Biochemical Basis for the Differences in Plant Protein Utilization

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The biological utilization of protein depends upon factors such as protein content, protein quality, and protein digestibility. Amino acid(s) deficiency or excess, which is exaggerated at low protein intake, affects protein utilization by either limiting the amino acid(s) for tissue protein synthesis or by creating an extra burden on liver and kidney for degradation of the excessive amino acid(s). The digestibility of protein is considerably influenced by the presence of enzyme-resistant peptides and enzyme-inhibiting substances. The structural features and amino acid sequence of proteins may also influence the availability of amino acids. For example, the protein component of many plant foodstuffs with high cystine content has been found to be refractory to attack by trypsin, an affect which is attributed to the stability of the molecule produced by a large number of disulfide bonds. Other specific examples and data on the subject matter are presented and discussed.

Because of economic reasons, it is expected that plant foodstuffs will play a major role in supplying the protein needs of an ever-increasing world population. To meet this challenge, there is little doubt that much of the knowledge concerning the nutritional quality of these plant materials must come from the basic biochemical studies on the proteins themselves. This will enable us not only to select plants as a source of life-sustaining protein, but also to expand our future world supply of proteins.

The protein nutritive value or protein utilization as it will be referred to in this paper may be defined as the ability of a protein to provide a pattern of amino acids in proper concentrations similar to body proteins. This, usually, involves a series of steps in the animal by which a dietary protein is subjected to digestive enzymes in the gastrointestinal tract to liberate the constituent amino acids (process of digestion), followed by transport of these amino acids from the intestine through the cell walls to the blood stream (absorption), and finally complex biochemical reactions at the cellular level which synthesize body proteins. Although factors such as calories, vitamins, minerals in the diet, as well as species, age, and sex of the animal affect the protein nutritive value, the present discussion will be limited to explain, from a biochemical viewpoint, just how and why a protein of one plant is utilized differently from a protein of another plant origin.

PROTEIN QUANTITY

The plant foodstuffs such as potatoes, cassava, yam, various cereals, and millets constitute an important dietary source of protein for many segments of the world's population, particularly where animal protein is in short supply or is forbidden by cultural or religious practices. One of the most serious disadvantages of these types of foodstuffs is their low protein content. The situation can be further intensified, especially in case of tuber roots where about 50% of total nitrogen is nonprotein nitrogen, some of which may not be utilizable for metabolic purposes. The consumption of low protein containing plant foodstuffs appears to be the single most important factor contributing to the problem of protein malnutrition prevalent in those parts of the world which are mostly developing countries.

It is well established that for maximum protein utilization a certain level of dietary protein is essential, below which tissue proteins (*e.g.*, muscle) are broken down to supply amino acids for the synthesis of more essential body proteins required for maintenance. Numerous reports indicate that an excess or deficiency of amino acid is exaggerated at low protein intake (Harper and Benevenga, 1970). This may have some practical significance since many cereals are known to contain disproportionately high amounts of leucine, *e.g.*, corn, sorghum, etc. It has been suggested that pellagra observed in certain parts of India, where jowar (a millet *Sorghum vulgare*) is a staple food, may be caused by its high content of leucine, thereby creating an amino acid imbalance (Gopalan, 1969).

Although a quantitative aspect of protein quality has been recognized for a long time, it has been only recently reemphasized in evaluating the utilizable protein from various sources as shown in Table I (Hegsted, 1969; Rosenfield, 1973). The data presented in Table I clearly indicate the dependence of utilizable protein on the protein content of a given protein source and show that cereals have low utilizable protein as compared to protein-rich foodstuffs even though the relative nutritive value may be similar, *e.g.*, rice and peanut flour.

PROTEIN QUALITY

Since protein utilization is a function of the amino acids present, the amino acid composition of protein plays an important role in determining the nutritive value of plant protein foodstuffs. Two recent review articles (Swaminathan, 1967; Bressani and Elias, 1968) gave a complete account of the amino acid composition as it relates to protein quality of various plant foodstuffs. Suffice it to say that plant proteins, in general, are deficient in one or more amino acids, specifically, cereals are mainly deficient in lysine while legumes and leaf proteins are deficient in methionine. The primary deficiency of an amino acid, namely lysine or methionine, in many instances is further intensified by a secondary deficiency of amino acid(s), *e.g.*, threonine and/or tryptophan. It is not surprising, therefore, to find that supplementation of deficient amino acid(s) to the diets containing these plant proteins greatly improved the protein utilization over unsupplemented diets (Swaminathan, 1967).

Certain plant proteins are also characterized by the presence of some excessive amino acids which could affect the protein utilization under the conditions described by Harper and Benevenga (1970). These effects are classified as: (i) amino acid imbalance, (ii) amino acid antagonism, and (iii) amino acid toxicity. The reader should refer to a review article by Harper (1964) for further details. For example, it was demonstrated that when wheat gluten (poorly balanced in amino acids) was included in the diet as a source of lysine, the lysine requirement of the rat for maximum growth was also increased (Munaver and Harper, 1959). In amino acid antagonism, excess of one amino acid depressed the utilization of a structurally similar amino acid as in the case of corn protein where an excess of leucine was found to depress the utilization of isoleu-

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Table I. Relative Nutritive Value and Utilizable Protein in Plant Foodstuffs[a]

Plant foodstuff	Protein content, %	Rel nutritive value,[b] %	Utilizable[c] protein, %
Soy flour heated	51.90	60	31.1
Peanut flour	48.40	54	26.1
Cottonseed flour	37.70	65	24.5
Rice flour, high protein	19.10	44	8.4
White flour	13.75	28	3.8
Sorghum	9.80	31	3.0
Rice	8.30	50	4.2
Corn meal	7.95	37	3.0

[a] Data taken from Hegsted (1969). [b] Ability of a test protein to promote growth as compared to lactalbumin taken as 100%. [c] Per cent protein × relative nutritive value.

cine (Harper *et al.*, 1955). Ganapathy and Chitre (1970) recently reported that excessive arginine in relation to lysine is a factor contributing to the poor utilization of millet protein. In this connection, it should be pointed out that sesame protein also contained large amounts of arginine compared to lysine (Table II). Many cereals contain a high amount of proline. Although proline is not an essential amino acid, its level in proportion to lysine and arginine content could be a factor contributing to poor digestibility of cereal proteins. This aspect will be discussed further under the section on Protein Digestibility. In view of the known toxicity of methionine (Harper and Benevenga, 1970) a high methionine content of brazil nuts warrants further investigation. The amount of disproportionate amino acids present in the various plant foodstuffs is shown in Table II. The effect of these disproportionate amino acids on the efficient utilization of plant protein is still to be evaluated in many instances, perhaps because of a difficulty in defining and measuring a disproportionate amino acid.

Although supplementation of deficient amino acid(s) to plant proteins generally results in an increased protein utilization, there still seem to exist some differences in protein quality of various plant sources. This is particularly true for cereals and cassava types of foods where the ratio of total essential amino acids to the total amino acids or nitrogen is low (FAO, 1965). This ratio recognizes not only the importance of adequate amounts of all essential amino acids, but also the role of nonessential amino acids in protein synthesis.

TYPES OF PROTEINS

Plant proteins may be divided into two groups: the reserve or storage proteins of seeds and functional proteins of vegetative parts of the plant (leaf, stalk, and root). Since the amino acid composition of functional or metabolic proteins is similar, only reserve proteins are considered in this section.

Seed proteins can be separated into four different types by successive solvent extractions: (i) water, albumin; (ii) salt solutions, globulins; (iii) 70% ethanol, prolamins; and (iv) dilute alkali (or acid), glutelins. Prolamins and glutelins constitute the bulk of the proteins of most cereals, while in legumes globulins represent about 80% of the total protein. Since prolamins contain only small amounts of lysine, the poor protein quality of many cereals is often attributable to their prolamin content. The possibility of such a relationship is quite evident from the data presented in Table III. Indeed, the superior protein quality of opaque-2 mutant corn over the normal corn lies in the gene which suppresses the prolamin formation (Mertz, 1971). It is, therefore, clear that the high lysine content of

Table II. Disproportionate Amino Acids in Selected Plant Protein Foodstuffs[a]

Name	Grams per 16 g of nitrogen				
	Arg	Leu	Lys	Met	Pro
Wheat	3.8	5.6	3.2	1.6	7.2
Corn	4.5	11.4	2.7	2.0	8.5
Rice	9.6	6.9	3.5	2.1	3.2
Barley	4.1	6.0	3.5	1.2	7.8
Millet	3.2	11.1	1.6	2.3	5.4
Navy beans	5.6	7.7	6.5	1.2	4.0
Peanuts	11.6	6.6	3.6	0.9	
Soybeans	6.2	7.4	5.6	0.9	5.5
Sesame seeds	12.4	6.6	2.6	4.0	
Brazil nut[b]	13.6	6.8	2.7	8.8	

[a] Taken from Bandemer and Evans (1963) and Evans and Bandemer (1967a). [b] Orr and Watt (1957).

Table III. Relationship between Prolamin and Lysine Content and Its Influence on Utilizable Protein of Some Cereals

Name	Prolamin[a] % of protein	Lysine[b] % of protein	Utilizable protein[c] % of lactalbumin
Sorghum	60	2.2	3.0
Corn	50–55	2.8	3.0
Wheat	40–50	3.4	3.8
Barley	35–40	3.6	
Rye	30–40	4.3	
Rice	1–5	4.4	4.2

[a] Brohult and Sandegren (1954). [b] Mertz (1971). [c] Hegsted (1969).

opaque-2 corn is a result of simple change in the relative amounts of protein fraction (Mossé, 1966).

As mentioned previously, the predominant and most characteristic type of protein found in legumes (peas and beans) is the globulin type which can be further fractionated into two or more types. For example, it was reported that two globulins, legumin and vicilin, which differ in solubility and in sulfur content, have been isolated from peas (Danielsson, 1950). Similarly, two distinct globulin components have been found in peanuts, namely arachin and conarachin (Irving *et al.*, 1945). The arachin fraction which represents about 63% of the total protein was found to contain 1.51% cystine and 0.67% methionine as compared with 2.92% cystine and 2.12% methionine in conarachin (Brown, 1942). It is, therefore, not surprising to find the marked differences in the protein quality of these two globulins, arachin being utilized poorly by the animals, while conarachin by itself or mixed with arachin (1:3) was utilized to the same extent as the parent meal (Woodham and Dawson, 1968). Recently Roberts and Briggs (1965) reported that one of the soybean globulins which comprises 30% of the total protein has an extremely low methionine content (0.18 g/100 g of protein) while the entire globulin fraction has a methionine content of 1.4 g/100 g of protein. Evans (1963) also studied the amino acid content of the two globulin fractions of navy beans, namely conphaseolin and phaseolin. Conphaseolin contained 3.5 times as much cystine and 7 times as much tryptophan as phaseolin, and phaseolin contained more isoleucine, leucine, and phenylalanine than conphaseolin.

PROTEIN DIGESTIBILITY

The factor which is most likely to affect the amino acid availability is the protein digestibility. Essentially, it is a rate measurement of protein hydrolysis by digestive enzymes and is influenced by the nature of linear amino

Table IV. Protein Digestibility and Amino Acid Availability from Soybeans and Navy Beans as Measured in Rats

Amino acid	Soybeans[a]		Navy beans[b]	
	Raw	Heated	Raw	Heated
Methionine	49.4	56.9	21.8	68.7
Cystine			36.6	80.6
Lysine	64.1	71.1	58.8	85.0
Leucine	65.9	76.3	47.6	85.7
Valine	61.7	80.8	46.0	84.8
Protein digestibility	82.9	89.7	43.5	80.9

[a] de Muelenaere (1964). [b] Kakade and Evans (1966).

Table V. Relative Nutritive Value of Legume Protein[a]

Legume	Met, g/16 g of N	Met + cystine, g/16 g of N	Growth of rats, % of casein	
			Without added Met	With added Met
Alaska peas	0.7	1.8	51	102
First and best peas	0.9	2.0	41	91
Soybeans (heated)	1.1	2.3	87	109
Blanco beans (heated)	1.0	1.9	0	0
Borro sweet blue (heated)	1.0	2.5	0	85
Navy beans (heated)	1.3	2.0	50	98

[a] Data taken from Evans and Bandemer (1967b).

acid sequence near the reactive site and to a greater extent by the nature of the tertiary structure of protein. A considerable amount of work has been done to show that there are marked differences in the protein digestibility and availability of amino acids from the various plant protein sources (Bressani and Elias, 1968). However, the reasons for these differences remain to be adequately explored or explained.

Primary Structure and Amino Acid Content. Gupta *et al.* (1958) found that lysine availability to the weanling rat was only 50% for corn, 70% for wheat, and 85% for rice. The studies of Josheph *et al.* (1959) and that of Kurien *et al.* (1960, 1961) indicate that the apparent protein digestibility coefficient was greatest for rice and decreased as millet, maize, or ragi was partially substituted for rice. These studies indicate that rice protein is better utilized than other cereal proteins, a conclusion which has been also reached by de Muelenaere *et al.* (1967).

A reasonable biochemical explanation for such differences in the protein digestibility and availability of amino acids among cereal proteins may be evident from the data shown in Table II. It is well known that trypsin exhibits a strict specificity for arginyl and lysyl peptide bonds (the rate of splitting of the arginyl peptide bond being twice as fast as the lysyl bond), and, therefore, it follows that the maximum number of peptide bonds split in the protein molecule should equal the sum of arginine and lysine residues (Milhalyi, 1972). As can be seen from Table II, the sum of arginine and lysine content in rice protein is almost twice as much as that of other cereals. Moreover, it was found that lysylprolyl (or by analogy arginylprolyl) linkages are completely resistant to trypsin (Bell, 1954). It is quite conceivable that high proline coupled with the low arginine and lysine content of many cereals may favor the formation of lysylprolyl and/or arginylprolyl bonds in their proteins. Hence, the ratio of arginine plus lysine to proline (for rice the ratio is nearly equal to 4, while for other cereals it is 1 or less) could be an important factor in determining the protein utilization of plant foodstuffs, especially that of cereals, a possibility which remains to be explored. Other factors which would influence the rate of tryptic hydrolysis of protein would be the amino acid residue forming the scissible bond adjacent to the N-terminal side and the presence of side-chain carboxyl groups on either side of the susceptible bond (Milhalyi, 1972).

It has been suggested (Jones and Waterman, 1922) that the poor quality of arachin may partly be due to the enzyme-resistant peptide since disproportionately large amounts of the total lysine and histidine were found in that fraction of arachin which was resistant to *in vitro* hydrolysis. Geiger *et al.* (1952) and de Muelenaere *et al.* (1967) also indicated the presence of certain threonine containing peptides which are refractory to enzymatic attack as a factor contributing to the poor utilization of corn proteins. Almquist *et al.* (1966) recently reported the presence of amino acid structures involving cystine which are enzyme resistant as a part of an explanation for the poor quality of unheated soybeans.

Tertiary Structure and Bonding Forces. Besides the influence of primary structure, the rate of protein hydrolysis is considerably affected by its tertiary structure. Native proteins occur as highly organized folded compact structures stabilized by hydrogen and hydrophobic bondings. Their susceptibility to proteolytic digestion depends upon the availability of amino acid residues which are compatible with the enzyme specificity. It follows, therefore, that any change in the tertiary structure of a protein molecule through denaturing agents such as heat which will expose the enzyme-susceptible bonds will result in an increased rate of protein hydrolysis (Grau and Carroll, 1958). This would explain, at least in part, the improvement in protein quality usually observed with heat-treated plant foodstuffs (Liener, 1958). The data presented in Table IV clearly indicate the improved protein digestibility and amino acid availability from heated soybeans or navy beans as compared to unheated beans. However, it should be pointed out that only part of the improvement in protein digestibility and amino acid availability produced by heat is due to the effect of heat *per se*, and that trypsin inhibitor and possibly hemagglutinins also play a role as will be described later. Fukushima (1968) also reported that most soybean proteins are globular molecules which are completely folded, and these show little susceptibility to proteinases unless the internal structure is disrupted by denaturation.

An interesting set of data is presented in Table V which shows, among other things, the marked difference in the availability of methionine from various legume proteins fed to rats despite the fact that all contain equal or nearly equal amounts of methionine and/or cystine and that all were heat treated. It is only after the addition of methionine at a 0.2–0.3% level to the diets that the rat growth was similar in all cases.

One may explain these results assuming that certain bean proteins are more resistant to heat denaturation although the presence of enzyme-resistant methionine containing peptide cannot be ruled out. The work of Seidl *et al.* (1969) indicates that a globulin fraction isolated from black beans, which represents about 30% of the total bean protein, is resistant to the action of a number of proteolytic enzymes. Heat or urea denaturation caused only a slight improvement in the pepsin hydrolysis of the globulin fraction, but no change was noted using trypsin. According to Jirgensons (1963) there is considerable variation in the conformation of plant proteins (high, very little, or no degree of helical structure). The lack of α helix in this globular protein may explain the ineffectiveness of heat or urea denaturation which is known to disrupt the

Table VI. Growth Depression in Animals Fed Trypsin Inhibitor or Hemagglutinin Isolated from Various Plant Sources

Source	Trypsin inhibitor or hemagglutinin	Test animal	Reference
Soybean (*Glycine max*)	Trypsin Inhibitor	Rat	Borchers *et al.* (1948)
			Liener *et al.* (1949)
			Haines and Lyman (1961)
			Rackis *et al.* (1963)
			Khayambashi and Lyman (1966)
		Mice	Westfall *et al.* (1948)
		Chick	Borchers *et al.* (1948)
			Garlich and Nesheim (1966)
	Hemagglutinin	Rat	Liener (1953)
Navy bean (*Phaseolus vulgaris*)	Trypsin Inhibitor	Rat	Kakade and Evans (1965)
			Kakade *et al.* (1970)
		Chick	Hewitt *et al.* (1973)
	Hemagglutinin	Rat	Kakade and Evans (1965)
			Kakade and Evans (1966)
			Evans *et al.* (1973)
		Chick	Hewitt *et al.* (1973)
Lima bean (*Phaseolus lunatus*)	Trypsin Inhibitor	Mice	Tauber *et al.* (1949)
		Rat	Klose *et al.* (1948)
Black bean (*Phaseolus vulgaris*)	Hemagglutinin	Rat	Honavar *et al.* (1962)
		Mice	Jaffé (1949)
Kidney bean (*Phaseolus vulgaris*)	Hemagglutinin	Rat	Honavar *et al.* (1962)
Peanut (*Arachis hypogea*)	Trypsin Inhibitor	Rat	Kwaan *et al.* (1968)
Field bean (*Dolichis lablab*)	Hemagglutin	Rat	Salgarkar and Sohonie (1965)
			Manage *et al.* (1972)
Double bean (*Phaseolus lunatus*)	Hemagglutinin	Rat	Manage *et al.* (1972)

hydrogen bonding, an important feature of α helix structure. It was indicated that the hydrophobic bonds may be playing an important role in the structural stability of the globular proteins with little or no helical conformation. It appears that the inability of the hydrophobic side chains of the protein to form bonds with water may be the major cause of compact, tightly folded conformations of the long polypeptide chains (Kauzman, 1954; Tanford *et al.*, 1960). The importance of "available" water in the enzymatic reaction or in the process of denaturation can hardly be overemphasized. In fact, one wonders whether the beneficial effect of preliminary soaking prior to the heat treatment in improving the protein quality of black beans and kidney beans (Jafé, 1949; Honavar *et al.*, 1962) is related to the hydration of protein molecule, thereby increasing its sensitivity to heat denaturation.

Inhibitory Effects of Cellulosic Supports and Protein Complexes. It is well known that one of the prerequisites for an enzyme reaction is that the enzyme must come in contact with the surface of the protein molecule. Since many plant proteins are located inside cellulosic supportive structures, the limited contact of protein with the enzyme may influence the protein digestibility (Booher, 1948). The poor digestibility of chloroplastic proteins as compared to cytoplasmic soluble proteins may be due to the inability of protease to reach the protein which is located inside the chloroplastic membrane (Byers, 1971).

Many plants contain substances such as phytic acid, saponins, phenolic compounds, various sugars, and metals which may complex with proteins. Such complex formation may affect the rate of protein hydrolysis through conformational changes, a possibility discussed in detail by Milhalyi (1972). Indeed, Feeney (1969) reported the ability of oak leaf tannin to bind casein or nettle leaf proteins and that the complex thus formed was resistant to trypsin hydrolysis. In this connection a reference should be made to the work of Ishwaya and Birk (1965) who showed saponins to have a nonspecific inhibitory effect on the digestion of proteins by various proteolytic enzymes.

In any event, the overall effect resulting from the above-mentioned factors is a reduction in the rate of protein hydrolysis, which in turn affects the protein digestibility as well as amino acid availability. One interesting and possibly a significant aspect of reduced protein digestibility is the increased secretion of pancreatic enzymes in response to undigested proteineous materials as suggested recently by Green *et al.* (1973). Since pancreatic enzymes are rich in sulfur-containing amino acids, the preferential synthesis of pancreatic enzymes would create an increased requirement for methionine and/or cystine for the synthesis of other tissue proteins, thus accentuating the deficiency of sulfur-containing amino acids which already exist in many plant proteins. Indeed, the work of Kakade *et al.* (1973) indicates that unheated soy extract from which the trypsin inhibitor was specifically removed (passing through immobilized trypsin column) contributed up to 60% of the total pancreatic enlargement and growth depression in rats, while the remaining 40% was caused by the trypsin inhibitor. It is, therefore, tempting to suggest that the increased requirement of sulfur-containing amino acids (and other limiting amino acids) resulting from the increased synthesis of pancreatic enzymes in response to the undigested protein as well as the trypsin inhibitor is the primary cause of poor utilization of soybean and possibly other bean proteins.

PROTEINS WITH BIOLOGICAL ACTIVITY

Two proteins, present in a number of plant materials, which display a certain type of biological property *in vitro* will be discussed here. The first one is referred to as trypsin inhibitor because it inhibits the action of trypsin and the other one is called hemagglutinin because of its ability to agglutinate red blood cells. The nutritional significance of these proteins has been the subject of a number of studies for the last 30 years. The reader should refer to recent review articles on trypsin inhibitor (Liener and Kakade, 1969) and on hemagglutinins (Jaffé, 1969) for further details. Suffice it to say that trypsin inhibitors or hemagglutinins isolated from various plant sources, when included in the diet, caused definite growth inhibition in the experimental animals (see Table VI).

Table VII. Cystine and Trypsin Inhibitor (TI) Content of Navy Beans (*Phaseolus vulgaris*) and Lima Beans (*Phaseolus lunatus*)[a]

	Cystine content		TI content of protein, %	Contrib. of TI cystine to total	
	Beans, g/16 g of N	TI, g/16 g of N		Protein, %	Cystine, %
Navy beans	1.0	15.5	2.6	0.40	40
Lima beans	1.4	18.5	2.5	0.45	32

[a] Data taken from Kakade *et al.* (1969).

It has been suggested that trypsin inhibitor adversely affects the utilization of proteins in two ways, depending upon the experimental conditions and species of animals. In chicks it inhibits the intestinal proteolysis by reducing the effective level of trypsin to form an inactive trypsin–trypsin inhibitor complex. In rats, however, trypsin inhibitor increases the requirement of sulfur-containing amino acids thus accentuating the deficiency of these amino acids which already exist in the plant protein foodstuffs (Kakade *et al.*, 1970).

With the recent isolation and characterization of pure trypsin inhibitors rich in cystine from a number of legumes (Liener and Kakade, 1969) a hypothesis was presented by Kakade *et al.* (1969) that a dietary loss of cystine derived from the inhibitor itself could contribute in a significant fashion to the poor protein quality of these legumes. From the data compiled in Table VII it can be estimated that the trypsin inhibitors, although comprising only about 2.5% of the bean protein, contribute approximately 32 and 40% of the total cystine of the protein of lima and navy beans, respectively. Experimental results (Table VIII) designed to test the above-mentioned hypothesis support the contention that in the case of navy bean trypsin inhibitor, the cystine which it contains is not available to chicks for growth (Kakade *et al.*, 1969). It was further demonstrated that the unavailability of cystine from navy bean trypsin inhibitor was due to its resistance to enzymatic attack. This resistance to enzyme attack is probably due to the stability of a molecule produced by a large number of disulfide bonds (Liener and Kakade, 1969).

Recently Holm *et al.* (1973) found differences in the net protein utilization, true digestibility, and biological value for the two soybean flours whose chemical score was similar. In an attempt to explain these differences, the authors suggested that a high level of heat-stable trypsin inhibitor activity, which contains disproportionately large amounts of cystine for one soy flour, may be a causative factor for the observed differences. Kakade *et al.* (1972) were unable to establish any correlation between the trypsin inhibitor activity of different varieties of soybean and their protein efficiency ratio (PER). However, recalculation of the data presented in Table III (see Kakade *et al.*, 1972) did reveal an inverse correlation between the cystine content of five soybean varieties and their PER value, the significance of which, if any, must await further experimentation.

A hypothesis regarding the mechanism by which hemagglutinin exerts its effect is advanced by Jaffé (1969). According to Jaffé, the action of hemagglutinin is to combine the cells lining the intestinal wall, resulting in an alteration of cell function, thus causing a nonspecific interference of intestinal absorption of all nutrients. A possibility that hemagglutinin may interfere in the protein synthesis is suggested by the work of Kakade *et al.* (1968). These workers found that intraperitoneal injection of navy bean hemagglutinin into young rats reduced the incorporation of leucine-^{14}C into proteins of skeletal muscle. Jayne-Williams and Hewitt (1972) proposed that hemagglutinins may interfere with normal body defense mechanisms, thereby allowing the normal intestinal bacteria to pass through lumen to other body tissues.

Since the biological activity of these proteins can be destroyed by heat, the practical significance of such types of proteins may be questionable or debatable. Nevertheless, they serve the purpose of bringing to our attention the complexity involved in considering the biochemical differences in plant protein utilization.

CONCLUDING REMARKS

One compelling fact emerges out of this presentation and that is that the rate of protein hydrolysis is a limiting factor in determining the efficacy of various plant protein utilizations. Such factors as enzyme-resistant peptides, tightly folded protein conformation, and trypsin inhibitor can adversely affect the rate of protein hydrolysis. The situation is further accentuated by the fact that various plant proteins are deficient in one or more essential amino acids, *e.g.*, methionine and lysine. Although the use of high protein foodstuffs or supplementation with deficient amino acids may improve the plant protein utilization, from the practical standpoint (taking into consideration the problems such as social, political, economical, local eating habits, and availability of food materials), it may be desirable to understand the basic biochemical reasons involved affecting protein digestibility. Therefore, more research efforts are needed to measure and improve the protein digestibility of plant foodstuffs.

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Table VIII. Effect of Feeding Navy Bean Trypsin Inhibitor (NBTI) on Growth of Chicks and Protein Digestibility[a]

		Protein digestibility				
		In vivo[b]		*In vitro*[c]		
Diet	Wt gain	Protein	Crystine	Pepsin	Trypsin	Chromotrypsin
Basal (amino acid mixture–cystine)	−1.0					
Basal + 0.15% cystine	4.6					
Basal + 2% unheated NBTI	−1.8	53.8	44.5	0.115	0.050	0.050
Basal + 2% heated NBTI	3.3	63.1	76.3	0.055	0.320	0.420

[a] Kakade *et al.* (1969). [b] (Protein (cystine) intake minus protein (cystine) execreted in feces)/(protein (cystine) intake) × 100. [c] Expressed as unit of enzyme activity where one unit is defined as an increase of 1.0 absorbance unit at 280 mμ.

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