Induction of Intestinal Malabsorption Syndrome in Rats Fed with *Agaricus bisporus* Mushroom Lectin†

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Young Sprague-Dawley rats were fed rat chow containing 25–75% dried *Agaricus bisporus* mushroom or 100 mg of purified lectin. Animals fed mushroom concentrations of ≥50% or purified lectin showed a significant decrease in body weight and protein digestibility. Their small intestine showed a flattened mucosa with fused villi as well as alterations from the columnar to the cuboidal type of absorptive surface cells. We have identified in the intestinal mucosa saccharidic structures containing galactose-(β1,3)GalNAc that might be involved in the lectin–intestinal surface interaction.

INTRODUCTION

Lectins, which are present in both plants and animals, are proteins or glycoproteins of nonimmune origin that recognize and interact specifically with saccharidic structures (Goldstein et al., 1980). Certain lectins derived from beans belonging to the genus *Phaseolus* have proved to be toxic to animals and human beings fed with either the raw bean or the purified lectin (Jaffe, 1981; Pusztai et al., 1979). The red kidney bean lectin, phytohemagglutinin, has been found to impair protein digestion (Pusztai et al., 1981) and to cause inadequate absorption of nutrients, particularly amino acids (Palmer et al., 1987; Pusztai et al., 1981), thus resulting in protein malnutrition and, occasionally, death.

A more detailed analysis of the mechanism involved in this lectin-induced malabsorption has shown that small intestine impairment is secondary to the disruption of the intestinal enterocyte apical microvilli (King et al., 1980, 1982; Rouanet et al., 1985; Sotelo et al., 1983) due to the binding of the lectin with specific sugar structures on the gut surface. Nevertheless, the effect of the lectins contained in a normal diet upon the intestinal mucosa is difficult to evaluate since beans—so far the subject of the greater number of studies—are ingested only after a lengthy cooking period, which results in protein denaturation and dissipation of the malnutritional effect (Grant et al., 1982; King et al., 1982; Palmer et al., 1987).

To circumvent the latter and study the possible mechanism of lectin-induced small intestine mucosa damage, we have chosen to feed rats with the mushroom *Agaricus bisporus* or its purified lectin, a frequent uncooked ingredient in a variety of Mexican dishes due to its relatively low cost and high protein content (Chang, 1980). Our results showed that the mushroom's lectin induces histological abnormalities similar to those observed in the malabsorption syndrome, as a consequence of the structures that the intestinal mucosa mucin possesses which recognize and bind the lectin.

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† This work was supported by the Secretaria de Educación Pública, México; Consejo Nacional de Ciencia y Tecnologia (Grant P219CCOA-880695), México; and Programa Universitario de Investigación en Salud, UNAM, México.

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MATERIALS AND METHODS

Reagents. DEAE-cellulose, Sephadex G-25 fine, *Vibrio cholerae* neuraminidase, and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, MO. Biogel P-2 was purchased from Biotechnics, Clichy, France. Amicon P-10 membrane was obtained from Millipore Corp. Commercial rat chow was purchased from Purina, Mexico. All of the remaining reagents were purchased from Merck and were of analytical quality.

Lectin Purification. Greenhouse-cultured *A. bisporus* mushroom fruitbodies obtained at one of the local markets in the city of Cuernavaca, Mexico, were taken to the Centro de Investigaciones Biológicas at the Universidad Autónoma de Morelos, Mexico, where they were identified, dehydrated at 70 °C for 72 h and ground to a fine flour. The mushroom's lectin was extracted by incubating 1 g of the ground flour with 10 mL of isotonic saline solution (0.15 M NaCl in H_2O) with continuous stirring at room temperature for 24 h; the solution thus obtained (crude extract) was then applied to a column (3 × 15 cm) of DEAE-cellulose previously equilibrated with 0.9% NaCl at room temperature (Presant and Kornfeld, 1972). Unbound material was eluted with the same solution; retained material was eluted with a 0.15–0.5 M stepwise NaCl gradient at a 10 mL/h flow rate and tested for its hemagglutinating activity against human red blood cells. Those fractions with agglutinating activity were pooled and placed on a Sephadex G-25 fine column (5 × 20) containing stroma from desialylated human type O erythrocytes (Zenteno and Ochoa, 1988) previously equilibrated with 0.9% NaCl. Unbound material was eluted with the same buffer and the lectin was desorbed with 0.2 M lactose, dialyzed against 0.001 M NaCl, lyophilized, and kept at freezing temperature until use. SDS–PAGE (Laemmli, 1970) showed that the purification process ends with a preparation where only one band with a molecular mass of 16 kDa can be seen by Coomassie stain (Figure 1). To determine whether our purification process preserved the lectin biological activity, we compared results between experiments performed with our lectin and those performed with commercially available *A. bisporus* lectin (Sigma).

Hemagglutination Assay. The hemagglutinating activity of the crude extract and the purified lectin was assayed, by the 2-fold dilution test, in microtiter U plates (Nunc, Roskilde, Denmark), using 25 μL (2×10^6 red blood cells) of *V. cholerae* neuraminidase-treated (Seaman et al., 1977) human A, B, and O erythrocytes.

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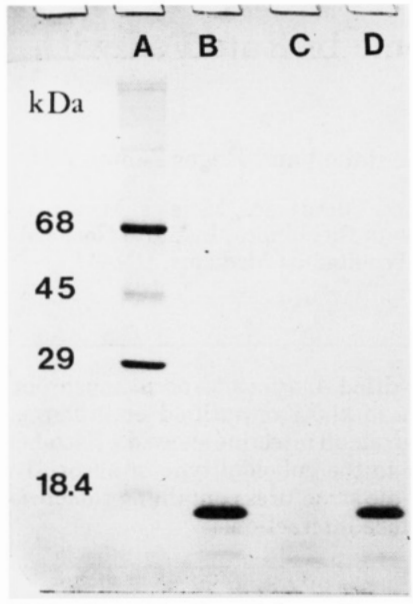

Figure 1. SDS–polyacrylamide gel electrophoresis analysis of purified *A. bisporus* lectin: (a) molecular mass markers (68 kDa, albumin bovine; 45 kDa, albumin egg; 29 kDa, carbonic anhydrase from bovine erythrocytes; 18.4 kDa, β-lactoglobulin from bovine milk); (b) commercially available lectin; (c) crude extract; (d) affinity-purified lectin. Each sample contained 15 μg of protein.

Analytical Methods. Whole dehydrated fruitbody flour as well as rat fecal samples were analyzed for protein content (N × 6.25) according to the AOAC (1984) procedure. Serum glucose concentration was determined by the method of Folin–Wu (Frankel et al., 1970). The purified lectin protein concentration was determined according to the method of Lowry (Lowry et al., 1951) with bovine serum albumin as standard.

Animals and Diets. Five groups of six female Sprague-Dawley rats, 82 ± 1 g each, grown and maintained in the animal facility of the Centro de Investigaciones Biológicas at the Universidad Autónoma de Morelos, were used. Each group was kept in a separate cage and fed ad libitum for 5 days with a total of 400 g of a diet based on commercial rat chow containing 16 different vitamins, 1.5% minerals, 58% carbohydrates, 23% protein, 2.5% fat, 7% crude fiber, and 8% ash. Experimental group diets 2, 3, and 4 were supplemented with 25, 50, and 75% (w/w) dehydrated *A. bisporus* flour, which corresponds to approximately 125, 250, and 375 mg of lectin and to 97, 102, and 107 g of total protein, respectively; group 5 diet was supplemented with 100 mg of purified *A. bisporus* lectin. Group 1 was fed with nonsupplemented basal diet.

A. bisporus mushroom contains 28% protein, 4% fat, 53% carbohydrates, 7% fiber, and 16% ash by dry weight as determined by AOAC (1984) procedures.

Rats were weighed twice a week, and their food intake was recorded daily. Fecal samples were collected daily, and their protein nitrogen content was also determined daily to establish rat protein digestibility. Rats were fed the above described diets for 5 days and were then sacrificed. Blood was collected in 0.2 M ethylenediaminetetraacetic acid (pH 7.4) (0.1 mL for each 6 mL of blood) and the plasma separated by centrifugation and kept at −20 °C. Each animal liver, kidney, pancreas, spleen, stomach, and small and large intestine was dissected, embedded in Bouin's solution, and frozen in dry ice. Sections 7 μm thick were cut and stained with hematoxylin–eosine.

Isolation of Intestinal Mucosa Glycoproteins. To determine the presence of specific receptors for the lectin on the surface of the small intestine, we isolated mucosal glycoproteins from the small intestine of two animals fed with the control diet. The intestinal mucosa was scraped with a surgical blade and homogenized twice in a Polytron with 20 mL of saline solution at 2 °C, 5 min each. After centrifugation at 10000*g* for 15 min at 4 °C, the clear supernatant was collected and divided in two aliquots, one of which was concentrated by ultrafiltration in an Amicon P-10 membrane and kept at 4 °C until use; the other was desialylated by incubation with 0.02 N H_2SO_4 at 90 °C (Spiro and Bhoyroo, 1974) for 1 h followed by chromatography on a 100 × 1.2 cm Biogel P-2 column equilibrated with 1% acetic acid in water. The material collected in the void volume was concentrated and kept at 4 °C until use. The protein and carbohydrate concentrations of the glycoproteins thus obtained were determined according to the method of Lowry (Lowry et al., 1951) and the phenol–sulfuric method (Dubois et al., 1956), respectively.

Native and desialylated glycoproteins from the intestinal mucosa were tested for their ability to inhibit the *A. bisporus* lectin hemagglutinating activity. Results are expressed as the minimal concentration of glycoprotein capable of inhibiting the agglutinating activity (Osawa and Matsumoto, 1972).

Statistical Analysis. Data from the different animal groups was compared by a two-way analysis of variance; the significance of the comparisons was determined by the multiple-range Student's *t*-test.

RESULTS

The intake of *A. bisporus* mushroom dehydrated flour or its purified lectin induced a significant weight loss, which was proportional to the amount of mushroom content in the animal diet (Table I). The differences between the mean of the control group and those of groups 3, 4, and 5 which were fed commercial chow containing 50 and 75% mushroom flour or 100 mg of purified lectin, respectively, reached statistical significance ($P < 0.01$). This difference was also observed in the daily nitrogen intake and in the protein digestibility ratio, which is basically influenced by the daily nitrogen intake.

The animals' general condition showed a very slight decline; it was more obvious in those animals fed with greater amounts of mushroom flour or the purified lectin. Moreover, the gain in weight of the animals in group 4 during the 5-day experiment was negative, whereas those in groups 3 and 5 showed a weight gain of only 14 and 23% compared to 50% in the control group. Nevertheless, no appetite or food consumption reduction was observed in any of the groups studied. The differences in plasma glucose concentration showed a tendency similar to that observed with nitrogen intake, apparent protein digestibility, and weight but did not reach statistical significance.

The kidneys, liver, pancreas, and stomach showed no histological alterations, independent of which group the animal was allocated to. The histological pattern of the small intestine from animals fed either the mushroom flour or the purified mushroom lectin showed important alterations. Figure 2 shows a histological picture of the small intestine belonging to a control animal, and Figure 3 is a typical example of the histological changes observed in animals fed on the mushroom lectin (flattened mucosa, shortening and fusion of the villi, alterations of the absorptive surface cells from the columnar to the cuboidal type). Histologically demonstrated mucosal damage was greater in those animals fed with diets containing higher quantities of mushroom flour. Five rats were fed with a 75% supplemented diet consisting of *A. bisporus* fruitbodies heated at 100 °C for 1 h before they were incorporated into the chow. The histological lesions already described were not found.

The presence of lectin activity in the mushroom flour was determined by hemagglutination assays with desialylated human type A, B, and O erythrocytes, all of which were agglutinated with 0.3 mg/mL of mushroom crude extract or 0.001 mg/mL of, either ours or commercially available, purified lectin. The hemagglutinating ability of both the crude extract and the purified lectin was

Table I. Nutritional Parameters of *A. bisporus* Fed Rats[a]

group	wt gain, g	N intake, mg/day	fecal N, mg/day	N digestibility[b]	glucose concent, mg/mL
1	41 ± 3	175 ± 15	23 ± 3	86.9	115 ± 4
2	30 ± 3	147 ± 7	22 ± 2	85.0	101 ± 4
3	12 ± 2	125 ± 9	21 ± 2	83.2	99 ± 3
4	−7 ± 3	99 ± 6	14 ± 2	81.8	97 ± 3
5	19 ± 3	115 ± 5	20 ± 3	82.6	100 ± 4

[a] Results are expressed as the mean ± standard error of the mean per group; each group consisted of six animals. Group 1, control group; groups 2, 3, and 4 were fed with 25, 50, and 75%, respectively, of dried *A. bisporus*; group 5 was fed with 100 mg of mushroom lectin. [b] Nitrogen digestibility = [(N intake − fecal N)/N intake] × 100.

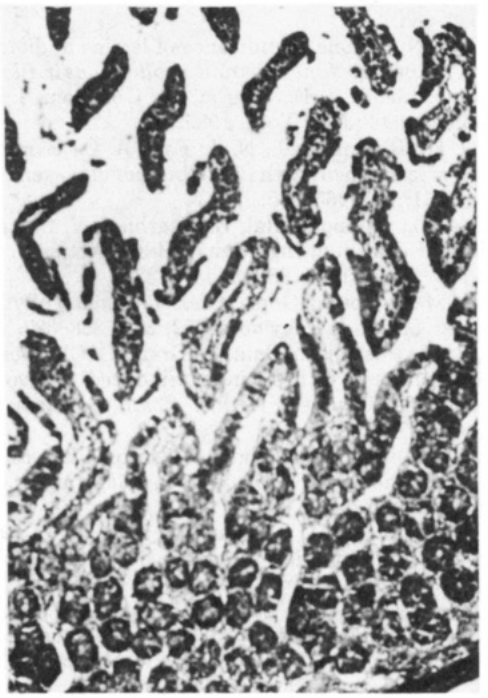

Figure 2. Photomicrograph of a control rat small intestine with prominent and abundant intestinal villi; the prominence is responsible for the impression given by some villi of being isolated inside the intestine. H–E [×25]. (Figure is reproduced here at 75% of the original.)

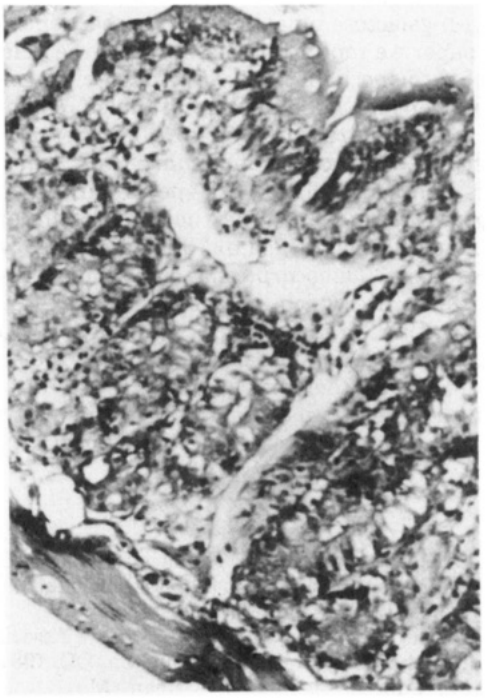

Figure 3. Photomicrograph of the small intestine of a rat fed with 0.1% *A. bisporus* lectin where the mucosal thickness is clearly reduced, the intestinal villi are merged, giving the appearance of a meset, and the superficial covering epithelium is of the low cylindrical type. H–E [×63]. (Figure is reproduced here at 75% of the original.)

completely inhibited with 0.5 mg/mL of nondesialylated or 0.002 mg/mL of desialylated rat intestinal mucosa glycoproteins.

DISCUSSION

The ingestion of crude dehydrated mushroom *A. bisporus* or its purified lectin produced a reduced absorption of glucose and an important weight loss. As has already been shown in the case of other lectins, the effect is dose-dependent (Goldstein et al., 1980; Jaffe, 1981; King et al., 1980; Palmer et al., 1987; Pusztai et al., 1981; Rouanet et al., 1985; Sotelo et al., 1983). The alterations with the purified lectin were slightly less pronounced than those observed with a high concentration of mushroom flour, probably as a result of feeding the animals a lesser quantity of lectin (100 vs 350 mg). It has already been established that the lectin content is much lower in the fruitbody of several macromycetes than in the raw bean seeds of leguminosae, where the lectin content by weight is between 2 and 10% (Presant and Kornfeld, 1972). Nevertheless, we decided to include a lectin concentration that varied from 100 to approximately 350 mg as a diet supplement to compare the effects of our mushroom with those reported with soybean (Alvarez and Torres-Pinedo, 1982) or *Phaseolus vulgaris* (Bulajic et al., 1986) lectins which induced, when used at similar concentrations, growth impairment and nitrogen uptake.

Our results showed that the nitrogen digestibility of the protein contained in the diet was slightly, although non-significantly, reduced in those animals receiving the mushroom fruitbody or its purified lectin; the fact that lectins from *P. vulgaris* induced a far more important reduction in the same parameter (Jaffe, 1981; Pusztai et al., 1981; Palmer et al., 1987) could be secondary to a higher amount of protein in the diet, as has already been described by some authors (Bulajic et al., 1986; Rouanet et al., 1985). Similarly to *Phaseolus* (Grant et al., 1982), heating the *A. bisporus* fruitbodies abolished the hemagglutinating activity as well as the effect on protein digestibility.

The histological analysis of the organs that might be involved in deficient nitrogen digestibility indicated that the mushroom or its purified lectin primarily affects the small intestine; the light microscopy examination of this organ showed a shortening of the villi and a lengthening of the crypts. These alterations have also been reported with *Lathyrus odoratus*, *Dolichos biflorus* (Liener, 1986), soybean (Jindal et al., 1984; Alvarez and Torres-Pinedo, 1982), *Phaseolus acutifolius* (Sotelo et al., 1983), and *P. vulgaris* (Oliveria et al., 1989) lectins; in the case of all of these lectins, membrane shedding has been proposed as the possible mechanism of action.

The hemagglutinating activity of *A. bisporus* lectin was specifically inhibited by the glycoproteins isolated from the rat small intestine. The inhibition was enhanced when

desialylated glycoproteins were used; this observation is fully in agreement with that of Presant and Kornfeld (1972). *A. bisporus* lectin is specific for Gal(β1,3)GalNAc-containing structures (Presant and Kornfeld, 1972), and as it has been shown (VanHalbeek et al., 1982) intestinal mucin contains similar structures. It should be kept in mind that previous studies on lectins and nutrition have been performed either with *Phaseolus* genus lectins, which have a broad sugar specificity, or with lectins, which have a more restricted sugar specificity (concanavalin A, glucose/mannose; wheat germ, *N*-acetyl-D-glucosamine; soybean, *N*-acetyl-D-galactosamine) [for a review see Liener (1986)]. In this paper we report for the first time the deleterious effect of *A. bisporus* lectin, which is specific for the *O*-glycan present in the inner core of mucin, composed of highly complex structures (VanHalbeek et al., 1982). Nevertheless, the lectin from *Amaranthus leucocarpus* which is also Gal(β1,3)GalNAc specific, did not induce histological alterations or differences in protein consumption (Calderon et al., 1985a,b). This discrepancy might be the result of *A. leucocarpus* having a far more restricted lectin specific site which, in some special way (Zenteno and Ochoa, 1988), induces the recognition of the GalNAc structure in the inner core of the *O*-glycan moiety.

To sum up, the deleterious effect of the interaction between the *A. bisporus* mushroom lectin and the small intestine mucosa endows this lectin with great value for the study of enteropathy.

LITERATURE CITED

Alvarez, J. R.; Torres Pinedo, R. Interaction of soybean lectin, soya saponins and glycinin with rabbit jejunal mucosa in vivo. *Pediatr. Res.* **1982**, *16*, 728–731.

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.

Bulajic, M.; Cuperlovic, M.; Movsesijian, M.; Borojevic, D. Interaction of dietary lectin (phytohemagglutinin) with the mucosa of rat digestive tract—immunofluorescence studies. *Period. Biol.* **1986**, *88*, 331–336.

Calderon, A. M.; Ochoa, J. L.; Valencia, M. E. Effect of the extraction of a hemagglutinin on the nutritive value of *A. leucocarpus* seeds. *J. Food Sci.* **1985a**, *50*, 1700–1708.

Calderon, A. M.; Zenteno, E.; Carvajal, R.; Valencia, M. E.; Ochoa, J. L. Biological properties of *Amaranthus leucocarpus* lectin and its relation to the nutritional value of the seed meal. In *Lectins, Biology, Biochemistry, Clinical Biochemistry*; Bog-Hansen, T. C., Breborowicz, J., Eds.; de Gruyter: Berlin, 1985b; Vol. 4.

Chang, S. T. Mushrooms as human food. *Bioscience* **1980**, *30*, 399–401.

Dubois, M. A.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugar and related substances. *Anal. Chem.* **1956**, *28*, 350–356.

Frankel, S.; Reitmanane, S.; Sonnenwirth, A. *Gradwohl's Clinical Laboratory Methods in Diagnosis*; Mosby: New York, 1970.

Goldstein, I. J.; Hughes, R. C.; Monsigny, M.; Osawa, T.; Sharon, N. What should be called a lectin? *Nature* **1980**, *285*, 66.

Grant, G.; Moore, L. J.; McKenzie, N. H.; Pusztai, A. The effect of heating on the hemagglutinating activity and nutritional properties of bean (*Phaseolus vulgaris*) seeds. *J. Sci. Food Agric.* **1982**, *33*, 1324–1326.

Jaffe, W. G. Haemagglutinins. In *Toxic constituents of plant foodstuffs*; Liener, I. E., Ed.; Academic Press: New York, 1981.

Jindal, S.; Soni, G. L.; Singh, R. Biochemical and histopathology studies in albino rats fed with soybean lectin. *Nutr. Rep. Int.* **1984**, *29*, 95–106.

King, T. P.; Pusztai, A.; Clarke, E. M. W. Kidney bean (*Phaseolus vulgaris*) lectin induced lesions in rat small intestine: 1, Light microscope studies. *J. Comp. Pathol.* **1980**, *90*, 585–595.

King, T. P.; Pusztai, A.; Clarke, E. M. W. Kidney bean (*Phaseolus vulgaris*) lectin induced lesions in rat small intestine: 3, ultrastructural studies. *J. Comp. Pathol.* **1982**, *92*, 357–353.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **1970**, *277*, 680–685.

Liener, I. E. Nutritional significance of lectins in diet. In *The lectins, properties, functions and applications in Biology and Medicine*; Liener, I. E., Sharon, N., Goldstein, I. J., Eds.; Academic Press: New York, 1986.

Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. S. Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

Oliveria, A. C.; Campos Vidal, B.; Sgarbieri, V. C. Lesions of Intestinal Epithelium Ingestion of Bean Lectins in Rats. *J. Nutr. Sci. Vitaminol.* **1989**, *35*, 315–322.

Osawa, T.; Matsumoto, I. Gorse (*Ulex europaeus*) phytohemagglutinins. *Methods Enzymol.* **1972**, *28B*, 323–327.

Palmer, R. M.; Pusztai, A.; Bain, P.; Grant, G. Changes in rates of tissue protein synthesis in rats induced in vivo by consumption of kidney bean lectins. *Comp. Biochem. Physiol.* **1987**, *88C*, 179–183.

Presant, C. A.; Kornfeld, S. Characterization of the cell surface receptor for the *Agaricus bisporus* haemagglutinin. *J. Biol. Chem.* **1972**, *247*, 6937–6945.

Pusztai, A.; Clarke, E. M. W.; Grant, G.; King, T. P.; Stewart, J. C. Nutritional evaluation of kidney beans (*Phaseolus vulgaris*). *J. Sci. Food Agric.* **1979**, *30*, 843–848.

Pusztai, A.; Clarke, E. M. W.; Grant, G.; King, T. P. The toxicity of *Phaseolus vulgaris* lectins. Nitrogen balance and immunochemicals studies. *J. Sci. Food Agric.* **1981**, *32*, 1037–1046.

Rouanet, J. M.; Lafont, J.; Claet, M.; Creppy, A.; Besancon, P. Effects of dietary kidney bean (*Phaseolus vulgaris*) lectins in growing rats. *Nutr. Rep. Int.* **1985**, *31*, 237–241.

Seaman, G. V. F.; Knox, R. J.; Nordt, F. J.; Reagan, D. H. Red cell aging. 1. Surface charge density and sialic acid content of density-fractionated human erythrocytes. *Blood* **1977**, *50*, 1001–1011.

Sotelo, A.; Gonzalez, A.; Gonzalez-Garza, M. T.; Velasco, E.; Feria-Velasco, A. Ultrastructural changes of epithelial intestinal cells induced by the ingestion of raw *Phaseolus acutifolius*. *Nutr. Rep. Int.* **1983**, *27*, 865–873.

Spiro, R. G.; Bhoyroo, V. D. Structure of the O-glycosidically linked carbohydrate units of fetuin. *J. Biol. Chem.* **1974**, *249*, 5704–5717.

VanHalbeek, H.; Dorland, L.; Vliegenthart, J. F. G.; Kotchkov, N. K.; Arbatsky, N. P.; Derevitskaya, A. Characterization of the primary structure and the microheterogeneity of the carbohydrate chains of porcine blood-group H substance by 500-MHz ^{1}H-NMR spectroscopy. *Eur. J. Biochem.* **1982**, *127*, 21–29.

Zenteno, E.; Ochoa, J. L. Isolation and purification of *Amaranthus leucocarpus* lectin on stroma column. *Phytochemistry* **1988**, *27*, 313–317.

Received for review March 30, 1992. Accepted May 28, 1992.

Registry No. Galactose (β1,3)GalNAc, 3554-90-3.