

Effect of kidney bean (*Phaseolus vulgaris*) toxin on tissue weight and composition and some metabolic functions of rats

BY FIONA GREER, ALAN C. BREWER AND ARPAD PUSZTAI*

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

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1. Inclusion of raw kidney bean (*Phaseolus vulgaris*) proteins in the diet for rats was shown to affect the weight of some internal organs. Of these, in addition to the well-known hypertrophy of the pancreas attributable to dietary trypsin inhibitors, the observed atrophy of the thymus and the doubling in weight of the small intestine are related to the protein or lectin content of the bean diet, or both.

2. Changes in tissue composition of the small intestine were also recorded. Its protein content increased by about 40–50% and carbohydrate content doubled suggesting the occurrence of increased mucinous glycoprotein secretion. Increased DNA content (by about 30–40%) however also indicated mucosal hyperplasia.

3. Changes were also observed in mineral content, urea concentration and some enzyme activities in sera and urine, possibly as a result of disturbances in systemic metabolism or hormone levels, or both.

4. The results gave further support to previous suggestions that the oral toxicity of kidney-bean lectins involves local reactions in the small intestine in combination with their effects on the systemic immune system and general metabolism.

Inclusion of raw kidney bean (*Phaseolus vulgaris*) in animal diets reduces growth and may cause death (Johns & Finks, 1920; Evans *et al.* 1973; Jayne-Williams & Burgess, 1974; Jaffe, 1980; King *et al.* 1983). Similarly, inadequately cooked beans are known to be toxic for humans (Griebel, 1950; Noah *et al.* 1980). More recently toxic effects were also shown to occur in ruminants (Williams *et al.* 1984). The toxic factor in these beans has been shown to be identical with the constituent lectins and the extent of toxicity related to the lectin content of the diets (Pusztai & Palmer, 1977; Pusztai *et al.* 1981).

The mechanism of lectin toxicity is not yet clear. However, rats fed on diets containing kidney bean lectins are apparently unable to utilize dietary N fully, while at the same time they also have an increased rate of tissue catabolism (Pusztai *et al.* 1981). Accordingly both faecal and urinary N outputs are elevated, and if kidney beans provide the main source of dietary N the animals would be in a negative N balance. In addition to these disturbances in digestion and absorption, kidney bean lectins are known to bind to and be transported through the intestinal epithelium (Pusztai *et al.* 1979, 1981; King & Pusztai, 1982).

In the present paper the effects of systemically-absorbed lectins on the weight of internal organs are described. These and other results on the composition of affected tissues and body fluids are discussed in relation to other systemic effects previously observed.

MATERIALS AND METHODS

Kidney bean

The variety of kidney bean 'Processor' was obtained from Hurst Gunson Cooper Taber Ltd (Witham, Essex). The seeds were ground in a Christy and Norris Laboratory Mill fitted with a 1 mm pore diameter grid. Cooked bean samples were prepared by soaking dry beans for 18 h at room temperature. After draining and replacing the water, the hydrated beans were boiled for 20 min. Boiling water was discarded. The cooked beans were freeze-dried and ground in a Moulinex Junior coffee grinder. When tested, cooked beans had no residual haemagglutinin activity and supported the growth of rats (Grant *et al.* 1982).

* For reprints.

Diets, experimental animals and design of experiments

Diets based on maize starch, glucose, maize oil and vitamin and mineral mix were prepared as described previously (Palmer *et al.* 1973). Diets A and B contained 100 g protein/kg diet as casein and cooked bean protein respectively. Diet C contained the same concentration of protein as a mixture of equal parts by weight of casein and raw bean protein. Diet D was the protein-free control diet with an equal amount of maize starch replacing the protein. The protein sources were supplemented with L-methionine and L-tryptophan to bring their concentration to target requirements for rats (Coates *et al.* 1969). The concentration of raw bean protein in the test diet was made to 50 g/kg as the food intake of rats on diet C at this level was the same (5.6 g/rat per d) as that of rats on diet D (Pusztai *et al.* 1981). The corresponding intake of rats on diets A and B was 8.3 g/rat per d in these experiments. These diets also provided an adequate input of minerals, most of which came from the mineral mix. The actual mineral intakes in mg/rat per d for rats fed on the diet containing raw beans (diet C) and the comparable non-protein control (diet D) respectively were sodium 9.0 and 8.4, potassium 60 and 44, calcium 33 and 31, magnesium 10 and 8, phosphorus 23 and 19, copper 0.18 and 0.17. The daily Cu intake (0.20) was a little higher for diet A.

Male rats of the Hooded Lister (Rowett) strain were weaned at 19 d of age and fed on a stock diet (Oxoid Ltd) for the next 12 d. For the various experiments a minimum of four to a maximum of sixteen rats between 70 and 80 g weight were selected and housed singly.

In balance experiments the rats were kept in metabolism cages based on the design of Schiller (1960) using glass separators for urine and faeces collection (see Table 4). Food and water were available *ad lib.* and their consumption was recorded daily (Pusztai *et al.* 1981). After the first 2-d adaptation period, urine and faeces collection was made for 8 d. This collection period was arbitrarily divided into two parts of 3 and 5 d respectively. Analyses were carried out separately for the samples collected in these two periods and the results were combined and recorded only if there were no disproportionately large differences between them.

For experiments in which the weights of tissues were measured (Tables 1 and 2) or blood samples were taken (Table 5), in addition to the balance experiments described previously, rats were fed on diets A, B, C and D (in Makralon cages) for periods of up to 11 d but no urine or faeces were collected. At the end of feeding for 11 d a blood sample was obtained by cardiac puncture under light anaesthesia with diethyl ether and the animals were killed with an injection of pentobarbitone. Immediately the spleen, thymus, pancreas and kidneys were excised, rinsed in ice-cold distilled water, blotted dry and weighed. The entire small intestine was also removed, cut into two parts to facilitate handling and each half was rinsed out three times with ice-cold distilled water, 10 ml each time, with the aid of a syringe. Between each rinse the contents of the small intestine were emptied by gentle squeezing between the fingers. The tissue was then blotted dry, its length measured and weighed. Finally, after freeze-drying, it was dried to constant weight over phosphorus pentoxide.

Chemical analyses

Diets and ground faeces samples were analysed for moisture by freeze-drying and drying to constant weight at 105° for 16 h, and total nitrogen was determined by the Kjeldahl method (Davidson *et al.* 1970). Urine samples were analysed for N (Davidson *et al.* 1970), P (Young, 1966), Ca (Gitelman, 1967) Mg (Gitelman *et al.* 1966) and glucose (Trinder, 1969). Na and K were estimated by standard Technicon Instruments Co. Ltd (1971) methods. Blood samples were collected in centrifuge tubes, cooled to 4° and sera separated from the clot by centrifugation at 3000 g for 20 min. Sera were analysed for P (Young, 1966)

and urea (Marsh *et al.* 1965) and Na and K using standard Technicon Instruments Co. Ltd (1971) methods. Determinations by atomic absorption spectrophotometry (Varian-Techtron AA5) of Cu and Zn were carried out on serum samples collected in acid-washed tubes and after precipitation with trichloroacetic acid (TCA) at 50 g/l concentration. Alkaline phosphatase (EC 3.1.3.1) activity (Sigma, 1982), aspartate transaminase (EC 2.6.1.1) and alanine transaminase (EC 2.6.1.2) enzyme activities were measured by using commercial colorimetric assay kits (Sigma, 1977).

Small intestinal tissue composition

Samples of freeze-dried small intestinal tissue were homogenized with 0.1 M-citrate-phosphate buffer of McIlvaine (1921), pH 3.0 at 0°. After treatment with TCA at a final concentration of 100 g/l the precipitate, collected by centrifugation and washed with TCA solution (100 g/l), was homogenized in 0.2 M-sodium hydroxide and the clear supernatant fraction used for protein estimations. Colorimetric determination of protein was carried out by the Schacterle & Pollack (1973) variation of the Lowry *et al.* (1951) method or by the ninhydrin assay of Moore & Stein (1954). The results are expressed in bovine serum albumin equivalent. The TCA supernatant fraction was used for the estimation of neutral sugars by the orcinol-sulphuric acid method of Johansen *et al.* (1960) using glucose as a standard.

For DNA estimations, acid-soluble compounds and lipids were removed from homogenized intestinal tissue by the method of Curtis-Prior *et al.* (1975) using ice-cold diethyl ether-ethanol (3:1, v/v) containing TCA (100 g/l). The residue was then freeze-dried. DNA was extracted from the dried powder according to either the Schmidt & Thannhauser (1945) or Schneider *et al.* (1950) procedures and was estimated by the diphenylamine method of Burton (1956).

Statistical analysis

The results in Tables 1, 2 and 4 have been derived from values obtained from a number of different experiments which were conducted over an extensive period of time. For each variable examined the values used in the analysis were from those experiments on which the variable was measured. However, not all the experiments in the series recorded all the variables examined in the present paper. The experiments had differing numbers of observations and not all the diets examined here were included as entries into all experiments. However, in most experiments at least three diets were present (except in one which contained two diets only) and since greater interest was placed on the raw bean diets (diet C) than on the controls, the raw bean treatments have in general been given greater replication. The number of observations on each diet are given in the Tables and the numbers of experiments used in the analyses were nine in Table 1, six in Table 2 (except for the wet weight of the small intestinal tissue for which there were twelve experiments) and fifteen in Table 4. Imbalances in the data have arisen because the study was sequential in nature and the selection of treatment entries into individual trials were determined at the time of the trial on the basis of which treatment comparisons were of primary interest at that stage. These imbalances have been accounted for in the results by using non-orthogonal analyses of variance in which the treatment means given in Tables 1, 2 and 4 have been adjusted for differences in the means of individual experiments. The diet \times experiment interaction term has been used as the error term in calculating standard errors and significance tests of the adjusted treatment means on those variables for which this interaction was significantly different from experimental error, otherwise the interaction and experimental error have been pooled to form a combined error term. The results given in Tables 3 and 5 have been obtained from a single experiment and the treatment means were compared by Student's *t* test.

Table 1. *Wet weight (g/kg body-weight) of internal organs from rats fed on various diets, including kidney bean (Phaseolus vulgaris) proteins, for 11 d (total)*

(Values are means with their standard errors. All means have been adjusted for differences in experiment means. SE values and significance tests are based on the diet x experiment interaction, except for thymus and spleen for which the interaction was not significant and is pooled with the experimental error)

Diet ...	D (non-protein)			A (casein)			B (boiled-bean)			C for 7 d followed by diet A for 4 d			C (casein + raw bean)		
	No. of observations	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
Thymus	6	2.6**	0.30	19	3.5	0.11	13	3.3	0.13	5	3.2	0.26	24	1.4***	0.10
Spleen	7	2.6†	0.20	20	3.0	0.09	13	3.0	0.11	5	3.3	0.21	25	2.5***	0.08
Pancreas	7	3.3	0.65	20	3.7	0.25	11	3.7	0.40	5	3.7	0.70	23	5.6***	0.28
Kidneys	7	10.8	0.80	20	9.8	0.36	13	9.5	0.45	5	11.5	0.86	26	12.2***	0.32

Significantly different from value for diet A: † $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$.

Table 2. *The weight (g/kg body-weight) and length (mm) of small intestinal tissue from rats fed on various diets, including kidney bean (Phaseolus vulgaris) protein, for 11 d (total)*

(Values are means with their standard errors. All means are adjusted for differences in experiment means. SE values and significance tests are based on the diet x experiment interaction mean square)

Diet ...	D (non-protein)			A (casein)			B (boiled-bean)			C for 7 d followed by diet A for 4 d			C (casein + raw bean)		
	No. of observations	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
Wet wt (g/kg)	10	30.6	4.8	23	31.0	2.1	20	32.8	2.3	20	49.5***	2.7	28	74.3***	1.9
Dry wt (g/kg)	7	6.1	1.93	20	6.7	0.05	14	6.5	0.58	9	8.9*	0.79	21	13.8***	0.49
Length (mm)	—	—	—	19	860	20	16	850	22	7	900†	37	21	940*	19

Significantly different from value for diet A: † $P < 0.1$, * $P < 0.05$, *** $P < 0.001$.

Table 3. Contents of protein, carbohydrate and DNA of small intestines obtained from rats fed on various diets, including kidney bean (*Phaseolus vulgaris*) protein, for 11 d (total)

(All chemical analyses were carried out simultaneously on freeze-dried small intestines collected from the balance experiments during the work. Values are means with their standard errors)

Diet ...	A (casein)			B (boiled-bean)			C (casein + raw bean)		
	No of observations	Mean	SE	n	Mean	SE	n	Mean	SE
Protein (mg)†	14	289	10	8	291	21	18	381***	10
Protein (mg)‡	12	291	11	6	256	16	12	385***	12
Neutral carbohydrate (mg glucose equivalents)	13	428	18	8	464	45	16	836***	29
DNA (mg)	7	8.65	0.80	—	nd	—	9	11.02†	0.79
DNA (mg)	3	9.12	0.15	—	nd	—	9	12.52†	1.09

nd, not determined.

Significantly different from value for diet A: † $P < 0.01$, *** $P < 0.001$.

‡ Determined by the Folin method (Schacterle & Pollack, 1973).

§ Determined by the Ninhydrin method (Moore & Stein, 1954).

|| Extracted by the Schmidt & Thannhauser (1945) method.

||| Extracted by the Schneider *et al.* (1950) method.

RESULTS

The effect on internal organ weights of the various diets is shown in Table 1. Since the body-weights of the animals varied the results are expressed as a percentage of the body-weight. Animals fed on the raw bean-casein diet (diet C) for 11 d had significantly lower thymus ($P < 0.001$) and spleen ($P < 0.001$) weights than those fed on the casein control diet (diet A). On the other hand, the weight of both the pancreas and the kidneys of the raw-bean-fed rats (diet C) was significantly ($P < 0.001$) higher than that of the casein-fed controls (diet A). There was no significant difference in the weights of organs obtained from rats which were given cooked beans (diet B) or casein (diet A). However, animals fed on a non-protein diet (diet D) had a somewhat smaller thymus and spleen than those on the casein diet (diet A).

The tissue weights (wet and dry) and lengths of the small intestines of rats fed on various diets are shown in Table 2. There was a considerable increase in the wet weight of the small intestine from animals fed on raw-bean-containing diets (diet C) over that of all the controls. The increase, although less than that in rats fed on beans for 11 d, was still significant with animals which, after being given raw bean diets for 7 d, were fed on casein for the last 4 d. The substantial increase was also clearly reflected in the doubling of the dry weight values of the small intestine from raw-bean-fed animals. The length of the small intestine of raw-bean-fed rats (diet C) was also significantly increased ($P < 0.05$) although not to the same extent as its weight (Table 2).

The increase in weight of the small intestine from raw-bean-fed rats was reflected in changes of its protein, carbohydrate and DNA contents (Table 3). While there was no significant difference in these values between the rats given casein or cooked bean (diets A and B), the protein and the DNA content of the small intestine from raw-bean-fed (diet C) rats increased by a minimum of between 30 and 40% and its carbohydrate content doubled.

Table 4. *Urinary constituents (mg) of rats fed on diets containing casein and raw kidney bean (Phaseolus vulgaris) protein (C) and non-protein control (D) for 10 d (total)*

(The results are given as mg constituent (total) in the 8 d collection period of the balance experiments. Values are means with their standard errors. All means are adjusted for experiment means. SE values and significance tests are based on the diet \times experiment interaction except for K for which the interaction was not significant and is pooled with experimental error)

Diet ...	D (non-protein)			C (casein + raw bean)		
	No. of observations	Mean	SE	<i>n</i>	Mean	SE
K	8	326*	24	20	401	15
Na	9	68.3*	5.8	20	48.5	4.0
P	8	51.4†	4.8	17	39.4	3.3
Ca	8	1.15	0.45	20	1.88	0.29
Mg	8	19.2	1.86	20	19.7	1.21

Significantly different from value for diet C: † $P < 0.01$, * $P < 0.05$.

Table 5. *Serum constituents of rats fed on diets containing casein or casein + raw kidney bean (Phaseolus vulgaris) proteins for 11 d*

(Values are means with their standard errors)

Diet ...	A (casein)			C (casein + raw bean)		
	No. of observations	Mean	SE	<i>n</i>	Mean	SE
K (mg/l)	5	212.2	17.7	7	191.3	5.3
Na (mg/l)	7	3287.8	126.5	7	3330.2	171.6
P (mg/l)	6	75.9	3.7	4	78.4	10.0
Urea (mg/l)	15	351.4	41.9	20	433.9†	25.5
Alkaline phosphatase (EC 3. 1. 3. 1) (Sigma units/l)§	6	3540	330	7	2780*	110
Aspartate transaminase (EC 2. 6. 1. 1) (SF units/l)	4	19400	380	8	21500	800
Alanine aminotransferase (SF units/l)	4	39400	4550	8	39300	2580
Cu (μ g/l)	8	180.0	9.9	8	231.0**	15.6
Zn (μ g/l)	8	175.0	16.3	8	200.0	9.5

Significantly different from value for diet A: † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$.

§ 1 Sigma unit liberates 1 μ mol *p*-nitrophenol/h.

|| 1 SF unit forms 4.82×10^{-4} μ mol glutamate/min at pH 7.5 and 25°.

Changes were also observed in the mineral content of the urine of rats (Table 4) fed on the raw bean diet (diet C) when compared with that of urine samples obtained from controls including that obtained from rats fed on non-protein diets (diet D). Sera from such animals, however, showed no such differences in mineral composition (Table 5) although the Cu concentration from raw-bean-fed rats was significantly ($P < 0.01$) higher than that in the casein-fed control group (diet A). As expected, the blood urea level was elevated somewhat

in raw-bean-fed rats. Of the enzymes investigated in sera only the alkaline phosphatase activity was depressed significantly ($P < 0.05$) when the rats were given raw bean in their diets (diet C).

DISCUSSION

Feeding raw kidney beans to rats causes significant changes in the weight of some internal organs. The decrease in the weight of the spleen and the increase in that of the kidneys in rats fed on uncooked beans confirmed the earlier findings of Kakade *et al.* (1965). The weight of the spleen is, however, known to be dependent on the nutritional state of animals. For example, protein inadequacy, particularly when coupled with increased stimulation of the immune system, results in atrophy of the spleen in humans (Chandra & Newberne, 1977). Thus the effect on the spleen may not necessarily be directly due to components of the raw bean diet. Hypertrophy of the pancreas on the other hand is well-established in animals fed on legume-seed meals which contain trypsin inhibitors (Pusztai, 1967).

The most striking anatomical differences due to feeding rats on raw-bean proteins were found in the thymus and the small intestine. In some cases the thymus atrophy was such that the organ nearly disappeared after 10 d feeding, thus confirming earlier observations (Tedeschi *et al.* 1965). The significance of this finding is not apparent. However, thymus atrophy may be linked to stress or systemic immunological reactions of the absorbed kidney bean lectin (Pusztai *et al.* 1981, 1982).

As expected, the ingested raw kidney bean proteins had an appreciable effect on the small intestine, which increased its size and weight and its protein, carbohydrate and DNA contents. A part of this increase may be the result of a lectin-induced hypersecretion of mucinous glycoproteins, similar to that observed on oral administration of the lectin concanavalin A (Freed & Buckley, 1978; Freed, 1982). Stimulation of increased endogenous N secretion was also observed when rats received relatively large doses of the purified main reserve glycoprotein of the seeds of kidney bean (Sgarbieri *et al.* 1982). Moderate increases in relative intestinal weight may in fact be a general response to the presence of some vegetable proteins in the diet. For example, the intestinal weights per unit length increased appreciably when, in the diet of preruminant calves, milk proteins were replaced by those from soya bean (Roy *et al.* 1977). In this case it was suggested that the muscle layer of the intestines thickened to deal with the increased mass of food. Indeed increased luminal nutrition and the accompanying local and systemic hormonal changes are generally thought to be the main factors responsible for intestinal hyperplasia and enhanced mucosal enzyme functions (Dowling & Booth, 1967; Tutton, 1973; Klein & McKenzie, 1983). However, diets containing raw beans reduce appetite and the animals lose weight (Pusztai *et al.* 1981, 1982), thus the increase in the relative weight of the small intestine in rats fed on raw beans is apparently not attributable to the effects of increased luminal nutrition. Furthermore, although rats fed on non-protein diets have similarly low food intake and consequently suffer a weight loss comparable with those fed on raw beans (Pusztai *et al.* 1981), these animals show no similar increase in intestinal weight. Thus the effects on the small intestine of feeding uncooked kidney beans are apparently related specifically to the presence of bean proteins in the diet and not to the amounts of nutrients in the lumen. As kidney bean lectins have a high reactivity with cellular membranes and receptors (King *et al.* 1980; Pusztai *et al.* 1982) they may mimic the effect of hormones. Indeed, pure kidney bean lectins are known to have an insulin-like activity on isolated fat cells (Pusztai & Watt, 1974). Thus dietary lectins may also trigger off the release of hormones and other intestinal stimulants locally or in the systemic circulation. The increase in DNA content found in the present study indicates that, in addition to the effect on secretions, there is an increase in the actual number

of intestinal cells. This may possibly be due to the mitogenic effect of kidney bean lectins on some type of intestinal cells, similar to that on lymphocytes. It is, however, well-established that by a direct binding to the brush-border enterocytes the lectins speed up the replacement of the damaged epithelial cells (King *et al.* 1982).

Although differences in the composition of serum and urine from rats fed on diets containing raw kidney beans or control diets were observed (Tables 4 and 5), these could have been the result of many causes, some difficult to interpret. However, as the food intake of rats on raw bean and on non-protein control diets was very similar and both groups showed a net loss of body protein (Pusztai *et al.* 1981), a comparison of their urinary and serum constituents may be meaningful in the context of the present investigation. Thus the decreased output of Na and phosphate in rats fed on uncooked kidney bean may indicate the occurrence of disturbances in systemic metabolism. Moreover, as there was no difference (Table 5) in the concentration of these ions in the sera of the same animals and no change in pH either (A. Pusztai and E. M. W. Clarke, unpublished results), reduced urinary output of salts may be the result of a necessary adaptive mechanism to maintain homeostasis. The modulation of mineral concentration of body fluids may thus be due to the presence of native bean proteins in the diet and perhaps to the hormone-mimicking effects of the systemically absorbed lectin.

However, there were no changes observed in the activities of the two transaminase enzymes, thus the liver function appears to be undisturbed by ingestion of kidney bean lectins in contrast to that found when the lectins were injected (Ikegwuono & Bassir, 1976; 1977). The significance of the lower level of alkaline phosphatase activity in raw-bean-fed rats is not apparent yet. However, this activity was also depressed in raw-bean-fed pigs (Myer *et al.* 1982). The increased urea concentration in sera, although only significant at a low level ($P < 0.1$), does agree with the results in pigs (Myer *et al.* 1982) and is in accord with the substantially higher urinary urea excretion found previously (Pusztai *et al.* 1981), which suggests that the ingestion of kidney bean lectins causes a disruption of normal systemic protein metabolism.

In conclusion, apart from the hypertrophy of the pancreas, an effect most likely due to trypsin inhibitors, feeding rats with native kidney bean proteins including lectins causes relatively small effects on internal organs and most of these may not be related directly to components of the bean diet but rather to a general protein deficiency through metabolic disturbances. The effects on the thymus and the small intestine are however more extensive and likely to be due to the reactivity of the dietary kidney bean lectins with the mucosa and the subsequent transport through the epithelial cells of the small intestine. The ensuing toxic effects include the interference with local and systemic immune reactions (Pusztai *et al.* 1981, 1982) and it is suggested that disturbances in systemic mineral and N metabolism are the direct expression of kidney bean lectin reactivity with the cells controlling these reactions.

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