Effect of Carbohydrates on Adherence of *Escherichia coli* to Human Urinary Tract Epithelial Cells

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Adherence of *Escherichia coli* cells to voided uroepithelial cells from healthy women was measured by use of [3H]uridine-labeled bacteria filtered through a polycarbonate membrane filter (5-μm pore size). At a concentration of 2.5% (wt/vol), D-mannose, D-mannitol, α-methyl-D-mannoside, and yeast mannan completely inhibited adherence of the bacteria to the epithelial cells. At this same concentration, D-fructose, D-lyxose, D-arabinose, and D-glyceraldehyde partially inhibited adherence. Reducing the concentration of D-mannose, or its derivatives, to between 1.0 and 0.1% resulted in partial inhibition in the adherence of the bacteria; a further reduction in the concentration to between 0.01 and 0.001% caused an enhancement of adherence up to 160% of the control level. Bacterial preincubation in 2.5% D-mannose for 1 min before epithelial cells were added completely inhibited adherence; similar treatment of the epithelial cells had no significant effect on subsequent adherence of the bacteria. Bacteria that were preincubated for 1 h with D-mannose at concentrations between 0.1 and 0.75% showed enhanced adherence. The inhibitory effect of D-mannose was decreased if bacterial adhesive ability, or cell receptivity, increased. A variety of other carbohydrates tested had no effect on the adherence of *E. coli* to the uroepithelial cells. These results suggest that adherence can be altered by interaction(s) between specific carbohydrate molecules and receptors on the bacterial surface.

Attachment of bacteria to mucosal surfaces of a susceptible host is now recognized as an important step in colonization and infection (11, 21, 25). The ability of bacteria to adhere to epithelial cells in vitro appears to be mediated by the surface components of both cell types. To probe the biochemical nature of the structures involved, investigators have focused on the sensitivity of bacterial agglutinins to carbohydrates which compete with identical or related structures on the cell surface (5, 6, 17). Studies showing that D-mannose or its derivatives specifically inhibit adherence of *Escherichia coli* to epithelial cells support the hypothesis that *E. coli* ligands can recognize and bind D-mannose or D-mannose-like receptors on the epithelial cell surface (8, 13, 16, 20). Recent identification of D-mannose-insensitive strains of *E. coli* suggests that other bacterial ligands may also mediate attachment to epithelial cells (4, 9).

Investigations of *E. coli* strains isolated from urine suggest that several adherence factors are instrumental in the attachment of bacteria to human uroepithelial cells. Svannborg Edén and Hansson (26) demonstrated D-mannose- and α-methyl-D-mannose-sensitive *E. coli* agglutination to guinea pig erythrocytes, but D-mannose-insensitive adherence to uroepithelial cells. In subsequent studies which assessed adherence with radioisotopic rather than visual techniques, the addition of 2.5% D-mannose to *E. coli*-uroepithelial cell suspensions completely inhibited adherence, even when the epithelial cells were highly receptive (22). The purpose of this study was to further define D-mannose-sensitive adherence by investigating the effect of various carbohydrates on the adherence of *E. coli* to human uroepithelial cells. The results support the concept that adherence can be altered by specific carbohydrate molecules which compete with epithelial cell receptors for bacterial binding sites. This complex interaction is influenced by the ability of bacteria to adhere, the epithelial cell receptivity, and the concentration and time of exposure to the carbohydrates.

**MATERIALS AND METHODS**

**Bacteria.** The *E. coli* strain used in all experiments was isolated from infected urine. It was serotyped (O18) by the method of Brent and Vosti (3). The bacteria were grown in brain heart infusion broth (Difco Laboratories) for 72 h, harvested by centrifugation, and washed once in phosphate-buffered saline (Difco; pH 7.2). Variation between assays was minimized by suspending the bacteria in brain heart infusion broth plus dimethyl sulfoxide (Fisher Scientific Co.) at a final concentration of 8% (vol/vol) and freez-
RESULTS

Cell storage technique. The effect of the storage technique on adherence and viability was investigated by performing the assay with fresh cells and subsequently with samples from the same cell pools after the addition of dimethyl sulfoxide freezing. Adherence and viability were not affected when cells were stored in dimethyl sulfoxide at −20°C for up to 3 months. Cells stored at 4°C or in glycerol showed decreased adherence and viability.

Inhibition of adherence by carbohydrates and lectins. To determine the most effective carbohydrate and lectin inhibitor of bacterial binding to uroepithelial cells, we made suspensions of *E. coli* in MEM with twice the final carbohydrate concentration. After 1 min, the suspensions were diluted in half by the addition of the epithelial cells. Bacterial adherence was compared with that in nontreated controls. None of the compounds used were toxic to the epithelial cells, as determined by trypan blue exclusion, or to the bacteria, as determined by growth curves. Maximal inhibition of bacterial adherence occurred with yeast mannann and the six-carbon carbohydrates D-mannose and D-mannitol (Table 1). A threefold increase in the molar concentration of α-methyl-D-mannoside or D-fructose was required to bring about the same inhibitory effect. Five-carbon (D-arabinose, D-lyxose) and three-carbon (L-glyceraldehyde) carbohydrates were less effective inhibitors of bacterial adherence and required a 6-fold and 13-fold increase, respectively, in their concentrations to produce the 50% inhibition achieved with D-mannose or D-mannitol.

Adherence was inhibited completely by all carbohydrates with the hydroxyl group at carbon 2 in the same configuration as D-mannose.

<p>| Table 1. Inhibition of <em>E. coli</em> adherence to uroepithelial cells by carbohydrates or ConA |
|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th><strong>Compound</strong></th>
<th><strong>Concn required for 50% inhibition (mM)</strong></th>
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<tbody>
<tr>
<td>Six-carbon</td>
<td></td>
</tr>
<tr>
<td>D-Mannose</td>
<td>27.8</td>
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<tr>
<td>D-Mannitol</td>
<td>27.4</td>
</tr>
<tr>
<td>α-Methyl-D-mannoside</td>
<td>77.0</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>83.0</td>
</tr>
<tr>
<td>Five-carbon</td>
<td></td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>166.0</td>
</tr>
<tr>
<td>D-Lyxose</td>
<td>166.0</td>
</tr>
<tr>
<td>Three-carbon</td>
<td></td>
</tr>
<tr>
<td>L-Glyceraldehyde</td>
<td>350.0</td>
</tr>
<tr>
<td>Yeast mannann</td>
<td>0.05%*</td>
</tr>
<tr>
<td>ConA</td>
<td>0.0093</td>
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</table>

* Molecular weight not determined.
with the exception of L-fucose, which had no inhibitory effect. All carbohydrates with the hydroxyl group at carbon 2 in the opposite configuration (D-glucose, L-mannose, α-methyl-D-glucoside, L-rhamnose, D-ribose, D-galactose, D-xylose, D-glyceraldehyde, dihydroxyacetone) were ineffective inhibitors of adherence even at concentrations of 2.5%. The plant lectin ConA, which binds to D-mannose (23), was also a very effective inhibitor of adherence.

Effect of preincubation of E. coli or uroepithelial cells with MEM, D-mannose, or ConA. The site of D-mannose and ConA binding was investigated by adding these compounds to the bacterial or epithelial cell suspensions prior to performing the adherence assay. After a 30-min preincubation, the E. coli or uroepithelial cell suspensions were washed once with phosphate-buffered saline and then incubated with untreated epithelial or bacterial cells, respectively. The adherence was compared with that of control cells preincubated in MEM (Table 2). Preincubation of the bacteria with D-mannose completely prevented their adherence to epithelial cells, whereas preincubation of bacteria with ConA was much less inhibitory. When epithelial cells were preincubated with ConA, subsequent E. coli adherence was inhibited by 92.6%, whereas preincubation of epithelial cells with D-mannose inhibited adherence by 29.9%.

Influence of variation in bacterial or epithelial cell adhesive characteristics on D-mannose inhibition. The possibility that cell adhesive characteristics alter the inhibiting effect of D-mannose was investigated by incubating E. coli cells exhibiting different degrees of receptivity with a common epithelial cell pool and a fixed concentration of D-mannose. One strain of E. coli was repeatedly transferred in brain heart infusion broth to select for presumably heavily piliated organisms (7). Adherence to epithelial cells from a common pool increased from 8 to 80 bacteria per cell and was completely inhibited by 2.5% D-mannose. As shown in Fig. 1, inhibition by 0.1% D-mannose ranged from 1% for bacteria that were good adherers (presumably heavily piliated) to 100% for bacteria that adhered poorly. The possibility that the adhesive characteristics of epithelial cells alter the inhibiting effect of D-mannose was investigated by incubating epithelial cells with different degrees of receptivity with the same E. coli strain and a fixed concentration of D-mannose. Epithelial cells from different individuals have various degrees of receptivity (22). Epithelial cells were collected from several individuals, pooled, and tested with the same strain of E. coli to determine their receptivity. Adherence for the cell pools collected at different times ranged from 13 to 93 bacteria per cell and was completely inhibited by 2.5% D-mannose. As shown in Fig. 2, inhibition by 0.1% D-mannose ranged from 100% for cells with poor receptivity to 45% for cells to which large numbers of bacteria adhered in the absence of mannose.

Effect of various concentrations of carbohydrates on adherence. E. coli and epithelial cells were incubated with D-mannose, D-mannitol, α-methyl-D-mannoside, D-fructose, or D-glucose at concentrations of 2.5 to 0.001%. Depending on the carbohydrate, adherence could be completely inhibited, partially inhibited, or enhanced. For example, when the concentrations of D-mannose added to the suspensions of bacteria and epithelial cells was varied, adherence could be completely inhibited (2.5%), partially inhibited (between 1.0 and 0.1%), or significantly enhanced (between 0.01 and 0.001%) to up to 160% of control levels (P

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<table>
<thead>
<tr>
<th>Table 2. Effect on adherence of preincubation of E. coli or uroepithelial cells with MEM, D-mannose, or ConA</th>
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<tbody>
<tr>
<td>Cells and preincubation medium</td>
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<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
</tr>
<tr>
<td>MEM</td>
</tr>
<tr>
<td>D-Mannose (1%)</td>
</tr>
<tr>
<td>Concanavalin A (0.05%)</td>
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<tr>
<td><strong>Uroepithelial cells</strong></td>
</tr>
<tr>
<td>MEM</td>
</tr>
<tr>
<td>D-Mannose (1%)</td>
</tr>
<tr>
<td>ConA (0.05%)</td>
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*After a 30-min preincubation, the E. coli or uroepithelial cells were washed once with phosphate-buffered saline and then incubated with untreated uroepithelial or bacterial cells, respectively. The results are representative of three experiments.

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**Fig. 1. Influence of variation in E. coli adhesive characteristics on D-mannose (0.1%) inhibition of adherence.** Repeated subculturing of E. coli increased adherence to uroepithelial cells from a common pool from 8 to 80 bacteria per cell. Each point represents the mean of two experiments.
< 0.05, Fig. 3). It is important to note that glucose had no inhibitory or enhancing effect. The curves for α-methyl-D-mannoside and D-mannitol were similar to the D-mannose curve shown in Fig. 3, except that no enhancement was observed for D-mannitol.

The possibility that interactions of carbohydrates might modify their effects on bacterial adherence was investigated by adding combinations of inhibitory and non-inhibitory carbohydrates to the adherence assay. The combined effect of inhibitory carbohydrates appeared to be additive. For example, 1% D-mannose or 1% D-fructose inhibited adherence by 80% and 20%, respectively. When combined, 1% D-mannose and 1% D-fructose showed 100% inhibition. Non-inhibitory carbohydrates had no effect on the degree of inhibition of an inhibitory carbohydrate. When 1% glucose (which had no inhibitory effect on adherence) was added to 1% D-mannose or 1% D-fructose, the inhibitory effect was the same as that of 1% D-mannose or 1% D-fructose alone.

Effect of time of bacterial preincubation with D-mannose on adherence. The possibility of increasing the inhibitory effect of D-mannose was investigated by increasing the time of bacterial preincubation with various concentrations of the carbohydrate prior to the addition of uroepithelial cells. Adherence was compared with that observed for bacteria preincubated for the same length of time in MEM. As shown in Fig. 4, complete inhibition was observed with 2.5% D-mannose preincubated with bacteria for 30 or 60 min. Initially, concentrations of D-mannose (between 1.5 and 0.75%), adherence increased significantly to levels 120 to 260% of the control (P < 0.05). With 0.001% D-mannose, the initial level of enhanced adherence (190% of control) was maintained. The possibility that higher levels of adherence were due to an increased bacterial concentration was excluded by the observation that the colony-forming units remained virtually constant during the time of preincubation. Furthermore, bacteria preincubated in 2.5% glucose or in MEM for 60 min showed no increase in adherence compared with control bacteria that had not been preincubated. Lastly, the possibility of enhanced bacterial adherence through mannose metabolism was investigated. Preincubation of E. coli for 5 min with α-methyl-D-mannoside produced levels of enhancement similar to those observed for D-mannose, even though growth curves demonstrated that the test strain could not use α-methyl-D-mannoside as a sole carbon source.

DISCUSSION

Further insight into the stereochemical specificity of the bacterial ligand can be obtained by examining the extent to which a large number of monosaccharides, yeast mannan, and ConA inhibit the adherence of bacteria to uroepithelial cells. The carbohydrate combining site of the E. coli ligand investigated in these experiments appears to be directed primarily toward the unmodified hydroxyl group at carbon 2 of D-mannose and its derivatives. Thus, D-mannose, D-

![Fig. 2. Influence of variation in uroepithelial cell receptivity on D-mannose (0.1%) inhibition of adherence. Epithelial cells from different individuals have various degrees of receptivity. Adherence of the same strain of E. coli to epithelial cell pools from different individuals varied from 13 to 93 bacteria per cell. Each point represents the mean of two experiments.](http://iai.asm.org/)

![Fig. 3. Effect of various concentrations of D-mannose (●), D-fructose (□), and D-glucose (○) on adherence of E. coli to uroepithelial cells. Each point represents the mean ± standard deviation of four experiments.](http://iai.asm.org/)
mannitol, and yeast mannan (a polysaccharide that is polymer of mannose) were potent inhibitors of *E. coli* adherence to uroepithelial cells in vitro. D-Fructose was a twofold less effective inhibitor of adherence than D-mannose. Although the open-chain configuration of D-fructose has an aldehyde group at the second carbon, in solution rearrangement occurs such that D-fructose assumes the pyranose form with the same configuration as D-mannose at carbon 2 (12, 18). Although L-fucose has the same configuration at carbon 2 as D-mannose, it did not inhibit adherence. These findings are in agreement with those of other investigators who have reported inhibition with D-mannose and no inhibition with L-fucose (2, 15, 17, 19, 20). This could be due to the replacement of the hydroxyl group on carbon 6 with a hydrogen atom. Any modification of the carbon 2 hydroxy group completely abolished the inhibitory action of the carbohydrate. Thus D-glucose, which differs from D-mannose only in the hydroxy group at carbon 2, did not inhibit adherence even at a concentration of 2.5%. The length of the carbon chain also appears to be an important determinant of the inhibitory effect of a carbohydrate. Five-carbon and three-carbon carbohydrates, with the same configuration as D-mannose at the second carbon, were 6-fold and 13-fold less effective inhibitors of adherence, respectively. Presumably, the pyranose ring of D-mannose is not active in this interaction because the open-chain hexitol D-mannitol was a very effective inhibitor of adherence. The α linkage also seems to be unimportant since the α-methyl glycoside of D-mannose reduced the inhibitory effect observed for D-mannose and the α-methyl glycoside of D-glucose had no effect on adherence.

The concept that the hydroxyl group at carbon 2 in the D-mannose configuration may interfere with binding of *E. coli* to uroepithelial cells is supported by the inhibitory effect of D-mannose on *E. coli* adherence to buccal cells (16), renal cells (20), erythrocytes (27), and leukocytes (2, 13). Other investigators, using mannose-sensitive *E. coli* strains (2, 15, 20, 27), have reported results on the inhibitory effect of other carbohydrates, and even the mannose derivatives, which differ from our findings. These inconsistencies may be attributable to differences in the specific surface characteristics of the bacteria, as well as the eucaryotic cells, that were used.

Preincubation of bacteria with D-mannose completely inhibited their subsequent binding to epithelial cells. Conversely, preincubation of epithelial cells with the mannose-binding lectin ConA significantly reduced their receptivity by 92.6%. These observations support the concept that attachment of *E. coli* to uroepithelial cells is mediated by a mannose-specific bacterial ligand which binds to mannose or mannose-like receptors on the epithelial cell surface. Studies with ConA suggest that mannose is widely distributed on the surface of other mammalian cells (23) and may be an integral part of the receptor site required for *E. coli* attachment to epithelial cells (2, 16).

When bacteria were incubated in broth to increase their binding capacity (and presumably pilation), the inhibitory effect of a fixed concentration of D-mannose was inversely proportional to the degree of bacterial adherence. These results suggest that the mannose-binding ligand can be altered by growth conditions which are conducive to increased piliation formation and may be associated with pil. Ofek and Beachey (15) showed that the bacterial population which adhered to epithelial cells consisted of heavily piliated cells that possessed increased mannose binding activity, and Salit and Gotschlich (20) demonstrated mannose-sensitive binding of purified *E. coli* type I pili to monkey kidney cells. More recent evidence suggests, however, that other mannose-sensitive adhesions, biochemically distinct from type I pili, may also be involved in the mannose-specific adherence of *E. coli* to eucaryotic cells (8, 13). Mannose-insensitive adhesions also appear to participate in *E. coli*–epithelial cell interactions. Svanborg Eden (26) described mannose-insensitive, pilus-associated adherence of *E. coli* to uroepithelial cells, and mannose-insensitive binding of enteropath-
ogenic E. coli to intestinal cells has been identified (4, 10).

We also observed that the inhibitory effect of D-mannose was influenced by the degree of receptivity of the epithelial cells. When 0.1% D-mannose was added to incubation systems containing the same bacteria and epithelial cells with different degrees of receptivity, the inhibitory effect of D-mannose decreased as the receptivity of the epithelial cells increased. This suggests that D-mannose inhibits bacterial adherence by competing with mannose-like receptors on the epithelial cell surface and that there may be a difference among uroepithelial cells in the number of receptor sites or the degree of affinity, or both, that these receptors have for bacteria.

The inhibitory effect of D-mannose, and its derivatives, on the adherence of E. coli to uroepithelial cells was dose related and was linear between concentrations of 2.5 and 1%. The observation that adherence was enhanced by low concentrations (between 0.01 and 0.001%) of D-mannose, D-fructose, or α-methyl-D-mannoside is intriguing and may be representative of physiological conditions. That this phenomenon was due to the production of additional receptor sites on the epithelial cells is unlikely because only approximately 20% of the epithelial cells used in the assay were viable. Furthermore, enhancement was observed when the bacteria were preincubated in D-mannose prior to incubation with the epithelial cells. It also seems unlikely that bacterial metabolism of a carbohydrate during preincubation is necessary for enhancement since enhancement was observed after preincubation with α-methyl-D-mannoside, a carbohydrate that cannot be utilized as a sole carbon source by the E. coli strain used in these experiments. Enhancement of adherence in the presence of 0.001% D-mannose or after prolonged bacterial preincubation with 0.75 to 1.5% D-mannose may be due to cross-linking of bacteria prior to their attachment to the epithelial cell receptors. Thus, many bacteria could attach to a single binding site, increasing the total number of bacteria per epithelial cell. Cross-linking could be due to nonspecific effects of mannose, such as alterations in the electrostatic charge of pili, which make the bacteria more susceptible to binding with epithelial cell receptors. Alternatively, bacteria could link themselves to one another through shared mannose molecules. McMichael and Ou (14) observed that piliated E. coli cells formed aggregates in the presence of lysozyme (one lysozyme molecule per two pili subunits) or divalent cations such as Ca²⁺ or Mg²⁺. Although high concentrations of mannose might induce maximal cross-linking of bacteria, all available bacterial ligands would be blocked, thereby resulting in complete inhibition of adherence to the epithelial cells.

The relationship between mannose-mediated adherence of E. coli to uroepithelial cells and urinary tract infections is not clear, but some studies have suggested that it may have clinical significance. Duguid (6) screened 108 strains of E. coli isolates from humans by hemagglutination and mannose-sensitive inhibition of hemagglutination. He found that the majority (74%) of the strains possessed mannose-binding activity and that all of the mannose-sensitive strains were pilated. Aronson et al. (1) found that α-methyl-D-mannoside prevented urinary tract infections with E. coli in mice, presumably by blocking bacterial adherence to the urinary tract. Although it appears that receptors other than mannose could bind E. coli to uroepithelial cells, continued investigation of the mannose-binding activity of E. coli to uroepithelial cells should greatly increase our insight into the pathogenesis and treatment of urinary tract infections.

ACKNOWLEDGMENTS

We are grateful to Byron Anderson, Department of Biochemistry and Otolaryngology and Maxillofacial Surgery, Northwestern University Medical School, for helpful advice. This investigation was supported by Public Health Service grant RR-05370 from the National Institutes of Health.

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