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# Heat-Labile Growth-Inhibiting Factors in Beans (*Phaseolus vulgaris*)

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*ABSTRACT* A comparative study in vitro was made of enzyme-inhibiting and hemagglutinin activities, and the effect on rat growth of 5 varieties of kidney beans (*Phaseolus vulgaris*). Extracts of 2 bean samples were active in agglutinating rabbit blood cells and toxic when fed to growing rats. Diets prepared with these seeds supplemented with methionine caused weight loss and death when fed to rats alone or with a supplement of 10% enzymatically or acid-digested casein. Hemagglutinating activity was observed in the feces of rats fed the raw bean diets. The possibility that the hemagglutinins are at least partly responsible for the toxic effects was examined. Three other samples of kidney beans had no significant hemagglutinating or lethal effect. Rats fed the raw seed meals supplemented with methionine did not gain weight but grew well with a similar diet supplemented with enzymatically digested casein. Supplements of 10% casein, 1% Na glutamate, or 10% acid-digested casein did not improve growth significantly but the latter did when tryptophan was added. Antitrypsin and antiamylase activities were low or absent in some of the seeds and high in others, and did not appear to be directly related to the growth inhibition observed. The low growth-promoting action of the hemagglutinin-free beans might be explained by low digestibility and an enzyme-inhibiting activity of the bulk proteins different from that of the trypsin or amylase inhibitors.

The existence of a marked inhibitory action on the growth of experimental animals fed diets containing various raw legumes, especially beans or soybeans, has been well-established. Biochemically active factors defined by their in vitro action have been observed and related to the anti-nutritional effect, notably trypsin inhibitors and hemagglutinins or lectins. The extensive literature has been reviewed by Liener (1).

The relative importance of these 2 factors and the existence of other ill-defined growth inhibitors is still subject to controversy. Although a number of papers have been published in recent years on this problem, progress in this field has been slow. Several factors may be responsible for this situation: The isolation of purified fractions with only one biochemical activity in amounts large enough for toxicological studies is difficult; a purified fraction incorporated into a non-toxic diet may have a different effect from that of a combination of active principles occurring naturally in the seeds; the raw legumes or fractions used were not always sufficiently defined with respect to the different biochemical activities present; and different legume species and even varieties of one species may vary considerably in respect to different biochemical and biological activities, a fact often neglected in this type of investigation.

In the present experiments we investigated the different varieties of one species of legumes that are free from or low in one or the other of the biochemically active factors suspected of being related to toxicity. The growth-promoting or toxic effects of diets prepared with equal amounts of the different ground seeds with known hemagglutinin and enzyme inhibitor content were compared in an attempt to relate these factors and the nutritional properties without the need of fractionation.

## MATERIALS AND METHODS

The legume seeds were purchased at a local market and finely ground in a hammer mill. One part of them was soaked in water for 2 to 3 hours, autoclaved for 30 minutes at 118°, fan-dried, and then reground. The experimental diets contained, unless stated otherwise, the following ingredients: (in percent) ground legume seed meal, 40; corn oil with 0.2% percomorphum oil, 5; salt mixture USP XVI, 4; DL-methionine, 0.3; and the following vitamins (2) per

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TABLE 1

*Biochemical activities in extracts of different beans*

| | Rabbit blood | Rat blood | Trypsin inhibitor | Amylase inhibitor |
|---|---|---|---|---|
| | *hemagglutination units* [1] | | *units* | *units* |
| Red bean (*Phaseolus vulgaris*) | 500 | 20 | 3.8 | 0.77 |
| Black bean (*P. vulgaris*) | 400 | 8 | 32.1 | 0.65 |
| White bean (*P. vulgaris*) | 3 | 0 | 3.3 | 2.44 |
| Tapiramo bean (*P. vulgaris*) | 0 | 0 | 20.2 | 1.86 |
| Mottled bean (*P. vulgaris*) | 0 | 0 | 46.0 | 0 |
| Soybean (*Glycine maxima*) | 80 | 0 | 28.3 | |

[1] All activities are expressed as units/milligram of extracted protein (N × 6.45); hemagglutination activity is expressed as the number of milliliters of a 0.2% erythrocyte suspension agglutinated by 1 mg protein (N × 6.45) of the corresponding extract.

100 g: (in milligrams) thiamine·HCl, 0.3; riboflavin, 0.3; pyridoxine·HCl, 0.2; Ca pantothenate, 0.2; niacin, 2; folic acid, 0.025; biotin, 0.010; inositol, 10; choline·HCl, 100; and cassava starch to make 100. A commercial enzymatic casein digest,[2] an acid casein digest,[3] or other supplements were added at the expense of starch. Three of the diets contained no seed meal. The kidney bean diets without added casein contained 8 to 9% protein (N × 6.25).

The rats were descendants of the Sprague-Dawley strain bred in this laboratory for over 20 years. In the first experiment, of 2 weeks' duration, male animals weighing 45 to 55 g were used. In experiments 2 and 3, three males and 3 females, weighing 35 to 45 g, were fed the respective diets for 28 days, with the exception of those dying before the end of the experiment. Young from different litters were distributed at random among the different groups. All animals were kept in individual screen-bottom cages and received food and water ad libitum. The surviving animals were killed at the end of each experiment and the pancreas and spleen removed and weighed immediately.

For the in vitro tests, extracts were prepared by allowing 10 g of finely ground seeds to stand in 100 ml of 0.85% NaCl solution for 2 hours with occasional stirring, and then filtering. Nitrogen content of these extracts was determined by the micro-Kjeldahl method.

Hemagglutination activity was studied in the ground seeds and in the feces of some of the experimental animals with washed rabbit or rat blood cells by the serial dilution technique used in earlier experiments (2) and expressed as the number of milliliters of a 0.2% erythrocyte suspension agglutinated by 1 mg of protein (N × 6.25) of the corresponding extract. Trypsin inhibitor activity was measured by the method of Kunitz (3), using casein as substrate. The method described by Bernfeld for the measurement of amylase activity (4) was used for the determination of amylase inhibitors. One unit was defined as the number of milligrams of protein in a seed extract that reduced to 50% the activity of the quantity of pancreatic amylase [4] capable of releasing 15 mg maltose from 1 ml of 1% starch solution in 3 minutes at 25° after 8 minutes of pre-incubation of amylase and inhibitor.

Nitrogen absorption was calculated in each animal of experiments 2 and 3 by measuring food consumption and fecal nitrogen excretion. Nitrogen was determined by macro-Kjeldahl method.

## RESULTS

The results of several of the in vitro tests on biochemical activities of the bean varieties used are presented in table 1. Extracts of red and black kidney beans agglutinated both rat and rabbit blood cells, whereas soybean extracts were active only with rabbit blood; the other extracts had little or no hemagglutinating activity under the present experimental conditions.

Mottled and black kidney beans and soybeans had considerable trypsin inhibitor

[2] Bacto-casitone, Difco Laboratories, Detroit.
[3] Bacto-casamino-acids, Difco Laboratories.
[4] Pangestin, E. Merck, Darmstadt.

TABLE 2

*Performance of rats fed different experimental diets* [1]

| No. | Diet | Wt change/day | Pancreas wt/ body wt × 100 | Spleen wt/ body wt × 100 |
|---|---|---|---|---|
| | | *g* | | |
| 1 | Raw black beans | − 1.8 ± 0.58 [2,3,4] | 0.47 ± 0.13 [4] | 0.15 ± 0.02 [4] |
| 2 | Raw black beans + casitone [5] | − 1.1 ± 0.22 [4,6] | 0.56 ± 0.09 [4] | 0.16 ± 0.01 [4] |
| 3 | Raw black beans + casamino acids [7] | − 1.0 ± 0.38 [3,4] | 0.54 ± 0.10 [4] | 0.16 ± 0.02 [4] |
| 4 | Autoclaved black beans | + 3.0 ± 0.39 | 0.32 ± 0.05 | 0.25 ± 0.04 |
| 5 | Raw red kidney beans | − 2.1 ± 0.60 [3,4] | 0.34 ± 0.04 | 0.11 ± 0.01 [4] |
| 6 | Raw red kidney beans + casitone | − 2.0 ± 0.09 [3,4] | 0.50 ± 0.14 [4] | 0.12 ± 0.04 [4] |
| 7 | Autoclaved red kidney beans | + 3.1 ± 0.34 | 0.37 ± 0.05 | 0.26 ± 0.03 |
| 8 | Raw white beans | + 0.8 ± 0.43 [4] | 0.40 ± 0.07 | 0.20 ± 0.05 |
| 9 | Raw white beans + casitone | + 2.5 ± 0.37 | 0.32 ± 0.12 | 0.24 ± 0.04 |
| 10 | Autoclaved white beans | + 2.7 ± 0.05 | 0.32 ± 0.08 | 0.23 ± 0.04 |
| 11 | Raw mottled beans | + 0.1 ± 0.37 [4] | 0.25 ± 0.04 | 0.24 ± 0.03 |
| 12 | Raw mottled beans + casitone | + 2.5 ± 0.42 | 0.51 ± 0.09 [4] | 0.21 ± 0.02 |
| 13 | Autoclaved mottled beans | + 2.5 ± 0.35 | 0.33 ± 0.02 | 0.23 ± 0.04 |
| 14 | Raw tapiramo beans | + 0.4 ± 0.33 [4] | 0.37 ± 0.03 | 0.18 ± 0.02 [4] |
| 15 | Raw tapiramo beans + casitone | + 2.9 ± 0.40 | 0.49 ± 0.10 | 0.16 ± 0.02 [4] |
| 16 | Autoclaved tapiramo beans | + 3.0 ± 0.44 | 0.41 ± 0.10 | 0.26 ± 0.04 |
| 17 | Raw soybeans | + 4.4 ± 0.39 | 0.50 ± 0.08 [4] | 0.30 ± 0.05 |
| 18 | Raw soybeans + casitone | + 5.6 ± 0.46 | 0.57 ± 0.05 [4] | 0.40 ± 0.04 |
| 19 | Autoclaved soybeans | + 5.2 ± 0.38 | 0.34 ± 0.03 | 0.37 ± 0.05 |
| 20 | Stock diet (without seed meal) | + 5.1 ± 0.24 | 0.35 ± 0.06 | 0.34 ± 0.03 |
| 21 | 10% casitone diet (without seed meal) | + 2.8 ± 0.36 | 0.34 ± 0.04 | 0.30 ± 0.03 |
| 22 | Protein-free diet (without seed meal) | − 2.3 ± 0.20 | 0.24 ± 0.07 | 0.16 ± 0.02 |

[1] All diets with the exception of nos. 20 and 22 were supplemented with 0.3% DL-methionine; the stock diet used was Purina Laboratory Chow, Ralston Purina Company, St. Louis.
[2] SD.
[3] All animals died or were killed in moribund condition within 2 weeks.
[4] Significantly different from group fed autoclaved legume diet, $P < 0.05$, calculated from $t$ value.
[5] Bacto-casitone (trypsin-digested casein), Difco Laboratories, Detroit.
[6] Two animals died within 2 weeks.
[7] Bacto-casamino-acids (acid-digested casein), Difco Laboratories, 0.1% D-tryptophan added.

activity. Four of the 5 kidney bean samples showed amylase inhibitor action, but mottled beans lacked this activity. It was not possible to detect amylase inhibitors in soybeans with the technique used because of the strong amylase activity present in the corresponding extracts.

In growth experiments the rats fed the black or red kidney beans with high hemagglutinating activity lost weight rapidly and did not survive with this diet for more than 2 weeks. Supplementation with 10% casein digested with acid or trypsin did not improve the performance to a significant degree but autoclaving abolished the toxic effect (table 2, diets 1–7; and table 3, diets 1–3).

The diet prepared with the non-agglutinating white, mottled, or tapiramo beans did not produce weight loss and death comparable to that of the previous experiments. Growth was very poor, however, with these diets unless supplemented with tryptic casein digest. In this case growth was similar to that observed in the rats fed the autoclaved seeds (table 2, diets 8–16; table 3, diets 4–9).

Data reported in table 3 show that food intake and nitrogen absorption was low in all animals consuming unsupplemented diets containing raw beans.

Further experiments were performed to study in more detail the possible reason for the lack of growth in rats fed the unsupplemented diet of white kidney beans containing little hemagglutinin and trypsin inhibitor activity, but which was the most active in amylase inhibition (table 1). Several experiments with soybeans were also included for comparison (table 4).

Supplementation of a white bean diet with enzymatically digested casein brought growth to near-optimal values (diet 3), while casein had no significant effect (diet 4). An acid-digested casein enhanced growth significantly only after having been

TABLE 3

*Performance of rats fed various methionine-supplemented diets*

| No. | Diet | Wt change/day | Food intake/day | N absorbed [1] | PER [2] | Pancreas wt/body wt × 100 | Spleen wt/body wt × 100 | Pancreas wt/spleen wt |
|---|---|---|---|---|---|---|---|---|
| | | *g* | *g* | % | | | | |
| 1 | Raw black beans | − 1.2 ± 0.14 [3,4,5] | 2.9 ± 0.5 [5] | 13.3 ± 9.9 [5] | — | 0.42 ± 0.07 [5] | 0.15 ± 0.02 [5] | 2.9 ± 0.81 [5] |
| 2 | Raw black beans + casitone [6] | − 0.57 ± 0.61 [4,5] | 4.0 ± 1.0 [5] | 47.7 ± 4.5 [5] | — | 0.49 ± 0.09 [5] | 0.18 ± 0.06 [5] | 3.1 ± 1.3 [5] |
| 3 | Autoclaved black beans | + 3.2 ± 0.26 | 11.0 ± 1.3 | 66.8 ± 1.9 | 2.8 ± 0.09 | 0.30 ± 0.06 | 0.25 ± 0.02 | 1.20 ± 0.23 |
| 4 | Raw tapiramo beans | + 0.05 ± 0.12 [5,7] | 6.1 ± 0.7 [5] | 27.1 ± 4.7 [5] | 0.3 ± 0.09 [5] | 0.45 ± 0.16 [5] | 0.18 ± 0.05 | 2.6 ± 0.72 [5] |
| 5 | Raw tapiramo beans + casitone | 2.6 ± 0.55 | 10.5 ± 0.6 [5] | 31.8 ± 5.8 [5] | 2.5 ± 0.40 | 0.39 ± 0.05 [5] | 0.25 ± 0.03 | 1.6 ± 0.23 [5] |
| 6 | Autoclaved tapiramo beans | 2.9 ± 0.21 | 12.8 ± 0.7 | 64.3 ± 6.0 | 2.3 ± 0.16 | 0.29 ± 0.01 | 0.23 ± 0.02 | 1.27 ± 0.10 |
| 7 | Raw mottled beans | − 0.22 ± 0.28 [5,8] | 4.1 ± 0.6 [5] | 17.6 ± 6.5 [5] | — | 0.37 ± 0.07 | 0.20 ± 0.04 | 1.80 ± 0.45 [5] |
| 8 | Raw mottled beans + casitone | 3.4 ± 0.59 | 13.8 ± 1.9 | 53.1 ± 4.0 [5] | 2.4 ± 0.15 | 0.46 ± 0.08 [5] | 0.27 ± 0.06 | 1.76 ± 0.56 [5] |
| 9 | Autoclaved mottled beans | 3.6 ± 0.17 | 13.7 ± 0.8 | 63.0 ± 2.1 | 2.8 ± 0.43 | 0.31 ± 0.03 | 0.26 ± 0.03 | 1.20 ± 0.19 |

[1] Calculated: $\frac{\text{N intake} - \text{N excreted in feces}}{\text{N intake}} \times 100$.

[2] Protein efficiency ratio.

[3] SD.

[4] All animals died or were killed in moribund condition after 10 to 14 days.

[5] Significantly different from group fed autoclaved legume diet, $P < 0.05$, calculated from $t$ test.

[6] Bacto-casitone (trypsin-digested casein), Difco Laboratories, Detroit.

[7] One animal died after 27 days.

[8] Three animals died after 25 to 27 days.

TABLE 4

*Performance of rats fed diets containing white beans or soybeans*

| No. | Diet | Wt change/day | Food intake/day | N absorbed [1] | PER [2] | Pancreas wt/body wt × 100 | Spleen wt/body wt × 100 | Pancreas wt/spleen wt |
|---|---|---|---|---|---|---|---|---|
| | | *g* | *g* | % | | | | |
| 1 | Raw white beans | + 0.05 ± 0.21 [3,4] | 3.8 ± 1.10 [4] | 42.2 ± 5.07 [4] | 0.40 ± 0.14 [4] | 0.31 ± 0.005 [4] | 0.16 ± 0.04 | 1.73 ± 0.07 [4] |
| 2 | Raw white beans without methionine | − 0.32 ± 0.12 [4] | 3.0 ± 0.46 [4] | 35.8 ± 5.99 [4] | — | 0.30 ± 0.03 | 0.10 ± 0.01 [4] | 1.52 ± 0.17 [4] |
| 3 | Raw white beans + casitone [5] | + 3.2 ± 0.39 | 11.5 ± 0.89 | 51.7 ± 8.54 [4] | 2.8 ± 0.21 | 0.37 ± 0.09 [4] | 0.24 ± 0.02 | 1.55 ± 0.39 [4] |
| 4 | Raw white beans + casein | + 0.79 ± 0.42 [4] | 5.5 ± 0.84 [4] | 50.4 ± 2.87 [4] | 1.3 ± 0.55 [4] | 0.48 ± 0.55 [4] | 0.20 ± 0.03 | 2.43 ± 0.42 [4] |
| 5 | Raw white beans + casamino acids [6] | − 0.64 ± 0.05 [4] | 7.1 ± 0.59 [4] | — | — | 0.36 ± 0.04 [4] | 0.26 ± 0.06 | 1.38 ± 0.45 [4] |
| 6 | Raw white beans + casamino acids + tryptophan | + 1.93 ± 0.63 [4,7] | 10.2 ± 1.65 | — | 1.9 ± 0.62 | 0.42 ± 0.07 [4] | 0.30 ± 0.56 | 1.40 ± 0.72 [4] |
| 7 | Raw white beans + glucose | − 0.25 ± 0.24 [4] | 4.7 ± 0.72 [4] | 47.7 ± 7.72 [4] | — | 0.38 ± 0.05 [4] | 0.20 ± 0.02 | 1.83 ± 0.22 [4] |
| 8 | Raw white beans + Na glutamate | − 0.08 ± 0.10 [4] | 5.5 ± 1.10 [4] | 32.2 ± 9.34 | — | 0.35 ± 0.05 [4] | 0.17 ± 0.04 | 1.95 ± 0.71 [4] |
| 9 | Autoclaved white beans | + 2.8 ± 0.40 | 11.0 ± 0.93 | 71.7 ± 2.26 | 2.6 ± 0.23 | 0.20 ± 0.02 | 0.20 ± 0.10 | 1.0 ± 0.16 |
| 10 | Autoclaved white beans without methionine | + 0.87 ± 0.15 [4,7] | 6.9 ± 0.91 [4] | 68.7 ± 3.86 | 1.3 ± 0.77 [4] | 0.30 ± 0.03 | 0.20 ± 0.01 | 1.5 ± 0.17 [4] |
| 11 | Raw soybeans | + 3.96 ± 0.52 [4] | 12.3 ± 2.04 | 72.1 ± 3.05 [4] | 3.1 ± 0.59 [4] | 0.53 ± 0.08 [4] | 0.27 ± 0.05 | 1.94 ± 0.24 [4] |
| 12 | Raw soybeans without methionine | + 2.86 ± 0.37 [4,7] | 11.3 ± 1.11 | 73.1 ± 2.26 | 2.5 ± 0.18 [4] | 0.43 ± 0.07 [4] | 0.21 ± 0.02 [4] | 2.07 ± 0.49 [4] |
| 13 | Autoclaved soybeans | + 5.15 ± 1.24 | 12.9 ± 1.87 | 77.3 ± 2.40 | 4.0 ± 0.34 | 0.36 ± 0.02 | 0.29 ± 0.03 | 1.24 ± 0.10 |
| 14 | Autoclaved soybeans without methionine | + 4.98 ± 0.90 [8] | 12.8 ± 1.66 | 78.7 ± 1.63 | 3.8 ± 0.37 | 0.34 ± 0.03 | 0.27 ± 0.03 | 1.27 ± 0.23 |

[1] Calculated: $\frac{\text{N intake} - \text{N excreted in feces}}{\text{N intake}} \times 100$.

[2] Protein efficiency ratio.

[3] SD.

[4] Significantly different from previous group fed autoclaved legume diet, $P < 0.05$, calculated from $t$ value.

[5] Bacto-casitone (trypsin-digested casein), Difco Laboratories, Detroit.

[6] Bacto-casamino-acids (acid-digested casein), Difco Laboratories.

[7] Significantly different from previous group, $P < 0.05$.

[8] Significantly different from no. 12, $P < 0.05$.

supplemented with tryptophan (diets 5 and 6). Glutamate did not improve weight gain of rats receiving a white bean diet, although it improved food intake (diet 8). Substitution of glucose for starch did not affect growth performance (diet 7).

Only a slight difference in weight gain was observable between the groups of rats receiving the crude white bean diets with or without methionine (diets 1 and 2) in contrast with those fed the autoclaved beans whether supplemented or not with this amino acid (diets 9 and 10). With soybean diets the effect of a methionine supplement was most pronounced when the crude seed meal was used (diets 11 and 14).

Pancreas hypertrophy was most conspicuous in rats fed raw soybeans or black beans. The diets containing raw red kidney beans, mottled beans, tapiramo beans (tables 2 and 3), or white beans (table 4), also stimulated pancreas growth.

Spleen weights significantly lower than those of the corresponding controls were observed in the rats fed raw black, red, or tapiramo beans. Moreover, low spleen weights developed when a protein-free diet was fed (table 2, diet 22). In two other groups, namely, those receiving the diets of crude white beans and cooked soybeans, respectively, both without added methionine (table 4, diets 2 and 12), significantly low spleen weights were observed.

The ratio of pancreas weight to body weight is characterized by a large standard deviation. For this reason we have included in tables 2, 3 and 4, the ratios between pancreas and spleen weights which show less variation from the mean value.

## DISCUSSION

The results indicate that the 2 seed samples of black and red kidney beans containing hemagglutinin were much more toxic than the 3 samples of the same species devoid of significant hemagglutinating activity. This difference was shown by the weight changes of the respective experimental animals as well as by the mortality rates reported in tables 2 and 3. A casein digest did not overcome this toxic action in the diets prepared with the first 2 samples but produced nearly normal growth when added to rations prepared with any of the latter three; this is further evidence of an important difference in the nutritional properties of these groups of bean varieties.

Rats fed raw black beans showed the largest loss of fecal nitrogen of all groups studied. Increased fecal nitrogen excretion has been observed in animals with pancreatic hypertrophy and has been related to increased excretion of endogenous nitrogen (5). In the present experiments the pancreas of the rats consuming crude soybeans was as enlarged as that in the animals fed black beans, but fecal nitrogen excretion was much lower in the former. Reduced intestinal absorption would also result in a larger nitrogen excretion. In previously reported experiments we observed that a black bean diet, or isolated black bean hemagglutinin (Phaseolotoxin), interfered significantly with intestinal absorption (2, 6). The failure of tryptic, or tryptophan-supplemented, acid-digested casein to stimulate growth when added to black or red bean diets, could be explained by the existence of an absorption defect.

The present results are in accord with the hypothesis that oral toxicity of kidney bean agglutinin may be caused by interference with intestinal absorption (2). Kakade and Evans (7) recently observed reduction in the absorption of amino acids by rats fed navy beans and consider the interference of the hemagglutinin as a possible explanation.

A protein exhibiting a toxic effect when ingested by the oral route should be able to resist digestion in the gastrointestinal tract. To study this aspect, feces of rats (tables 3 and 4) were assayed for hemagglutinin activity. In those of all the animals fed the crude black bean diet definite blood-agglutinating activity was detected, indicating that at least part of the agglutinin had not been inactivated by digestion. No fecal excretion of agglutinating activity was observed in any of the rats fed raw soybeans. The parenteral toxicity of soybean agglutinin is similar to that of kidney beans (8) but the oral toxicity is much lower. It would be of interest to explore further whether the difference in oral toxicity between raw soybeans and kidney beans

is related to the difference in resistance to digestion of the respective agglutinins.

Stead et al. (9) and Kakade and Evans (10) have presented evidence for a partial separation of the hemagglutinating activity from the toxic activities in bean extracts. This is not necessarily in contradiction with the view that agglutinins may exhibit toxic action, because more than one hemagglutinin can be present in beans (11), and these fractions vary in their relative potencies as hemagglutinins as compared with their toxicity (12). Our previous observation that absorption of an extract from black kidney beans with stroma from human red blood cells reduces both the agglutinating and the toxic actions simultaneously (13) is in accord with this explanation. Nevertheless, the possibility of the existence of another toxic factor can not be ruled out.

The presence of a toxic hemagglutinin can not explain the poor growth-promoting capacity of diets containing white, mottled, or tapiramo beans which are devoid of this factor (table 1). Very little trypsin inhibitor activity could be found in white beans; nevertheless the growth of rats fed this material was not better than with diets made with tapiramo or mottled beans, which contain about 10 times more antitrypsin. Nitrogen absorption was low in the experiments with all 3 seeds; this is probably not due to an absorption defect, because addition of enzyme-digested casein permitted normal growth; acid-digested casein had no similar effect unless supplemented with tryptophan, an amino acid destroyed by acid treatment, but which must be present in adequate amounts in the bean seeds, because the rats did not require this supplement for growth when fed the autoclaved bean diet. This observation implies that tryptophan and probably also the other amino acids are not as available from the raw beans as from the properly heated seeds. That undigested casein, when added to a diet containing raw white bean meal did not allow for full growth (table 4, diet 4) may be an indication that the proteins from raw beans are not only poorly digested but also inhibit digestion of other nutritional proteins.

Unpublished studies in vitro showed that kidney bean proteins are very resistant to digestion and exhibit an inhibitory action on various proteolytic enzymes even after removal of the trypsin inhibitors.

The growth-preventing mechanism observed in rats receiving non-hemagglutinating white, mottled, or tapiramo beans may be present in agglutinin-containing black and red beans too, as these still show some growth-depressing action after repeated extractions with saline until the soluble hemagglutinins have been eliminated (2).

Two additional experiments were performed to rule out other possible explanations for the poor growth-promoting action of white beans. The effect of a supplement of sodium glutamate was investigated because food consumption was low in all rats fed bean diets and lack of palatibility has been cited as a possible explanation for the low growth rate (14). The result of the experiment of table 4, diet 8, does not support this view as no growth improvement was observed although food consumption was enhanced.

An amylase inhibitor was observed in beans by Bowman (15) but has not attracted the attention of the investigators concerned with the explanation of the low nutritional value of raw legumes. The action of this factor or factors was observed not only in the in vitro experiments (table 1) but also in some of the rats receiving the white bean or tapiramo bean diets causing the production of copious light colored feces; the presence of undigested starch could be detected easily by reaction with iodine solution. At autopsy these animals had bloated, white intestines similar to those described under conditions of refection in rats having ingested large amounts of raw potato starch (16). That mottled bean diets free of this amylase inhibitor did not allow better growth than white beans (table 2 and 3) and that substitution of glucose for starch in the experimental diet was without benefical effect (table 4, diet 7) would rule out the amylase inhibitor as a major factor to explain the anti-nutritional effect in the present experiments.

Pancreas hypertrophy has been observed in animals fed trypsin inhibitor containing

legumes (5). The relation between the ingestion of this enzyme inhibitor and excessive pancreas weight is accepted by most investigators in this field (17) but has not remained unquestioned (18). In the present experiments a hypertrophic pancreas was observed in all animals fed bean-containing diets, and all the legume samples had some trypsin inhibitor action. Crude soybeans and black beans were the most active in stimulating pancreas growth, although mottled beans had the highest trypsin-inhibiting activity in vitro. The white beans caused a moderate but significant increase of pancreas weight, but had little antitryptic action in vitro, whereas they were most active in amylase inhibition. No seed sample was found to be free of antitrypsin and rich in anti-amylase which could have helped to clarify the possible action of both enzyme inhibitors on pancreas growth.

The great variation in biochemical activities, both in vitro and in vivo, existing between different varieties of kidney beans as shown by the present results, should be taken as a demonstration of the importance of a clear definition of the factors present in a given legume sample for the correct interpretation of growth effects on experimental animals. These results also point to the feasibility of selecting legume varieties for low or high agglutinin or enzyme inhibitor content.

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