Modulation of *Salmonella* infection by the lectins of *Canavalia ensiformis* (Con A) and *Galanthus nivalis* (GNA) in a rat model *in vivo*

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P.J. NAUGHTON, G. GRANT, S. BARDOCZ AND A. PUSZTAI. 2000. The plant lectins, Concanavalin A (Con A) and Galanthus nivalis agglutinin (GNA) have been prefed to rats for 3 d pre- and 6 d postinfection with *Salmonella typhimurium* S986 or Salm. enteritidis 857. Con A significantly increased numbers of Salm. typhimurium S986 in the large intestine and in faeces, and severely impaired growth of the rats, more severely than is the case of infection with *Salmonella typhimurium* alone. Con A had much less effect on rats infected with Salm. enteritidis 857 only showing a significant increase in numbers in the colon, accompanied by intermittent increases of *Salmonella* in the faeces during the study. GNA significantly reduced pathogen numbers in the lower part of the small bowel and the large intestine of rats infected with Salm. typhimurium S986 and significantly improved rat growth. GNA had little effect on infection by Salm. enteritidis 857 with slight decreases in Salmonella numbers in the small intestine and large intestine and transient increases in the faeces.

**INTRODUCTION**

Adherence is probably an important factor in bacterial colonization (Melhem and LeVerde 1984), and numerous surface carbohydrates of various forms on animal cells provide a plethora of attachment points for bacterial lectins (Karlsson *et al*., 1992; Sharon 1993). Cell surface-bound carbohydrate-binding proteins (animal lectins) may also act as attachment points for micro-organisms carrying the appropriate sugars on their surface (Sheriff *et al*., 1994). This mechanism is used by macrophages for nonopsonic phagocytosis for clearance of micro-organisms in tissues (Ofek *et al*., 1992). Cell surface lectins (Sharon and Lis 1989) are also involved in cell-to-cell attachment and/or signalling. Thus, lectins could be involved in the binding of bacteria to target cells by a variety of mechanisms. Numerous components have been proposed for inhibiting or promoting bacterial binding in the gastrointestinal (GI) tract. Various sugars (Allen *et al*., 1997), bacterial antigens (Thorns 1995), probiotics (MacFarlane and Cummings 1999) and symbiotics (Collins and Gibson 1999) have been investigated.

Plant lectins can bind to the GI tract *in vivo* and induce changes in the gut structure, glycosylation and function. Therefore, they have the potential to influence adherence and gut colonization by pathogens. Beuth *et al.* (1993) highlighted the importance of lectins in the prevention of bacterial infections. Work by Giannasca *et al.* (1996) suggests that one receptor exploited by *Salmonella* on Caco-2 cells is the Gal-β(1–3) GalNAc structure which is also recognized by peanut agglutinin (PNA). Consequently, PNA has been shown to inhibit *Salmonella* binding to Caco-2 cells. More recently Poschet and Fairclough (1999) have shown that a number of lectins can prevent the invasion of Caco-2 cells by *Salmonella in vitro*. Lectins may also interact directly with bacteria and can be been used for typing bacterial strains (Kellens *et al.* 1995) and for the targeted separation of Enterobacteriaceae *in vitro* (Porter *et al.* 1998).

The lectin GNA from the snowdrop (*Galanthus nivalis*) bulb is specific for terminal β-1-3-linked mannose. Orally administered GNA inhibited kidney bean lectin-induced *Escherichia coli* growth in the rat small intestine *in vivo* (Pusztai *et al.* 1990; Pusztai *et al.* 1993). GNA has, however, little or no effect on intestinal metabolism itself (Pusztai *et al.* 1995) and may therefore have uses in preventing or interfering with colonization by pathogenic micro-organisms.

Seeds of Jack bean (*Canavalia ensiformis*) contain Concanavalin A (Con A), a mannose/glucose binding lec-
tin. Con A can induce or inhibit cell-mediated lympholysis in murine lymphocytes in vitro (Sitkovsky et al. 1982) and can activate macrophages in vitro (Maldonado et al. 1994). Con A facilitates the clearance of Candida albicans by macrophages from tissues (Felipe et al. 1995). Summer and Howell (1936) showed that Con A agglutinated bacteria from the genera Mycobacterium and Actinomyces, a property of the lectin which has been repeatedly confirmed (Pistole 1981). Con A also binds to viruses such as Newcastle disease V4 strain (Rehmani and Spradbrow 1995) and has been shown to inhibit attachment of Salm. pullorum to chicken gut (Zhou et al. 1995) and to block attachment of Giardia lamblia trophozoites to Caco-2 cells (Katelaris et al. 1995). However, Con A has been found to promote adherence of Salmonella to isolated viable intestinal cells and to promote adherence to intestinal loops (Abud et al. 1989). The lectin might therefore promote bacterial adherence to the small intestine. The effects of dietary GNA and Con A on Salmonella infection have now been studied in vivo using a rat model.

**MATERIALS AND METHODS**

**Bacterial strains**

*Salm. enteritidis* 857 phage type 4 (Van Asten et al. 1995) and Salm. typhimurium S986 phage type 49 (Naughton et al. 1996) were used in this study. These wild type strains were grown under static conditions at 37°C for 48 h in nutrient broth (CM1, Unipath, Basingstoke, UK) (for studies in vivo).

**Lectins**

Concanavalin A (specificity D-mannose/glucose) was obtained from Sigma (St Louis, MO, USA). A strictly mannose-specific lectin, GNA, was isolated from snowdrop (Galanthus nivalis) bulbs by affinity chromatography on mannose-agarose (Van Damme et al. 1987).

**Lectin aggregation of Salmonella in vitro**

This method was based on the procedure of Abud et al. (1989). Bacterial cells were grown without shaking for 48 h in nutrient broth (CM1; Unipath) at 37°C. Bacterial cells were collected by centrifugation (1300 g, 10 min), washed twice and resuspended in PBS (pH7.4). The absorbance of the suspensions as measured at 620 nm in a spectrophotometer (Varian, Mountain View, CA, USA; DMS 200 u.v. visible spectrophotometer), and the suspensions were adjusted to an A620 equivalent of 5·0. Lectin solutions were prepared at a concentration of 1 mg ml$^{-1}$ (w/v) in PBS (pH7.4). Agglutination assays were carried out in glass tubes (12 by 75 mm). Assays consisted of 10 μl lectin and 90 μl cell suspension. A negative control consisted of 10 μl buffer instead of lectin solution. Sugar inhibition tests were carried out with 2 mol$^{-1}$ D-glucose and D-mannose. Tubes were left at room temperature (20°C) for 1 h and agglutination was viewed under the microscope.

The degree of aggregation was estimated visually as follows:

- 3+: 80–100% of cells clumped (under a microscope)
- 2+: 40–60% of cells clumped (under a microscope)
- 1+: 10–20% of cells clumped (under a microscope)
- tr: < 10% of cells clumped (under a microscope)

**Animal handling**

 Newly weaned 19 d-old male Hooded Lister-specific pathogen-free rats of the Rowett colony were housed singly in metabolism cages and fed a good quality semisynthetic diet based on lactalbumin (100 g kg$^{-1}$ diet) as the sole protein source (Grant et al. 1995). Food was freely available for 12 d. Once the rats reached 80±1 g, they were transferred to a flexi-film isolator (Moredun Animal Health, Edinburgh, UK) were kept singly in metabolism cages and fed the above diet at 6 g/rat d$^{-1}$ (given as two feeds over the day) for 4 days. Water was available *ad libitum*.

**Experimental procedures in vivo**

The small animal unit of the Rowett Research Institute is licensed under the UK Animals (Scientific Procedures) Act 1986, and its operation is regulated by both the animal welfare unit of the institute and the appropriate government inspectorate. All management and experimental procedures conducted during this study were done in strict accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986 by staff personally licensed under this act to perform such procedures.

Prior to infection with either *Salm. typhimurium* or *Salm. enteritidis*, the rats were prefed for 3 d either Con A or GNA incorporated into the control lactalbumin diet or control diet without lectin. The diet was given as two feeds of 3·3 g diet over each day. On day one the rats received 10 mg lectins and on days two and three, the rats received 20 mg lectin d$^{-1}$ in their diet. Overnight the lectin treated groups received lectin (2 mg ml$^{-1}$) in their drinking water. Control rats were given water without lectin. Control groups treated with Con A alone and GNA alone were included.

On the eve of the fourth day rats were fasted to reduce the effects of stomach pH and then given either 1 ml saline (controls) or 1 ml saline containing 10 mg lectin by intra-gastric intubation. Two hours later they were given 1 ml broth of either *Salm. enteritidis* 857 or *Salm. typhimurium
S986 (1 × 10⁸ CFU/ml⁻¹) by gavage. A further 1-5 h later the test group was fed 3.3 g control diet containing 20 mg lectin while the control group received 3.3 g control diet alone. This feeding protocol was repeated in the evening and for the remaining 5 d of the experiment. Lectin (2 mg ml⁻¹) were also available in the water throughout the experiment for the lectin-treated groups. On day 6, the rats were fed 1.5 g of diet with or without 10 mg of lectin. Post mortems were performed exactly 2 h later and the animals dissected. Tissues and organs were removed aseptically for the enumeration of bacteria.

Bacteriological examination

The tissues were weighed and homogenized in maximum recovery diluent (Unipath; CM733). A 10-fold dilution series was prepared for the homogenates to a final dilution of 1 × 10⁻⁷. Salmonella were enumerated on XLD agar (Unipath; CM469) after incubation at 37 ºC for 16 h. Rogosa agar (Unipath; CM627) was used for the enumeration of Lactobacilli, and a selective medium consisting of Columbia agar (Unipath; CM331) and 0.5% (v/w) propionic acid (Fisons, Loughborough, UK) was used for the isolation of Bifidobacteria. These cultures were incubated for 48 h at 37 ºC in an anaerobic cabinet (Don Whitley Scientific, Shipley, UK) and colonies were counted (Miles and Misra 1938). The presence of Salmonella was confirmed by specific antisera (Murex, Berkshire, UK) and API 20E (BioMerieux, Marcy l’Etoile, France). Lactobacilli and Bifidobacteria were presumptively identified by Gram-stain, their ability to grow on selective media, colonial morphology and catalase reaction. The detection limit was 500 cfu g⁻¹ wet weight or per ml. Spleen and liver samples were plated out according to the pour-plate method (Collins and Lyne 1995); the detection limit was 10 cfu g⁻¹ wet weight.

Statistics

Data were analysed using one-way analysis of variance by the Minitab computer program (Minitab, State College, PA, USA), and multiple comparisons were done by the Tukey test using Instat statistical package (Graphpad Software, San Diego, CA, USA).

RESULTS

Lectin aggregation of Salmonella in vitro

Two lectins, Con A and GNA were tested for their ability to aggregate Salmonella strains (Table 1). Most Salm. typhimurium strains we have tested were aggregated by Con A. In contrast, there was virtually no aggregation of these Salm. typhimurium strains by GNA. The Con A-mediated aggregation was due to the specific carbohydrate binding properties as binding of lectin to the bacteria was prevented by sugars. Aggregation of Salm. enteritidis occurred in a majority of strains examined in the presence of GNA (5/6) and Con A (4/6).

Effects of Con A in vivo

Apart from the colon there were no significant increases in the Salm. enteritidis 857 population in the GI tract of infected rats as a result of the inclusion of Con A in the diet (Table 2). Con A did not alter the Lactobacilli or Bifidobacteria populations (Lactobacilli (log₁₀ counts per gram: jejunum 3.1 ± 0.5, ileum 4.8 ± 1.2, caecum 6.3 ± 0.4, colon 6.0 ± 0.8. Bifidobacteria (log₁₀ counts g⁻¹): jejunum 2.8 ± 0.4, ileum 3.0 ± 0.7, caecum 4.3 ± 1.3, colon 4.0 ± 2.4) in noninfected control rats. Dietary Con A had a marked effect on Salm. typhimurium S986 infection (Table 2). Most tissues had increased numbers of Salmonella with differences in the numbers present in the caecum, colon and liver reaching significance (P < 0.05).

Effects of GNA in vivo

Inclusion of GNA in diets of infected rats did not significantly alter the numbers of Salm. enteritidis 857 in the gut or internal organs (Table 2). In contrast, GNA-treatment reduced numbers of Salm. typhimurium S986 in the ileum with levels reaching significance (P < 0.05) in the caecum and colon of infected rats (Table 2).

Effects of lectins on Salmonella numbers in the faeces

Control rats infected with Salm. typhimurium or Salm. enteritidis excreted high amounts of the pathogen in their faeces through the 6-d trial (Table 3). In both cases, faecal excretion of the bacteria tended to be further increased when Con A was included in the diet. Dietary GNA also tended to increase faecal excretion of Salm. enteritidis but appeared to have only transient effects on faecal outputs of Salmonella typhimurium.

Weight gain

Rats infected with Salm. typhimurium S986 grew more slowly than noninfected controls (Fig.1). This growth retardation was exacerbated when Con A was included in the diet of the infected rats. Indeed these rats grew little over the 6 d. In contrast, the growth rates of rats infected with Salm. typhimurium S986 were similar to those of noninfected controls when GNA was added to the diet. Salm.
Table 1  Aggregation of Salmonella by lectins

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serovar</th>
<th>Source</th>
<th>GNA</th>
<th>Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wharton pt104*</td>
<td>Salm. typhimurium</td>
<td>Chicken</td>
<td>tr</td>
<td>3+</td>
</tr>
<tr>
<td>2 Acres pt20*</td>
<td>Salm. typhimurium</td>
<td>Bovine</td>
<td>tr</td>
<td>tr</td>
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<tr>
<td>Gloucester pt1*</td>
<td>Salm. typhimurium</td>
<td>Human</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>Swindon pt32*</td>
<td>Salm. typhimurium</td>
<td>Human</td>
<td>tr</td>
<td>3+</td>
</tr>
<tr>
<td>S986 pt49*</td>
<td>Salm. typhimurium</td>
<td>Chicken</td>
<td>tr</td>
<td>3+</td>
</tr>
<tr>
<td>F98*</td>
<td>Salm. typhimurium</td>
<td>Chicken</td>
<td>tr</td>
<td>3+</td>
</tr>
<tr>
<td>195*</td>
<td>Salm. cholerasuis</td>
<td>Procline</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>76 Sa 88†</td>
<td>Salm. enteritidis</td>
<td>Chicken egg</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>The Neale*</td>
<td>Salm. typhimurium</td>
<td>Chicken</td>
<td>tr</td>
<td>2+</td>
</tr>
<tr>
<td>A50*</td>
<td>Salm. cholerasuis</td>
<td>Procline</td>
<td>tr</td>
<td>3+</td>
</tr>
<tr>
<td>181 Sa 91†</td>
<td>Salm. typhimurium</td>
<td>Bovine</td>
<td>tr</td>
<td>2+</td>
</tr>
<tr>
<td>Bangor 0T44*</td>
<td>Salm. typhimurium</td>
<td>Bovine</td>
<td>tr</td>
<td>1+</td>
</tr>
<tr>
<td>p132344*</td>
<td>Salm. enteritidis</td>
<td>Chicken</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>p125589*</td>
<td>Salm. enteritidis</td>
<td>Chicken</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Beau ville*</td>
<td>Salm. typhimurium</td>
<td>Chicken</td>
<td>tr</td>
<td>2+</td>
</tr>
<tr>
<td>S1339*</td>
<td>Salm. dublin</td>
<td>Bovine</td>
<td>tr</td>
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</tr>
<tr>
<td>1116*</td>
<td>Salm. typhimurium</td>
<td>Chicken</td>
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<td>2+</td>
</tr>
<tr>
<td>LAS‡</td>
<td>Salm. enteritidis</td>
<td>Chicken</td>
<td>3+</td>
<td>tr</td>
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<tr>
<td>Cambridge*</td>
<td>Salm. typhimurium</td>
<td>Turkey</td>
<td>tr</td>
<td>3+</td>
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<tr>
<td>857‡</td>
<td>Salm. enteritidis</td>
<td>Chicken</td>
<td>3+</td>
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<tr>
<td>WI118§</td>
<td>Salm. typhimurium</td>
<td>Human</td>
<td>tr</td>
<td>3+</td>
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<tr>
<td>SAL.33+¶</td>
<td>Salm. enteritidis</td>
<td>Human</td>
<td>tr</td>
<td>3+</td>
</tr>
<tr>
<td>TML§</td>
<td>Salm. typhimurium</td>
<td>Human</td>
<td>tr</td>
<td>3+</td>
</tr>
</tbody>
</table>

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Table 2  Viable Salmonella counts detected in tissues after 6 d

<table>
<thead>
<tr>
<th></th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Caecum</th>
<th>Colon</th>
<th>Spleen</th>
<th>Liver</th>
<th>MLN*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salm. enteritidis 857 (SE†)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SE + LA</td>
<td>3-1 ± 0-0</td>
<td>4-9 ± 0-3</td>
<td>5-3 ± 0-5</td>
<td>3-9 ± 0-7</td>
<td>3-0 ± 0-4</td>
<td>2-9 ± 0-4</td>
<td>4-8 ± 0-3</td>
</tr>
<tr>
<td>SE + GNA</td>
<td>3-5 ± 0-7</td>
<td>5-6 ± 0-3</td>
<td>4-6 ± 1-8</td>
<td>5-1 ± 1-4</td>
<td>3-4 ± 0-7</td>
<td>2-9 ± 0-5</td>
<td>5-2 ± 0-2</td>
</tr>
<tr>
<td>SE + Con A</td>
<td>3-9 ± 0-8</td>
<td>5-3 ± 0-2</td>
<td>5-7 ± 0-6</td>
<td>5-1 ± 0-7</td>
<td>3-2 ± 0-2</td>
<td>2-7 ± 0-6</td>
<td>5-0 ± 0-2</td>
</tr>
<tr>
<td>Salm. typhimurium S986 (STM‡)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>STM + LA</td>
<td>5-8 ± 0-7</td>
<td>6-8 ± 0-3</td>
<td>8-8 ± 0-9</td>
<td>8-4 ± 0-8</td>
<td>4-1 ± 0-6</td>
<td>4-1 ± 0-6</td>
<td>5-9 ± 0-4</td>
</tr>
<tr>
<td>STM + GNA</td>
<td>4-7 ± 0-3</td>
<td>6-4 ± 0-1</td>
<td>7-1 ± 0-8</td>
<td>6-5 ± 1-6</td>
<td>3-7 ± 1-9</td>
<td>3-6 ± 1-9</td>
<td>5-8 ± 0-1</td>
</tr>
<tr>
<td>STM + LA</td>
<td>4-5 ± 1-1</td>
<td>5-9 ± 1-0</td>
<td>7-1 ± 1-6</td>
<td>6-3 ± 1-7</td>
<td>3-6 ± 0-7</td>
<td>3-3 ± 0-6a</td>
<td>5-8 ± 0-2</td>
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<tr>
<td>STM + Con A</td>
<td>5-4 ± 0-3</td>
<td>6-8 ± 0-4</td>
<td>8-8 ± 0-3b</td>
<td>8-9 ± 0-4b</td>
<td>4-3 ± 0-4</td>
<td>4-3 ± 0-6b</td>
<td>6-3 ± 0-6</td>
</tr>
</tbody>
</table>

Values are means, log cfu g⁻¹ faeces (with S.D.). Groups (n = 5) of rats were gavaged with either Salm. enteritidis 857 or Salm. typhimurium S986 and fed on different diets: LA – control lactalbumin for 6 d; Con A or GNA were included in the diet for 3 d pre- and 6 d postinfection. For Salm. typhimurium each lectin treatment has a control. For each experimental group mean values with different superscripts in vertical columns are significantly different (P < 0.05).

*Mesenteric lymph nodes.
†SE–Salm. enteritidis.
‡STM–Salm. typhimurium.
ab represent significance at P < 0.05.
Effects of lectins alone in the diet and in the presence of *Salm. enteritidis* 857 impaired the growth of rats (Fig. 1). This was not affected by addition of Con A to the diet. However, GNA did cause a slight improvement in weight gain of rats infected with *Salm. enteritidis* 857.

Con A caused a slight (approximately 11%) but not statistically significant reduction in weight gains when fed to noninfected control rats (Fig. 1). GNA did not alter rat growth.

**DISCUSSION**

Adherence of bacteria to epithelial cells appears to be a key initial step in infection of the gut by a pathogen (Ofek and Sharon 1988). This attachment of the bacteria to enterocytes is primarily mediated by the fimbrial adhesins/lectins which recognize and bind to specific carbohydrate structures on the epithelial surface. Plant lectins, with the same carbohydrate specificity as bacterial lectins, can also bind to these carbohydrate structures on the gut epithelium. Thus, if they are present in the gut lumen in excess, they have the potential to modulate colonization by micro-organisms. Thus, we have shown that GNA reduces *Salm. typhimurium* numbers in *vivo*, a property previously shown for GNA but in a *Chlamydia trachomatis* infection in *vitro* (Amin et al. 1995). Con A has been shown to increase *Salmonella* numbers in the rat in *situ* (Abud et al. 1989). We have now shown that Con A increases *Salmonella* numbers in the rat in *vivo*.

**Aggregation of Salmonella by lectins in vitro**

Lectin–bacterial interaction studies have concentrated on the use of lectins as a means of characterizing bacterial populations or bacterial structures. Plant lectins are used routinely for mapping glycosylation patterns of mammalian tissues (Pusztai et al. 1995). Basu et al. (1994) used the sialic acid-binding lectin from *Cepaea hortensis* as a means of characterization of *Salm. djukaria* and *Salm. isaszeg*. Thus, it was not unexpected that we found certain lectins were capable of causing aggregation of *Salmonella*. The extent of aggregation was affected by changes in the carbohydrate composition of the bacterial membrane (Pusztai and Sharon 1988).

### Table 3 Viable *Salmonella* counts obtained in faeces samples taken on different days

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
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<tbody>
<tr>
<td><em><em>Salm. enteritidis (SE</em>)</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SE + LA</td>
<td>7.5 ± 0.6</td>
<td>5.8 ± 0.8</td>
<td>5.2 ± 0.6</td>
<td>5.7 ± 0.9</td>
<td>5.1 ± 0.8</td>
<td>4.6 ± 1.4</td>
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<tr>
<td>SE + GNA</td>
<td>5.7 ± 1.8</td>
<td>6.8 ± 1.0</td>
<td>7.6 ± 0.5b</td>
<td>6.6 ± 1.3</td>
<td>6.7 ± 0.5b</td>
<td>6.9 ± 1.3b</td>
</tr>
<tr>
<td>SE + Con A</td>
<td>7.4 ± 0.5</td>
<td>6.8 ± 1.1</td>
<td>6.4 ± 0.5b</td>
<td>7.0 ± 1.0</td>
<td>6.5 ± 0.5b</td>
<td>6.7 ± 0.8b</td>
</tr>
<tr>
<td><strong>Salm. typhimurium (STM†)</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STM + LA</td>
<td>7.7 ± 1.8</td>
<td>6.1 ± 0.7a</td>
<td>7.9 ± 1.1</td>
<td>7.1 ± 1.2</td>
<td>8.1 ± 0.9</td>
<td>7.9 ± 0.9</td>
</tr>
<tr>
<td>STM + GNA</td>
<td>7.7 ± 1.7</td>
<td>7.5 ± 0.8b</td>
<td>7.6 ± 1.1</td>
<td>6.6 ± 2.5</td>
<td>7.7 ± 1.6</td>
<td>7.5 ± 1.5</td>
</tr>
<tr>
<td>STM + LA</td>
<td>5.4 ± 0.6a</td>
<td>5.9 ± 0.8a</td>
<td>5.9 ± 0.6a</td>
<td>7.1 ± 1.2</td>
<td>6.8 ± 1.4</td>
<td>7.7 ± 1.8a</td>
</tr>
<tr>
<td>STM + ConA</td>
<td>8.3 ± 1.4b</td>
<td>7.8 ± 0.8b</td>
<td>8.6 ± 1.0b</td>
<td>7.9 ± 1.5</td>
<td>7.8 ± 1.3</td>
<td>9.8 ± 0.6b</td>
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</tbody>
</table>

Values are means, log cfu g⁻¹ faeces (with S.D.). Groups (n = 5) of rats were gavaged with either *Salm. enteritidis* 857 or *Salm. typhimurium* S986 and fed on different diets: LA – control lactalbumin for 6 d; Con A or GNA were included in the diet for 3 d pre- and 6 d postinfection. For *Salm. typhimurium* each lectin treatment has a control. For each experimental group mean values with different superscripts in vertical columns are significantly different (P < 0.05).

a SE – *Salm. enteritidis*.

b STM – *Salm. typhimurium*.

*ab* represent significance at P < 0.05.

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Fig. 1 Effects of lectins alone in the diet and in the presence of *Salmonella* on the weight gain of rats 6 d postinfection. Data are expressed as mean (n = 5) with S.D. of mean.
the aggregation was highly dependent on the specificity of the lectin used. We found that GNA did not aggregate the Salm. typhimurium strains tested in this study but did bind to Salm. enteritidis strains (Table 1). In contrast, Con A caused the aggregation of most, but not all strains of Salm. typhimurium and Salm. enteritidis tested in this study.

Con A, which is mannose/glucose specific, aggregates a variety of Gram-negative bacteria especially Salmonella spp. (Le Minor et al. 1973). The sites of Con A-binding are thought to be the exposed sugars of the bacterial lipopolysaccharide and the O-antigen factor 1 (Liener 1976). Early studies on Con A reactivity showed that the lectin bound to lipopolysaccharide from some Enterobacteriaceae but not that from others (Doyle et al. 1968). Since Con A aggregated Salmonella strains and was also found to increase the attachment of Salm. typhimurium to gut epithelium in situ (Abud et al. 1989), the effects of Con A on gastrointestinal infection merited study.

Effect of lectins on rat growth

Con A (20 mg rat\(^{-1}\) \(\text{d}^{-1}\)) caused a slight reduction in weight gain when it was fed to noninfected rats whereas GNA (20 mg rat\(^{-1}\) \(\text{d}^{-1}\)) had little effect on growth. This was consistent with earlier findings with higher dietary intakes of these lectins (Oliveira et al. 1994; Pusztai et al. 1996).

Modulation of Salmonella infection in vivo

Con A appeared to increase the severity of infection in rats dosed with strain Salm. typhimurium S986 but did not alter the numbers of Salm. enteritidis 857. Con A is highly resistant to proteolysis in vivo and is stable during its passage through the gastrointestinal tract (Nakata and Kimura 1985). At high levels in the diet it disturbs brush border membrane function and interferes with reformation of brush border membranes (Nakata and Kimura 1986). There may also be some cytotoxic effects (Lorenz-Meyer et al. 1985), particularly in young animals (Weaver and Bailey 1987). The lectin binds to brush borders in vitro (Etzler and Branstrator 1974) and in vivo and thus leads to increased shedding of brush border membranes, accelerated cell loss and slight villus atrophy (Lorenzsonn and Olsen 1982). These changes require the presence of bacteria in the gut since Con A has been shown to have no effect on the internal structure and function of germ-free animals (Pusztai et al. 1995).

Some polyvalent lectins can recognize receptors on bacteria as well as on the enterocyte, such as mannose residues located on the O-antigen of Salm. typhimurium and glycoproteins on the enterocyte (Alexander 1981). For example, afimbriate bacteria were shown to bind to Con A-agarose beads in vitro (Abud et al. 1989). Bar-Shavit and Goldman (1976) have shown Con A-mediated attachment and ingestion of yeasts cells by macrophages. Gallily et al. (1984), studied the wheat germ agglutinin-mediated uptake of bacteria by murine peritoneal macrophages. Con A may therefore form a bridge between the brush border and the bacterium and thus promote bacterial adherence. The observation that the presence of Con A in the diet did not alter numbers of lactobacilli or bifidobacteria populations suggests that the increase in Salm. typhimurium S986 numbers may be specific, at least to this serotype.

Con A can stimulate histamine release and activation of epidermal growth factor (EGF). Con A binds to the EGF receptor on mammalian cells (Carpenter and Cohen 1977) and inhibits the dimerization and tyrosine phosphorylation of the receptor leading to inhibition of mitogenicity of EGF (Hazen et al. 1995). Salm. typhimurium has similar effects on many of these pathways (Galán et al. 1992). Con A and Salm. typhimurium S986 by acting together may have a synergistic effect on the mechanism by which colonization and invasion take place and thereby increase infectivity. This is borne out by a further significant reduction in the weight gain of rats infected with Salm. typhimurium S986 by giving Con A in the diet (Fig. 1).

Previous studies in this laboratory have shown that the incorporation of GNA alone in the diet of rats at 42 mg rat\(^{-1}\) \(\text{d}^{-1}\) for 6 d did not alter the numbers of lactose fermenters, non-lactose fermenters or lactobacilli from LA control levels (Pusztai et al. 1993). Far from having a deleterious effect, GNA reduced Salm. typhimurium S986 numbers in the ileum, caecum and colon (Table 2). GNA has a selective specificity for terminal mannose structures. High-mannose glycans are ubiquitous components of bacterial pathogens, parasites, yeasts and some viruses (Ezekowitz and Stahl 1988), and are also present in many other cells particularly immature or damaged cells. It is therefore possible that GNA can agglutinate Salmonella in vivo and in so doing, prevent attachment to the substratum. However, we have shown in the case of Salm. typhimurium S986 that GNA does not aggregate the bacterium in vitro (Table 1) whereas it will aggregate Salm. enteritidis 857. Therefore the ability of GNA to decrease the numbers of Salm. typhimurium S986 is unlikely to be due to the aggregation of bacteria. It is possible that GNA may alter cellular metabolism and reduce the level of expression of enterocyte membrane glyconjugates essential for attachment of Salm. typhimurium S986.

Salm. typhimurium S986 causes major changes to gut epithelium. In particular it triggers high rates of epithelial cell division and turnover which can then facilitate infection by the bacteria (Naughton et al. 1995). GNA may bind to these mannose-rich sites on enterocytes and thus prevent association of the bacterium, alternatively GNA may slow down the rate of cell turnover and limit the
damaging effects of *Salm. typhimurium* S986 by reducing the numbers of immature cells reaching the villus surface.

**CONCLUSIONS**

The lectins studied had different effects on infection by *Salmonella*. Con A appeared to promote adherence by *Salm. typhimurium* S986 whereas GNA reduced adherence of the pathogen. This suggests that lectins are likely to be selective in their effects on the gut flora. Specific lectins might be used to promote or suppress particular bacterial types within the gut flora and thereby improve the health of the host.

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