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Studies of the Poor Utilization by the Rat of Methionine and Cystine in Heated Dry Bean Seed (*Phaseolus vulgaris*)

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Growing rats fed autoclaved bean four did not absorb 50% of the bean methionine and 41% of the bean cystine. When the bean flour was supplemented with synthetic methionine, the supplemental methionine was completely absorbed. Methionine plus cystine availability in bean flour and bean flour fractions was determined by weight gains of rats fed diets containing them and by PER of diets. Potassium hydroxide (0.2%) extracts of autoclaved bean flour depressed rat growth. Methionine and cystine of water extracts of raw bean flour were poorly utilized. An undialyzed water extract of autoclaved bean flour depressed growth of rats fed casein as principal protein, but a dialyzed extract did not. Bean flour prepared from beans boiled in water or in 0.1% sodium bicarbonate solution supported similar growth to autoclaved bean flour. Water in which beans had been boiled depressed growth of casein-fed rats, but the 0.1% sodium bicarbonate did not.

Legume seeds, including navy beans (*Phaseolus vulgaris*) are generally deficient in the sulfur-containing acids, methionine, and cystine (Evans and Bandemer, 1967). Optimum growth of rats was obtained when they were fed properly cooked navy beans supplemented with methionine, and this was not improved by supplementation with other amino acids which might be limiting (Kakade and Evans, 1965a). Soybean protein and navy bean protein contained similar levels of methionine and cystine, but soybeans promoted much better growth than navy beans when fed to rats at the same protein level (Evans and Bandemer, 1967). Soybeans required less supplemental methionine to promote optimal growth of rats than did navy beans (Evans et al., 1974). Evans et al. (1974) observed that rats fed cooked beans excreted as much as 49% of the methionine and 25% of the cystine in the feces. Dry beans also appeared to interfere with the utilization of supplemental methionine.

The present studies were undertaken to determine the reason for the poor utilization of dry bean methionine and cystine by the rat. Beans were fractionated and the fractions fed to growing rats to determine whether the methionine and cystine which are poorly utilized occur in certain proteins or fractions of the beans, or whether one of the fractions contains a heat-stable substance which interferes with methionine utilization.

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EXPERIMENTAL SECTION

Methionine and cystine balance studies were conducted, in the first experiment, with two groups of six litter-mate 28-day-old rats which had been fed rat pellets for 1 week. The three experimental diets fed contained autoclaved bean flour (Sanilac Foundation seed which contained 23.8% crude protein), the autoclaved bean flour plus 0.15% synthetic DL-methionine, or a commercial soybean meal of good quality (contained 47.1% crude protein) to supply 10% of crude protein. Each rat was fed each of the experimental diets randomly for 1 week, and during the last 4 days of each period, the weight of each diet consumed and of feces and urine excreted were determined, and all were analyzed for methionine and cystine. From the data obtained, methionine and cystine excreted and methionine and cystine utilized were calculated.

The second through sixth experiments were growth studies with weanling rats (21-days old), housed in individual cages and distributed into groups of six rats each

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so that the rats of each group were comparable in weight to those of the other groups. The diet was composed of 30% sucrose, 6% corn oil, 4% Hegsted salt mixture (Hegsted et al., 1941) (ICN Pharmaceuticals, Inc., Life Sciences Group), 2% vitamin mixture (ICN Pharmaceuticals, Inc., Life Sciences Group, containing 4.5 g of vitamin A concentrate, 200 000 units/gram, 0.25 g of vitamin D concentrate, 400 000 units/gram, 5.0 g of α-tocopherol, 45.0 g of ascorbic acid, 5.0 g of inositol, 75.0 g of choline chloride, 2.25 g of menadione, 5.0 g of *p*-aminobenzoic acid, 4.5 g of niacin, 1.0 g of riboflavin, 1.0 g pyridoxine hydrochloride, 1.0 g of thiamin hydrochloride, 3.0 g of calcium pantothenate, 20 mg of biotin, 90 mg of folic acid, and 1.35 mg of vitamin B_{12} in 1000 g), 0.05% zinc oxide, with bean flour (Commercial Michigan-grown Sanilac beans), bean flour fractions, casein or casein plus bean fraction to furnish 10% protein, and cornstarch to make to 100%, and was fed ad libitum. All bean flour or fractions, if they were not autoclaved or boiled in preparation, were autoclaved and ground before mixing in the diets. Autoclaving was at 121 °C for 15 min. Methionine was added to each diet to bring it to a level of 0.3% methionine and cystine unless otherwise noted. Autoclaved bean flour and casein diets were included in each experiment for comparison. Diets containing 10% protein were used for comparison with previous studies (Evans et al., 1974).

The rats were weighed at the start of each experiment and twice weekly thereafter, and the weekly consumption of diet by each rat was determined. The protein efficiency ratio (PER) was calculated as the grams of gain in weight per gram of protein consumed.

Rat growth, feed consumption, and PER, as well as methionine and cystine excreted and methionine and cystine utilized in the first experiment, were analyzed statistically by analysis of variance (Snedecor, 1956), with significant difference between means determined by the Duncan (1955) multiple range test.

The bean flours, each bean flour fraction, and the soybean meal were analyzed for crude protein (N × 6.25) by the Kjeldahl procedure (AOAC, 1965) and for methionine and cystine by the microbiological assay procedure described previously (Evans et al., 1974). Feces and urine excreted by the rats in the first experiment were analyzed for methionine and cystine by the microbiological assay procedure.

Trypsin inhibitor activities of some of the autoclaved and boiled beans were determined by titrimetric method of Pusztai (1972) with the synthetic substrate, tosyl-L-arginine methyl ester (TAME). A trypsin solution, or a mixture of the trypsin solution and an extract of raw or cooked beans, was reacted with a TAME solution, and the speed of hydrolysis was determined by titration in a Radiometer pH–Stat. Trypsin inhibitor activity was expressed as percent of that of raw beans.

Preparation of Bean Fractions. In the second experiment, bean flour which had been heated in the autoclave was reground and extracted with 0.2% potassium hydroxide solution. The mixture was centrifuged, the residue was again extracted in the same manner, and the two extracts were combined. The residue was dried to give the fraction designated in Table II as *bean residue*. The combined extracts were adjusted with HCl to pH 3.8, at which point a precipitate formed. This precipitate was removed by centrifugation, dried, and ground to give the traction designated as the *pH 3.8 precipitate*. The remaining solution was dried by lyophilization to give the *pH 3.8 soluble* fraction. The diets were fed for 4 weeks.

One group of rats was fed a diet containing casein as the sole protein source. Another group of rats was fed a diet containing casein plus 0.5% phytic acid.

In the third experiment, bean flour was extracted with distilled water and the mixture centrifuged. The residue was extracted again and the two extracts combined. The dried residue was designated as *residue raw beans* (Table IV). The combined extracts were divided into two parts. One was lyophilized to give the *extract of raw beans*. The other was dialyzed against distilled water and lyophilized to give the *extract of raw beans (dialyzed)*.

Bean flour was heated in the autoclave, reground and extracted by the same procedure as was the raw bean flour, and the fractions were designated as *residue autoclaved beans*, *extract autoclaved beans*, and *extract autoclaved beans (dialyzed)*.

Preparation of Cooked Beans. For the fourth experiment, whole beans were cooked for 2 h in boiling distilled water, and the water was decanted and evaporated to dryness, yielding the *boiled bean water* (Table V). The cooked beans were dried and finely ground to give the *boiled beans*. Diets were prepared in which boiled beans furnished 10% protein, or casein furnished 6.57% protein and boiled bean water 3.43% protein. These diets contained no added methionine and were fed to weanling rats for 3 weeks.

Bean flour was cooked in boiling water in a fifth experiment, the mixture was centrifuged to separate the water-soluble bean proteins from the insoluble residue, and bean residue was reground to give the *boiled beans* (Table VI). The *bean water* was evaporated to dryness, and the dry solids were ground. Rats were fed diets containing 10% protein from boiled beans, or 8.57% protein from casein plus 1.45% protein from the boiled bean water solids.

In a sixth experiment, whole beans were cooked in boiling water or in boiling 0.1% sodium bicarbonate solution (Tables VII and VIII). Rats were fed diets which contained the following: (a) 10% protein from beans boiled in water, (b) 10% protein from beans boiled in 0.1% sodium bicarbonate solution, (c) 8.2% protein from casein and 1.8% protein from boiled bean water, or (d) 8.2% protein from casein and 1.8% protein from boiled bean water containing 0.1% sodium bicarbonate solution. Rats were fed for 4 weeks (Table VIII), but because of the poor performance of rats fed the autoclaved bean flour, data obtained at 2 weeks (Table VII) are presented for comparison with data from the fifth experiment (Table VI).

RESULTS AND DISCUSSION

Methionine and Cystine Balance Studies with Rats Fed Soybean Meal, Beans, and Beans Plus Supplemental Methionine. The results are presented in Table I. Previous data indicated that beans may contain something which interferes with the utilization of methionine added to the diet (Evans et al., 1974). The present experiment was designed to determine whether methionine added to a bean-containing diet would be absorbed and utilized by the growing rat. In calculating the data for line 4 of the table, it was assumed that all of the added methionine was absorbed and utilized. If it were not completely absorbed, the undigested methionine of the beans in line 4 would have been larger, and the amount of utilized methionine smaller, than for the beans alone (line 2). However, since the two values were not significantly different, we may conclude that all of the supplemental methionine was absorbed by the rats, and further, that the poor utilization of methionine in dry beans is not caused by something in the beans which interferes with the ab-

Table I. First Experiment: Methionine and Cystine Digested and Utilized or Metabolized by Rats Fed Soybean Meal, Dry Bean Flour, or Dry Bean Flour Plus Methionine as the Sole Source of Protein in the Diet (Rats Were on Experiment for 1 Week)

Diet	Undigested		Utilized	
	Methionine, %	Cystine, %	Methionine, %	Cystine, %
Soybean meal	$29.8_a \pm 7.8$[a]	$15.3_a \pm 3.2$	$69.4_b \pm 7.8$	$82.3_a \pm 4.1$
Bean flour	$49.6_b \pm 9.2$	$40.8_b \pm 8.2$	$49.5_c \pm 9.2$	$53.1_b \pm 10.0$
Bean flour plus methionine	$27.4_a \pm 2.5$	$38.5_b \pm 4.2$	$71.4_a \pm 2.3$	$53.8_b \pm 5.2$
(Bean flour plus methionine)-methionine[b]	$50.3_b \pm 4.6$	$38.5_b \pm 4.2$	$47.8_c \pm 4.4$	$53.8_b \pm 5.2$

[a] The means within each column which are followed by the same subscript letter are not statistically significantly different ($P > 0.5$) from each other, but are different from those means which are followed by a different letter. [b] To calculate these values, it was assumed that all of the added methionine was absorbed and utilized.

Table II. Second Experiment: Weight Gains of Rats and Protein Efficiency Ratios (PER) of Diets Containing Autoclaved Bean Flour, Bean Fractions, Casein, and Casein Plus Phytic Acid (Rats Were on Experiment for 4 Weeks)

Protein source	Weight gain, g	Feed intake, g	PER
Bean flour	$80_b \pm 22$[a]	$338_b \pm 66$	$2.35_{bc} \pm 0.23$
Bean residue	$138_a \pm 29$	$439_a \pm 93$	$3.16_a \pm 0.42$
pH 3.8 precipitate	$82_b \pm 6$	$333_b \pm 20$	$2.45_{ab} \pm 0.10$
pH 3.8 soluble	$-5_d \pm 3$	$139_d \pm 11$	$0.00_d \pm 0.24$
Casein	$37_c \pm 6$	$224_c \pm 18$	$1.64_c \pm 0.17$
Casein plus phytic acid[b]	$42_c \pm 7$	$240_c \pm 19$	$1.75_{bc} \pm 0.19$

[a] Values within a column not having the same subscript letter differ significantly ($P < 0.05$). [b] Phytic acid (Sigma, Type III, calcium salt from corn) was added to give 0.5% in the diet.

sorption of free methionine.

Separation of Bean Flour into Fractions on the Basis of Solubility. To test the hypothesis that beans may contain a protein which is not digested by the rat, in the second experiment, bean flour was separated into three fractions on the basis of differences in solubility. Each of these fractions was then fed to weanling rats for 4 weeks. The data on weight gain, feed consumption, and PER are presented in Table II. Whole beans and the pH 3.8 precipitate fraction promoted similar growth and PER. The bean residue promoted better growth than either whole beans or the pH 3.8 precipitate and higher PER than whole beans. Bean residue gave a higher PER than the pH 3.8 precipitate, although the difference was not statistically significant.

Rats fed the whole bean diet supplemented with methionine, to give a level of 0.30% methionine plus cystine, gained 80 g with a PER of 2.35. Rats fed the pH 3.8 precipitate gained about the same amount (82 g) as rats fed whole beans and had nearly the same PER (2.45). This would indicate that these diets contained similar levels of unavailable methionine and cystine. Rats fed the bean residue gained 138 g with a PER of 3.16.

The diet which contained the pH 3.8 supernatant contained 0.05% methionine and 0.07% cystine, or 0.12% methionine plus cystine from the pH 3.8 supernatant and 0.18% of added methionine. Rats fed this diet lost weight, but the 0.18% of added methionine should have been sufficient to support some growth even if none of the methionine or cystine in the fraction were available. There could be several explanations for the poor performance of the rats fed the pH 3.8 soluble fraction. The protein of the fraction could have been deficient in one or more of the other essential amino acids, but even a highly deficient protein should still have supported some growth. There could have been a heat-stable growth-inhibiting protein in the beans which was concentrated in the pH 3.8 soluble fraction. There also could have been a nonprotein substance in the beans which depressed growth and which was concentrated in the pH 3.8 soluble fraction. The pH 3.8 soluble fraction did contain much nonprotein material, as shown by its analysis of 20.9% crude protein, 0.10% methionine, and 0.15% cystine. The proteins were extracted from bean flour with a potassium hydroxide solution, which was neutralized when the solution was adjusted to pH 3.8 with hydrochloric acid, and the filtrate was lyophilized, so that the pH 3.8 soluble fraction would contain a high level of potassium chloride, which might have contributed to the weight loss of the rats fed this diet.

A third experiment was carried out to see if the toxicity of the pH 3.8 soluble fraction was caused by the potassium chloride present, by a low molecular weight nonprotein constituent, or by a nondialyzable protein component of the fraction. Raw beans or beans which had been heated in the autoclave were extracted with water, and the extracts were divided into two portions, one of which was dialyzed against distilled water and lyophilized and the other lyophilized without dialysis. The results of feeding these fractions to growing rats are presented in Tables III and IV. The fractions which were prepared from the raw beans were fed to the rats for 3 weeks (Table IV) and those from the autoclaved for only 2 weeks (Table III). None of the fractions from the raw beans depressed growth to

Table III. Third Experiment: Weight Gains of Rats and Protein Efficiency Ratios (PER) of Diets Containing Bean Flour and Bean Fractions (Rats Were on Experiment for 2 Weeks)

Protein source	Weight gain, g	Feed intake, g	PER
Bean flour	$63_{bc} \pm 7$[a]	$174_a \pm 7$	$3.63_{cd} \pm 0.28$
Residue raw beans	$58_{cd} \pm 13$	$180_a \pm 24$	$3.21_d \pm 0.44$
Extract raw beans	$17_e \pm 3$	$109_{bc} \pm 9$	$1.52_f \pm 0.22$
Extract raw beans (dialyzed)	$16_e \pm 4$	$98_c \pm 7$	$1.62_f \pm 0.26$
Residue autoclaved beans	$78_{ab} \pm 1$	$199_a \pm 20$	$3.89_{bc} \pm 0.20$
Extract autoclaved beans[b]	$44_d \pm 5$	$133_b \pm 16$	$3.31_d \pm 0.33$
Extract autoclaved beans (dialyzed)[c]	$83_a \pm 6$	$186_a \pm 15$	$4.48_a \pm 0.10$
Casein	$84_a \pm 10$	$180_a \pm 15$	$4.49_a \pm 0.14$

[a] Values within a column not having the same subscript letter differ significantly ($P < 0.05$). [b] Diet contained 3.28% protein from the extract and 6.74% from casein. [c] Diet contained 1.15% protein from dialyzed extract and 8.87% from casein.

Table IV. Third Experiment: Weight Gain of Rats and Protein Efficiency Ratios (PER) of Diets Containing Bean Flour and Bean Fractions (Rats Were on Experiment for 3 Weeks)

Protein source	Weight gain, g	Feed intake, g	PER
Bean flour	$102_{bc} \pm 8^a$	$304_a \pm 9$	$3.35_b \pm 0.20$
Residue raw beans	$89_c \pm 18$	$295_a \pm 43$	$3.00_c \pm 0.24$
Extract raw beans	$29_d \pm 5$	$175_b \pm 16$	$1.67_d \pm 0.28$
Extract raw beans (dialyzed)	$29_d \pm 5$	$154_b \pm 15$	$1.87_d \pm 0.14$
Residue autoclaved beans	$113_{ab} \pm 17$	$328_a \pm 42$	$3.43_b \pm 0.14$
Casein	$134_a \pm 13$	$326_a \pm 25$	$4.11_a \pm 0.10$

[a] Values within a column not having the same subscript letter differ significantly ($P < 0.05$).

the extent that the pH 3.8 soluble fraction of the first experiment did, which indicates that at least some of the toxicity of the pH 3.8 soluble fraction was caused by potassium chloride. The dialyzed and undialyzed soluble fractions promoted similar growth and PER, and both gave significantly poorer results than did whole beans or the bean residue. It would thus appear either that the methionine and/or cystine in the water-soluble proteins was much less available than that in the water-insoluble proteins, or that a heat-stable growth depressant was extracted from the beans with water.

The amount of protein extracted from the autoclaved beans was very small, so that only enough of the undialyzed extract was obtained to feed at a level of 3.28% protein, and of the dialyzed extract at a level of 1.15% protein for a period of 2 weeks. Both diets were supplemented with casein to bring the total protein to 10% (6.74 and 8.87%, respectively). The data for the 2-week growth and PER are presented in Table III. Growth of rats fed the residue of autoclaved beans was significantly better than growth of rats fed whole beans, but the PER was not. Growth of rats fed the diet and PER of the diet with 8.87% casein protein and 1.15% protein from the dialyzed extract of autoclaved beans were not significantly different from growth of rats fed the diet and PER of the diet with 10% casein protein, which indicated that there were no toxic substances in this bean fraction. However, when the undialyzed extract from autoclaved beans supplied 3.28% casein and 6.74% protein, both growth and PER were significantly less than growth and PER when casein supplied 10% protein, which indicated a poor utilization of the protein from the bean extract. Rats fed the diet which contained 10% protein from casein gained 84 g, and if one assumes a linear growth rate with protein level in the diet, rats fed a diet containing 6.74% protein from casein should have gained $0.674 \times 84 = 57$ g. Rats receiving that level of casein plus the bean extract gained only 44 g, so there was a growth-depressing effect by the undialyzed bean extract. This would indicate that the growth depressant was a low molecular weight (dialyzable) substance. The growth depressant in the raw bean extract was not removed by dialysis and was thus different from the low molecular weight substance. The low molecular weight substance was probably responsible for part of the toxicity of the pH 3.8 soluble fraction of the second experiment.

Influence of Phytic Acid upon the Utilization of Methionine and Cystine. Dry beans contain phytate (inositol hexaphosphate). Makower (1970) reported levels of 0.94–1.9% in dry beans, and Lolas and Markakis (1975) reported levels of 0.54–1.58%. Some phytic acid of beans appears to be present as a protein–phytic acid complex, where the protein represents at least 16% of the total protein of the seeds (Bourdillon, 1951). This protein contained 0.30% sulfur, which would be equivalent to 1.2–1.4% of methionine plus cystine compared to 1.9–2.0% in total proteins (Evans and Bandemer, 1967). Since phytic acid is not available to humans (McCance and Widdowson, 1935), the protein associated with it might also be unavailable, but the protein so associated is relatively poor in methionine and/or cystine.

Table V. Fourth Experiment: Weight Gains of Rats and Protein Efficiency Ratios (PER) of Diets Containing Dry Beans Cooked by Different Methods[a] (Rats Were on Experiment for 3 Weeks)

Protein source	Weight gain, g	Feed intake, g	PER
Bean flour	$33_c \pm 7^b$	$202_{bc} \pm 24$	$1.64_c \pm 0.17$
Boiled beans	$32_c \pm 11$	$194_c \pm 40$	$1.61_c \pm 0.32$
Boiled bean water[c]	$62_b \pm 8$	$258_{ab} \pm 35$	$2.42_b \pm 0.14$
Casein	$109_a \pm 14$	$309_a \pm 45$	$3.36_a \pm 0.22$

[a] The diets did not contain any added methionine or cystine. [b] Values within a column not having the same subscript letter differ significantly ($P < 0.05$). [c] This diet contained casein to supply 6.57% protein and boiled bean water to supply 3.43% protein.

The question might be asked whether the phytic acid in beans interferes with methionine and cystine availability. To study this, rats were fed diets with casein as the protein, both with and without 0.5% added phytic acid. The results of the experiment are given in Table II. The addition of phytic acid did not decrease rat growth or PER. Therefore, phytic acid did not appear to interfere with utilization of the methionine or cystine casein. However, the rats which were fed casein in this experiment did not grow normally, which may have influenced the results. A different batch of casein was used in the remainder of the experiments.

Influence of Method of Cooking Dry Beans on Methionine and Cystine Availability. The apparent presence of a low molecular weight substance in autoclaved beans which depressed growth suggested the possibility that if beans were boiled in water and the cooking water discarded, the boiled beans would be a better nutritional source of protein (methionine and cystine) than if the water were not discarded, and would be better than autoclaved beans. Weanling rats were fed diets which contained autoclaved beans, boiled beans, casein, or casein to supply 65.7% of the dietary protein and dried bean water to supply 34.3% of the protein in the fourth experiment. The diets contained no added methionine. Boiled beans did not support better growth and PER than autoclaved beans (Table V). The diet which contained casein plus water from boiled beans supported growth and PER between those of the casein and the beans. Assuming linear growth from different levels of casein, the casein contained in the casein plus bean water diet should have promoted $109 \times 0.657 = 72$ g of growth. The rats gained only 62 g, which indicates (but does not prove) a toxic action of the bean water (the difference between 62 and 72 was not statistically significant).

In a fifth experiment, the beans were ground before they were cooked in boiling water. The bean water was separated from the bean residue by centrifugation. One diet contained 1.45% protein from the boiled bean water and

Table VI. Fifth Experiment: Weight Gains of Rats and Protein Efficiency Ratios (PER) of Diets Containing Dry Beans Cooked by Different Methods (Rats Were on Experiment for 2 Weeks)

Protein source	Weight gain, g	Feed intake, g	PER
Bean flour	$63_b \pm 7^a$	$174_a \pm 7$	$3.63_b \pm 0.28$
Boiled beans	$64_b \pm 8$	$180_a \pm 13$	$3.57_b \pm 0.24$
Boiled bean water[b]	$72_{ab} \pm 11$	$172_a \pm 21$	$4.18_a \pm 0.14$
Casein	$84_a \pm 10$	$180_a \pm 19$	$4.49_a \pm 0.14$

[a] Values within a column not having the same subscript letter differ significantly ($P < 0.05$). [b] This diet contained casein to supply 8.57% protein and boiled bean water solids to supply 1.45% protein.

Table VII. Sixth Experiment: Weight Gains of Rats and Protein Efficiency Ratios (PER) of Diets Containing Dry Beans Cooked by Different Methods (Rats Were on Experiment for 2 Weeks)

Protein source	Weight gain, g	Feed intake, g	PER
Bean flour	$11_d \pm 4^a$	$90_c \pm 13$	$1.21_d \pm 0.41$
Boiled beans (H_2O)	$60_b \pm 12$	$162_{ab} \pm 39$	$3.73_b \pm 0.17$
Boiled bean water (H_2O)	$31_c \pm 9$	$130_{bc} \pm 20$	$2.34_c \pm 0.41$
Boiled beans ($NaHCO_3$)	$52_b \pm 11$	$145_{ab} \pm 22$	$3.58_b \pm 0.28$
Boiled bean water ($NaHCO_3$)	$54_b \pm 12$	$141_{ab} \pm 25$	$3.78_b \pm 0.33$
Casein	$88_a \pm 12$	$187_a \pm 23$	$4.69_a \pm 0.28$

[a] Values within a column not having the same subscript letter differ significantly ($P < 0.05$).

8.57% from casein. The diets were fed to growing rats for 2 weeks. The results of this experiment are presented in Table VI. There were no significant differences in the weight gain or PER between rats fed the autoclaved beans and those fed the boiled beans. Boiled bean water added to the casein diet decreased weight gain and PER, but not significantly so.

Whole beans were cooked either in boiling water or boiling 0.1% sodium bicarbonate solution in a sixth experiment. Diets were fed for 4 weeks. Data for 2-week growth are given in Table VII, and those for 4-week growth in Table VIII. The boiled bean water was added to diets to furnish 1.8% protein, with 8.2% protein from casein. Comparison of diets containing boiled beans with those containing autoclaved beans was difficult, because rats fed the autoclaved beans did not grow well (Table VIII). These rats had the poorest growth and PER of any in this experiment and grew much less than rats fed similarly in earlier experiments. Growth of rats fed beans boiled in water and PER of diets were not significantly different than those of rats fed beans boiled in 0.1% sodium bicarbonate solution. However, rats fed water from boiled beans had significantly poorer growth and PER than rats fed the sodium bicarbonate solution from beans boiled in it. Assuming a linear relationship between level of casein in the diet and rat growth, rats fed a diet containing 8.2% protein from casein should have gained 157 g, but rats fed this level of casein plus boiled bean water ($NaHCO_3$) gained 143 g, and those fed casein plus boiled bean water gained only 78 g. The latter value definitely indicates the presence of a growth inhibitor in the boiled bean water.

Table VII presents the 2-week growth and PER for rats in experiment 6. These data for rats fed the autoclaved beans can be compared with data for rats fed autoclaved beans in the fifth experiment (Table VI). Comparison of data for rats fed casein shows that rats in the fifth experiment gained 84 g with a PER of 4.49, compared to 88 g gain and 4.69 PER for rats in the sixth experiment. Now compare the 63 g gain and 3.63 PER of rats fed the autoclaved beans in the fifth experiment with the 11 g gain and 1.21 PER of rats fed the autoclaved beans in the sixth experiment. If values of 63 g gain and 3.63 PER are substituted for the 11 g gain and 1.21 PER in Table VII, there are no significant differences in gain and PER among rats fed autoclaved beans, beans boiled in water, and beans boiled in 0.1% sodium bicarbonate solution.

Table VIII. Sixth Experiment: Weight Gains of Rats and Protein Efficiency Ratios (PER) of Diets Containing Dry Beans Cooked by Different Methods (Rats Were on Experiment for 4 Weeks

Protein source	Weight gain, g	Feed intake, g	PER
Bean flour	$39_e \pm 10^a$	$224_c \pm 30$	$1.72_d \pm 0.32$
Boiled beans (H_2O)	$128_{bc} \pm 20$	$409_{ab} \pm 63$	$3.14_b \pm 0.09$
Boiled bean water (H_2O)[b]	$78_d \pm 20$	$326_b \pm 56$	$2.34_c \pm 0.30$
Boiled beans ($NaHCO_3$)	$110_c \pm 17$	$370_b \pm 47$	$2.95_b \pm 0.14$
Boiled bean water ($NaHCO_3$)[b]	$143_b \pm 12$	$398_{ab} \pm 43$	$3.59_a \pm 0.14$
Casein	$191_a \pm 21$	$482_a \pm 44$	$3.95_a \pm 0.17$

[a] Values within a column not having the same subscript letter differ significantly ($P < 0.05$). [b] This diet contained casein to supply 8.2% protein and boiled bean water to supply 1.8% protein.

Beans contain a heat-labile growth inhibitor, phytohemagglutinins, and trypsin inhibitors, all of which have been associated with growth depression in rats fed raw beans (Kakade and Evans, 1965b). In an attempt to explain the poor results obtained for rats fed autoclaved beans in the sixth experiment, trypsin inhibitor activities of the beans were determined by the procedure of Pusztai (1972). Beans boiled in water had 3% of the trypsin inhibitor activity of raw beans, beans boiled in 0.1% sodium bicarbonate solution had 5%, autoclaved beans used in the fifth experiment had 5%, and autoclaved beans used in the sixth experiment had 24%. This indicated that, for some reason, the beans used in the sixth experiment had not been heated sufficiently to destroy all of the trypsin inhibitors and, probably, all of the growth inhibitors as well.

Properties of the Dialyzable Portion of Boiled Bean Water. Beans were extracted with a 2% sodium chloride solution. The extract was evaporated to a small volume by boiling and the precipitate removed by centrifugation. The supernatant was filtered through a UM-10 membrane in the DiaFlo apparatus. The filtrate from which large molecules (>10 000 mol wt.) had been removed was separated by high-voltage electrophoresis at pH 6.7 into 15 ninhydrin stained bands and by paper chromatography with butanol–acetic acid–water (4:1:5) into 18 ninhydrin stained bands. The ultraviolet absorption maximum of the solution was at 260 nm rather than at 280 nm, with a 280/260 ratio of 0.54, indicating the presence of pyrimidines, purines, nucleosides, and/or nucleotides.

Causes for the Poor Growth of Rats Fed Autoclaved Beans Supplemented with Methionine to Bring the Level to That Required for Good Growth of Rats If All Were Utilized. The residue from the 0.2% potassium hydroxide extraction of autoclaved dry beans used in the second experiment contained 16.2% crude protein, 0.18% methionine, and 0.11% cystine. When it was fed to rats to supply 10% protein in the diet, it furnished 0.11% methionine and 0.07% cystine, or 0.18% methionine plus cystine. It was necessary to add 0.12% methionine to the diet to bring the level of methionine plus

cystine to 0.30%. Since the supplemental residue supported optimal growth and PER, it appears that all of the methionine and cystine of this residue was utilized by the rat. However, neither water nor 0.1% sodium bicarbonate extraction in the sixth experiment removed all of the nonutilizable methionine and cystine from either raw or autoclaved beans. All extracted less protein than did 0.2% potassium hydroxide.

Autoclaved or boiled beans appeared to contain a water-soluble dialyzable substance which inhibited rat growth. This substance did not appear to be present in water extracts of raw beans, or in water from beans boiled in 0.1% sodium bicarbonate solution. The nature of the inhibitor is not known, but the fraction containing the inhibitor contained many peptides and/or free amino acids and substances which absorbed in the ultraviolet at 260 nm.

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LITERATURE CITED

Association of Official Agricultural Chemists, "Official Methods of Analysis", 10th ed, Washington, D.C., 1965, p 16.
Bourdillon, J., *J. Biol. Chem.* **189**, 65 (1951).
Duncan, D. B., *Biometrics* 11, 1 (1955).
Evans, R. J., Bandemer, S. L., *J. Agric. Food Chem.* **15**, 437 (1967).
Evans, R. J., Bauer, D. H., Sisak, K. A., Ryan, P. A., *J. Agric. Food Chem.* **22**, 130 (1974).
Hegsted, D. M., Mills, R. C., Elvehjem, C. A., Hart, E. B., *J. Biol. Chem.* **138**, 459 (1941).
Kakade, M. L., Evans, R. J., *Br. J. Nutr.* **19**, 269 (1965a).
Kakade, M. L., Evans, R. J., *J. Agric. Food Chem.* **13**, 450 (1965b).
Lolas, G. M., Markakis, P., *J. Agric. Food Chem.* **23**, 13 (1975).
Makower, R. U., *Cereal Chem.* **47**, 288 (1970).
McCance, R. A., Widdowson, E. M., *Biochem. J.* **293**, 2694 (1935).
Pusztai, A., *Planta* **107**, 121 1972).
Snedecor, G. W., "Statistical Methods Applied to Experiments in Agriculture and Biology", 5th ed, Iowa State College Press, Ames, Iowa, 1956.

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Susceptibility of the Major Storage Protein of the Bean, *Phaseolus vulgaris* L., to in Vitro Enzymatic Hydrolysis

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The in vitro susceptibility to enzymatic hydrolysis of affinity-isolated G1, the major storage protein of the bean, has been examined. The extent of hydrolysis of G1 by a number of enzymes was less than that of native bovine serum albumin under similar conditions. Sequential treatments with different enzymes resulted in more complete hydrolysis. Discontinuous SDS gel electrophoresis of G1 after exposure to trypsin confirmed the susceptibility of the molecule to tryptic hydrolysis and indicated the presence of a number of extremely trypsin-labile peptide bonds. The existence of a number of relatively large trypsin-resistant peptides in G1 has also been observed. The effects of heat treatment on G1 suggest that there may exist some conformational constraints on hydrolysis of the native molecule. The concentration of tannins in different seed lines did not correlate with the in vitro susceptibility to hydrolysis of the affinity-isolated protein but added tannins readily decreased the in vitro susceptibility to hydrolysis.

Several factors are likely to limit the nutritional quality of legume seed proteins. Low content of sulfur-containing amino acids and the presence of a number of potentially antinutritive proteins are the most frequently discussed factors. However, sulfur content (cystine + methionine) does not correlate well with protein efficiency ratio for cooked legumes (Liener, 1976) which suggests that other factors may be dominant. In addition, the proteinase inhibitors and phytohemagglutinins which were once thought to be major determinants of nutritional quality in legumes may not play dominant roles (Kakade et al., 1973; Turner and Liener, 1975). As a result, the role that protein digestibility may play in determining nutritional quality has been referred to with increasing frequency in recent years (e.g., Kakade, 1974).

Susceptibility to in vitro enzymatic hydrolysis appears to correlate with in vivo measurements of nutritional quality even without any corrections for absorption or other complicating metabolic factors. Several procedures for in vitro hydrolysis with pepsin or with pepsin plus pancreatin were developed before 1965 and were shown to correlate better with biological value than did a number of chemical scores based only on amino acid analysis (Akeson and Stahman, 1964; Sheffner, 1967). Subsequent work with other enzymes or with simplified procedures has not substantially improved correlations with in vivo

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