Lesions of Intestinal Epithelium by Ingestion of Bean Lectins in Rats

Admar C. de OLIVEIRA,1 Benedicto de Campos VIDAL,2 and Valdemiro C. SGARBIERI1

1 Departamento de Planejamento Alimentar e Nutrição, Faculdade de Engenharia de Alimentos,  
2 Departamento de Biologia Celular, Instituto de Biologia, Universidade Estadual de Campinas, 13081 Campinas,  
São Paulo, Brasil  
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Summary Wistar rats were submitted to the action of active lectins from common dry beans (Phaseolus vulgaris) and from jack beans (Canavalia ensiformis, DC). Raw common bean was offered to the rats in an otherwise balanced diet to make 10% protein as the sole protein source. A single dose of 20 mg of jack bean lectin (concanavalin A) was given by gastric intubation. Half of the rats receiving raw bean died within 22 days of experiment. Histological findings showed ulceration and necrosis of the intestinal villi in the surviving rats. In some cases the lesions reached also the submucosa. Gastric intubation of concanavalin A caused intense scaling off in the apical portion of the villi.

Key Words bean lectins, intestinal epithelium, concanavalin A, gastric intubation, terminal rats

Lectins (from Latin lectio, ōnis meaning to select, to choose) are proteins capable of binding to cellular surfaces through specific oligosaccharides or glycopeptides. Several review articles have been reported in the literature (1–4). The toxicity of native lectins to the rat has been already studied by Jaffe and Camejo (5), Pusztai et al. (6), King et al. (7), and Miyoshi et al. (8), among others. However, none of the reported work was done on rats under terminal conditions which have received substantial amounts of bean lectins by oral ingestion. Pusztai et al. (9) and King et al. (7) reported on alteration of rat intestinal microvilli in the initial phase of degradation. In the present report, 50% of the animals had died, and the toxic effect of lectins was observed on rats fed a diet containing raw bean (Phaseolus vulgaris, L.) as the sole source of protein and also on rats which had received purified lectins isolated from jack beans (Canavalia ensiformis, DC) by stomach intubation in an acute experiment.
EXPERIMENTAL

**Experimental animals.** Twenty-one albino male rats of the Wistar strain, weighing $66.4 \pm 6.7$ g (mean $\pm$ SD), were utilized in the experiments.

**Source of lectins.** Raw bean (*Phaseolus vulgaris*, L.) cultivar Carioca 80 was obtained from Agronomic Institute at Campinas, São Paulo, Brasil. The beans were ground until they were a 20-mesh flour, which contained 21.34% crude protein ($\%N \times 6.25$) and hemagglutinating activity of 239.9 hemagglutination units per mg of protein. The activity was calculated from the reciprocal of the smallest quantity, in mg of sample protein, still capable of agglutination of swine trypsinized erythrocytes (10).

Concanavalin A (Con A), extracted from jack bean (*Canavalia ensiformis*, DC), was from Sigma Chemical Company, St. Louis, MO, USA, and had an activity of 2.0 hemagglutination units per mg of protein.

**Diets utilized.** A diet was prepared with 10.60% crude protein ($\%N \times 6.25$) from raw dry bean (*Phaseolus vulgaris*) as the only protein source. The other constituents were as follows: corn oil 8%; salt mixture, according to Hegsted et al. (11), 4%; vitamin mixture, according to Nutritional Biochemicals Corporation (12), 2%; and carbohydrate mixture (1:3, w/w) sucrose and cornstarch to complete 100%.

A diet was also prepared with 10.18% casein (Tacrigy Comercial Ltda.) containing 81.43% protein ($\%N \times 6.38$). The other dietary components were in the same proportion as described for the raw bean diet. A commercial diet (Dieta Comercial Anhanguera, Duratex S.A., Campinas, São Paulo, Brasil) was used with 17.0% crude protein ($\%N \times 6.25$), 2.5% ether extract, 10.0% minerals, vitamin supplementation (manufacturer's specifications), and otherwise adequate for the growing rat.

**Biological assays.** The weaned rats, average weight 35g, were caged individually and fed commercial diet and water *ad libitum* for 10 days. The temperature of the animal laboratory was maintained at 23 ± 2°C. After the 10 days acclimation period, the animals were randomly distributed in groups of six. One group received the casein diet; another group received the commercial diet; the third group received the diet containing raw bean. All three groups received their diet and water *ad libitum* and the rats were weighed at the beginning of the experiment and at the fifth and eleventh days on the diets.

On the eleventh day of the experiment, the rats fed on the raw bean diet were shifted to the commercial diet and the rats fed the commercial diet were shifted to the raw bean diet. The rats were further weighed at 20 days and at the end of the experiment. After 50% of the rats fed the raw bean diet had died, the remaining animals were sacrificed by diethyl ether atmosphere and dissected for tissue examination. An equal number of rats fed the casein (control) diet were treated in the same way.

**Gastric intubation of concanavalin A.** A group of three rats was fasted for 24 h with water *ad libitum* to empty the stomach and intestinal contents (13). After
fasting, 20 mg of Con A suspended in 2 ml of sodium chloride 0.9%, was intubated in the rat stomach after mild anesthesia with diethyl ether (7). One and a half hours after intubation, the animals were sacrificed by diethyl ether atmosphere and the intestinal tissue was dissected for histological examination.

**Histological preparation and examination.** After dissection, the duodenum and medial portions of the mid-jejunum of the rat were removed and fixed in formaldehyde, followed by embedding in paraffin. Sections of 8 μm thickness were stained with hematoxylin and eosin (HE) for microscopic examination.

**RESULTS**

The results of the growing assays with three different types of diets are shown in Fig. 1, expressed as the evolution of the body weight mean of the rats as a function of time. The rats fed the casein diet and commercial diet had the same body weight gain of approximately 27.0 g in the first 11 days of feeding. On the other hand, the rats fed the raw bean containing diet lost an average of 16.3 g per rat in the same period.

From the eleventh day of experiment the rats fed the commercial diet were shifted to the raw bean diet. On the raw bean diet these rats lost weight continuously until the end of the experiment (33 days of feeding) when the average body weight was 55.2 ± 6.7 g per rat and half of the animals had already died.

![Fig. 1. Evolution of the body weight mean of Wistar rats fed diets containing casein (▲), raw bean (Phaseolus vulgaris, L.) (●), and commercial diet (□), during 33 days of experiment. At the eleventh day of experiment the rats fed the diet containing raw bean were shifted to commercial diet, and vice versa.](image-url)
The rats fed the raw bean diet in the first 11 days were then shifted to the commercial diet. These rats showed a rapid recovery, reaching an average body weight of $137.0 \pm 8.6\text{g}$ per rat at 33 days of feeding as compared with $142.1 \pm 4.8\text{g}$ for the rats maintained on the casein diet all the time.

Histological observation with a light microscope were made on preparations from the intestinal duodenum (Figs. 2 to 8). Figure 2 is a photomicrographic plate taken from a preparation of duodenal mucosa of a rat maintained 33 days on the casein diet. Sections of villi at different levels show a normal morphology including the absorptive cell with brush border (microvilli) with perfect continuity, the goblet cell, the lamina propria, the glands of Lieberkuhn, the submucosa and muscular layers.

By comparing the morphology of the duodenum of rats fed the raw bean diet with active lectins, or intubated with Con A, with those fed the casein control diet, the following alterations could be observed on the rats that suffered the action of lectins: a) lack of continuity of the brush border; b) ulceration and collapse of villi, exposing the lamina propria; c) reduction in the number of goblet cells, particularly in the region of damaged villi. In the rats fed the raw bean diet, after 22 days of feeding, necrosis of the absorptive cells (Figs. 3 and 4) and other deleterious effects such as disappearance of villi and parts of the lamina propria were observed. Only remaining residues of the villi and parts of the Lieberkuhn glands could be observed in Fig. 5. A great deal of cell debris could be observed in the lumen of the small intestine, which originated from collapse of the villi. Gastric intubation of pure Con A caused an intense desquamation of absorptive cells in the apical region of the rat duodenum villi, as illustrated in Figs. 6 and 7. Histological preparation of the jejunum of rats fed the raw bean diet showed alterations similar to those already described for the duodenum. On the other hand, the rats which had been fed the raw bean diet and had then subsequently recovered after feeding of the commercial diet showed morphological characteristics similar to those fed the casein diet (Fig. 8).

**DISCUSSION**

Loss of body weight of rats fed a diet containing raw bean has been described by various investigators (9, 14). The toxicity of raw bean has been attributed to lectins by De Muelenaere (15), by Pusztai et al. (9), among others. Although the cultivar
Fig. 2.

Fig. 3.

Fig. 4.
utilized in this study has been shown by Sgarbieri et al. (14) to be less toxic than other Brazilian cultivars, the rats still lost weight. The subsequent body weight recovery of rats which had initially been given the diet containing raw bean, therefore active lectins, suggests that the toxic effect of raw bean and possibly raw bean lectin is reversible. Similar observations were described by Pusztai et al. (9).

Previous reports (7, 9) describe lesions on the intestinal epithelium of rats fed raw Phaseolus vulgaris affecting only the microvilli. The present experiment permits the conclusion that prolonged action of Phaseolus lectins or intubation of Con A caused devastating degradative effects on the rat small intestinal tissue, which begin in the microvilli and progress through villi, epithelial and goblet cells and finally reach the submucosa. The ulceration of villi, the necrosis of absorptive cells and consequent loss of cell materials to the intestinal lumen would explain, to a great extent, the pronounced loss of fecal endogenous nitrogen by rats fed diets containing raw bean as the source of protein, as demonstrated by Oliveira and Sgarbieri (16, 17).

The subsequent restoration of the normal growth of the recovered rats by feeding the commercial diet, after being fed for 11 days on the raw bean diet, associated with the fact that the intestine showed normal morphological characteristics, permitted the conclusion that the main toxic effect of bean lectins occurs at the rat intestine epithelium and it ceases when the lectins have been removed, as already suggested by Durigan et al. (18). The reduction in the number of goblet cells in the damaged portion of the villi seems to indicate that the exhaustion of goblet cells could be one starting point of the villi destruction, but the precise mechanism has not been clarified.

REFERENCES


Fig. 5. Photomicrographic plate showing an advanced stage of degradation of duodenal tissue of a rat fed the diet containing raw bean and active lectins. The degraded stage reached the submucosa in the vicinity of the muscular layer. HE, 58×.

Fig. 6. Photomicrographic plate showing the effect of gastric intubation of 20 mg of purified Con A on the rat duodenal villi, 1 1/2 h post cibum. It shows intense desquamation of absorptive cells and reduction in the number of goblet cells in the apical region of the villi. HE, 111×.

Fig. 7. Amplification of Fig. 6, showing the region with desquamation of mucosal epithelium (arrow), ulceration, and exposure of the lamina propria. HE, 367×.

Fig. 8. Photomicrographic plate of the duodenum of a rat fed the diet containing raw bean for 11 days and then shifted to commercial diet for 22 days, showing the recovered intestine. HE, 147×.


