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# Inhibitory Activity of Cranberry Juice on Adherence of Type 1 and Type P Fimbriated *Escherichia coli* to Eucaryotic Cells

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**Inhibition of bacterial adherence to bladder cells has been assumed to account for the beneficial action ascribed to cranberry juice and cranberry juice cocktail in the prevention of urinary tract infections (A. E. Sobota, J. Urol. 131:1013–1016, 1984). We have examined the effect of the cocktail and juice on the adherence of *Escherichia coli* expressing surface lectins of defined sugar specificity to yeasts, tissue culture cells, erythrocytes, and mouse peritoneal macrophages. Cranberry juice cocktail inhibited the adherence of urinary isolates expressing type 1 fimbriae (mannose specific) and P fimbriae [specific for α-D-Gal(1→4)-β-D-Gal] but had no effect on a diarrheal isolate expressing a CFA/I adhesin. The cocktail also inhibited yeast agglutination by purified type 1 fimbriae. The inhibitory activity for type 1 fimbriated *E. coli* was dialyzable and could be ascribed to the fructose present in the cocktail; this sugar was about 1/10 as active as methyl α-D-mannoside in inhibiting the adherence of type 1 fimbriated bacteria. The inhibitory activity for the P fimbriated bacteria was nondialyzable and was detected only after preincubation of the bacteria with the cocktail. Cranberry juice, orange juice, and pineapple juice also inhibited adherence of type 1 fimbriated *E. coli*, most likely because of their fructose content. However, the two latter juices did not inhibit the P fimbriated bacteria. We conclude that cranberry juice contains at least two inhibitors of lectin-mediated adherence of uropathogens to eucaryotic cells. Further studies are required to establish whether these inhibitors play a role in vivo.**

Bacterial adherence to mucosal cells is an important step in the development of infection (3). This has been amply demonstrated, especially for urinary tract infections (19, 28). Since the adherence of many bacterial species to epithelial cells is mediated by lectin-sugar interactions (21, 31), consumption of foods containing lectins or carbohydrates might affect the infection process (15, 25).

There is a wealth of anecdotal evidence, as well as several published reports, on the use of cranberry juice and cranberry juice cocktail for the prevention of recurrent urinary tract infections (4, 23, 27, 35). In an attempt to clarify the possible mode of action of cranberry juice, Sobota (34) has demonstrated that it inhibits the adherence of urinary tract isolates of *Escherichia coli* to human buccal and uroepithelial cells. The strains used by Sobota were, however, not defined with respect to the type of fimbriae they might have expressed or the sugar specificities of these fimbriae. *E. coli* is the most frequent urinary isolate from patients with urinary tract infection (19). Virtually all *E. coli* isolates are capable of expressing a mannose-specific lectin associated with type 1 fimbriae, which mediates the adherence of the bacteria to uroepithelial cells (6, 9, 32). In addition to type 1 fimbriae, most pyelonephritogenic isolates of *E. coli* express an α-Gal(1→4)β-Gal (abbreviated as Gal-Gal)-specific lectin associated with P fimbriae, which also mediates the adherence of the bacteria to uroepithelial cells (20, 38) (all sugars are of the D configuration). We therefore undertook this investigation to examine the effect of cranberry juice or cocktail and its constituents on the adherence to eucaryotic cells of *E. coli* mediated by type 1 and type P fimbriae. As shown in this report, the inhibitory effect of cranberry juice and cocktail on adherence of *E. coli* to eucaryotic cells is due to two (or more) different constituents. One of these is the fructose present in the juice and the cocktail, which inhibits the adherence of type 1 fimbriated *E. coli*, and the other is a nondialyzable substance (or substances) which inhibits binding of P fimbriated *E. coli*.

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## MATERIALS AND METHODS

**Juices.** Cranberry juice cocktail and cranberry juice (made from the American cranberry, *Vaccinium macrocarpon*) were obtained from Ocean Spray Cranberries Inc. The major constituents of cranberry juice are glucose (3.1%), fructose (1%), citric acid (1.1%), quinic acid (1.1%), and malic acid (0.8%). Cranberry juice cocktail is a 25% dilution of the native juice to which glucose and fructose have been added to concentrations of about 7 and 5%, respectively, and ascorbic acid to 0.32 mg/ml (values are according to the manufacturer and reference 17). Orange juice and pineapple juice were purchased in a local supermarket. All juices and the cocktail were adjusted to pH 7.0 by the addition of 1 M NaOH. For some of the experiments, the cocktail was dialyzed against phosphate-buffered saline (PBS; 150 mM NaCl and 20 mM phosphate, pH 7.2) in dialysis bags with a cutoff point of $M_w$ 15,000 in the cold for 3 days against six changes of PBS. In other experiments, the cocktail was similarly dialyzed against distilled water and lyophilized.

**Bacteria.** The *E. coli* strains used are listed in Table 1. The bacteria were grown under conditions optimal for the production of their surface lectins. Type 1 fimbriated bacteria were grown in tryptic soy broth under static conditions at 37°C for 48 h. After being harvested, the bacteria were washed once in PBS and suspended in PBS or in the tested juice to a concentration of $5 \times 10^9$ cells per ml, corresponding to 1.0 optical density (OD) units, measured on a Coleman junior spectrophotometer at 540 nm. P fimbriated *E. coli* and *E. coli* CFA/I were grown on Casamino Acids yeast extract agar at 37°C for 24 h; the bacteria were collected from the agar into 5 ml of PBS, washed once, and suspended to a

TABLE 1. *E. coli* strains used in this study

| Strain | Serotype | Origin[a] | Source (reference) | Fimbriae after serial passage in agar[b] |
|---|---|---|---|---|
| 47 | O8 | UTI | H. L. T. Mobley (22) | Type P |
| 267 | O8:K?:H30 | Diarrhea | J. Goldhar (16, 40) | |
| 334 | O78:K?:H− | Diarrhea | J. Goldhar (16, 40) | |
| 346 | O25 | UTI | F. J. Silverblatt (33) | |
| 347 | O8:K⁻:H4 | Diarrhea | J. Goldhar (16, 40) | |
| 349 | O8:col:H− | Diarrhea | J. Goldhar (16, 40) | |
| 569 | O75 | UTI | H. L. T. Mobley (22) | Type P |
| 801 | O4:H40 | Diarrhea | J. Goldhar (16, 40) | |
| 827 | O83:K1:H4 | UTI | J. Goldhar (16, 40) | NFA |
| 913 | O6 | UTI | H. L. T. Mobley (22) | Type P |
| 4877 | O8 | UTI | H. L. T. Mobley (22) | Type P |
| CSH50 | O:K12 | Laboratory derivative | B. I. Eisenstein (14) | |
| H-10407 | O78:K⁻:H12 | Diarrhea | D. G. Evans (12) | CFA/I[c] |
| IHE-1002 | O1:K1:H7 | UTI | T. Korhonen (18) | Type P |

[a] UTI, Urinary tract infection.
[b] After serial passage in broth, all strains except IHE-1002 had type 1 fimbriae. NFA, Nonfimbrial agglutinin, blood group N-specific.
[c] This fimbrial adhesin is specific for polysialic acid (37).

concentration of $1.5 \times 10^{10}$ cells per ml (3.0 OD units) in PBS or in the tested juice.

**Type 1 fimbriae.** Type 1 fimbriae were purified from *E. coli* 346 by the method of Eshdat et al. (11). The fimbrial preparation was dissolved in 0.05 M Tris hydrochloride buffer (pH 7.0) to a concentration of 350 μg of protein per ml as determined by the method of Bradford (5) with bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) as a standard. To obtain better agglutinating activity, the fimbrial preparation was cross-linked by adding glutaraldehyde to a final concentration of 0.5% for 10 min at room temperature.

**Tissue cultures.** The animal cell lines used were Y1 mouse adrenal cortex tumor cells and Chinese hamster ovary (CHO) cells (both from the American Type Culture Collection). They were grown in 1.5-mm-diameter wells (in 24-well semi-micro plates; Costar, Cambridge, Mass.) in 1 ml of Eagle minimal essential medium supplemented with 10% fetal calf serum, 1.0 M L-glutamine, and 40 U of penicillin and 40 μg of streptomycin per ml and incubated at 37°C in a humidified atmosphere of 5% $CO_2$ in air for 48 h (39). Confluent monolayers of $10^5$ cells per well were obtained.

**Mouse peritoneal macrophages.** Resident mouse peritoneal macrophages were obtained as described elsewhere (2). The cells were distributed in wells of a 96-well plastic microdilution plate (Nunclon-Delta; A/S Nunc, Roskilde, Denmark). To each well, 50 μl of the macrophages ($5 \times 10^5$ cells per ml) was added. The monolayers were incubated at 37°C for 30 min in a 5% $CO_2$ atmosphere. The supernatants containing the nonadherent cells were removed by aspiration, and the monolayers of the adherent cells consisting of macrophages were washed three times with PBS-CaMg (154 mM NaCl, 7.6 mM $Na_2HPO_4$, 7.6 mM $KH_2PO_4$, 1 mM $CaCl_2$, 1 mM $MgCl_2$), pH 7.4. To block nonspecific binding of the bacteria to the plastic, the macrophage monolayers were incubated with 100 μl of 1% bovine serum albumin in PBS-CaMg per well at 37°C for 30 min in 5% $CO_2$, and the supernatants were aspirated and discarded just before the bacterial suspension was added.

**Yeast aggregation assay.** *Saccharomyces cerevisiae* (bakers' yeast; Standard Brands Inc., New York, N.Y.) cells were suspended in PBS to a concentration of 0.5 mg/ml. Yeast aggregation was monitored in a Payton Aggregometer, model 300B (Payton, Scarborough, Canada) as described previously (24). The rate of aggregation (change in transmittance as a function of time) was calculated from the tangent of the steepest slope of the curve produced by the increase in light transmittance as a result of aggregate formation after addition of 10 to 20 μl of the bacterial suspension. In each test the activity of the bacteria in PBS was considered 100%; usually it was 7 to 14 U/min. The concentrations of test inhibitors needed to give 50% inhibition were calculated from the linear curves of inhibition as described previously (13).

**Yeast agglutination.** Agglutination was performed by mixing 20 μl of cross-linked fimbriae (350 μg of protein per ml) and 20 μl of yeast cells (0.5 mg/ml of PBS) on a glass slide; the results were scored visually after 2 min at room temperature. For inhibition assays, the yeast cells were suspended in serial dilutions of cranberry juice cocktail or 5% fructose, and the highest dilution giving complete agglutination was noted. Each experiment was done in triplicate.

**Hemagglutination tests.** To serial twofold dilutions in 50 μl of the bacterial suspension (starting with 1.0 OD units) in 96-well (U-shaped) microdilution plates were added 50-μl volumes of a 2% suspension of erythrocytes prepared from freshly drawn blood. For type 1 fimbriated bacteria, guinea pig erythrocytes were used as indicator cells. For all other bacteria, human group A erythrocytes were used. The results were recorded visually after 45 to 60 min at room temperature. In some experiments, hemagglutination was assayed on a glass slide by mixing 20 μl of bacteria (3.0 OD units) with 20 μl of human group A erythrocytes (25% in PBS). Hemagglutination was recorded after 3 min of horizontal shaking at room temperature.

**Gal-Gal beads agglutination test.** Latex beads coated with Gal-Gal (Bach-test; Kabivitrum, Stockholm, Sweden) were used as a test kit for the assay of P fimbriated *E. coli* as described by de Man et al. (8). A drop (20 μl) of the bead suspension was placed on a slide. An equal volume of the bacterial suspension (3.0 OD units) was added and mixed with the end of a toothpick. Agglutination was recorded after 3 min of horizontal shaking at room temperature.

**Adherence tests.** The tested bacteria ($5 \times 10^9$ cells per ml of PBS), without or with inhibitors at the desired concentration, were added in duplicate to wells containing washed tissue culture cells and incubated at 37°C for 30 min. The

TABLE 2. Effect of cranberry juice cocktail on yeast aggregation by type 1 fimbriated *E. coli*[a]

| *E. coli* strain | Aggr. rate in PBS | Nondialyzed CJC | | Dialyzed CJC | |
|---|---|---|---|---|---|
| | | Aggr. rate | % Inhibition | Aggr. rate[b] | % Inhibition |
| 267 | 8.1 ± 0.82 | 0.6 ± 0.07 | 93 | | NT |
| 334 | 6.28 ± 0.16 | 0.48 ± 0.13 | 92 | 4.9 ± 0.25 | 22 |
| 346 | 14.6 ± 0.52 | 0 | 100 | 12.2 ± 1.49 | 16 |
| 347 | 3.65 ± 0.21 | 0.41 ± 0.02 | 89 | | NT |
| 349 | 2.15 ± 0.02 | 0.27 ± 0.02 | 88 | | NT |
| 801 | 7.6 ± 1.27 | 0.34 ± 0.02 | 95 | 6.7 ± 0.26 | 12 |
| 827 | 2.5 ± 0.03 | 0.4 ± 0.02 | 84 | | |
| CSH50 | 11.5 ± 1.27 | 0.8 ± 0.03 | 93 | | NT |

[a] In all experiments, the yeast cells were suspended in PBS or in a 1:2 dilution of cranberry juice cocktail (CJC) which was nondialyzed (neutralized) or dialyzed against PBS. All tests were run in triplicate. To 0.5 ml of yeast suspension, 10 to 20 μl of bacteria in PBS or test solution was added, and aggregation (aggr.) rate was measured as described in the text. NT, Not tested.

[b] Values in this column are not significantly different from those obtained with PBS ($P > 0.05$, Student's $t$ test).

supernatants were decanted and the monolayers were washed five times with PBS before they were stained with Giemsa stain. The cells were then examined under a light microscope, and the percentage of tissue cells which bound more than 10 bacteria per cell was calculated. In experiments with macrophages, 100 μl of the bacterial suspension ($10^8$/ml) was added in duplicate to each well containing the macrophage monolayers. The microdilution plates were then kept on ice for 30 min. The unbound bacteria were removed by aspiration followed by three washes with PBS-CaMg. To estimate the number of bacteria bound, the monolayers were lysed by sterile double-distilled water (100 μl per well) followed by incubation for 1 h at room temperature. The lysates were diluted (1:$10^3$, 1:$10^4$, 1:$10^5$), and samples (5 μl) of each dilution were plated in petri dishes on nutrient agar for counts of CFU.

## RESULTS

**Effect of cranberry juice cocktail on yeast aggregation by type 1 fimbriated *E. coli*.** Yeast aggregation by bacteria is a measure of the mannose-specific activity of the bacteria and correlates with their ability to adhere to epithelial cells via type 1 fimbriae (24). We found that a 1:2 dilution of cranberry juice cocktail almost completely inhibited yeast aggregation by eight strains of type 1 fimbriated *E. coli* of different serotypes (Table 2) and that dilutions of 1:12 to 1:50 gave 50% inhibition (Table 3). Different lots of the cocktail gave similar inhibition (data not shown). The inhibitory effect was completely lost after dialysis of the cocktail against PBS, as shown for the three strains tested (Table 2). Preincubation of the bacteria with cranberry juice cocktail diluted 1:2 followed by washing with PBS did not affect their yeast-aggregating activity (data not shown), suggesting that the inhibitor acts as a hapten. As demonstrated with three strains of type 1 fimbriated *E. coli* (334, 346, and CSH50), the inhibitory activity is dose dependent in the range of dilutions assayed (Fig. 1). Since fructose (~5%) and glucose (~7%) are the major constituents of the cocktail (17) and since both are dialyzable, their effects on yeast aggregation by the bacteria was examined. It was found that glucose at a concentration of 5% was not inhibitory (data not shown), while the inhibitory activities of dilutions of 5% fructose

TABLE 3. Comparison of inhibitory activities of cranberry juice cocktail (CJC), fructose, and methyl α-mannoside (αMM) on yeast aggregation by type 1 fimbriated *E. coli*

| *E. coli* strain | Concn for 50% inhibition[a] | | | |
|---|---|---|---|---|
| | Fructose (%) | αMM (%) | CJC | |
| | | | Dilution | % Fructose[b] |
| 334 | 0.35 | 0.039 | 12 | 0.41 |
| 346 | 0.11 | 0.0123 | 52 | 0.09 |
| CSH50 | 0.20 | 0.017 | 27 | 0.18 |

[a] Data for fructose and cocktail were derived from Fig. 1; for methyl α-mannoside, the data were obtained from similar curves (not shown). Glucose (at 5%) was not inhibitory for any of the strains tested.

[b] Calculated on the basis of 5% fructose present in the undiluted cocktail.

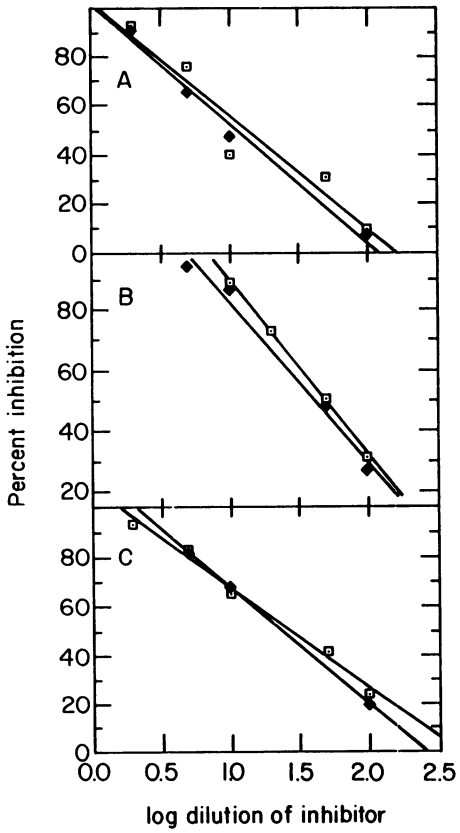


FIG. 1. Inhibition by cranberry juice cocktail (⊡) and fructose (5%) (◆) of yeast aggregation by *E. coli* 334 (A), 346 (B), and CSH50 (C).

TABLE 4. Effects of fructose and cranberry juice cocktail (CJC) on the adherence of *E. coli* 346 to tissue culture cells

| Cell line | Relative extent of adherence in[a]: | | | |
|---|---|---|---|---|
| | CJC | | Fructose | |
| | 1:2 | 1:5 | 2.5% | 1% |
| Mouse adrenal (Y1) | 35 | 90 | 40 | 80 |
| CHO | 20 | 40 | NT | NT |

[a] Calculated as percentages, with adherence in PBS as 100%. NT, Not tested.

were similar to those of the same dilutions of the cocktail for each of the three strains of *E. coli* tested (Fig. 1). The concentrations of cranberry juice cocktail, fructose, glucose, and methyl α-mannoside giving 50% inhibition of yeast aggregation by the three strains of *E. coli* listed above are different for the various strains (Table 3), a phenomenon previously observed with type 1 fimbriated enterobacterial species (13). Nevertheless, for each of the strains tested, the fructose concentration giving 50% inhibition was about the same as that present in the dilution of cranberry juice cocktail giving 50% inhibition and about 10 times that of methyl α-mannoside. It was also found that both cranberry juice cocktail and a 5% solution of fructose completely inhibited yeast agglutination by type 1 fimbriae, purified from *E. coli* 346, up to a dilution of 1:100.

**Effect of cranberry juice cocktail on the adherence of type 1 fimbriated *E. coli* strains to animal cells.** The adherence of *E. coli* 346 to Chinese hamster ovary cells and to mouse adrenal cells was inhibited similarly by dilutions of cranberry juice cocktail and by fructose at corresponding concentrations (Table 4). Inhibition of binding of strain 827 to mouse peritoneal macrophages by cranberry juice cocktail was similar to inhibition by fructose at concentrations present in the juice (Fig. 2). Methyl α-mannoside was about 10 times more inhibitory than fructose also in this assay system (data not shown).

**Effect of cranberry juice cocktail on hemagglutination by type 1 and P fimbriated *E. coli*.** The hemagglutinating activity of type 1 fimbriated *E. coli* and of P fimbriated *E. coli* was inhibited by cranberry juice cocktail, whereas the cocktail had no effect on the hemagglutinating activity of *E. coli* CFA/I (Table 5). The dialyzed cocktail did not inhibit the activity of the type 1 or the CFA/I fimbriated bacteria, but it was fully active against the P fimbriated bacteria. Fructose

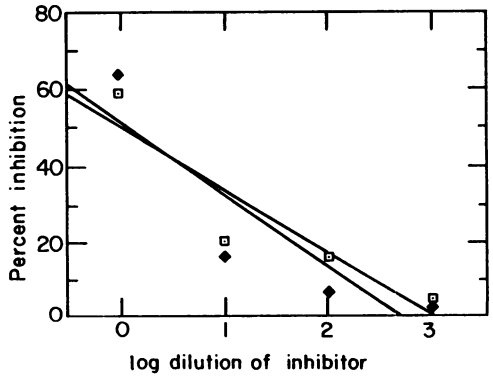


FIG. 2. Inhibition by cranberry juice cocktail (⊡) and by fructose (5%) (◆) of adherence of *E. coli* 827 to mouse peritoneal macrophages.

TABLE 5. Inhibitory effects of nondialyzed and dialyzed cranberry juice cocktail (CJC) on hemagglutination caused by strains of *E. coli*

| *E. coli* strain | Fimbrial lectin | Hemagglutination[a] titer of bacteria in: | | | |
|---|---|---|---|---|---|
| | | PBS | CJC[b] | | Fructose (2.5%) |
| | | | 1:2 | Dialyzed[c] | |
| CSH50 | Type 1 | 1:16 | 1:1 | 1:16 | 1:1 |
| H-10407 | CFA/I | 1:8 | 1:8 | 1:8 | 1:8 |
| IHE | Type P | 1:4 | <1:1 | <1:1 | 1:4 |
| 47 | Type P | 1:4 | <1:1 | <1:1 | 1:4 |
| 569 | Type P | 1:4 | <1:1 | <1:1 | 1:4 |
| 4877 | Type P | 1:4 | <1:1 | <1:1 | 1:4 |

[a] Hemagglutination by type 1 fimbriated *E. coli* was assayed with guinea pig erythrocytes, and that by other types of *E. coli* was assayed with human blood group A erythrocytes by using microdilution plates.
[b] Cranberry juice cocktail was neutralized to pH 7.0.
[c] Against PBS.

did not inhibit hemagglutination caused by the P fimbriated strains tested (Table 5).

The inhibitory effect of the dialyzed cranberry juice cocktail on hemagglutination by P fimbriated bacteria was time dependent (Table 6). The lower the concentration of the dialyzed cocktail needed to inhibit hemagglutination, the longer the preincubation period needed. The activity of the P inhibitor in the cocktail varied from one lot of cranberry juice cocktail to another. For example, with a lot different from that used in the experiment described in Table 6, no inhibition was observed after 30 min of incubation with the bacteria. However, after 90 min of incubation, inhibition of hemagglutination was observed. In a separate experiment, samples of the two lots of cranberry juice cocktail mentioned above were dialyzed against water; the nondialyzable material was lyophilized; and the dry residues were dissolved in PBS, each at a concentration of 2 mg/ml. Dilutions of these solutions were incubated with the P fimbriated *E. coli* for 30 min. Whereas the solution from the first lot was inhibitory up to 1:32 dilution, the solution of the second lot was inhibitory up to 1:8 dilution only.

**Effect of cranberry juice cocktail on agglutination of Gal-Gal beads by P fimbriated *E. coli*.** To examine more specifically the effect of cranberry juice cocktail on P fimbriae, we used indicator beads coated with Gal-Gal (8). In agreement with the results obtained in the hemagglutination experiments, the cocktail inhibited agglutination of the beads by the P fimbriated bacteria, and the inhibitory activity was nondialyzable (Table 7). Inhibition was also observed after preincubation of the P fimbriated bacteria with cranberry juice cocktail followed by washing with PBS. Preincubation

TABLE 6. Effect of preincubation with cranberry juice cocktail (CJC) on hemagglutinating activity of P fimbriated *E. coli* IHE

| Preincubation time (min) | Hemagglutinating activity[a] in dialyzed CJC (dilution) | | | | |
|---|---|---|---|---|---|
| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 |
| 0 | − | + | + | ++ | ++ |
| 2 | − | − | ± | ++ | ++ |
| 10 | − | − | − | + | ++ |
| 15 | − | − | − | ± | ++ |
| 30 | − | − | − | − | ++ |

[a] Hemagglutination was assayed with human type A erythrocytes by the glass slide technique. Symbols: ++, very strong agglutination; +, strong agglutination; ±, weak agglutination; −, no agglutination. Agglutination in PBS was very strong at all preincubation times.

TABLE 7. Inhibitory effect of nondialyzed and dialyzed cranberry juice cocktail (CJC) on agglutination of Gal-Gal beads by P fimbriated *E. coli* IHE

| Preincubation time (min) | Agglutinating activity[a] in CJC | | | |
|---|---|---|---|---|
| | pH 7.0 | | Dialyzed[b] | |
| | 1:2 | 1:5 | 1:10 | 1:2 |
| 0 | + | + | + | − |
| 2 | − | ± | + | − |
| 10 | − | ± | + | − |
| 15 | − | − | + | − |
| 30 | − | − | − | − |

[a] Symbols: +, strong agglutination; ±, weak agglutination; −, no agglutination. Agglutination in PBS was strong at all preincubation times.
[b] Against PBS.

of the beads with the cocktail followed by washing, however, did not inhibit their ability to be agglutinated by P fimbriated bacteria (data not shown). The minimal concentration of cranberry juice cocktail needed to inhibit the agglutination of the Gal-Gal-coated beads was dependent on the preincubation time of the bacteria with the cocktail (Table 7). The longer the preincubation time, the lower the concentration of the cocktail needed to inhibit agglutination.

The nondialyzable inhibitor for P fimbriated *E. coli* contains only small amounts of protein (4.8% as estimated by the Bradford method [5] with bovine serum albumin as the standard). The inhibitory activity, however, is heat stable (100°C, 30 min) and is unaffected by incubation with trypsin (1:30 enzyme/substrate ratio, 30 min, 37°C) or treatment with acid (1 M HCl, 30 min, room temperature). These findings make it highly unlikely that the inhibition observed is due to an enzyme.

**Inhibitory effect of cranberry juice and other fruit juices.** Cranberry juice, orange juice, and pineapple juice inhibited yeast aggregation by type 1 fimbriated *E. coli* in a manner similar to that of cranberry juice cocktail (Table 8), most probably because of their fructose content. Dilutions (1:2) of orange or pineapple juice, however, did not inhibit either the agglutination of Gal-Gal beads by P fimbriated bacteria or the hemagglutination caused by *E. coli* expressing CFA/I lectin.

TABLE 8. Effects of different fruit juices on yeast aggregation[a] by *E. coli* 346

| Juice | Result at juice dilution of: | | | | | |
|---|---|---|---|---|---|---|
| | 1:2 | | 1:5 | | 1:10 | |
| | Aggr. rate | % Inhibition | Aggr. rate | % Inhibition | Aggr. rate | % Inhibition |
| Cranberry Cocktail | 0 | 100 | 0.13 | 98 | 0.35 | 95 |
| Juice | 0.13 | 98 | 2.0 | 73[b] | 3.1 | 59[b] |
| Orange[c] | 1.6 | 80 | 3.7 | 53 | 5.5 | 31 |
| Pineapple[d] | 0 | 100 | 0.5 | 92 | 0.6 | 90 |

[a] Aggregation (aggr.) rate of the yeast by the bacteria in PBS was 7.5 U/min and was taken as 100%.
[b] 73 and 59% inhibition, according to a standard inhibition curve of fructose (Fig. 1), correspond to 0.33 and 0.14% fructose, respectively, indicating that cranberry juice contains an average of 1.45% fructose, which is close to the 1% reported by the producer.
[c] Israeli orange juice contains a total of 8% sugar, of which 4% is fructose.
[d] Pineapple juice contains a total of 4% reducing sugars, of which 2% is fructose.

## DISCUSSION

In this study, the effect of cranberry juice cocktail on the activity of fimbrial lectins which mediate adherence of *E. coli* to different sugars on animal cells was examined. It was found that the cocktail contains two different inhibitors, a dialyzable one and a nondialyzable one. The dialyzable component of the cocktail inhibited the activity of the mannose-specific type 1 fimbriated bacteria in a hapten-like manner, as assayed by yeast aggregometry, hemagglutination, adherence to tissue culture cells, and attachment to mouse peritoneal macrophages. The finding that both cranberry juice cocktail and 5% fructose inhibited yeast agglutination by purified type 1 fimbriae proves that the fimbriae are the target of inhibitory action.

It is highly unlikely that the decrease in inhibitory activity for type 1 fimbriated *E. coli* after dialysis is due to volume expansion, since even at a dilution of 1:2 the dialyzed material gave poor, if any, inhibition, whereas the dilution of cranberry juice cocktail causing 50% inhibition of type 1 fimbriated *E. coli* was in the range of 1:12 to 1:52. At such dilutions, cranberry juice cocktail contains 0.25 to 0.1% fructose as calculated from the reported analysis (17). Cranberry juice was also inhibitory to type 1 *E. coli*, most probably because of the presence of fructose in the juice. Indeed, the concentration of fructose required to inhibit 50% yeast aggregation by the bacteria or attachment of the bacteria to macrophages was also in the range of 0.25 to 0.1%.

As shown in Table 3, fructose is about a 10 times weaker inhibitor of type 1 fimbriae than methyl α-mannoside. Fructose was shown previously by Old (26) to inhibit weakly type 1 fimbriated *Salmonella typhimurium* and *Shigella flexneri*. Subsequently, Salit and Gottschlich (30) found that fructose inhibits hemagglutination of guinea pig erythrocytes by purified type 1 fimbriae from *E. coli* and that this inhibitory activity of fructose was 7.5 times less than that of methyl α-mannoside, a value in agreement with that obtained by us. Taken together, our data show that most or all of the inhibition of yeast aggregation by the cocktail or juice is due to the fructose content. Moreover, although glucose is present in the cocktail at about the same concentration (~7%), this sugar does not inhibit the aggregation of yeast by the type 1 fimbriated bacteria. The only other low-molecular-weight, dialyzable, organic constituents present in cranberry juice cocktail at significant levels are quinic acid (0.3%), citric acid (0.3%), and malic acid (0.22%). The cocktail also contains vitamin C (0.32 mg/ml) (17). Neutralized solutions of these compounds, at the concentrations listed above, did not inhibit yeast aggregation by type 1 fimbriated *E. coli* (data not shown).

The cocktail also inhibited the P fimbriated *E. coli*, as assayed by agglutination of human erythrocytes and of Gal-Gal-coated beads. These bacteria were, however, not inhibited by fructose. The inhibitor for P fimbriae was nondialyzable, suggesting that it is of high molecular weight. This activity is dependent on the level of the inhibitor and the time of preincubation of the bacteria with the cocktail. Evidence that the inhibitor is adsorbed by the bacteria was also obtained, since inhibition was observed even when bacteria which had been preincubated in the cocktail were extensively washed with PBS. Until the chemical nature of the inhibitor is defined, it is too early to speculate on the mechanism by which it binds to P fimbriated *E. coli* surfaces and interferes with the ability of the P fimbrial lectin to react with Gal-Gal residues. Moreover, at this stage, the possibil-

ity that the nondialyzable constituent(s) may inhibit adherence mediated by adhesions other than P fimbriae cannot be excluded.

Prevention of urinary tract infections by *E. coli* in mice and primates has been achieved by blocking bacterial adherence with inhibitory sugars (1, 29, 36). It is thus reasonable to assume that consumption of foods containing inhibitors of bacterial adherence might affect the infectious process.

Sobota (34), who found that the cranberry juice and cocktail inhibited bacterial adherence but lacked any bactericidal effect, suggested that they may act by preventing adherence to and colonization of mucosal surfaces. If so, the possibility should be considered that the cocktail acts either in the gut, the source of most uropathogens, or in the bladder, or at both sites, by preventing adherence to and subsequent colonization of the mucosa by *E. coli* strains with mannose-specific and Gal-Gal-specific lectins. The former mode of action is compatible with the suggestion that cranberry juice cocktail may act primarily as a preventive agent in urinary tract infection (35).

In connection with this possibility, we should consider the information available on the adsorption of fructose in the alimentary tract. This sugar is absorbed much more slowly than glucose (7). Thus, since fructose is present in the cocktail at levels that are at least 10 times higher than those required for the inhibition of type 1 fimbriated *E. coli*, it is conceivable that inhibitory levels of the sugar are attained in the colon, where most *E. coli* reside. Furthermore, it has been shown that a diet rich in fructose may result in secretion of fructose in the urine. Whether or not the same argument may apply for the putative inhibitor of P fimbriated lectin must await further study.

Another possibility is that drinking cranberry juice cocktail may affect the urinary concentrations of Tamm-Horsfall glycoprotein, which is known to interfere with the adherence of type 1 *E. coli* to human kidney cells (10).

Carefully conducted clinical studies are required to establish whether the cocktail indeed functions in vivo and whether any of the above mechanisms is operational.

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