
77. Duthrie, I.F., P.D. Porter, and B. Gadaly, Proc. Nutr. Soc. 31:80A (1972).
78. Flohé, L., G. Niebch, and H. Rieber, Z. Klin. Chem. Klin. Biochem. 9:431 (1971).