Concanavalin A Promotes Adherence of *Salmonella typhimurium* to Small Intestinal Mucosa of Rats (42960)

R. L. ABUD, B. L. LINDQUIST, R. K. ERNST, J. M. MERRICK, E. LEBENTHAL, AND P. C. LEE
International Institute for Infant Nutrition and Gastrointestinal Disease, Children's Hospital of Buffalo, Buffalo, New York 14222 and Department of Microbiology, State University of New York at Buffalo, Buffalo, New York 14214

Abstract. **A number of dietary lectins have been shown to resist proteolytic digestion. These lectins interact with the small intestinal mucosa causing structural and functional changes. Concomitant to these changes, bacterial overgrowth was reported and a possible interaction between lectins and bacteria in the small intestine was postulated. The aim of this study was to investigate the effect of various lectins on adherence of *Salmonella typhimurium* to both isolated small intestinal enterocytes and ligated intestinal loops. Isolated intestinal cells or ligated intestinal loops were incubated with [^{3}H] adenine-*S. typhimurium* in the presence or absence of concanavalin A, phytohemagglutinin, peanut agglutinin, and wheat germ agglutinin. Only concanavalin A promoted the adherence of various strains of nonfimbriated *S. typhimurium* to isolated viable intestinal cells. Other lectins showed no effect on the adherence. *In situ* studies showed that bacterial binding was increased in concanavalin A-treated intestinal loops, supporting the significance of the experiments *in vitro*. These data suggest that lectins may act by promoting bacterial adherence to the small intestine, thereby facilitating colonization and infection, and leading to bacterial overgrowth.** [P.S.E.B.M. 1989, Vol 192]

The widespread presence of lectins in dietary foods has prompted a number of investigators to examine their potential toxicity on our diet. There are several reports in the literature of cases of human intoxication in which lectins appear to have been the causative agent (1). Studies have shown that continuous exposure of the small intestine to active food lectins can alter the microenvironment of mucosal surfaces and result in morphologic, physiologic, and biochemical changes (2). The inclusion of raw kidney beans (*Phaseolus vulgaris*) in animal diets can reduce the growth rate and sometimes even cause death (3). Intraluminal administration of wheat germ agglutinin (WGA) and concanavalin A (Con A) caused increased shedding of brush border membranes, shortening of the villi, and accelerated cell loss (4). The inclusion of raw kidney beans or lectins purified from these beans caused severe damage to the enterocyte brush border, resulting in abnormal development of microvilli (5). Simultaneously, a dramatic overgrowth of *Escherichia coli* in the small intestine was observed. Since adherence is believed to play an important role in bacterial colonization (6, 7), it is possible that lectins may mimic and/or exacerbate the action of *E. coli* adhesins.

In a previous study (8), we have shown that mannose-sensitive fimbriated (fim$^+$) strains of *Salmonella typhimurium* adhered to isolated small intestinal enterocytes of rats in significantly higher numbers than did nonfimbriated strains. The present study was designed to assess whether Con A, a lectin with mannose specificity, could mimic the action of mannose-sensitive fimbriae and induce adherence of nonfimbriated (fim$^-$) strains of *S. typhimurium*. The effects of Con A on bacterial adherence were examined both with isolated small intestinal enterocytes *in vitro* and in ligated loops of the small intestine *in situ*.

Materials and Methods

Animals. Young adult male rats of the Sprague-Dawley strain (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing approximately 200–250 g were used throughout the study.

[1] To whom correspondence and reprint requests should be addressed at Department of Pediatrics, Medical College of Wisconsin, MACC Fund Research Center, Milwaukee, WI 52236.

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Lectins. Con A and fluorescein isothiocyanate (FITC)-Con A (D-mannose and glucose specific) were obtained from Sigma Chemical Co. (St. Louis, MO). Other lectins used in this study—WGA (*N*-acetylglucosamine disaccharide specific) and peanut agglutinin (PNA) (galactose *N*-acetylgalactosamine specific)—were also obtained from Sigma Chemical Co. Phytohemmagglutinin (PHA) (*N*-acetylgalactosamine specific) was prepared from red kidney beans according to the procedure described by Felsted *et al.* (9).

Bacteria and Cultivation Conditions. The following *S. typhimurium* strains were used: S7471ϕF, S7471N, LT2, and CR6600. Their characteristics are listed in Table I. Stock cultures were grown in 10 ml of Luria broth (10) at 37°C under aerobic and shaking conditions to inhibit expression of fimbriae. Stock cultures were also grown under aerobic and static conditions in order to promote fimbriae formation. To evaluate the expression of fimbriae, hemagglutination studies were performed by mixing bacteria and erythrocytes in a ratio of approximately 500:1 on a microscopic slide or as a tile test (11) and were evaluated after 3-min incubation at room temperature (25°C). Stock cultures were preserved at −80°C in 10% glycerol. For adherence studies (8), cultures were grown shaking for 12 hr at 37°C in Luria broth and subsequently transferred and grown for 3 hr in the presence of [8-^{3}H]adenine (10 μCi/ml). The broth culture was centrifuged and the pellet was washed twice in phosphate-buffered saline (PBS) and finally suspended in the fresh isolation buffer described below. The initial sp act of the radiolabeled bacteria was approximately 1000 cpm/1–3 × 10^6 bacteria.

Enterocytes. Enterocytes were isolated from intestinal segments as previously described (8, 12). Twenty-five centimeters of the proximal small intestine were excised and trimmed of fat and mesentery. The segment was longitudinally split open for washing and isolation of enterocytes by mechanical shaking. Collected enterocytes were combined and filtered through four layers of cheesecloth. The cell suspension was washed twice and suspended in isolation buffer to an appropriate density (10^6 cells/ml). Viability of cells was assessed by the trypan blue exclusion test and release of LDH into the medium (DART LD; Coulter Diagnostics, Hialeah, FL) (13). The isolation/incubation (II) buffer consisted of 24.5 m*M* Hepes, 98 m*M* NaCl, 6 m*M* KCl, 2 m*M* KH_2PO_4, 5 m*M* sodium pyruvate, 6 m*M* sodium fumarate, 5 m*M* sodium glutamate, 11.5 m*M* glucose, 2 m*M* glutamine, 0.2% (w/v) bovine serum albumin, 2% amino acid mixture (containing all essential amino acids), and 1% (v/v) vitamin mixture (standard mixture for minimal essential medium). The pH of the solution was adjusted to 7.4. All reagents were obtained from Sigma Chemical Co. except for the amino acid mixture (Gibco Laboratories, Grand Island, NY) and the vitamin mixture (M. A. Bioproducts, Walkersville, MD).

Table I. *S. typhimurium* Strains Used in This Study

Strain	Relevant characteristics	Fimbriated phenotype	Reference
S7471N	FIRN biotype	−	(8)
S7471ϕF	Transductant of S7471N	+ or −	(8)
LT2	Wild type	+ or −	(8)
CR6600	Wild type	+ or −	(9)

***In Vitro* Adherence Assay.** The adherence assay used for this study has been established in our laboratory and previously published (8). Briefly, a freshly prepared suspension of bacteria and enterocytes in a ratio of 500:1000 bacteria/enterocyte was incubated at 37°C on a slowly rotating wheel for 0 to 60 min in the presence of II buffer with or without lectin. Bacteria bound to enterocytes were separated from free bacteria by filtration through a polycarbonate membrane filter (5-μm pore size; Nuclepore Corp., Pleasanton, CA). After four washings with PBS, the radioactivity on the filter was counted in a scintillation spectrometer (LS 1800; Beckman). Binding results were expressed as the number of bacteria bound per enterocyte, which was calculated from the sp act of the labeled bacterial cells used in the assay. All experiments were run in duplicate and all results were corrected for nonspecific bacterial binding to the filter.

To determine whether Con A binds to enterocyte surfaces, freshly isolated enterocytes were divided equally into four treatment groups, each containing the same concentration of cells in incubation buffer: (1) control with no treatment; (2) FITC-Con A (0.2 mg/ml); (3) FITC-Con A (0.2 mg/ml) + mannose (0.1 *M*); and (4) FITC-Con A (0.2 mg/ml) + α-methylmannopyranoside (0.1 *M*). After a 30-min incubation at 37°C, the enterocytes were washed three times with the incubation buffer and then two more times with PBS. Final pellets were then suspended in 1 ml of PBS. All suspensions were then examined under fluorescence microscopy and measured by fluorescence spectrometry.

Bacterial aggregation by the lectins was evaluated by light microscopy and radioassay. Bacteria were incubated (from 15 to 120 min at 37°C) in the presence of varying concentrations of lectins (0.001–10.0 mg/ml). For light microscopy studies, quantitation was done with a hemocytometer by two of the investigators. For radioassay, we measured the radioactivity produced by bacterial retention on the 5-μm filter as a result of aggregation. Specificity of the Con A-induced binding was examined by competition assays using mannose at different concentrations.

***In Situ* Adherence Assay Using Cannulated Loops.** The following method is a modification of the one described by Ashkenazi and Mirelman (14). Rats were kept on a commercial rat chow upon arrival (Purina Lab Chow). Nonfasted animals were anesthe-

tized with intraperitoneal phenobarbitone (60 mg/kg). Their abdominal cavities were opened by a midline incision to expose the small intestine. Two loops of the small intestine were prepared. The first segment of the small intestine was cannulated 5 cm below the pylorus and flushed. A second cannula was inserted 10 cm below the first one to serve as a collection tube. The second segment was similarly prepared 5 cm below the collection tube of the first segment. Both segments were flushed with PBS containing 1 m*M* dithiothreitol (DTT) to remove the mucus and flushed again with PBS alone. Segments were incubated in the presence or absence of Con A (0.5 mg/ml) for 45 to 120 min with 1.0 ml of isolation buffer containing 5×10^8 bacteria labeled with [8-^{3}H]adenine. After 45 min the segments were flushed with PBS four times to remove the unbound bacteria. This time of incubation was chosen as preliminary studies indicated a plateau had been reached at 45 min. Segments were removed and three samples of 2-cm length each were collected for the quantitation of bacterial adherence. Segments were heated in 2% sodium dodecyl sulfate, and sample from the supernatant (0.5 ml) was collected and added to 4.5 ml of scintillation fluid for counting. The number of bacteria bound to the mucosa was expressed as the percentage of the total bacteria infused in each loop. Percentage of adherence was calculated as the ratio $cpm(T) \times d \times 100/cpm(b)$, where $cpm(T)$ = counts per minute contributed by the adhering bacteria; $cpm(b)$ = counts per minute contained in the bacterial inoculum; and d = dilution factor.

Statistical Analysis. Adherence was expressed as the mean number of bacteria bound per enterocyte ± SD. Student's *t* test was used to test the significance of difference between the two means.

Results

***In Vitro* Adherence Studies.** The effect of various lectins on the adherence of strain S7471ϕF Fim$^-$ are summarized in Figure 1. Con A was the only lectin that promoted adherence of this strain of Salmonella to isolated enterocytes from rat small intestine. Cell viability was similar in control and Con A-treated enterocytes as measured by the exclusion of trypan blue and release of LDH into the medium. WGA, PNA, and PHA had little effect on bacterial adherence. Although no change in bacterial binding was observed with PHA-treated enterocytes, these cells did not exclude the dye after 20 min of incubation. Binding of strain S7471ϕF fim$^-$ in the presence of Con A followed a kinetic pattern that resembled that of fim$^+$ strains in the absence of lectin.

Similar results were obtained with other Salmonella strains (LT2 and CR6600) grown under conditions in which fimbriae are not expressed. Con A had little effect on adherence of strain S7471N. The binding of *S. typhimurium* S7471ϕF fim$^-$ to rat enterocytes was dose dependent: the number of bound bacteria per enterocyte increased with increasing concentrations of Con A (Fig. 2). The same studies indicated that even at 10 mg/ml, Con A did not induce aggregation of nonfimbriated bacteria, whereas aggregation of fimbriated cells was readily observed even at low concentrations of Con A (Table II).

Membrane fluorescence following binding of FITC-Con A to enterocyte surfaces showed a strong fluorescence, whereas nontreated enterocytes showed no noticeable fluorescence. The binding is specific since mannose or α-methylmannopyranoside at a concentration of 0.1 *M* reduced the binding of FITC-Con A by 60–70% as judged by the quantitative drop in fluorescence from 1.13×10^3 fluorescent units (in FITC-Con

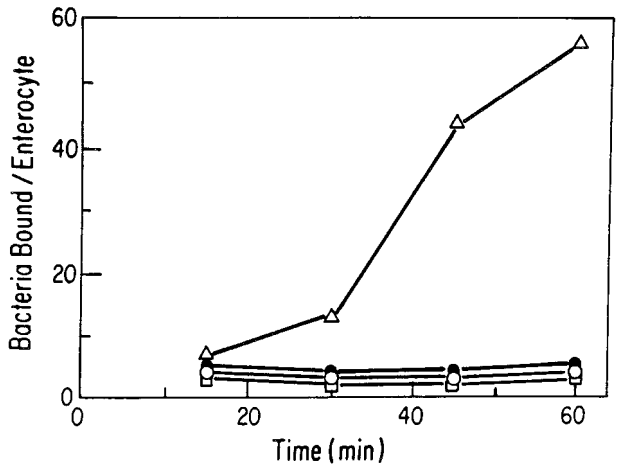

Figure 1. Effect of various lectins on the adherence of fim$^-$ *S. typhimurium* S7471ϕF to isolated rat enterocytes. □, PHA; ○, PNA; ●, WGA; △, Con A. Concentration of the lectins used in this experiment was 1.0 mg/ml. The values represent the mean number of bound bacteria after 10 experiments with Con A, and 3 experiments with other lectins. Similar results were obtained with higher concentrations of PHA, PNA, and WGA.

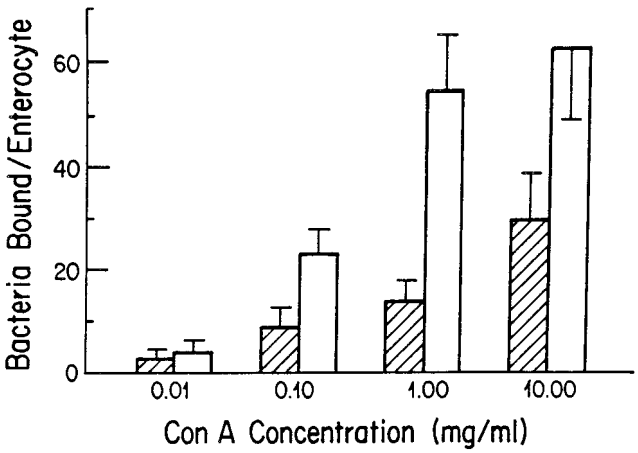

Figure 2. Dose response studies on Con A-induced adherence of fim$^-$ *S. typhimurium* S7471ϕF after 30 and 60 min of incubation. ▨, 30 min; □, 60 min. The values represent the mean number of bound bacteria per enterocyte after four experiments.

A alone) to 0.35×10^3 units (in FITC-Con A + mannose or α-methylmannopyranoside).

The specificity of con A-induced binding was determined by the addition of different carbohydrates before and during the incubation period (Table III). D-Mannose (1.0 mg/ml) added at time 0 inhibited the binding by 96%. Other carbohydrates (D-galactosamine and fucose) used in a similar concentration (1.0 mg/ml) showed a relatively low degree of inhibition compared to D-mannose.

To determine whether Con A-induced binding of Salmonella to rat enterocytes was reversible, D-mannose (1.0 mg/ml) was added at various times after the start of incubation. Con A was added at the same time as the bacteria. Figure 3 shows that the binding was partially reversible. Binding became progressively irreversible as the time of addition of Con A was delayed.

The influence of bacterial viability on binding was assessed by comparing the attachment of viable *S. typhimurium* to that of formalin-killed bacteria. Figure 4 shows that nonviable bacteria showed an initial binding phase, but the time-dependent additional increase was not observed.

***In Situ* Adherence Studies.** To determine whether the *in vitro* lectin-induced binding was of any physiologic significance, additional studies were carried out *in situ* with ligated loops. Results are summarized in Table IV. Con A-treated loops had an approximately 2-fold

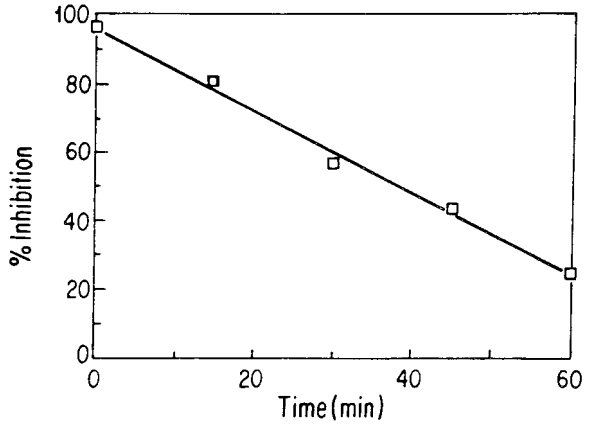

Figure 3. Time dependence of D-mannose addition on its inhibitory activity on Con a-induced adherence of *S. typhimurium* S7471φF. Experiments were run under standard conditions and D-mannose was added at 0, 15, 30, 45, and 60 min. Results are expressed as percentage of inhibition by D-mannose added at time 0. Concentration of D-mannose was 1.0 mg/ml.

Table II. Studies on Aggregation of *S. typhimurium* by Con A Using Light Microscopy (LM) and Radioassay (RA)[a]

Con A concentration (mg/ml)	*S. typhimurium* S7471φF			
	Fimbriated[b]		Nonfimbriated	
	LM	RA	LM	RA
0.01	−	−	−	−
0.1	−	+	−	−
1.0	+	++	−	−
10.0	+++	+++	−	−

[a] Bacteria were incubated with Con A for 15 to 120 min under standard conditions. Results are based on Con A-induced retention of aggregated bacteria in the polycarbonate filter.
[b] Relative degree of aggregation: −, none; +, low; ++, moderate; ++/+++, moderate to high.

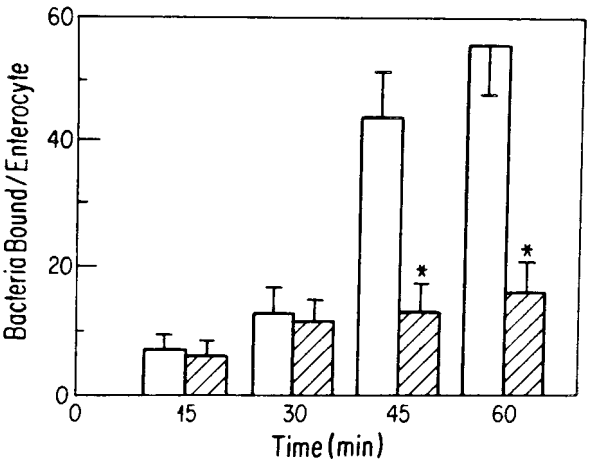

Figure 4. Effect of incubation time on Con A-induced adherence of viable and nonviable fim⁻ *S. typhimurium* S7471φF. □, viable bacteria; ▨, nonviable bacteria. Nonviable bacteria were obtained by treatment with 2% formalin. Note that after an initial adherence the time-dependent increase is not observed (*$P < 0.001$).

Table III. Effects of Various Carbohydrates on Con A-Induced Adherence of Non-Fimbriated *S. typhimurium* S7471φF to Enterocytes

Carbohydrate tested[a] (1.0 mg/ml)	Adherence[b]	% Inhibition
None	53 ± 5.2	0
D-Mannose	2 ± 0.3	96
D-Galactose	45 ± 2.7	15
Fucose	42 ± 2.8	21

[a] The tested carbohydrate was incubated at time 0 with Con A, bacteria, and enterocytes, and adherence assay was carried out under standard conditions.
[b] Adherence is expressed as the mean number ± SD of bound bacteria per enterocyte.

Table IV. Adherence of Fimbriated *S. typhimurium* S7471φF to Ligated Small Intestinal Loops

Group	Bacteria bound ($\times 10^6$)/cm (mean ± SD)	% Adherence[a]
Control	9.9 ± 3.7	1.99 ± 0.73
Con A	18.5 ± 5.6	3.70 ± 1.12[b]

[a] Results are expressed as percentage of the total bacteria infused in each loop.
[b] Significant difference ($P < 0.001$) between the Con A-treated loop and the control in the percentage of bacteria bound per centimeter of the intestinal segment.

higher percentage of *S. typhimurium* bound to the mucosa than untreated loops.

Discussion

Lectins are known to have deleterious effects in the gut, presumably by binding to carbohydrate structures present on the brush border membrane of the enterocyte (15). As postulated by Weiser (15), cells perturbed in this manner might possess an altered membrane structure and function. As a result, some lectins may be internalized and thereby impair metabolic processes, including protein synthesis. Such events might also alter the microenvironment in the immediate vicinity of the mucosal surface, hence promoting bacterial colonization and proliferation. The adherence of bacteria to enterocyte has been regarded as the crucial first step in the establishment of bacterial colonization. Thus, alteration of the enterocyte membrane surfaces might expose new binding sites for bacterial adhesins and facilitate bacterial binding. Alternatively, Wilson *et al.* (5) suggested that bacterial overgrowth in the small intestine due to lectin administration may have been a result of lectins mimicking the action of bacterial adhesins and promoting increased adherence of bacteria. It is quite possible that one or both of these mechanisms are operating in our system to account for the observed increase in adherence of Salmonella to enterocytes by Con A.

The present study demonstrated that Con A promoted the binding of phenotypically nonfimbriated strains of *S. typhimurium* to isolated small intestinal enterocytes. To the best of our knowledge, this is the first direct demonstration of lectin-induced bacterial adherence to intestinal epithelial cells. It is possible that lectins recognize the same ligands on the enterocyte surface as would bacterial adhesins. Most likely, lectin-induced adherence is due to lectin crosslinking bacteria to enterocyte. Polyvalent lectins may recognize similar receptors on bacteria as well as on the enterocyte, e.g., mannose residues located on the *O*-antigen of *S. typhimurium* and glycoproteins of the enterocyte. The ability of Con A to bind to fim$^-$ bacteria was shown by the binding of these cells to Con A linked to agarose beads, which was inhibitable by addition of D-mannose (unpublished observations). Our fluorescence studies using FITC-Con A also demonstrated the ability of Con A to bind to enterocyte surfaces.

Kinetics of Con A-induced binding of fim$^-$ bacteria to isolated enterocytes was similar to that reported for binding of fim$^+$ strains in the absence of lectin (8), suggesting that lectins may indeed mimic the action of fimbriae.

The action of Con A on the adherence of fim$^-$ *S. typhimurium* (S7471ϕF) to the small intestine was demonstrated in our *in situ* studies which showed an approximately 2-fold increase in bacteria bound to the intestinal mucosa. No difference could be observed on Con A-induced bacterial adherence between the proximal and distal segments of the small intestine using either isolated enterocytes or ligated intestinal loops.

Con A-induced adherence was apparently not related to changes in the membrane permeability as measured by exclusion of trypan blue and LDH release.

Lectins may have a direct and an indirect role on intestinal disease through their interaction with bacteria. In this respect, Banwell *et al.* (16) found that lectins such as PHA, administered to germ-free animals, do not seem to have the same antinutritional effects as those usually observed in animals with a normal intestinal flora. In addition, the same study reported increased numbers of *E. coli* colonies in the small intestine of rats after administration of PHA. Also, Jayne-Williams and Hewitt (17) found that the severity of the deleterious effects of dietary lectins is closely associated with the intestinal microflora. They demonstrated that Japanese quail chicks died within a few days when fed a diet containing raw navy beans, whereas the same diet was nonlethal to germ-free quail chicks.

Observations from the present study have important implications as adherence of bacteria to mucosal surfaces is an important factor in the pathogenecity of several human enteric pathogens (18, 19). Dietary lectins may enhance bacterial adherence. Several lectins have been found to be resistant to proteolytic digestion, e.g., WGA (20), tomato lectin (21), navy beans (22), and, even when cooked before eating are not completely destroyed (23, 24) or have been recovered intact from stool (3, 22, 24). A variety of lectins having different specificities to carbohydrate moieties have been shown to bind to a number of bacteria and other microorganisms (25). Given the significant exposure of the populace to these potentially harmful lectins, further studies are needed to provide additional information on their potential role in bacterial infection. Con A-induced adherence of *S. typhimurium* to small intestinal enterocytes should provide a useful model for future investigations to include other lectins and bacterial species.

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