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July 24, 1995

FOOD & DRUG ADMINISTRATION  
Office of Special Nutritionals  
200 "C" Street S.W.  
Washington, DC 20204

RE: Notification of Intention to make a Statement of Nutritional Support for  
AZO-CRANBERRY™ Cranberry Juice Powder Capsules

Pursuant to the "Dietary Supplement Health and Education Act of 1994," (DSHEA), this letter represents notification that our company intends to make a statement of nutritional support for our AZO-CRANBERRY capsules.

This statement will read as follows: "*Cranberry helps block the attachment of E. coli bacteria to the urinary bladder wall.*" In addition, the label will carry a bold disclaimer which will read "*This statement has not been evaluated by the Food & Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.*" These statements will be added to the existing packaging, a representation of which can be seen in Exhibit A.

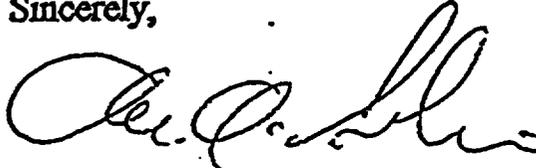
The above structure/function claim is supported by the following information:

- Exhibit B: - Paper entitled "Reduction of Bacteriuria and Pyuria After Ingestion of Cranberry Juice," J. Avorn, et al, JAMA 271, 751 (1994).
- Exhibit C: Paper entitled "Inhibition of Bacterial Adherence by Cranberry Juice: Potential Use for the Treatment of Urinary Tract Infections," A. E. Sobota, J. Urol. 131, 1013 (1984).
- Exhibit D: Article preprint entitled "Cranberry - America's Healthful Fruit," A. A. Siciliano.

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Exhibit E: Paper entitled "Inhibitory Activity of Cranberry Juice on Adherence of Type 1 and Type P Fimbriated *Escherichia coli* to Eucaryotic Cells," D. Zafriri, et al, Antimicrobial Agents and Chemotherapy, 33, 92 (1989).

Sincerely,



Arthur A. Siciliano, Ph.D.  
Executive Vice President

AAS/km

Enclosures

T:ADMIN\WPS\LTRS\JULY.95\FDA

In Convenient Capsule Form.

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WITHOUT THE TARTNESS

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# AZO-CRANBERRY™

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LOT #

**AZO-CRANBERRY™**  
NATURAL CRANBERRY SUPPLEMENT

NATURAL CRANBERRY GOODNESS  
3 CALORIES PER SERVING

# AZO-CRANBERRY™

**NEW**

CRANBERRY JUICE POWDER CAPSULES,  
450 mg., 50 Capsules

**AZO-CRANBERRY™**  
NATURAL CRANBERRY SUPPLEMENT  
3 Calories per Serving



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MANUFACTURED FOR: PolyMedica Pharmaceuticals (U.S.A.), Inc.  
Woburn, MA 01801 400.322.0994

**MANUFACTURED FOR:** PolyMedica Pharmaceuticals (U.S.A.), Inc.  
Woburn, MA 01801 400.322.0994

**NUTRITION FACTS:** Serving size: two capsules, 900 mg. Servings 25.  
Amount per serving: Calories 3, Total Fat 0g (0% DV), Sodium 0g (0% DV),  
Total carbohydrate 0g (0% DV), Protein 0g (0% DV), Not a significant source  
of calories from fat saturated fat, cholesterol, fiber, sugar, Vitamin A, Vitamin C,  
calcium or iron. Percent Daily Values (DV) are based on a 2,000-calorie diet.

**INGREDIENTS:** Natural cranberry juice powder, cellulose powder, and vegetable mono-  
resinate stearate.

**SUGGESTED USE:** Take two to four capsules at each meal time.

Two capsules are equivalent to approximately one ounce of cranberry juice concentrate.  
To help preserve the essential cranberry goodness.  
from native, fresh cranberries, they are dried, formulated into a powder, and encapsulated to  
provide a convenient, easy-to-take capsule form. Juice is requested  
Excess of cranberries in a convenient, easy-to-take capsule form. Juice is requested

**AZO-CRANBERRY™**  
Cranberry Goodness in a Capsule

## Inhibitory Activity of Cranberry Juice on Adherence of Type I 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 I fimbriae (mannose specific) and P fimbriae [specific for  $\alpha$ -D-Gal(1 $\rightarrow$ 4)- $\beta$ -D-Gal] but had no effect on a diarrheal isolate expressing a CFAd adhesin. The cocktail also inhibited yeast agglutination by purified type I fimbriae. The inhibitory activity for type I 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  $\alpha$ -D-mannoside in inhibiting the adherence of type I 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 I 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 I fimbriae, which mediates the adherence of the bacteria to uroepithelial cells (6, 9, 32). In addition to type I fimbriae, most pyelonephritogenic isolates of *E. coli* express an  $\alpha$ -Gal(1 $\rightarrow$ 4) $\beta$ -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 I 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 I fimbriated *E. coli*, and the other is a nondialyzable substance (or substances) which inhibits binding of P fimbriated *E. coli*.

### 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 I 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  $5 \times 10^7$  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* CFAd 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

\* Corresponding author.

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

Strain	Serotype	Origin	Source reference	Fimbriae after serial passage in agar*
47	O6	UTI	H. L. T. Mobley (22)	Type P
367	O8:K2:H30	Diarrhea	J. Goldfarb (16, 40)	
334	O78:K2:H-	Diarrhea	J. Goldfarb (16, 40)	
346	O25	UTI	F. J. Silverblatt (33)	
347	O8:K2:H4	Diarrhea	J. Goldfarb (16, 40)	
349	O8:ool:H-	Diarrhea	J. Goldfarb (16, 40)	
569	O75	UTI	H. L. T. Mobley (22)	Type P
801	O4:H40	Diarrhea	J. Goldfarb (16, 40)	
827	O83:K1:H4	UTI	J. Goldfarb (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:K2:H12	Diarrhea	D. G. Evans (12)	CFAT <sup>b</sup>
IHE-1002	O1:K1:H7	UTI	T. Korhonen (18)	Type P

\* UTI, Urinary tract infection.

<sup>b</sup> After serial passage in broth, all strains except IHE-1002 had type I fimbriae. NFA, Nonfimbrial agglutinin, blood group N-specific.

<sup>c</sup> This fimbrial adhesin is specific for polygalactonic acid (37).

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

**Type I fimbriae.** Type I 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  $\mu$ g 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  $\mu$ g of streptomycin per ml and incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> 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 (Nuncion-Delta; A/S Nunc, Roskilde, Denmark). To each well, 50  $\mu$ l 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<sub>2</sub> 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<sub>2</sub>HPO<sub>4</sub>, 7.6 mM KH<sub>2</sub>PO<sub>4</sub>, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>), pH 7.4. To block nonspecific binding of the bacteria to the plastic, the macrophage monolayers were incubated with 100  $\mu$ l of 1% bovine serum albumin in PBS-CaMg per well at 37°C for 30 min in 5% CO<sub>2</sub>, 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  $\mu$ l 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  $\mu$ l of cross-linked fimbriae (350  $\mu$ g of protein per ml) and 20  $\mu$ l 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  $\mu$ l of the bacterial suspension (starting with 1.0 OD units) in 96-well (U-shaped) microdilution plates were added 50- $\mu$ l volumes of a 2% suspension of erythrocytes prepared from freshly drawn blood. For type I 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  $\mu$ l of bacteria (3.0 OD units) with 20  $\mu$ l 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  $\mu$ l) 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*\*

<i>E. coli</i> strain	Aggr. rate in PBS	Nondialyzed CJC		Dialyzed CJC	
		Aggr. rate	% Inhibition	Aggr. rate <sup>b</sup>	% Inhibition
267	8.1 ± 0.82	0.6 ± 0.07	93		NT
334	6.38 ± 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 ± 1.37	0.34 ± 0.02	95	6.7 ± 0.26	12
827	2.5 ± 0.03	0.4 ± 0.02	84		
CSH50	11.5 ± 1.37	0.8 ± 0.03	93		NT

\* 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  $\mu$ l of bacteria in PBS or test solution was added, and aggregation (aggr.) rate was measured as described in the text. NT, Not tested.

<sup>b</sup> 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  $\mu$ l of the bacterial suspension ( $10^6$ /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  $\mu$ l 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  $\mu$ l) 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

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

<i>E. coli</i> strain	Concn for 50% inhibition <sup>a</sup>			
	Fructose (%)	$\alpha$ MM (%)	CJC	
			Dilution	Fructose <sup>b</sup>
334	0.35	0.039	12	0.41
346	0.11	0.0123	5	0.09
CSH50	0.20	0.017	27	0.18

<sup>a</sup> Data for fructose and cocktail were derived from Fig. 1; for methyl  $\alpha$ -mannoside, the data were obtained from similar curves (not shown). Glucose (at 5%) was not inhibitory for any of the strains tested.

<sup>b</sup> Calculated on the basis of 5% fructose present in the undiluted cocktail.

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 haptin. 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

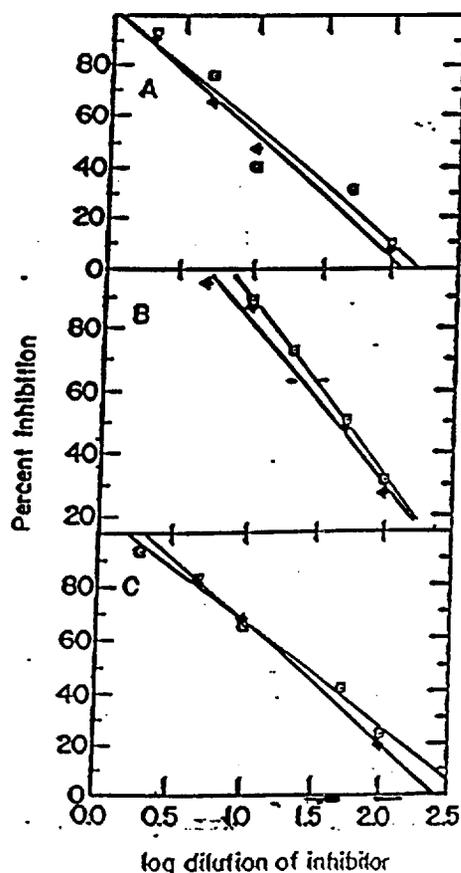


FIG. 1. Inhibition by cranberry juice cocktail ( $\square$ ) and fructose (5%) ( $\blacktriangle$ ) of yeast aggregation by *E. coli* 334 (A), 346 (B), and CSH50 (C).

TABLE 4. Effects of fructose and cranberry juice cocktail (CJC) on the adherence of *E. coli* 827 to tissue culture cells.

Cell line	Relative extent of adherence in:			
	CJC		Fructose	
	1:2	1:5	2.5%	1%
Mouse adrenal (Y1)	35	90	40	60
CHO	30	40	NT	NT

\* Calculated as percentages, with adherence in PBS as 100%. NT, Not tested.

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

<i>E. coli</i> strain	Fimbrial lectin	Hemagglutination* titer of bacteria in:			
		PBS	CJC*		Fructose 0.5%†
			1:2	Dialyzed*	
CSH50	Type I	1:16	1:1	1:16	1:1
H-10407	CFAM	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

\* Hemagglutination by type I 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.

† Cranberry juice cocktail was neutralized to pH 7.0.

\* Against PBS.

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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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* CFAM (Table 5). The dialyzed cocktail did not inhibit the activity of the type 1 or the CFAM fimbriated bacteria, but it was fully active against the P fimbriated bacteria. Fructose

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

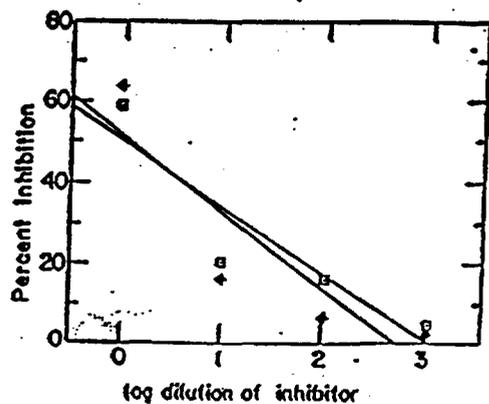


FIG. 2. Inhibition by cranberry juice cocktail (□) and by fructose (5%) (△) of adherence of *E. coli* 827 to mouse peritoneal macrophages.

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

Preincubation time (min)	Hemagglutinating activity* in dialyzed CJC dilution†				
	1:2	1:4	1:8	1:16	1:32
0	-	+	+	++	++
2	-	-	=	++	++
10	-	-	-	+	++
15	-	-	-	=	++
30	-	-	-	-	++

\* 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 <sup>a</sup> in CJC			
	pH 7.0		Dialyzed <sup>b</sup>	
	1:2	1:5	1:10	1:5
0	+	+	+	-
2	-	=	+	-
10	-	=	+	-
15	-	-	+	-
30	-	-	-	-

<sup>a</sup> Symbols: +, strong agglutination; =, weak agglutination; -, no agglutination. Agglutination in PBS was strong at all preincubation times.

<sup>b</sup> 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<sup>a</sup> 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 <sup>b</sup>	3.1	59 <sup>b</sup>
Orange	1.6	80	3.7	53	5.5	31
Pineapple <sup>c</sup>	0	100	0.5	92	0.6	90

<sup>a</sup> Aggregation (aggr.) rate of the yeast by the bacteria in PBS was 7.5 U/min and was taken as 100%.

<sup>b</sup> 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.

<sup>c</sup> Israeli orange juice contains a total of 8% sugar, of which 4% is fructose.

<sup>d</sup> 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  $\alpha$ -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  $\alpha$ -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 fimbrial *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.

Sobotta (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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## CRANBERRY

### *America's Healthful Fruit*

Arthur A. Siciliano, Ph.D.

Cranberries (*Vaccinium macrocarpon*; *V. oxycoccus*; *Oxycoccus quadripetalus*) are a member of the heath family and are one of three fruits native to North America. The plants yield pink flowers followed by small, red-black edible berries from June to July. They grow in the wild from the Carolinas to Canada. The first cultivation in North America occurred around 1816 (Henry Hall) and centered in sandy "bogs," marshes, or in rain-soaked salt meadows in Massachusetts, New Jersey, Wisconsin, Washington and Oregon. Current U.S. usage is about 350 million pounds, representing retail cranberry products valued at \$1.1 billion.

### **Chemistry/Nutrition**

Cranberries contain about 88% water. Among the other organic constituents are flavanoids, anthocyanins (odain), catechin, triterpinoids, B-hydroxybutyric acid, citric, malic, glucuronic and quinic acids, ellagic acid and vitamin C. They are a good source of fiber. Acid levels are high: pure cranberry juice is as tart as pure lemon juice.

Raw cranberries are fairly low in calories (209 calories/pound) and carbohydrates (11%). Cranberry juice "cocktail," the leading consumer product

prepared from cranberry juice, contains 140 calories per 8 ounce serving, primarily from the sweetener, corn syrup. Dried, unsweetened cranberry juice powder is available in capsule form (1½ calories/capsule).

## History

The word "cranberry" first appeared in a letter by Cape Cod missionary John Eliot (1647). The earliest description of a European wild cranberry plant (marsh wort/fenne berry) appeared in Lytes' "History of Plants" (1578). When the Pilgrims arrived in New England, the local native Americans had been consuming wild cranberries for centuries as a foodstuff called pemmican, a combination of crushed berries, fat and dried meat. Other tribes boiled the dried cranberries and seasoned them with maple sugar. The English settlers found the sweetened berries made an excellent sauce for meats. (1)

Most all cranberry sales in the early 1900's were of the fresh fruit. The crop was hand picked until recent times, although the first harvesting "machines" were patented in the late 1880's. In 1912, Marcus Urann began canning berries and producing cranberry jelly, a mixture of fruit and sugar. During the 1920's, cranberry sauce was introduced, and in the 1940's dehydrated cranberries and cranberry juice cocktail became available. Today, cranberries are marketed in a variety of forms (Table I).

## Medicinal Uses

Therapeutic applications of cranberries documented during the 17th century included the relief of blood disorders, stomach ailments, liver problems, vomiting, appetite loss, scurvy and cancer. Native Americans prepared wound dressings from the whole dried fruit. New England folk medicine used boiled cranberries and seal oil to reduce the severity of gall bladder attacks. Early scientists believed that cranberries beneficial effects, especially in urinary tract disorders, were due to its low pH (high acidity). German physicians in the mid-1800's noted hippuric acid excretion increased after cranberry ingestion (2). Since it was known that benzoic acid was metabolized to hippuric acid, researchers suggested that the beneficial effects of cranberries were due to these antimicrobial acids.

As early as 1914, a report proposed that bacterial suppression was not related to either pH or hippuric acid excretion (3). In 1966, researchers observed that the effects on the pH of urine by cranberry juice were transient and that acidification may not be the main mechanism of action (4). Current thinking suggests that cranberry juice contains a polymer that prevents the adherence of bacteria to the walls/lining of the bladder and urinary tract, thereby helping to prevent urinary infections (5-8). Other uses of cranberry include its role in a regimen to decrease the recurrence of urinary stones (9) and as a urinary deodorant (10).

Today, over 52 million households consume cranberry products. The early anecdotal use of cranberries to maintain urinary health is continually being substantiated by medical studies, and physicians are recommending cranberry products as an adjunct in avoiding urinary tract infections (11).

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## Cranberry Sources

Table I.

Form	Description	Use	Available to Consumer in Retail Stores	Unit "Strength" Compared to Cranberry Juice Cocktail
Cranberry Juice Cocktail	~25% juice, sweeteners (corn syrup), stabilizers	Beverage	Yes	1
Cranberry Juice Drinks	~10% juice content	Beverage	Yes	1/2
Cranberry Sauce	Sweetened/gelled berries	Food	Yes	1/2
Fresh or Frozen Whole Cranberries	Pure fruit	Food	Yes	4
Dried Cranberry Press Cake	Fiber from fruit hulls	Flavor, color, and fiber source	No	0
Cranberry Juice	Prepared from concentrate by dilution	Ingredient for drinks	No	4
Cranberry Juice Concentrate (frozen)	Frozen concentrated juice	Ingredient for cranberry juice cocktail/drinks	No	27
Cranberry Juice Concentrate Powder	Pure unsweetened dehydrated juice and carrier	Ingredient for capsules	No	32
Cranberry Powder ("90 MX")	Cranberry juice, partially neutralized and carrier	Food color	No	48
Cranberry Juice Capsules	Unsweetened cranberry juice concentrate powder in gelatin capsules	Dietary supplement	Yes	32

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**Human subjects.** Twenty-two healthy volunteers, 9 male and 13 female (age range 18 to 45) participated in this study. Controls for the adherence tests were included in which buffer was substituted for urine. In those instances where there was a significant difference between the urine samples and these controls, the results were not included.

### RESULTS

**Effect of cranberry juice on adherence.** Preliminary testing of the cranberry juice was performed on a clinical isolate that showed relatively high adherence to both uroepithelial cells, average 36 bacteria per cell and buccal cells, average 27 bacteria per cell. The pH of the 3 different preparations of cranberry juice ranged from 2.6 to 3.1. Normally, adherence tests are performed at a pH of about 7.0. To assess the potential effect of acid pH on bacterial adherence, tests were performed at intervals of 0.5 from pH 1.0 to 7.0. Minimal essential medium containing Earl's salts adjusted to the appropriate pH was used for the incubation medium.<sup>6</sup> Maximum adherence occurred at approximately pH 4 to pH 5 and a marked decrease in adherence only occurred below pH 2. Therefore, no attempt was made to adjust the pH of the various preparations of cranberry juice prior to testing. However, for each test, the pH of the juice was measured and controls were run at this pH using a citrate-phosphate buffer<sup>14</sup> containing 0.85 per cent NaCl.

The effect of the various preparations of cranberry juice on the adherence of *E. coli* to uroepithelial and buccal cells is presented in table 1. The degree of inhibition ranged from greater than 97 per cent in undiluted juice to approximately 30 per cent at a dilution of 1:100 for both kinds of cells. All 3 preparations of cranberry juice significantly inhibited adherence up to dilutions of 1:100.

The most palatable form of cranberry juice, the cranberry cocktail, contains, in addition to the juice, fructose, vitamin C and small amounts of additional vitamins and minerals. The concentration of the cranberry juice in the cocktail is approximately 33 per cent.<sup>11</sup> Vitamin C is added as 100 per cent of recommended daily allowance per 6 ounce serving or about 0.25 mg. per ml. The concentration of the added fructose was not available from Ocean Spray Cranberries, Inc. However, based on labeling information (26 gm. carbohydrate per 6 ounce serving) the concentration of fructose probably did not exceed 0.15 gm. per ml. Although it is clear from table 1 that cranberry juice alone can inhibit adherence, both fructose and vitamin C were also tested. Fructose concentrations ranging from 0.02 to 0.20 gm. per ml. were found to either inhibit or have no effect on bacterial adherence. The addition of 0.15 gm. per ml. of fructose to freshly prepared cranberry juice (33 per cent) showed no additive effect. When the concentration of cranberry juice was reduced to 10 per cent, a small additive effect could be observed. Vitamin C was tested both as ascorbic acid and sodium ascorbate in concentrations ranging from 0.1 to 10 mg. per ml. There was no observable effect on adherence.

**Mechanism of action.** Individual preparations of buccal, uroepithelial and *E. coli* cells were preincubated in freshly prepared cranberry juice, pH 2.7, for 30 minutes, then resuspended in citrate-buffered saline at pH 2.7, and then subjected to adherence tests. Preincubation of epithelial cells with cranberry juice showed only minor effects on normal adherence. In contrast, preincubation of *E. coli* in cranberry juice showed the typical previously observed, inhibition of adherence. The *E. coli* cells were then subjected to a series of washes with buffer and reassayed. After 1 wash, only 90 per cent of the inhibitory activity was lost and after 2 washes, the normal adherence pattern was restored. It was found that the preincubation of *E. coli* in the cranberry juice could be reduced to 30 seconds with no decrease in the inhibitory effect. Cranberry juice was also added for 30 minutes to epithelial cells showing adherent *E. coli*. The effect of the juice was variable. In some cases, the epithelial cells were completely freed of the adhering *E. coli*. However, at other times, with cells from the same individual very little release occurred.

The effect of cranberry juice on the growth of *E. coli* was also examined. *E. coli* was inoculated into BHI containing 33 per cent freshly prepared cranberry juice adjusted to a pH of 6.0 and a control medium containing 33 per cent glass distilled water. Growth was monitored at 24 and 48 hours. The control cultures contained  $4.5 \times 10^8$  and  $6.2 \times 10^8$  cfu per ml. while the treated contained  $2.4 \times 10^8$  and  $4.1 \times 10^8$  cfu per ml. After 48 hours the cultures were filtered through 0.23  $\mu$ m. filters to separate the bacteria from the culture medium. The culture medium was then tested for inhibitory activity via the adherence test. Uninoculated BHI was used as a baseline control and showed a mean adherence of 39.6 bacteria per uroepithelial cell and 33.1 bacteria for the buccal cells. The control medium was slightly inhibitory showing adherence values of 31.6 for uroepithelial cells and 27.7 for buccal cells. The medium containing the cranberry juice was still strongly inhibitory showing adherence values of 6.3 bacteria per uroepithelial cell and 9.3 bacteria per buccal cell. The *E. coli* cells collected from the 48-hour cultures were then assayed for adherence to uroepithelial cells. One half of each sample was 1st washed 2 times with PBS. Both washed and unwashed *E. coli* growing in BHI plus 33 per cent water showed the typical adherence pattern having mean adherence values of 32.8 and 36.1 bacteria per cell respectively. The unwashed *E. coli* cells growing in the medium containing 33 per cent cranberry juice had a mean adherence value of 3.71, while the washed cells had a mean value of 29.4 bacteria per uroepithelial cell.

**Effect of cranberry juice on clinical isolates of *E. coli*.** A total of 134 clinical isolates of *E. coli* were screened. A positive adherence to uroepithelial cells was demonstrable in 77 (57 per cent) of the isolates and to buccal cells in 71 (52 per cent) of the isolates. The strains showing positive adherence were then tested. The results are presented in table 2. Inhibition of bacterial adherence is presented as a percentage of control

TABLE 1. Effect of cranberry juice on adherence of *E. coli* to buccal and uroepithelial cells

Dilution of Cranberry Juice	Mean Bacteria per Cell*					
	Uroepithelial Cells			Buccal Cells		
	A	B	C	A	B	C
Control	36.1 ± 4.5	34.2 ± 5.2	36.6 ± 7.9	27.2 ± 2.9	24.9 ± 2.7	27.1 ± 2.4
0	0.94 ± 0.13	1.13 ± 0.21	0.81 ± 0.04	1.71 ± 0.20	0.56 ± 0.04	0.60 ± 0.13
1:2	1.86 ± 0.17	1.48 ± 0.16	1.16 ± 0.17	1.55 ± 0.16	1.70 ± 0.17	1.61 ± 0.13
1:5	0.75 ± 0.19	1.51 ± 0.14	1.28 ± 0.18	1.93 ± 0.17	0.72 ± 0.16	3.07 ± 0.51
1:5*	4.47 ± 0.25	6.39 ± 0.43	2.07 ± 0.41	6.10 ± 0.80	4.03 ± 0.44	2.66 ± 0.25
1:10*	5.25 ± 0.98	9.31 ± 1.2	7.11 ± 0.94	6.27 ± 0.60	8.67 ± 0.80	4.94 ± 0.54
1:100*	22.1 ± 4.0	25.1 ± 2.8	20.5 ± 2.3	19.7 ± 2.5	21.1 ± 1.6	17.6 ± 2.3
1:1000*	38.2 ± 3.9	33.1 ± 6.9	35.6 ± 7.4	24.1 ± 3.0	28.2 ± 4.5	23.4 ± 3.7

A, cocktail; B, concentrate; C, fresh juice.

\* ± Standard Error of Mean.

\* Significantly different from control ( $p < 0.01$ ).

\* Significantly different from control ( $p < 0.05$ ).

\* Not significantly different from control.

values and was arbitrarily divided into 5 cocktail inhibited adherence by 75 per cent more for over 60 per cent of the clinical strains.

**In vivo testing of the cranberry juice.** Fifteen mice were placed on a diet in which cranberry cocktail was substituted for their normal water supply for a period of 14 days. Fifteen controls retained their normal water supply. At 2-day intervals urine was collected from the mice for the adherence test. Since approximately 0.05 ml. of urine was collected from each mouse, the urine from 5 mice was pooled, diluted 1:1 with glass distilled water, and 0.2 ml. was used for each incubation with 0.2 ml. of *E. coli* and uroepithelial cells. The results appear in table 3. The urine from mice drinking cranberry juice significantly inhibited adherence when compared to control urines. It can also be observed that urine from control mice was not significantly different from the in vitro controls. The mice maintained on the cranberry cocktail were normally active and showed no outward signs of stress.

The antiadherence activity of cranberry cocktail was also examined in human urine. Initial trials were performed on 1 subject to establish guidelines for a larger study. Cranberry cocktail was 1st ingested in amounts ranging from 5 to 20 ounces before retiring and the urine was sampled the following morning and examined for antiadherence activity using uroepithelial cells. The results from these experiments were inconclusive. Antiadherence activity was observed but was not consistent. In a 2nd series of experiments the urine was sampled 1 to 3 hours after ingesting the cocktail. Urine taken prior drinking the cocktail was used as a baseline control. In a series of 6 experiments, it was found that ingestion of 15 ounces of the cocktail resulted in a consistent inhibition of bacterial adherence. On the basis of this observation an experiment was designed in which 22 volunteers, 9 male and 13 female, were asked to drink 15 ounces of cranberry cocktail and their urine was then sampled within 1 to 3 hours. Urine taken prior to

ingestion of the cocktail was used as a control. The results are presented in table 4. Urine from 15 of the 22 volunteers significantly inhibited bacterial adherence ( $p < 0.05$ ). Controls, in which buffer was substituted for urine, were also included. There was no significant difference between urine controls and these samples.

Increased excretion of hippuric acid has been reported to be associated with the feeding of cranberries.<sup>11</sup> The highest concentration of hippuric acid achieved in the urine was 0.02 M. Concentrations of hippuric acid ranging from 0.05 to 0.001 M were tested in the adherence assay and no inhibition was observed.

DISCUSSION

Previous investigations of the usefulness of cranberry juice in the treatment of urinary tract infections have focused on 1) the potential of cranberry juice to increase the acidity of the urine<sup>12,13</sup> and 2) the increased urinary excretion of hippuric acid, a strong bacteriostatic agent associated with the ingestion of cranberry juice.<sup>14</sup> It was found that increases in urine acidity were only slight and transient and hippuric acid rarely reached a concentration in the urine necessary for bacteriostasis of common urinary tract pathogens.

In the initial experiments performed using cranberry cocktail it was found that the cocktail was a very potent inhibitor of bacterial adherence. However, cranberry cocktail contains, in addition to cranberry juice, fructose and vitamin C as major components and has a pH of about 2.6, all of which could have individually or collectively contributed to the observed inhibition. The pH was ruled out as a factor when it was found that adherence was not affected above pH 2. This finding is consistent with the observations of other investigators.<sup>2,14</sup> Fructose is one of several carbohydrates that can inhibit adherence.<sup>15</sup> Fructose was tested at concentrations approximating that in the cocktail and was found to inhibit bacterial adherence in this range. However, the contribution of fructose to the observed inhibition of adherence by the cranberry cocktail appears to be minimal. No additive effect was observed except when very low concentrations of cranberry juice were tested in combination with fructose. Vitamin C, at the concentration occurring in the cocktail, had no observable effect on adherence. It therefore became apparent that some factor(s) in the cranberry juice, and not the pH or other constituents of the cocktail, was primarily responsible for the inhibition. This was confirmed when it was demonstrated that pure cranberry juice, either as a commercial concentrate or freshly prepared, is a strong inhibitor of bacterial adherence. Experiments designed

TABLE 2. Adherence of clinical isolates of *E. coli* to uroepithelial and buccal cells in the presence of undiluted cranberry juice

Percent Inhibition of Adherence	Number of Strains in Each Class	
	Uroepithelial Cells	Buccal Cells
>99	17	12
85-99	6	8
85-94	16	10
75-84	11	13
25-74	14	17
<25	13	11

TABLE 3. Inhibition of adherence by urine from treated mice

Treatment	Mean Bacteria per Cell*					
	Day					
	4	6	8	10	12	14
In vitro control	31.4 ± 3.5	30.7 ± 3.7	33.6 ± 5.6	29.5 ± 3.9	34.6 ± 6.2	29.1 ± 4.1
Urine from control mice <sup>b</sup>	27.8 ± 2.1	30.4 ± 4.6	29.8 ± 3.8	24.2 ± 3.2	32.1 ± 4.8	27.3 ± 3
Urine from treated mice <sup>c</sup>	9.44 ± 1.1	6.61 ± .94	6.32 ± .77	6.21 ± .51	4.22 ± .56	4.34 ± .42

\* ± Standard Error of Mean.  
<sup>b</sup> Not significantly different from control.  
<sup>c</sup> Significantly different from control ( $p < 0.01$ ).

TABLE 4. Antiadherence activity of urine of human subjects after drinking cranberry cocktail.

Treatment	Mean Bacteria per Cell*						
	4	6	8	10	12	14	16
control urine	41.2 ± 8.2	46.1 ± 9.6	39.6 ± 6.6	40.6 ± 4.3	36.4 ± 4.8	40.8 ± 9.3	40.8 ± 8.3
treated urine	*26.3 ± 3.4	48.2 ± 8.2	*22.7 ± 5.2	*17.7 ± 1.4	*13.5 ± 2.4	*11.3 ± 1.8	*11.3 ± 1.8
control urine	37.6 ± 3.6	34.3 ± 3.7	38.8 ± 6.8	45.6 ± 8.3	41.4 ± 4.9	33.7 ± 6.0	33.7 ± 6.0
treated urine	*16.6 ± 1.9	*14.4 ± 1.2	*17.3 ± 2.6	42.3 ± 5.1	36.3 ± 4.1	*14.3 ± 2.6	*14.3 ± 2.6
control urine	38.9 ± 4.9	27.6 ± 3.4	28.9 ± 2.7	37.4 ± 6.2	44.3 ± 9.3	33.7 ± 3.1	33.7 ± 3.1
treated urine	*12.0 ± 1.7	25.4 ± 6.1	*14.3 ± 1.5	*24.6 ± 4.9	*9.81 ± 1.6	*15.2 ± 1.8	*15.2 ± 1.8
control urine	31.4 ± 7.8	40.8 ± 7.5	38.4 ± 7.4	34.3 ± 4.5	34.3 ± 4.5	34.3 ± 4.5	34.3 ± 4.5
treated urine	33.6 ± 5.5	*21.2 ± 2.9	36.6 ± 4.8	36.1 ± 3.7	36.1 ± 3.7	36.1 ± 3.7	36.1 ± 3.7

\* ± Standard Error of Mean.  
<sup>b</sup> Significantly different from control ( $p < 0.05$ ).

to determine the concentration of juice necessary for inhibition produced the most striking results. At dilutions up to 1:100 a significant inhibition of adherence was observed and in the undiluted form inhibition in excess of 97 per cent was observed.

The mode of action of cranberry juice clearly seems to be associated with an interference of adherence and in particular with a surface component of *E. coli*. These conclusions are based on the following observations: 1) preincubation of the *E. coli*, but not epithelial cells, in cranberry juice strongly inhibited attachment, 2) washing of the *E. coli* cells restored the normal adherence pattern, 3) addition of the juice to epithelial cells with preattached *E. coli* caused a rapid release, 4) culture of the *E. coli* in cranberry juice for 48 hours had no effect on growth or permanent effect on adherence.

One of the problems that may be encountered in the clinical use of cranberry juice would be the delivery of the factor(s) in the juice responsible for the observed inhibition to the site of infection. We have evidence that this does occur. First, it was found that the factor(s) responsible for the inhibition of adherence are relatively stable since the inhibitory activity of the juice is still evident after 48 hours of culture with *E. coli*. Secondly, it was found that after ingestion of cranberry cocktail, the urine of mice and human subjects was able to significantly inhibit adherence of *E. coli* to uroepithelial cells. It thus appears that the active factor(s) in the juice can survive the normal metabolic degradative processes in man and in the mouse and accumulate in the urine, or alternatively, a metabolic byproduct of cranberry metabolism might accumulate in the urine. In regard to the latter possibility, urinary excretion of hippuric acid has been reported to increase with the ingestion of cranberries and cranberry juice.<sup>11</sup> It was found in the present study that hippuric acid in concentrations reported to accumulate in the urine did not inhibit bacterial adherence.

There have been several reports in the literature on the successful use of cranberry juice to treat urinary tract infections.<sup>16-18</sup> The most comprehensive investigation was undertaken by Prodromos and associates.<sup>17</sup> Their study included 44 females and 16 males. The patients were placed on 16 oz. of cranberry juice per day for 21 days. Thirty-two showed a positive clinical response, 12 were moderately improved and 16 showed no improvement. Six weeks after therapy was discontinued, 27 of the 44 showing improvement had recurring infection. Based on the evidence presented here, it would appear that the positive clinical results achieved in these investigations are probably related to the ability of the cranberry juice to prevent adherence. In particular, Prodromos<sup>17</sup> observed that withdrawal of the juice resulted in recurrence of infection in 61 per cent of the cases. This would be expected if the juice was acting to block adherence and thus would only be effective if continually present or until the normal defense mechanisms of the body could eliminate the pathogen.

The potential use of cranberry juice in the treatment of urinary tract infections might be particularly beneficial in the management of patients who suffer recurrent infections. There is presently a consensus that eradication of the infection following by long-term prophylactic therapy would be beneficial.<sup>19</sup> However, long-term prophylaxis with antimicrobial agents presents several problems including toxicity, side effects and the emergence of resistant populations. In contrast, Prodromos and associates<sup>17</sup> found cranberry juice was well accepted by their patients and no clinical side effects were observed. In this respect cranberry juice, or the factor(s) in the juice, might be of value as an adjunctive therapy and prophylaxis in patients with recurrent urinary tract infections, especially where previous long-term drug treatment has proven unsatisfactory. The observation that cranberry juice also inhibits bacterial adherence to buccal cells suggests that the juice, or the active factor

in the juice, may be beneficial for the treatment of other infections involving bacterial adherence.

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# Reduction of Bacteriuria and Pyuria After Ingestion of Cranberry Juice

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Igor Choodnovsky; Lewis A. Lipsitz, MD

**Objective.**—To determine the effect of regular intake of cranberry juice beverage on bacteriuria and pyuria in elderly women.

**Design.**—Randomized, double-blind, placebo-controlled trial.

**Subjects.**—Volunteer sample of 153 elderly women (mean age, 78.5 years).

**Intervention.**—Subjects were randomly assigned to consume 300 mL per day of a commercially available standard cranberry beverage or a specially prepared synthetic placebo drink that was indistinguishable in taste, appearance, and vitamin C content but lacked cranberry content.

**Outcome Measures.**—A baseline urine sample and six clean-voided study urine samples were collected at approximately 1-month intervals and tested quantitatively for bacteriuria and the presence of white blood cells.

**Results.**—Subjects randomized to the cranberry beverage had odds of bacteriuria (defined as organisms numbering  $\geq 10^5$ /mL) with pyuria that were only 42% of the odds in the control group ( $P=.004$ ). Their odds of remaining bacteriuric-pyuric, given that they were bacteriuric-pyuric in the previous month, were only 27% of the odds in the control group ( $P=.006$ ).

**Conclusions.**—These findings suggest that use of a cranberry beverage reduces the frequency of bacteriuria with pyuria in older women. Prevalent beliefs about the effects of cranberry juice on the urinary tract may have microbiologic justification.

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FOR DECADES cranberry-derived beverages have been thought to be useful in reducing bacterial infections of the bladder, but controversy exists as to whether this belief has any basis in scientific fact. In 1914, Blatherwick<sup>1</sup> reported that cranberries are particularly rich in benzoic acid, which is excreted as hippuric acid in the urine. Studies from the 1920s to the 1970s suggested that acidification of the urine was the mechanism through which cranberry juice produced a bacteriostatic effect,<sup>2-4</sup> but other studies have yielded conflicting results concerning urinary acidification.<sup>4-7</sup>

More recently, data have been presented on a different potential mechanism of action: the inhibition by cranberry juice of bacterial adherence to mucosal surfaces. Sobota<sup>8</sup> and Schmidt and Sobota<sup>9</sup> demonstrated that both cranberry juice and the urine produced by mice fed cranberry beverage inhibited adherence of *Escherichia coli* to uroepithelial cells by about 80%. Similar anti-adherence activity was found in human urine as well. Zabiri et al<sup>10</sup> identified two compounds in cranberry juice that inhibited lectin-mediated adherence of *E coli* to mucosal cells. One was fructose, common to many fruit juices, and the other was a nondialyzable polymeric compound that inhibited certain adhesins associated with pathogenic strains of *E coli*. Ofek et al<sup>11</sup> were able to isolate this compound from cranberry and blueberry juices, but not from grapefruit, orange, guava, mango, and pineapple juices. They hypothesized that exposure of pathogens to this compound in either the gut or the bladder produces a bacteriostatic effect by inhibiting specific adhesins present on the pilli of the bacterial surface.

Despite these intriguing findings and the widespread use of cranberry beverage ("cranberry juice cocktail") for its

supposed salutary effect on urinary tract infection, no adequately controlled, randomized clinical trial has been published evaluating its clinical utility in preventing urinary tract infection. Previous studies of the effect of cranberry juice on clinical urinary tract infections mostly have been uncontrolled, have been conducted on a small scale, and have yielded conflicting results.<sup>22-24</sup> Our study was designed to address this question.

Bacteriuria is common among elderly women both in and out of institutions.<sup>25,27</sup> Although much bacteriuria in this age group is asymptomatic and does not require treatment,<sup>28</sup> a large proportion of women older than 65 years will experience at least one urinary tract infection per year. Thus, this patient group presents an opportunity to learn whether the regular ingestion of cranberry juice beverage can influence the urinary flora or the host's granulocyte response to it.

## METHODS

After approval by institutional review boards, subjects were recruited from a large, multilevel long-term care facility for the elderly (the Hebrew Rehabilitation Center for Aged), as well as from nine housing complexes for elderly residents in the greater Boston, Mass, area. Those who were capable of giving informed consent were invited to participate in the study if they were willing to ingest at least 300 mL of cranberry juice cocktail per day throughout the 6-month study.

Subjects were excluded if they had terminal disease or severe dementia; only women were studied. In all sites, 153 eligible subjects agreed to participate and contributed at least one urine sample after baseline testing. A placebo beverage containing no juice was developed (Ocean Spray Cranberries, Inc, Lakeville, Mass) that used flavorings and color to simulate the taste and appearance of commercially available cranberry juice cocktail. Because glucose intolerance is common among elderly women, both the cranberry and placebo beverages were sweetened with saccharin. Beverages were delivered in identical containers except for coded lot num-

From the Program for the Analysis of Clinical Strategies, the Gerontology Division (Dr Avorn, Monane, Gurwitz, and Glynn, and Mr Choodnovsky), and the Division of Preventive Medicine (Dr Glynn), Department of Medicine, Brigham and Women's Hospital and Harvard Medical School; the Hebrew Rehabilitation Center for Aged (Dr Lipsitz); and the Geriatric Research and Training Center, Harvard Medical School, Boston, Mass. This work was funded through a research grant to Ocean Spray Cranberries, Inc, but the sponsor has no role at all in the development of the experimental design, analysis of the data, or interpretation and presentation of findings. No author serves as a consultant to Ocean Spray or has any financial relationship with the company.

Reprint requests to Program for the Analysis of Clinical Strategies, Brigham and Women's Hospital, 221 Longwood Ave, Boston, MA 02115 (Dr Avorn).

bers. Subjects providing informed consent were randomly assigned to receive standard, commercially available, low-calorie cranberry juice cocktail or indistinguishable placebo beverage, in most cases after a 1-month trial of placebo beverage to determine that daily intake would be acceptable throughout the study. Subjects were permitted to consume the beverage in single or divided doses.

We randomized subjects by means of odd vs even digits in the subject's institutional identification number or telephone number to determine which coded lot number of beverage would be used throughout the study. Neither participants nor investigators were aware of whether a given subject was receiving cranberry beverage or placebo beverage. Subjects were instructed not to consume any cranberry products other than those distributed to them during the study. To prevent the possibility that subjects in the institutional setting who were randomized to placebo beverage might inadvertently consume standard cranberry beverage elsewhere in the institution, all such beverage was converted to the placebo product throughout the institution during the study. To ensure that each subject continued to receive the correct beverage, patients were interviewed monthly. In addition, all bottle caps were collected from participants each month, and the coded lot number printed on each bottle cap was rechecked by a research assistant.

Sample size calculations were performed before the study began. We assumed that the overall rate of bacteriuria and pyuria in the control group would be about 0.50, and that the reduction attributable to the cranberry beverage in rate of bacteriuric-pyuric urine samples would be 40%, making the rate in the experimental group 0.30. Therefore, power of 0.80 and a confidence level of 95% would require a sample size of 92 subjects in each group; a total of 192 subjects were enrolled in the study. Data are presented on the 163 subjects who provided a baseline urine sample and at least one additional sample after randomization. No data were available for subjects who withdrew from the study without providing any urine samples after the baseline.

At study entry, a geriatric nurse obtained a complete medical history and instructed subjects in the proper method of obtaining a midstream, clean-voided specimen. In those subjects unable to collect an adequate clean-voided specimen themselves, the geriatric nurse assisted in performing the clean-catch procedure. Each month, the geriatric nurse or a research assistant, both of whom were blinded to subjects' study group

assignment, collected the urine samples. At the same time, the nurse collected an interval history to determine evidence of symptomatic urinary tract infection as well as any other symptoms occurring in the prior 30 days. If at study intake or during any monthly interval a subject had been prescribed an antibiotic for any indication, the nurse noted this in the study record. No urine was collected, and the subject was deferred from further study until 1 month after the end of any antibiotic course.

Standard urinalysis, bacterial culture, and antibiotic sensitivity testing were performed on each urine sample immediately after collection. Urine samples were collected until each subject had contributed a baseline specimen plus six monthly specimens or had withdrawn from the study. Data analysis included all urine samples contributed by subjects who provided at least one sample after the baseline collection, whether or not they remained enrolled for all six monthly collections. Samples of both the cranberry and placebo beverages were analyzed by Natan Sharon, PhD, Weizmann Institute, Rehovot, Israel, for their capacity to inhibit adhesion by *E coli* to human erythrocytes, as described previously.<sup>14,15</sup> These samples were identified by code number only to ensure blinded assessment.

A second research assistant, unaware of the experimental design or subject assignment, entered data into a relational database. The primary outcome was bacteriuria (organisms numbering  $\geq 10^5$ /mL, regardless of organism) with pyuria in a given study month. All subjects who continued to contribute urine samples were included in the analysis. We used logistic regression analysis to estimate the odds that this outcome was associated with assignment to the cranberry beverage group. Each participant contributed up to six assessments of this outcome, corresponding to six follow-up intervals. Because replicate assessments from the same participant were not statistically independent, SEs from the logistic regression model that treated assessments as independent were adjusted by the estimating equation approach of Liang and Zeger.<sup>16</sup> Logistic regression models with adjusted SEs were used to examine and adjust for the effect of bacteriuria with pyuria at baseline when examining the effect of cranberry beverage on study outcomes after randomization. An additional variable considered in the regression model was history of urinary tract infection in the 6 and 12 months before the study.

In secondary analyses we evaluated transition probabilities into and out of the state of bacteriuria with pyuria. The

goal was to measure separately whether cranberry beverage might promote recovery from existing infection or prevent the development of infection. Probabilities of recovery from and development of infection over each 1-month interval were estimated separately for subjects in the cranberry beverage and placebo beverage groups. Average 1-month transition probabilities in each group were weighted according to the denominators of the month-specific transition probabilities. We used logistic regression with SEs adjusted for replicate assessments to estimate odds ratios (ORs) and 95% confidence intervals for the transitions associated with treatment assignment. The unit of analysis in these logistic models was the 1-month study interval. First, we considered intervals without bacteriuria plus pyuria at the beginning of the interval and predicted the probability of bacteriuria with pyuria at the end of the interval. A second model evaluated intervals with bacteriuria and pyuria at the beginning and predicted the probability of no bacteriuria plus pyuria at the end of the month.

## RESULTS

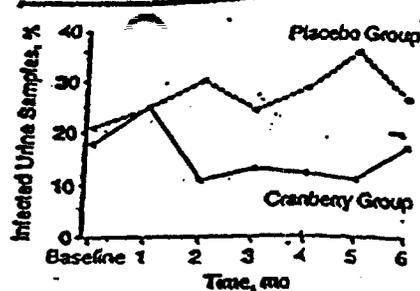
A total of 618 urine specimens were collected from the study subjects after baseline. About one third produced growth of  $10^6$  organisms per milliliter or more, about one third produced no bacterial growth, and the remainder yielded intermediate growth of organisms. *Escherichia coli* was the most commonly identified organism (43% of isolates), with *Klebsiella* the second most common single organism (7%); mixed flora accounted for 22% of bacteriuric-pyuric urine samples. As in other studies of urinary tract infection in the elderly,<sup>20-22</sup> the proportion of isolates represented by *E coli* was smaller than that seen in urinary tract infection in younger women.

The analyses that follow include data from all 163 subjects who contributed one or more urine samples after randomization; 109 subjects were community dwelling, and 44 were residents of a long-term care facility. Sixty of those randomized to the cranberry group and 61 of those in the placebo group completed the full 6 months of study. As shown in Table 1, both groups were similar in age, number of medications used, and number of medical problems, as well as bacteriuria, pyuria, and urinary tract symptoms at baseline, although subjects randomized to the cranberry group had a lower rate of previous urinary tract infection by history. Only four subjects used estrogen-containing compounds (two in each group). Slightly less than half of all urine samples revealed white blood cells on microscopic examination (44.4%) or had a positive

	Cranberry Group	Placebo Group
No. of participants	32	61
Mean±SD age, y	76.1±6.3	78.0±9.4
Mean±SD No. of diagnoses	2.5±2.1	2.5±2.2
Mean±SD No. of medications	3.3±2.7	3.4±2.7
Bacteriuria (organisms ≥10 <sup>4</sup> /mL) with pyuria present at baseline, No. (%)	13 (41)	17 (28)
Symptoms referable to urinary tract at baseline, No. (%)		
Oystritis or "burning"	1 (3)	1 (2)
Frequency	7 (22)	11 (18)
Flank pain	0	0
Incontinence	5 (16)	0
Foul odor of urine	3 (9)	7 (11)
History of genitourinary abnormalities, No. (%)		
Renal insufficiency	2 (6)	2 (3)
Cystocele	4 (12)	6 (10)
Pessary	1 (3)	1 (2)
Rectocele	3 (9)	1 (2)
Uterine prolapse	2 (6)	4 (7)
Urinary incontinence	11 (34)	16 (26)
Bladder stones	0	1 (2)
Solitary kidney	2 (6)	3 (5)
Renal stone	2 (6)	2 (3)
Urinary retention	2 (6)	0
Other genitourinary pathology	0	2 (3)
Previous urinary tract infection by history, No. (%)		
Ever	33 (100)	36 (59)
In past 12 mo	12 (38)	27 (44)
In past 6 mo	5 (16)	20 (33)
Antibiotic use in 6 mo before study	2 (6)	3 (5)

emical test for leukocyte esterase (10%). Symptoms referable to the urinary tract were noted on 22.0% of all month interval histories. Consumption study beverages measured by returned the caps and subject report exceeded 1% of assigned quantities. Bacteriuria with pyuria was found in 15.0% of urine samples in the placebo group and only 15.0% in the group randomized to the cranberry beverage. We found a difference in the mean proportion of bacteriuric-pyuric urine samples in each group for each individual (15.0% vs 16.5%, respectively). The difference was not present in the first month after randomization, but appeared strikingly between months 1 and 2 when remained fairly stable throughout the rest of the trial (Figure). Adding for replicate assessments of the individuals, we found an OR of 0.27 for bacteriuria with pyuria in subjects randomized to the cranberry beverage relative to control subjects (95% confidence interval, 0.23 to 0.76;  $P=0.04$ ). Effect persisted when we added to the model a variable describing a history of urinary tract infection in the 6 months prior to randomization (OR for infection in cranberry group, 0.53;  $P=0.01$ ) or 12 months prior to randomization (OR, 0.48;  $P=0.01$ ). Subjects in the placebo group also exhibited a trend toward less bacteriuria irrespective of

pyuria (34% of all urine samples in the control group vs 23% in the cranberry group), but this difference was not statistically significant in the replicate assessment analysis ( $P=0.09$ ). The average 1-month probability of change from bacteriuric-pyuric urine to a sample not meeting criteria for infection was 54% in the cranberry group and 28% in the placebo group (Table 2). The average 1-month probability of change from non-infected to bacteriuric-pyuric urine was 19% in the cranberry group and 12% in the placebo group. As a result, when odds of transition into and out of infection were studied, subjects randomized to the cranberry beverage group were far more likely than controls to make a transition from bacteriuric-pyuric to non-bacteriuric-pyuric urine on successive months (Table 2). For intervals beginning with a bacteriuric-pyuric urine sample, the OR for a bacteriuric-pyuric sample at the end of the interval in the cranberry group was 0.27 ( $P=0.006$ ), indicating that these subjects were only about a quarter as likely as controls to continue to have a bacteriuric-pyuric urine sample. These findings were also unaffected by adjusting for a history of urinary tract infection in the 6 months (OR, 0.31;  $P=0.02$ ) or 12 months (OR, 0.30;  $P=0.01$ ) before the study began. Most subjects with bacteriuria were asymptomatic, and many with symptoms referable to the urinary tract did not have bacte-



Percentage of urine samples at each month that had bacteriuria (organisms numbering ≥10<sup>4</sup>/mL) with pyuria for subjects randomized to the cranberry beverage (solid line) or the placebo beverage (dotted line).

riuria or pyuria. Of the 473 urine samples collected in the cranberry group, only 20 (4%) had bacteriuria and pyuria concurrent with the subject's reporting urinary tract symptoms, compared with 37 (7%) of 498 urine samples in the placebo group, although this did not reach statistical significance.

Antibiotic use after randomization included 16 instances of treatment for urinary tract infection in the control group (3.2 per 100 person-months) vs eight instances in the experimental group (1.7 per 100 person-months). All decisions to use antibiotics were made by subjects' own physicians, who were unaware of the study design or group assignment. No significant difference was observed in the acidification of urine in the cranberry group vs placebo group (median pH=5.0 and 5.5, respectively).

In analyses of the coded study beverages for their effect on bacterial adhesiveness<sup>11,12</sup> conducted by Dr Sharon and colleagues at the Weizmann Institute, 18 of 18 samples of the active cranberry beverage used in the study were found to inhibit *E. coli* adhesion *in vitro*; none of the 15 samples of placebo beverage used exhibited any inhibitory activity.

COMMENT

Despite decades of folk wisdom concerning the effects of cranberry juice on the urinary tract, to our knowledge this study represents the first placebo-controlled, large-scale clinical trial to document the *in vivo* effect of cranberries on bacteriuria with pyuria. While asymptomatic bacteriuria in elderly women is commonly observed, it does not represent a condition with a negative prognosis<sup>13</sup> or one that requires treatment.<sup>14,15</sup> However, demonstration of the capacity of cranberry beverage to reduce the occurrence of bacteriuria with pyuria in elderly women does lend credence to the belief that it contains a substance with biologic activity in relation to the urinary tract. The effect was not seen in the first study

	Month					
	1	2	3	4	5	6
Transitions From Bacteriuric-Pyuric to Non-Bacteriuric-Pyuric Urine						
Placebo group, %	24	16	38	35	19	33
Cranberry group, %	31	59	39	75	75	57
Transitions From Non-Bacteriuric-Pyuric to Bacteriuric-Pyuric Urine						
Placebo group, %	11	11	10	16	19	5
Cranberry group, %	15	2	7	10	9	13

month; it appeared only after 4 to 8 weeks of use of cranberry beverage and then persisted at about the same level. This time course could be compatible with modification of gut flora, which are the typical pathogens in urinary tract infections among women. The modest reduction seen in the rate of antibiotics prescribed by experimental group subjects' physicians to treat urinary tract infection suggests that this difference may have manifested itself in important clinical outcomes.

We did not find evidence that urinary acidification was responsible for the observed effect, since the median pH of urine samples in the cranberry group (6.0) was actually higher than that in the experimental group (5.5). While cranberry juice has been advocated as a urinary acidifier to prevent urinary tract infections, not all studies have shown a reduction in urine pH with cranberry juice ingestion, even with consumption of 2000 mL per day.<sup>13-15</sup> The amounts used in this trial (300 mL) may not have been large enough to produce this effect. Blatherwick,<sup>1</sup> in 1914, postulated that suppression of bacteriuria by cranberry juice consumption was not related to urine pH or hippuric acid formation but rather to some bacteriostatic properties of cranberry juice or its components.

Although the control group subjects had nearly identical rates of bacteriuria and pyuria at baseline, and baseline status was controlled for in all replicate assessments, control subjects were more likely to have histories of urinary tract infections. The effect of the cranberry beverage persisted even when this information was included in the multivariate regression equation. The rate of attrition differed slightly between the two groups: of those who contributed one or more urine samples following the baseline measurement, 60 (83%) of 72 subjects in the cranberry group completed all 6 months of the study compared with 61 (75%) of 81 in the placebo group. If subjects with sterile urine samples were more likely to drop out before contributing the full 6 months of study, this could have influenced our findings, although such an explanation seems unlikely.

Despite the popularity of use of cranberry juice for prevention of urinary tract infections, the probabilities-of-transition analyses indicate that its effect was more pronounced in converting urine samples out of a state of bacteriuria with pyuria, as compared with preventing the conversion of noninfected urine samples to bacteriuric-pyuric ones. While this does not imply that such a regimen should displace antibiotics as

needed, it does suggest important possibilities for the role of bacterial adhesion in the cause and treatment of urinary tract infection. The findings also indicate the need for a trial to determine whether treatment of urinary tract infections with antibiotics plus cranberry beverage would yield outcomes superior to those seen with antibiotics alone.

These findings, if replicated in other settings, would suggest evidence for the bacteriostatic property of cranberry beverage in the bladder. Further studies are needed on the biochemical properties of this substance. Future randomized trials of longer duration in younger women with symptomatic cystitis, as well as other patient groups with recurrent urinary tract infection, will help to clarify the role of cranberry beverage in the prevention and, as an adjunct to antibiotics, in the treatment of this common disorder.

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