Tolerance to Long-Term Feeding of Isolated Peanut Lectin in the Rat: Evidence for a Trophic Effect on the Small Intestines

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Summary Previously we have shown that rats fed a diet containing raw peanut meal as the sole source of protein exhibited alterations in enzyme activity and composition of certain organs. To determine the effects of isolated peanut lectin on body growth and on the intestines, experiments were carried out in weanling, male, Sprague-Dawley rats fed a casein diet incorporated with purified peanut lectin at three levels, 0.004, 0.04, and 0.2% for 23 days. Body weight gain was normal with all three diets. In rats fed the 0.004 and 0.04% peanut lectin, there were no changes in any of the small intestinal mucosal parameters under study. However, in rats consuming the 0.2% peanut lectin diet, the proximal, mid, and distal third regions of the small intestines all showed marked increases in mucosal weight, protein, and DNA contents, but without altered villus morphology. Of the 3 brush border enzymes studied, namely maltase, γ-glutamyltranspeptidase, and alkaline phosphatase, none was altered in activity in any region, suggesting that microvillus integrity was normal. These results are similar to the reported actions of red kidney bean lectin on the intestines. We conclude that peanut lectin at up to 0.2% of the diet does not inhibit food intake or growth of weanling rats and is apparently trophic for all areas of the small intestines.

Key Words peanut lectin, trophic agent, rat small intestines, intestinal mucosa

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Lectins are a group of naturally occurring proteins that are commonly found in most leguminous foods. Many lectins are able to agglutinate red blood cells in vitro and thus are also known as hemagglutinins. Lectins from red kidney bean, winged bean, and jack bean are known to disrupt different aspects of the gastrointestinal tract, cause poor growth, and in some cases cause death in experimental animals (1–6). Toxicity appears to be a result of lectin binding to the brush border surface of the small intestines leading to altered enzyme activities and malabsorption (3). There is also evidence of malabsorption due to bacterial overgrowth resulting from the lectin-mucosa interaction (2). Ulceration and necrosis of villi, including lesions reaching the submucosa, has recently been reported in rats ingesting bean lectins (6). Extraintestinal organs may also be affected (3, 7).

Although the toxicity of several different legumes has been characterized, there is little information concerning the tolerance for raw groundnut or peanut (Arachis hypogaea) or its antinutritional components. We previously found that weanling rats fed a diet containing the protein component solely from raw peanut meal for 4 weeks showed altered composition and enzyme activities of the liver and pancreas as well as altered blood chemistries (8, 9). It is not known which of the several antinutritional factors present in raw peanut accounted for these observed changes. Because of the reported toxicities of some lectins (10), and the similarities in chemical composition between peanut lectin and some of these other lectins, we thought it important to study the effects of purified peanut lectin in the rat.

Several chemical and biological properties of peanut lectin have been reported by several investigators (11–13). It has a molecular weight of 110,000, is composed of 4 apparently identical subunits, and is free of carbohydrate. It has a high specificity for receptors containing the disaccharide β-D-galactosyl(1-3)-N-acetyl-D-galactosamine. It agglutinates desialyzed ABO erythrocytes. Peanut lectins from some genotypes are mitogenic for lymphocytes and thymocytes.

In the current work, we report on the results of two experiments in which isolated peanut lectin was fed at various levels to weanling rats to assess growth and intestinal mucosa composition and enzyme activity.

**EXPERIMENTAL**

*Isolation and purification of peanut lectin.* The peanut lectin used for feeding was isolated and purified according to procedures adapted from Lotan et al. (12) and described in detail elsewhere (14). Briefly, raw peanuts of the Florunner variety were defatted by hydraulic press, ground, and extracted with saline. Protein was precipitated with ammonium sulfate, dialyzed, and purified by affinity chromatography. The column consisted of cyanogen bromide activated Sepharose 4B and N-E-aminocaproyl-β-D-galactopyranosylamine (Sigma Chemical, St. Louis, Mo., USA). The appropriate fractions were dialyzed and lyophilized. Yield was 0.08%. Purity was assessed by gel electrophoresis, extinction coefficient, UV absorption spectra, and subunit molecular weight (14). These properties were
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found to agree closely with those reported by other workers (11, 12) and were comparable to a commercially derived product (Sigma Chemical, Peanut Agglutinin, #L-0881, lot 82F-9575-1). Hemagglutinin titer was estimated by an in vitro assay utilizing fresh human type A desialyzed erythrocytes (12). Agglutination occurred at a lectin concentration of approximately 0.1 \( \mu \)g/ml of a 0.75% erythrocyte suspension (14).

**Animals.** Weanling, male Sprague-Dawley rats were utilized. Upon arrival, they were housed individually in stainless steel cages and fed a 15% casein diet ad libitum for 1.5 weeks before commencing the experiments. The diet formula was 15% purified casein, 25% sucrose, 8% corn oil, 1% cellulose, 0.2% methionine, 1% vitamin mix (15), 3.5% mineral mix (15), 0.2% choline bitartrate, and corn starch to 100%. Lectin was added at the expense of the corn starch.

**Experiment 1.** Two dietary concentrations of peanut lectin were fed. One group was fed the casein diet incorporated with 40 \( \mu \)g lectin/g diet (0.004%). This concentration was selected for the following reason. In previous work, we found that rats fed a diet containing raw peanut meal providing 10% protein showed alterations in food intake, growth, and pancreatic function (8, 9). This diet was found to contain lectin at a concentration of 40 \( \mu \)g/g diet. Thus, this amount of lectin was selected for incorporation into a casein diet in the present study. A second group was fed the casein diet containing 400 \( \mu \)g lectin/g (0.04%). This level was utilized to assess the effects of excessive lectin exposure. A third group (controls) ingested the casein diet without added lectins. Food and water were provided ad libitum. After 23 days, rats were sacrificed without fasting. The entire mass of the small intestines was rapidly isolated and divided into 3 equal segments. A 1 cm piece from the proximal ends of each segment was fixed in buffered formalin and glutaraldehyde for subsequent histologic evaluation. The remainder of each segment was rinsed with ice-cold 0.9% saline, everted, scraped, and the mucosa homogenized in ice-cold saline. Homogenates were assayed for protein, DNA, and the activities of maltase [EC 3.2.1.20], \( \gamma \)-glutamyl transpeptidase [\( \gamma \)-GT, EC 2.3.2.2], and alkaline phosphatase [AP, EC 3.1.3.1]. Methodologies have been previously reported (16, 17).

**Experiment 2.** Because the results from experiment 1 showed only minimal effects from ingestion of diets containing peanut lectin at 0.004 and 0.04%, a second experiment was carried out utilizing a higher lectin concentration. Rats were fed the casein diet to which lectin was added at a concentration of 2,000 \( \mu \)g/g (0.2%). This level is of a magnitude in the range employed by various other investigators studying other legume lectins (2, 3, 5, 18-20). A control group received the lectin-free casein diet. Because this high level of lectin could possibly affect food intake, the control group was pair-fed to the lectin group. After 23 days, rats were treated as described for experiment 1.

Within each experiment, data were subjected to analysis of variance and the means compared by \( t \)-test at \( p \leq 0.05 \).
RESULTS AND DISCUSSION

**Experiment 1**

Rats that ingested the casein diet to which purified peanut lectin was added at 0.004 and 0.04% showed no differences in any parameter studied in comparison with rats fed the lectin-free diet (controls). Weekly weight gains were similar among the groups. Weight gain over the 23-day study was 123 g for the control group, 121 g for the 0.004% group, and 125 g for the 0.04% group. Small intestinal mucosal composition (Table 1) did not differ in weight, protein, or DNA content within any region of the intestines. Likewise, the enzymes maltase, γ-GT, and AP also did not show any changes in the 2 treatment groups compared with controls. Histologic examination in blinded fashion showed no abnormal appearance of the villi in any region of the small intestines nor in the cecum. Thus, peanut lectin incorporated into a casein diet at levels of 40 or 400 μg/g resulted in no observed or measured toxic response. The lower concentration was equivalent to that present in a 10% raw peanut protein diet which was previously found to induce growth.

Table 1. Experiment 1: Effects of feeding low and moderate levels of peanut lectin on small intestinal mucosa of rats.

| Area            | Dietary lectin concentration (%) | 0     | 0.004 | 0.04  |
|-----------------|----------------------------------|-------|-------|-------|
| Proximal, per cm| Weight (mg)                      | 15.6±0.3 | 14.9±2.1 | 17.0±1.7 |
|                 | Protein (mg)                     | 0.56±0.02 | 0.68±0.07 | 0.65±0.09 |
|                 | DNA (μg)                         | 143±9 | 167±28 | 158±17 |
|                 | Maltase (μmol/min)               | 1.6±0.2 | 1.3±0.3 | 1.5±0.8 |
|                 | γ-Glut. transpeptidase (μmol/min)| 0.15±0.02 | 0.20±0.05 | 0.17±0.03 |
|                 | Alk. phosphatase (μmol/min)      | 5.0±0.4 | 4.2±0.8 | 5.9±0.9 |
| Mid, per cm     | Weight (mg)                      | 15.5±1.6 | 15.3±1.5 | 18.0±2.4 |
|                 | Protein (mg)                     | 0.66±0.09 | 0.67±0.06 | 0.82±0.12 |
|                 | DNA (μg)                         | 138±24 | 140±21 | 154±21 |
|                 | Maltase (μmol/min)               | 2.5±0.3 | 2.3±0.4 | 2.7±0.4 |
|                 | γ-Glut. transpeptidase (μmol/min)| 0.18±0.01 | 0.14±0.03 | 0.21±0.02 |
|                 | Alk. phosphatase (μmol/min)      | 1.8±0.3 | 1.6±0.2 | 1.6±0.2 |
| Distal, per cm  | Weight (mg)                      | 9.4±1.8 | 9.3±0.7 | 11.7±1.2 |
|                 | Protein (mg)                     | 0.32±0.08 | 0.35±0.03 | 0.40±0.16 |
|                 | DNA (μg)                         | 112±24 | 104±11 | 119±15 |
|                 | Maltase (μmol/min)               | 1.6±0.1 | 1.2±0.2 | 1.5±0.2 |
|                 | γ-Glut. transpeptidase (μmol/min)| 0.05±0.02 | 0.06±0.01 | 0.06±0.01 |
|                 | Alk. phosphatase (μmol/min)      | 0.4±0.1 | 0.7±0.1 | 0.6±0.2 |

*Mean±SE; no significant differences (p>0.05) among groups within each area.

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retardation as well as other abnormalities (8, 9). The results of the present study show that the toxicity observed in the earlier study was apparently due not to the presence of lectins but to other antinutrients, probably protease inhibitors. In studies describing toxicity from other lectins, such as from red kidney bean, soybean, and black bean, the concentration of dietary lectin utilized was in the range of 0.2 to 5%. These levels are substantially higher than the concentrations we studied in this experiment. Therefore, a second experiment was carried out in which the amount of peanut lectin in the casein diet was increased to 0.2%. A higher concentration was not considered due to the low yield from the lengthy and time-consuming extraction procedure.

Experiment 2

As was also observed in experiment 1, rats ingesting the lectin-containing diet showed no changes in food intake or growth rate. Weight gain was 127 g during the 23-day feeding period while the control (lectin-free diet) group gained 128 g. However, unlike the results in experiment 1, there were a number of definite alterations in the composition of the mucosa in all three regions of the small intestines (Table 2). These changes generally involved mucosal weight, protein, and DNA rather than enzymes activities. In all 3 regions of the small intestines, rats ingesting the 0.2% peanut lectin diet showed an approximately 40% increase in mucosal weight and a 50 to 60% increase in protein. DNA increased by 24% in the proximal region, 39% in the mid-region, and 48% in the distal segment. As was also found in experiment 1, histologic evaluation by light microscopy revealed no consistent abnormal findings in villus structure in any of the regions.

These results reveal that peanut lectin appears capable of inducing a mild hyperplasia of the small intestines. Mitogenicity of certain cell types, typically lymphocytic, has been reported for certain lectins (10, 21, 22). Recently, Tajiri et al. (23) reported for the first time that feeding of purified red kidney bean lectin at 0.1% of diet can stimulate rat small intestinal mucosal DNA synthesis and crypt cell division. In that study, rats were examined at periods ranging from 1 to 6 days after commencing the diet. At 6 days, the mucosa from the proximal bowel showed increases in weight of 43%, in protein of 32%, and in DNA of 50%. Mucosal thickness and villus height were only slightly altered. Sucrase and enterokinase activities was markedly diminished but leucine aminopeptidase was unchanged. More pronounced changes in these parameters occurred during earlier sampling times, suggesting that animals recover or adapt to some degree.

Our results generally support and extend the findings of Tajiri et al. (23) that lectins may be trophic for the small intestines. At low doses (0.004 and 0.04%), as used in experiment 1, no effects on the intestinal mucosa were observed. However, at a higher dose (0.2%), there was a pronounced enhancement of mucosal weight, protein, and DNA throughout the length of the small intestines. This was found even after 23 days of feeding, a duration expected to allow full adaptation. In the

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work of Tajiri et al. (23), the distal small intestines showed no increase in DNA content whereas we found a trophic effect in all areas of the small bowel. These workers suggest that red kidney bean lectin may become inactivated during passage down the intestines so that there is insufficient material remaining to interact with the distal region. If true, then this suggests that in our work, peanut lectin is either more resistant to luminal inactivation or the amount ingested exceeded any possible inactivation mechanisms. The latter is unlikely since the hyperplastic response was greatest in the distal area. Alternately, peanut lectin may bind more strongly to the distal region and thereby exert a more pronounced trophic effect.

The mechanism by which peanut lectin appears to induce a trophic effect is unknown. The mitogenic effect on enterocytes from kidney bean lectin is thought to occur via attachment to specific carbohydrate binding sites of crypt cells which then leads to an increased proliferation rate of the crypt cells (23).

In our study, none of the three brush border enzymes representing a disaccharidase, a peptidase, and a phosphatase, deviated from normal activity even in rats fed 0.2% dietary peanut lectin. This suggests that no microvillus damage occurred. It is possible, however, that enzyme activities were lower at some point in the

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### Table 2. Experiment 2: Effects of feeding a high level of peanut lectin on small intestinal mucosa of rats.

| Area            | Dietary lectin concentration (%) |       |
|-----------------|---------------------------------|-------|
|                 | 0                               | 0.2   |
| Proximal, per cm|                                 |       |
| Weight (mg)     | 14.4±1.6*                       | 19.7±1.2* |
| Protein (mg)    | 0.87±0.09                       | 1.32±0.23* |
| DNA (μg)        | 165±18                          | 204±21 |
| Maltase (μmol/min) | 1.9±0.3                        | 2.1±0.1 |
| γ-Glut. transpeptidase (μmol/min) | 0.22±0.03       | 0.24±0.02 |
| Alk. phosphatase (μmol/min) | 5.8±0.7                        | 6.6±0.4 |
| Mid, per cm     |                                 |       |
| Weight (mg)     | 16.4±0.8                        | 22.5±0.8* |
| Protein (mg)    | 1.18±0.14                       | 1.75±0.13* |
| DNA (μg)        | 174±15                          | 241±23* |
| Maltase (μmol/min) | 2.8±0.2                        | 2.4±0.1 |
| γ-Glut. transpeptidase (μmol/min) | 0.21±0.02       | 0.21±0.01 |
| Alk. phosphatase (μmol/min) | 1.4±0.1                        | 1.8±0.3 |
| Distal, per cm  |                                 |       |
| Weight (mg)     | 10.7±1.7                        | 15.3±1.3* |
| Protein (mg)    | 0.67±0.12                       | 1.08±0.09* |
| DNA (μg)        | 108±21                          | 160±14* |
| Maltase (μmol/min) | 2.0±0.3                        | 1.9±0.2 |
| γ-Glut. transpeptidase (μmol/min) | 0.10±0.01       | 0.14±0.01* |
| Alk. phosphatase (μmol/min) | 0.5±0.2                        | 0.7±0.0 |

*Mean±SE. *Significantly different at p ≤ 0.05 within each area.
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experiment but then recovered to normal levels by the end of the 23-day feeding period. One might predict that enzyme activities would rise in support of the observed increases in mucosal weight, protein, and DNA. However, hyperplasia can occur in the crypt region rather than the villus, as was previously reported to result from feeding red kidney bean lectin (23). Inasmuch as brush border enzymes are immature on the crypt cells, no increase in microvillus enzyme activity would occur. In any event, it is probably that, notwithstanding the different specificities of the lectins to bind to receptors on the villus-crypt surface (24), the response to lectin ingestion is dependent, in large part, on the amount ingested and on the length of the feeding period. In the work of Tajiri et al. (23), we estimate that daily lectin ingestion amounted to approximately 15–20 mg for 6 days. This intake was not toxic and was mitogenic for the proximal small intestines without increasing brush border enzyme activities. Acute challenge by gastric lavage from 300 mg of raw kidney bean [calculated to contain about 10.5 mg lectin, assuming a lectin concentration of about 3.5% (25)], results in damage to the microvillus but not to other villus structures (20). Moreover, the damage became repaired within 20 h. This suggests that the enterocyte brush border may be repeatedly damaged and then repaired after ingestion of red kidney beans. Banwell et al. (2) fed a casein diet adulterated with 0.5% red kidney bean lectin to weanling rats for up to 3 weeks. Food intake was reduced to about one-third normal and rats lost weight. The average amount of lectin ingested was approximately 19 mg/day, similar to the intake reported by Tajiri et al. (23). Specific activities of several disaccharidase enzymes were markedly reduced in the proximal small intestine but not in the distal region when compared with pair-fed controls. Further, the histologic appearance evaluated by both light and electron microscopy was normal. The lack of any observable morphologic changes, even of the microvilli, may have been due to the marked diminution of food intake, thereby reducing lectin intake and allowing rapid repair of microvilli (20). Rouanet et al. (19) reported on intestinal changes in rats fed a semi-purified diet containing 0.25% kidney bean lectin for 17 days. Compared with pair-fed controls, lectin-fed rats showed no significant change in jejunal villus length but crypt depth significantly increased. Mucosal protein increased by 60% but activities of sucrase and \( \gamma \)-glutamyl transpeptidase were unchanged. These results are consistent with those of Tajiri et al. (23) as well as our findings concerning enzyme activities.

In summary, the feeding of isolated peanut lectin to weanling rats at low to moderate levels is without effect on growth and small intestinal morphology and biochemistries, whereas at a high dietary concentration of 0.2%, growth remains normal but the small intestine shows a hyperplastic response.

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