Comparison of the Effect of Cranberry Juice on Urinary and Son-urinary Bacterial Isolates

by

Debra Schmidt

Submitted in Partial Fulfillment of the Requirements
for the Degree of
Master of Science
in the
Biology
Program

Youngstown State University

June, 1985

THESIS APPROVAL FORM

THESIS TITLE: Comparison of the Effect of Cranberry Juice on Urinary and Non-Urinary Bacterial Isolates

AUTHOR: Debra Schmidt

DEGREE: Master of Science - Biology

ADVISOR: Dr. Anthony E. Sobota

COMMITTEE MEMBERS:	ACCEPT	REJECT
[signature]	✓	
Leonard B. Spiegel	✓	
James R. Taylor	✓	
Paul VanLandt	✓	

DATE 28 May 85

1 copy to accompany library copy

1 copy to advisor

1 copy to Departmental Files

ABSTRACT

Comparison of the Effect of Cranberry Juice on
Urinary and Non-urinary Bacterial Isolates

Debra Schmidt

Master of Science

Youngstown State University, 1985

The administration of cranberry juice has been used successfully for the prevention and treatment of urinary tract infections. This investigation demonstrates cranberry juice to be a potent inhibitor of bacterial adherence. Since the initial event in colonization and invasion by microorganisms is the adherence to the epithelial cells of mucosal surfaces, it would appear this is the mechanism by which cranberry juice combats urinary tract infections. In this investigation it is demonstrated that adhesiveness to human urinary tract epithelial cells was higher for bacterial strains isolated from patients with diagnosed urinary tract infection than for organisms isolated from sputum, stool and wound sources. Bacteria incubated in cranberry juice showed a 70% decrease in adherence, In addition Escherichia coli that were allowed to attach to uroepithelial cells were rapidly released when cranberry juice was added. Urine collected after the ingestion of 12 ounces of cranberry juice inhibited adherence of E.coli to uroepithelial cells by approximately 58%. Antiadherence

activity was also detected on uroepithelial cells collected after ingestion of the cranberry juice. There was a 64% decrease in bacteria adhering to these uroepithelial cells. Antiadherence activity remained in the urine 4 hours after ingestion of the cranberry cocktail. This study has demonstrated that cranberry juice has a strong inhibitory effect on the adherence of bacterial isolates to epithelial cells.

ACKNOWLEDGMENTS

I would like to thank Erma Hastings MT (ASCP) of the Microbiology laboratory at Alliance City Hospital for providing the clinical specimens and Rebecca L. Wilson MT (ASCP) for her support. I am also very grateful to Dr. Anthony E. Sobota for his counsel and criticism of various portions of the thesis.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

IYTRODUCTION

During the past few years it has been demonstrated that urinary tract infections are the most common bacterial infections of humans of all ages. Urinary tract infections are most common in females with about 1% developing urinary tract infections by age 10, and that increases to 3% to 5% by adolescence: some 65% of the cases in women are recurrent. The rate of incidence increases about 1% per decade in women, suggesting that 20% of all women will have urinary tract infections at some time in their lives. The higher rate of infection in females can be attributed to a number of factors: a short urethra, anomalies or obstructions of the urinary tract, sexual intercourse, and pregnancy. By early adolescence, about 1% of males have urinary tract infections which recurs in about 20% (Wright and Matsen, 1984). For this reason a great deal of research both presently and in the past has dealt with bacterial adherence since it appears that attachment of bacteria to the mucosal surface is a prerequisite for infection.

Studies have shown that most urinary tract infections are due to gram negative aerobic 'bacilli that originate in the gut. Even though anaerobic fecal flora are also present in concentrations from 100 to 1000 times greater in the stool, they are rarely **involved in urinary tract infections, The**

most common pathogens causing urinary tract infections are the Enterobacteriaceae. Escherichia coli is the most common of these, accounting for approximately 80% of all urinary tract infections; Klebsiella, Proteus and Enterobacter follows, Pseudomonas, staphylocci and group *3* streptococci account for about 5% to 10% of the remainder infections (Kunin, 1975).

The urinary tract is normally resistant to infection. In the healthy individual a variety of normal functions act to continually reduce the body's bacterial burden. Desquamation and other forms of epithelial cell turnover at body surfaces remove large numbers of adherent bacteria. Defeaation results in the elimination of 10^{12} bacteria daily, and urination eliminates microorganisms colonizing the urethral epithelium (Durtz and Mills, 1982). Also the mucus layer overlying epithelial surfaces hinders contact betweeneepithelial cells and agents in the lumen: it also acts as a carrier of defense factors secreted from epithelial cells, including secretory IgA antibodies (Svanborg Eden, et al., 1981). Successful pathogens are those that are capable of penetrating these defense mechanisms, They attach to the mucosal surface and are also capable of colonizing the new surface as cells are desquamated, The bacteria may also penetrate the epithelial barrier, (Beachey, 1981).

In spite of these host defenses most infections occur via the mucous membranes. In recent years, evidence has accumulated to suggest that with urinary tract infections the

initial invasion is the adherence of bacteria to the epithelial cells of the mucosal surfaces (Beachey, 1981). This ability to attach may be considered a prime determinant of bacterial pathogenicity. Thus a successful infection depends on an interaction of the virulence factors of the bacteria and the host susceptibility and defense mechanisms.

Most recurrent urinary tract infections are caused by the reintroduction of bacteria from the fecal reservoir followed by the colonization of the vaginal and urethral mucosa which precedes the bacteriuria (Schaeffer, et al., 1979). The resulting urinary tract infection may be symptomatic, as in acute pyelonephritis or acute cystitis, or may be only detected at screening, as in asymptomatic bacteriuria (Svanborg Eden and Jodal, 1979). In symptomatic patients the same bacterial organism can be detected in both the feces and urine at the onset of the urinary tract infection. In the asymptomatic patient, which is only picked up by screening, the correlation between urinary and fecal bacterial strains is poor (Svanborg Eden, et al., 1979). The difference between the various forms of urinary tract infections appears to be related to the expression of the virulence factors in the bacterial strains. For example, it has been found that certain E. coli serotypes predominate in acute pyelonephritis. They possess complete lipopolysaccharide (O antigen) and capsular polysaccharide (K antigen) which seems to enhance adherence in these bacterial strains (Svanborg Eden, et al., 1980). In the same report Svanborg Eden, et al. (1980) also observed

that once pathogenic bacteria: attach, they increase their concentration of nutrients and they may form a polysaccharide capsule in order to prevent phagocytosis. Secretion of the liposaccharide may be of importance for the inflammatory reaction and onset of symptoms. Bacterial strains then which have low bacterial adherence may still be able to survive in the urinary tract but cannot cause major symptoms in the host. Also Svanborg Eden, *et al.* (1980) reported that *E.coli* strains that were isolated from the urine of symptomatic patients were not drawn randomly from the fecal reservoir. The distribution of O and K antigens differed from fecal *E. coli*, which indicated selection. Bacterial strains causing pyelonephrititis were richer in K antigen than strains causing cystitis or those isolated from the feces.

The concept that adherence of bacteria to both vaginal and uroepithelial cells plays an important role in urinary tract infections has been both supported and questioned by *in vitro* studies comparing adherence in controls and patients. In a study done in 1976 by Svanborg Eden and L.A. Hanson, *et al.*, they determined that *E.coli* causing symptomatic urinary tract infections differ from *E.coli* causing asymptomatic urinary tract infections. *E.coli* that were isolated from the urine of patients with acute pyelonephritis and acute cystitis attached in larger numbers to human urinary tract epithelial cells *in vitro* than did *E.coli* from patients with asymptomatic bacteriuria. They concluded that bacterial attachment may be one of the virulence factors determining the efficacy of

urinary tract invasion. A similar study was done by Svanborg Eden and Jodal in 1979 this time using epithelial cells obtained from the urinary tract of infection-prone and healthy children. They combined these epithelial cells with E.coli isolated from a patient with a recurrent urinary tract infection. Once again there was higher bacterial adherence to epithelial cells derived from patients with infection than for epithelial cells obtained from the children with no prior history of urinary tract infection. In vitro studies also demonstrated that more E.coli bacteria attached to vaginal and periurethral epithelial cells from patients with recurrent urinary tract infections than to cells from subjects without such infections (Svanborg Eden and Janson, 1979). All of these studies imply a relationship between the adhesive capacity in vitro and the severity of urinary tract infections in vivo. A study, however, carried out by M.J. Harber, et al., (1982) suggested that adherence is not a virulence factor for bacteria within the urinary tract. They found, that of the bacteria tested, the symptomatic and asymptomatic producing strains showed no difference in their ability to adhere to either buccal cells or uroepithelial cells. They felt that previous claims for the existence of a correlation between adherence of urinary pathogens and clinical severity of urinary tract infections was unfounded.

Whereas E.coli in general causes most urinary tract infections, Proteus mirabilis is common in young boys and in patients with recurrent urinary tract infections, An investi-

gation by Svanborg Eden, P. Larson, et al., (1980) demonstrated that the attachment of P. mirabilis to human uroepithelial cells is different from that of E.coli. A major difference is in the antigenic composition of the cell wall of the two species, P.mirabilis lacks a polysaccharide capsule (Svanborg Eden, L. Hagberg, et al., 1980). Most of the 335 P. mirabilis strains tested attached in large numbers to the uroepithelial cells regardless of the bacteria's origin. There was no difference in mean adherence of strains isolated from individuals with urinary tract infections and strains from other sources. P.mirabilis strains also only attached to squamous but not transitional epithelial cells, whereas E.coli adhered to both types, These results suggest that P.mirabilis is less efficient in colonizing the bladder in patients, since the bladder epithelium only contains transistional epithelial cells.

There also seems to be some debate over the method of choice for studying bacterial adherence. Svanborg Eden, et al.,in their studies relied on microscopic inspection of incubated cell preparations. A.J. Schaeffer, et al., (1979) used bacteria labeled with [^{3}H] uridine. The use of these radioisotopically labeled bacteria eliminated problems associated with identifying or removing indigenous bacteria that frequently colonize epithelial cells. K.J. Harber, et al., (1981) felt that these methods did not allow for a distinction between attachment to urinary mucus and attachment to epithelial cells per se. They used a stain which

contained 0.25% toluidine blue in borax which allowed them to visualize uromucoid. Svanborg Eden, et al. (1977) also added a trypan blue stain to the cell suspension, before counting, to exclude dead epithelial cells. Dead cells appear not to bind as many bacteria as viable cells. However, Chick, et al. (1981) found trypan blue an unreliable index of cell viability and their test regarding adherence differences was inconclusive.

A series of studies were also conducted to determine the optimal conditions for bacterial adherence (A.J. Schaeffer, et al., 1979). It was found that adherence was not appreciably affected by temperature and was maximal at a pH of 4 to 5. However, Parsons and Schmidt (1980) found no significant differences in mean adherent bacteria at a pH of 4 and 6.4. They also noted that maximal adherence occurred within one minute and rather than increasing with time, as other investigators had reported , adherence decreased gradually to a stationary level of adherence approximately 50% of that observed initially, No relationship was found between the age of the individual who donated the epithelial cells and the capacity of E.coli to attach (Svanborg Eden and Jodal, 1979). Adherence was however, correlated with the day of the menstrual cycle and was found to be highest during the estrogen dependent phase and diminish after ovulation (Schaeffer, et al., 1979). ddherence was also found to be enhanced by bacterial incubation in broth for 72 hours and inhibited by α-D-mannose (Schaeffer, et al., 1979).

The adherence of bacteria to uroepithelial cells is dependent on specific recognition systems between bacteria and epithelial cells. Bacteria are thought to possess certain molecular structures on their surfaces that are capable of binding in a stereospecific fashion with specific molecular structures on the surfaces of the tissue cells of the host. Pili and fimbriae are believed to mediate the attachment of gram negative bacteria to various host tissues. Several experiments have supported this hypothesis, In a study done by Svanborg Eden and Hansson (1978), they showed a significant correlation between the presence of pili or fimbriae on E.coli and the ability of the bacteria to adhere to uroepithelial cells. Of the 12 strains tested, none with adhesive ability lacked pili and only a few of the non-adhering strains contained pili. When they treated the bacteria to remove the pili a loss in adhesion paralleled the loss of pili, It was then concluded that pili or substances coappearing with pili were likely responsible for the ability of E.coli to adhere to human uroepithelial cells, and thus initiating various forms of urinary tract infection.

In a study of experimental ascending infection with Proteus, Silverblatt and Ofek (1975) showed that rats inoculated with heavily piliated organisms developed significantly more pyelonephritic kidneys than did animals challenged with lightly pilated Froteus. Furthermore, when they examined the rat kidney by electron microscopy 24 hours later, many heavily piliated bacteria had become bound to the renal

pelvic mucosa, Another study using exfoliated rabbit bladder cells and heavily and lightly piliated Proteus also confirmed their results, once again the heavily piliated Proteus bound more readily than did the lightly piliated strain (Silverblatt and Ofek, 1975). A similar test was conducted using human buccal epithelial cells and the results were once again the same, showing that binding was not specific for cell type, nor for animal species (Silverblatt and Ofek, 1975). Schaeffer, et al. (1979) noted that pili could be washed off the bacterial surface when subjected to several washings prior to incubation. Adherence decreased by 10 to 25%. Also bacteria that were grown in broth for 72 hours to stimulate pili formation adhered more than organisms grown on agar, a medium not conducive to piliation (Schaeffer, et al., 1979).

In order for adherence to occur between bacteria and epithelial cells, the epithelial cell must also contain specific receptor sites that are recognized by a specific molecule on the bacteria. The inhibition of bacterial adherence by purified receptor or ligand material serves to demonstrate directly its role in adherence, unfortunately, in most cases neither the ligand nor the receptor have been identified or purified. Investigators have resorted to using receptor or ligand analogues. The receptor site for the Enterobacteriacae appears to be similar to the simple sugar mannose. The ligand and receptor for other bacteria vary according to the species.

Ofek, et al. (1977) performed a series of experiments

demonstrating that saturation of binding sites on the bacterial surface by mannose or mannose-like sugars prevented the attachment of these organisms to epithelial cell receptors. When they added 3-mannose or its derivatives to the epithelial cells to which E.coli was preattached, this caused rapid release of the organism from the epithelial cells. They concluded that the binding of E.coli to epithelial cells is mediated by a mannose specific lectin-like structure present on the surface of the E.coli which binds to a mannose-like receptor on the epithelial cell. Schaeffer, et al. (1979) also showed that adherence was markedly enhanced by bacterial incubation in broth for 72 hours and inhibited by α-D-mannose. These results suggest that adherence can be considered a complex phenomenon and appears to be mediated by pili on the bacteria and mannose residues on uroepithelial cells.

Uroepithelial cells appear to have a limited number of receptors on their surface. With, increasing concentrations of bacteria, the number of bacteria adhering per uroepithelial cell increased but at high concentrations, adherence tended to stabilize; at this point the epithelial cells were saturated (Schaeffer, et al., 1979).

An indication of the possible role of sugar binding activity in infectivity was obtained by an in vivo study performed by Aronson, et al. (1979) using mice, In these experiments infective strains of E.coli were injected into the bladder of mice in the presence or absence of different sugars, It was found that methylα-D-mannoside, but not

methyl α-D-glucoside, caused a marked reduction in the number of bacteriuric mice, Similar experiments were obtained in a rabbit model of gastrointestinal infection where colonization of E.coli was specifically blocked by 3-mannose but not by other sugars (Hirschberger, et al., 1977).

Since the attachment of certain bacterial pathogens is mediated by bacterial surface structures, antibacterial agents altering the bacterial surface are likely to affect attachment, Small amounts of antibacterial agents that decrease attachment may be sufficient to prevent colonization and may be sufficient for prophylaxis against recurrent urinary tract infections. E.coli that were still viable, were found to adhere less after treatment with sub-inhibitory concentrations of ampicillin and amoxycillin (Svanborg Eden, et al., 1978), Eisenstein, et al. (1980) found a decrease in the mannose binding capacity of E.coli after streptomycin, tetracycline and trimethoprim treatment, It appears that certain properties of bacterial adherence (piliation, mannose binding, and adherence) appear to be more sensitive than others to suppression by different antibiotics.

Growth of E.coli in streptomycin, gentamicin or tetracycline, but not chloramphenicol or streptinomycin, resulted in a reduction in the degree of piliation of those cultures (Eisenstein, et al., 1980). Penicillin G inhibits the expression of the mannose specific ligands in E.coli by distorting cell wall biosynthesis. Streptomycin suppresses the formation and expression of the mannose specific ligand

in E.coli by acting on the bacterial ribosome to induce misreading of messenger RNA, which leads to abnormal protein synthesis (Beachey, 1981).

Although there are numerous antimicrobial agents available for use in the treatment of urinary tract infections only a few are suitable for long term prophylaxis. The treatment of choice for recurrent urinary tract infections is a continuous, low dosage prophylaxis. The agent used must cover a wide range of pathogens, be of low toxicity, have minimal side effects and should not produce a fecal reservoir filled with resistant strains,

Cranberry juice, for some time, has been considered a folk remedy for the relief of dysuria and urinary tract infections. A study done by Sobota (1984) has now shown cranberry juice to be a potent inhibitor of bacterial adherence, In his study, a total of 77 clinical isolates of E.coli showing a positive adherence were tested. Cranberry juice inhibited adherence by 75% or more for over 60% of these strains. Also urine from mice and humans drinking cranberry juice significantly inhibited the adherence of E.coli to uroepithelial cells. These results demonstrate cranberry juice contains an active factor(s) that interferes with bacterial adherence.

The aim of this study is to continue to investigate the potential role of cranberry juice in the treatment of urinary tract infections. The effects of cranberry juice on the adherence of various strains of bacteria were studied. In addition the adhesive ability of the five bacterial

pathogens most commonly causing urinary tract infections were compared to see if strain or isolation source affects adherence.

CHAPTER II

MATERIALS AND METHODS

Bacteria and Culture Conditions

A total of 145 gram negative bacterial isolates consisting of 15 Klebsiella, 5 Enterobacter, 30 Pseudomonas 32 Proteus and 63 Escherichia coli were used in this study. All urinary bacterial isolates were obtained from hospital patients with a known urinary tract infection (greater than 10^5 bacteria per mL of urine), Other sources of bacteria ranged from blood, stool, sputum, wound, throat and ear. All bacteria were obtained from the microbiology laboratory at Alliance City Hospital, Upon receipt, the organisms were streaked on MacConkey agar plates and cultured for a minimum of 24 hours at 37°C. They were then identified using API 20E strips which identify. gram negative organisms on the basis of their specific biochemical reactions, Once identified, the organisms were transferred to Brain Heart Infusion agar deeps (BHI Difco) and grown at 37°C for 48 hours and then stored at 2° - 6°C. When bacteria were needed for testing, they were transferred from the BHI agar deeps to BHI broth (BHI Difco) and grown at 37°C for 48 hours. At the end of this time period, 2 ml of the culture was removed and centrifuged at 2,500 rpm for 10 minutes. The supernatant was discarded and the bacterial pellet was resuspended in 2 ml of 0.01 M phosphate buffered saline (PBS) at a pH of

7.2. The pH of the PBS was determined using an Owens Corning pH and gas analyzer. The concentration of bacteria was approximately 10^9 bacteria per ml.(Sobota, 1984).

Uroepithelial Cells

Human urinary tract epithelial cells were obtained from the sediment of freshly voided midstream urine specimens. Urine specimens were obtained from one healthy individual with no prior history of urinary tract infection. A sterility control was done by inoculating one calibrated loopful (0.001 ml) of urine onto blood and MacConkey agar plates and incubating them at 37°C for 48 hours. Urine samples were also randomly screened for the presence of abnormal amounts of white blood cells and albumin, both of which indicate urinary tract infection.

A 10 ml sample of urine was used for each unknown to be tested. The 10 ml of urine was centrifuged at 2,500 rpm for 10 minutes to harvest any cells. The supernatant was discarded and the epithelial pellet was washed one time with 10 ml of PBS buffer, centrifuged for 10 minutes and the supernatant was discarded. The cell pellet was then resuspended in PBS buffer to a final concentration of 10^5 cells per ml using a hemocytometer. Uroepithelial cells were mostly of the squamous cell type (Sobota, 1984).

Bacterial Adherence Test

The method used in these experiments was that of Sobota (1984) and is a modification of the technique described by Parsons, et al..(1980). Two ml of uroepithelial cells at

10^5 cells per ml was added to 2 ml of bacteria at 10^9 organisms per ml.. The resulting sample was then vortexed and allowed to incubate for 30 minutes at 37°C in a water bath. The samples were mixed every 5 minutes by inversion.. After incubation, 1 ml of the cell-bacteria mixture was removed using a tuberculin syringe. The tuberculin syringe containing the 1 ml sample was then attached to a filter apparatus containing a 8 μm pore size polycarbonate membrane filter (Nucleopore). The sample was filtered and then immediately washed with 30 ml of distilled water, thus removing any non-adherent bacteria present on the filter. The epithelial cells with any adhering bacteria were trapped on the nucleopore filter. While still wet, the filter was removed from the holder and pressed against a glass slide (topside down) and allowed to dry, After drying the filter was removed leaving the epithelial cells adhering to the slide. Samples were then gram stained. The Gram stain method used was 10 seconds of Gram Crystal Violet, followed by 10 seconds of Grams Iodine, followed by 10 seconds of 95% ethanol and finally 10 seconds of Grams Safranin.. This stain distinguished gram negative bacteria from gram positive bacteria, while it also stained the cytoplasm of the epithelial cells a slight pink color and the nucleus a purple color. The gram negative bacteria on the first 50 intact epithelial cells were counted via oil emersion using a bright field microscope. Adherence was recorded as the average number of bacteria per epithelial cell on the 50 counted cells. A control of epithelial cells

without added bacteria was run concurrently with the unknown samples. Results of the adherence tests were omitted if the background counts exceeded five bacteria per epithelial cell (Eisenstein, Beachey and Ofek, 1980).

Cranberry Juice

The cranberry juice used in these experiments was one of two typesr Either Ocean Spray Cranberry Cocktail juice or cranberry juice obtained from fresh cranberries. The juice from the fresh cranberries was obtained by crushing the berries in a blender and then centrifuging this mixture at 4,000 rpm for 15 minutes to express the juice, The cranberry juice was used full strength unless otherwise indicated, If the cranberry juice was to be diluted it was diluted with distilled water. The pH of the Cranberry Cocktail juice used in these experiments was 3.1 while the fresh cranberry juice's pH was 2.7.

Statistical Analysis Methods

Standard statistical methods were used in this study. Location and dispersion were estimated using the arithmetic mean and the standard error, The two tailed Student "t" test was used for all statistical comparisons. The coefficient of variation was used to assess any reproducibility studies,

CHAPTER III

RESULTS

Adherence of Bacterial Pathogens to Uroepithelial Cells

Each of the 145 gram negative bacterial isolates were incubated with uroepithelial cells according to the bacterial adherence method described. Bacteria which had the capacity to adhere were seen on the surface of the epithelial cells, Each of the bacterial isolates was run in conjunction with a control. The control for this experiment consisted of uroepithelial cells minus the bacterial isolate.. The results are presented in Table 1, All of the bacterial pathogens tested showed significant adherence to uroepithelial cells when compared to the control, E.coli exhibited the greatest amount of adherence with a mean of 11.4 bacteria per cell. Klebsiella, Enterobacter, Pseudomonas, and Proteus all exhibited significant adherence but considerably less than E.coli isolates,

When urinary and non-urinary E.coli isolates were studied, there were significant differences between the two. The 32 urinary E.coli isolates tested had a mean of 17.3 bacteria per cell in comparison to the mean of 5.4 bacteria per cell for the 31 non-urinary E.coli. This can be seen in Table 2.

To determine if the source from which the bacteria were collected affected the adherence to uroepithelial cells,

bacterial isolates were cultured from four different origins urine, sputum, wound and stool. Bacterial adherence testing was carried out as previously described. Bacteria isolated from the urine had a mean adherence of 13.7 bacteria per cell in comparison to bacteria isolated from sputum, wound and stool that had an average mean of 5.7 bacteria per cell. These data are presented in Table 3.

Effect of Cranberry Juice on Adherence

To determine whether cranberry cocktail affects the adherence of bacteria to epithelial cells, five different experiments were performed. Each experiment tested two organisms, one showing strong adherence and one showing weak adherence, belonging to the following groups: urinary Proteus isolates, urinary Pseudomonas isolates, urinary E.coli isolates, and E.coli isolated from non-urinary tract sources. For ease of comparison a mean adherence for each of the five different experiments was determined.

Bacteria and/or uroepithelial cells not exposed to cranberry juice were used as a control for the first three methods (Procedures RC, UC, and PC). Epithelial cells and bacteria were both resuspended in PBS buffer to their desired concentrations. Each mixture was combined together forming a 1:1 ratio. Bacterial adherence was allowed to occur for 30 minutes in a 37°C water bath with mixing of the controls occurring every 5 minutes. At the end of this time, 1 ml volumes were removed and filtered as previously described. Bacteria adhering to the first 50 intact epithelial cells

was determined for each control. The reproducibility of the technique was assessed by determining the adherence of 5 seperate controls prepared from the same pools of bacteria and uroepithelial cells. The coefficient of variation was 15.2%.

In the first experiment , designated BC, fresh cranberry juice was used to resuspend the bacterial pellet to a final concentration of 10^9 bacteria per ml. The bacteria suspended in cranberry juice were incubated at $37^{\circ}C$ for 30 minutes in a water bath with agitation occurring every 5 minutes. At the end of the 30 minutes the mixture was centrifuged at 2,500 rpm for 10 minutes and the supernatant containing the cranberry juice was discarded. The bacterial pellet was then resuspended in PBS to a final concentration of 10^9 bacteria per ml. The adherence assay was then carried out using the treated bacteria and non-treated uroepithelial cells. The treated bacteria adhering to the first 50 intact uroepithelial cells was determined for each organism. The results can be seen in Table 4 under the heading Procedure BC. The mean adherence for the control group was 8.3 bacteria per uroepithelial cell while the mean adherence for the cranberry treated bacteria was significantly different with a mean adherence of 2.5 bacteria per uroepithelial cell.

The second inhibition test, designated UC, involved the incubation of fresh cranberry juice with uroepithelial cells. Uroepithelial cells, collected from a fresh midstream urine, were suspended in fresh cranberry juice to a final

concentration of 10^5 cells per ml. The epithelial-cranberry juice mixture was allowed to incubate for 30 minutes at 37°C in a water bath with manual mixing of the samples occurring every 5 minutes. At the end of the 30 minutes, the mixture was centrifuged and the cells were harvested and resuspended in PBS buffer to a final concentration of 10^5 epithelial cells per ml. The adherence assay was performed using the treated uroepithelial cells and non-treated bacteria. Mean adherence values were determined for each of the organisms tested. The results are presented in Table 4. The control group's mean adherence was 8.3 bacteria per cell while the mean adherence for Procedure UC was 5,2 bacteria per cell. To determine whether the inhibition of adherence tests were reproducible, four organisms (2 urinary and 2 non-urinary E.coli) were tested 5 times using Procedures BC and UC. Coefficient of variation for Procedure BC was 22.3% while Procedure UC had a coefficient of variation of 26.9%

The third method, designated PC, involved the incubation of both the uroepithelial cels and bacteria in fresh cranberry juice. The bacteria, at a concentration of 10^9 cells per ml, and the uroepithelial cells, at a concentration of 10^5 cells per ml, were allowed to incubate separately for 30 minutes in a 37°C water bath. At the end of this time the two were combined, both still suspended in fresh cranberry juice, and allowed to incubate at 37°C for 30 minutes. The rest of the adherence assay was carried out as previously described. Adherence values were obtained for each of the

organisms tested and are presented in Table 4 under the heading Procedure PC. Compared to the control of 8.3 bacteria per cell, the treated bacteria and treated uroepithelial cells showed significantly reduced adherence, a value of 2.8 bacteria per cell. Reproducibility studies were once again carried out for this experiment, showing a coefficient of variation of 24.4%.

The fourth method, designated C, involved testing of the urine before and after ingestion of cranberry cocktail. An initial zero time sample of urine was collected and then 12 ounces of cranberry cocktail juice was ingested. The urine taken at time zero served as the control. After 2 hours a second urine sample was collected. Both samples were centrifuged at 2,500 rpm to recover the epithelial cells. The epithelial cells were then washed in PBS buffer and adherence tests were performed. The following combinations were tested:

a) zero time epithelial cells incubated with each of the bacteria

b) 2 hour epithelial cells incubated with each of the bacteria.

The results appear in Table 5. Note that in all eight bacteria tested there was a significant decrease in adherence on the epithelial cells collected 2 hours after ingestion of the cranberry coctail. The mean adherence for Procedure C was 3.0 bacteria per epithelial cell compared to 8.3 bacteria per cell for the control.

The fifth method, designated U, used the urine

collected at time zero and at 2 hours after ingestion of the cranberry cocktail. The urine was centrifuged for 10 minutes at 2,500 rpm. The urine from the 2 hour sample and epithelial cells from the zero time sample were mixed and incubated at 37°C for 30 minutes. The urine from the zero time sample and the epithelial cells from the zero time sample were mixed and incubated so as to serve as a control. Bacterial adherence tests were performed on both groups. The results appear in Table 6. The urine from the 2 hour sample significantly decreased the amount of bacteria adhering to the epithelial cells. The mean adherence for Procedure U was 3.6 bacteria per epithelial cell compared to 8.5 bacteria per epithelial cell for the control group. To determine whether the inhibition of adherence tests was reproducible, fourorganisms (2 urinary E. coli and 2 non-urinary E. coli) were tested 5 times using the two in vivo methods just described. Procedure C had a coefficient of variation of 21.5% while Procedure U had a coefficient of variation of 19.0%.

Pre-Attached Bacteria Test

Two urinary E. coli isolates were tested, one showing a high degree of adherence, designated EcH, and one showing a lower level of bacterial adherence, designated EcL. Bacteria and uroepithelial cells were incubated for 30 minutes at 37°C, and then an equal volume of fresh cranberry ,juice was added, and placed back into the 37°C water bath. At times of 0, 5, 10, 30, 60, 120, and 240 minutes a 1 ml volume of sample was

removed and processed in the usual manner. A control consisting of 2 ml of 10^9 bacteria per ml and 2 ml of 10^5 uroepithelial cells per ml was included. The results can be seen in Table 7. It can be seen that the fresh cranberry juice significantly reduced the attachment of the bacteria to the uroepithelial cells. Isolate EcH had an initial adherence of 19.5 bacteria per cell, while at 30 minutes after incubation with the cranberry juice that adherence dropped to 6.1 bacteria per cell. The same situation occurred with isolate EcL.

Time Study

Urinary E.coli isolates, EcH and EcL, were used in this study. Twelve ounces of cranberry cocktail juice was ingested and at time intervals of 0, 30, 60, 90, 120, and 240 minutes urine specimens were collected, Ten ml of each specimen was centrifuged at 2,500 rpm for 10 minutes to harvest the uroepithelial cells. The uroepithelial cells were washed with 10 ml of PBS buffer, centrifuged and resuspended in PBS buffer to a final concentration of 10^5 cells per ml and adherence tests were performed. A control was included using epithelial cells from untreated urine. The seven urine samples collected above, free of epithelial cells, were also incubated with epithelial cells from urine collected at time zero. At the end of the 30 minutes incubation in a 37^oC water bath the samples were subjected to the adherence assay. The results can be seen in Table 8. Both the epithelial cells and urine collected after ingestion of the twelve ounces of

cranberry cocktail juice significantly inhibited the adherence of the bacteria. The time interval of 90 to 120 minutes after ingestion of the cranberry juice showed the greatest amount of inhibition for both E.coli isolates tested,

TABLE 1

Comparison of the Adhesive Ability of Bacterial Pathogens to Human Urinary Tract Epithelial Cells

Bacterial Isolate	Number of Isolates	Mean Bacteria per Cell	Standard Error
Control	22	0.9	0.13
Klebsiella[a]	15	6.6	1.50
Enterobacter[a]	5	5.7	1.34
Pseudomonas[a]	30	7.6	0.71
Proteus[a]	32	5.2	0.51
E.coli[a]	63	11.4	1.41

a) Significantly different from contro ($p < 0.01$)

TABLE 2

Comparison of the Adhesive Ability of Urinary E.coli and Non-urinary E.coli to Human Urinary Tract Epithelial Cells

Bacterial Isolate	Number of Isolates	Mean Bacteria per Cell	Standard Error
Non-urinary E.coli	31	5.4	0.45
Urinary E.coli [a]	32	17.3	2.31

a) Significant difference from non-urinary E.coli ($p < 0.01$)

TABLE 3

Attachment of Bacterial Isolates from Various Sources to Human Urinary Tract Epithelial Cells

Bacterial Isolates Origin	Number of Isolates	ean Bacteria per Cell	Standard Error
Urine	53	13.7	1 55
Sputum[a]	29	6.1	0 85
Wound[a]	30	5.7	0 51
Stool[a]	17	5.4	0 73

a) Significantly different from urine isolates ($p < 0$ 01)

TABLE 4

Effect of Cranberry Cocktail Juice on Bacterial Isolates

Bacterial Isolate	Control	Procedure UC	Procedure BC	Procedure PC
#5160 E.coli (urinary)	16.8 ± 5.42	7.0 ± 1.77	1.1 ± 0.34	2.6 ± 0.38
#4796 E.coli (urinary)	9.8 ± 3.92	5.1 ± 0.83	2.7 ± 0.62	2.4 ± 0.62
#811 Proteus	7.1 ± 1.09	4.7 ± 1.60	2.0 ± 0.41	1.9 ± 0.37
#560 Proteus	4.3 ± 1.97	3.8 ± 0.76	2.1 ± 0.47	3.2 ± 0.52
#9112 Pseudomonas	11.0 ± 2.90	4.5 ± 0.48	2.5 ± 0.40	1.7 ± 0.33
#7285 Pseudomonas	5.8 ± 0.91	4.9 ± 0.75	4.0 ± 0.55	2.6 ± 0.45
#7175 E.coli (non-urinary)	4.4 ± 1.09	3.9 ± 0.54	2.1 ± 0.40	4.1 ± 0.66
#648 E.coli (non-urinary)	7.5 ± 1.43	7.3 ± 1.97	3.7 ± 0.59	3.6 ± 0.61
Mean Adherence for Each Procedure	8.3 ± 1.48	5.2 ± 0.47[b]	2.5 ± 0.34[a]	2.8 ± 0.29[a]

a) Significantly different from control ($p < 0.01$)

b) Significantly different from control ($p < 0.05$)

TABLE 5

Effect of Ingestion of Cranberry Cocktail Juice on Bacterial Isolates

Bacterial Isolate	Control	Procedure C
#5160 E.coli (urinary)	16.8 ± 5.42	1.4 ± 0.40
#4796 E.coli (urinary)	9.8 ± 3.92	4.5 ± 0.71
#811 Proteus	7.1 ± 1.09	1.4 ± 0.44
#560 Proteus	4.3 ± 1.97	1.8 ± 0.44
#9112 Pseudomonas	11.0 ± 2.90	5.2 ± 0.99
#7285 Pseudomonas	5.8 ± 0.91	3.8 ± 0.75
#7175 E.coli (non-urinary)	4.4 ± 1.09	2.4 ± 0.95
#648 E.coli (non-urinary)	7.5 ± 1.43	3.6 ± 0.27
Mean Adherence for Each Procedure	8.3 ± 1.48	3.0 ± 0.52[a]

a) Significantly different from control ($p < 0.01$)

TABLE 6

Inhibition of Adherence by Treated Urine

Bacterial Isolate	Control	Procedure U
#5160 E.coli (urinary)	14.5 ± 2.31	2.3 ± 0.30
#4796 E.coli (urinary)	7.2 ± 1.05	5.2 ± 0.99
#811 Proteus	9.1 ± 0.99	1.7 ± 0.42
#560 Proteus	5.5 ± 0.90	4.1 ± 1.19
#9112 Pseudomonas	13.3 ± 2.67	5.4 ± 0.72
#7285 Pseudomonas	6.0 ± 0.89	3.9 ± 1.00
#7175 E.coli (non-urinary)	4.3 ± 0.91	3.2 ± 0.53
#648 E.coli (non-urinary)	8.1 ± 2.00	2.9 ± 0.37
Mean Adherence for Each Procedure	8.5 ± 1.47	3.6 ± 0.47[a]

a) Significantly different from control ($p < 0.01$)

TABLE 7

Effect of Cranberry Juice on Pre-attached Urinary E.coli to Uroepithelial Cells

Minutes	Mean Bacteria per Cell*	
	EcH	EcL
Control	19.5 ± 3.30	8.6 ± 2.70
0	13.6 ± 2.85[b]	7.0 ± 1.09[c]
5	7.8 ± 1.72[a]	5.5 ± 0.66[c]
10	7.5 ± 1.07[a]	3.5 ± 0.49[b]
30	6.1 ± 0.96[a]	3.0 ± 0.41[a]
60	5.0 ± 0.94[a]	3.5 ± 0.65[b]
120	7.4 ± 1.36[a]	4.4 ± 1.39[b]
240	6.9 ± 1.19[a]	2.8 ± 0.43[a]

* ± Standard error of the mean

a) Significantly different from control ($p < 0.01$)

b) Significantly different from control ($p < 0.05$)

c) Not significantly different from control

TABLE 8

Time Study Results

Minutes	Mean Bacteria per Cell*			
	Treated Uroepithelial Cells		Treated Urine	
	EcH	EcL	EcH	EcL
0	11.8 ± 3.09	13.2 ± 2.19	15.1 ± 2.02	11.1 ± 2.42
30	6.9 ± 1.28[a]	10.3 ± 3.33[c]	11.2 ± 1.69[c]	11.1 ± 1.83[c]
60	5.7 ± 1.52[a]	5.1 ± 0.67[a]	7.2 ± 1.22[a]	5.2 ± 0.69[a]
90	2.5 ± 0.86[a]	4.0 ± 0.87[a]	1.9 ± 0.96[a]	3.0 ± 0.74[a]
120	2.7 ± 0.54[a]	3.6 ± 0.70[a]	2.9 ± 0.73[a]	3.1 ± 1.10[a]
240	5.1 ± 0.60[a]	6.4 ± 0.65[a]	7.7 ± 1.60[a]	8.1 ± 1.07[b]

* ± Standard error of mean

a) Significantly different from control ($p < 0.01$)

b) Significantly different from control ($p < 0.05$)

c) Not significantly different from control

CHAPTER IV

DISCUSSION

Previous investigations have suggested that the initial event in colonization and invasion is the adherence of bacteria to epithelial cells of the mucosal surface. All of the 145 bacterial isolates in this study were shown to adhere to uroepithelial cells in varying degrees. Particular attention was focused on E.coli since it causes approximately 80% of all urinary tract infections. It was observed in this study that E.coli isolates adhered more readily than Klebsiella, Proteus, Enterobacter, and Pseudomonas isolates. When E.coli was divided into urinary E.coli and non-urinary E.coli, ~~E.coli~~ isolated from patients with diagnosed urinary tract infection adhered 70% more readily than did E.coli isolated from non-urinary sources. Similar results were found by Svanborg Eden, et al. (1976) when they noted that E.coli isolated from the urine of patients with acute symptomatic pyelonephritis or cystitis attached to normal uroepithelial cells in larger amounts than did E.coli isolated from the urine of patients with asymptomatic bacteriuria. Since the asymptomatic bacteria screening strains adhered poorly in Svanborg Eden's study (1976), the importance of attachment as a mechanism in urinary tract colonization remained uncertain especially in these patients, However, in my particular study the comparison being made was between non-urinary E.coli

and urinary E.coli and not E.coli isolated from asymptomatic patients. Asymptomatic bacteria may still be capable of adherence in the urinary tract. When comparing urinary E.coli to the unrelated non-urinary E.coli a significant difference was noticed. These results suggest that attachment is an important mechanism in urinary tract colonization and may be an important virulence factor for E.coli causing urinary tract infections.

Organisms isolated from patients with known urinary tract infections, adhered approximately 60% more readily than did bacteria isolated from sputum, wound and stool cultures. These results support previous studies which indicate that adhesive molecules on the surface of the bacteria are recognized by specific receptor molecules on animal cells. Bacterial isolates lacking these specific adhesive molecules appear to be either unable to attach to uroepithelial cells or may not form enough bonds to allow the bacterial isolate to remain attached. Beachey (1981) has shown that bacterial adherence involves the simultaneous binding of a large number of bacterial ligand molecules with a large number of receptor molecules to form multiple, independent bonds, therefore the attachment of bacteria becomes virtually irreversible. If only a few bonds are formed this interaction is relatively weak, thus the adherent bacteria may not be able to effectively overcome the physiologic cleansing mechanism of the tissue cell surface.

A great deal of variation was observed throughout this

study on the adherence of bacteria to uroepithelial cells. Some cells from a single individual were covered with bacteria while other cells from the same sample were free of bacteria. This phenomenon was also noted by other researchers (Schaeffer, et al., 1979) (Svanborg Eden, et al., 1977) (Parsons and Schmidt, 1980). One possible explanation for this variation could depend on the maturity of the cells, resulting in variations in the density of receptor sites, The population beinp studied probably contains both viable cells and cells in various stages of cell death, If the number of receptor sites varies for each of these cell types this could explain the degree Sf variation seen in this study. A dead cell may not bind as many bacteria as a more viable uroepithelial cell. Also adherence may be affected by changes in hormone levels in the body, It may be that receptor sites are more available during certain stages of cell development as well as hormonal influence. Schaeffer, et al. (1979) found that when adherence was correlated with the day of the menstrual cycle, a repetitive pattern developed. Adherence was maximal during the estrogen dependent phase and diminished after ovulation. However, Svanborg Eden, et al. (1980) found no correlation between hormone levels and bacterial adherence. Beachey (1981) postulated that in some cases it is the arrangement of the membrane receptors on the epithelial cell that determines the ability of certain tissues to be colonized by a particular bacterial pathogen. This suggests that receptor and ligand molecules must be accessible and arranged in a fashion that

many bonds are produced.

The reproducibility of each technique was assessed by determining the adherence of 5 different samples for each of the bacterial adherence experiments. When the samples were prepared using the same pool of bacteria and uroepithelial cells, the average coefficient of variation for the 5 bacterial adherence experiments and their controls was 21.6%. Schaeffer, et al. (1979) noted that the coefficient of variation for his technique was 22%. A 22% coefficient of variation was also noted by Schaeffer, et al. in his 1981 bacterial adherence experiments. These comparisons show that the bacterial adherence assay is quite reproducible not only between experiments but also between different researchers.

The administration of cranberry juice has been used in the past as a urinary acidifier for the prevention and treatment of urinary tract infections, It was believed that quinic acid, found in cranberries, was the precursor of the hippuric acid which is a strong antibacterial agent (Moen, 1962). Bodel, et al. (1959), however, demonstrated that cranberry juice is not an effective acidifier of urine and it rarely increases the hippuric acid concentration in the urine to the minimum necessary for bacteriostasis of common urinary pathogens. Similar results were found by Sobota (1984).

Cranberry cocktail, used in these experiments, also contains fructose and Vitamin C in addition to cranberry juice, Sobota (1984) has shown that the amount of fructose

present in the cranberry cocktail had only a minimal effect on inhibition of adhernnce. He also found that Vitamin C, at concentrations found in cranberry cocktail juice, had no profound effect on adherence. The pH of the cranberry juice was ruled out as a factor when it was determined that adherence was not affected above a pH of 2 (Sobota, 1984). Therefore cranberry cocktail was used exclusively in these studies since it is the cranberry juice in the cocktail that contains the active factor.

In order to determine the concentration of cranberry juice necessary for adherence a dilution study was performed. These results indicate that dilutions up to 1:500 of the cranberry cocktail still showed significant inhibitory effects. Sobota (1984) had noted that at dilutions up to 1:100 had a significant effect on adherence. Thus it appears that the cocktail is effective at dilutions 5 times greater than reported by Sobota (1984).

The results presented in this study confirmed cranberry juice to be an inhibitor of bacterial adherence. It appears that cranberry juice prevents bacterial adherence by interfering primarily with the surface properties of the bacteria that may be pertinent for their ability to colonize and infect human mucosal surfaces and does not alter surface characteristics on epithelial cells. This is based on two observations: 1) organisms preincubated in cranberry juice had significantly reduced adherence whereas uroepithelial cells preincubated in cranberry juice were not as strongly inhibited, The preincu-

bated bacteria showed a 70% decrease in the number of adhering bacteria while the preincubated uroepithelial cells had a 37% decrease in the number of adhering bacteria, The cranberry juice seems to interfere more with the bacterial adherence factors than it does with the surface features of the uro-epithelial cell, 2) E.coli that were allowed to attach to uro-epithelial cells were rapidly released from these cells after the addition of fresh cranberry juice, It appears that cranberry juice affects the bonds formed between the bacteria and epithelial cell either by weakening them or by completely breaking these bonds causing the release of the bacteria. This release is rapid, as shown in this study, where a significant decrease in the number of attached bacteria occurred 5 minutes after the addition of the cranberry juice. It was noted that even after the cranberry juice was removed and replaced with P3S buffer the inhibitory effect on the bacteria still remained. Sobota (1984) had found in his study that after 1 wash 90% of the inhibitory activity was lost and after 2 washes the normal adherence pattern was restored. It was found in this study, however, that only 11% of the inhibitory activity was lost after 1 wash. This result suggests that whatever factor(s) is present must bind in a very specific manner either to the bacteria or epithelial cell so that it is not easily removed after one wash. After a series of washings this factor may be removed and normal adherence once again restored as indicated by Sobota's (1984) findings. Differences between these two studies could be due to species or type differences.

Of the eight strains of bacteria tested, all were significantly inhibited after incubation in cranberry juice. This suggests that cranberry juice may have universal effects. Further studies would be needed to verify this suggestion.

~~In vivo~~ results from this study showed a significant decrease in adherence of bacteria to uroepithelial cells collected after the ingestion of 12 ounces of cranberry cocktail, E.coli failed to adhere to uroepithelial cells collected two hours after ingestion of the cranberry cocktail, In contrast uroepithelial cells collected before ingestion were capable of bacterial adherence. This phenomenon occurred for all eight strains of bacteria tested. These results had not been previously reported. It was noted earlier that in the in vitro study the juice had only a minimal effect (37% decrease in adherence) on the epithelial cells while in the in vivo study bacterial adherence decreased by 64%. This indicates that a metabolite of the cranberry juice may have a more profound affect on the mucosal surface lining the urinary tract than did the non-metabolized factor present in the cranberry juice. Also it must be noted that it is uncertain to what extent epithelial cells in the urine can be representative of the intact mucosal surface, Similar results were observed when untreated uroepithelial cells were incubated in urine collected two hours after ingestion of the cranberry cocktail juice. Uroepithelial cells incubated in treated urine showed a 58% decrease in mean bacterial adherence when compared to uroepithelial cells incubated in

untreated urine. It was also observed that there was no significant difference between urine controls (urine collected prior to ingestion and controls in which PBS buffer was substituted for urine). These results indicate that substances present in the urine, before ingestion of cranberry juice, had no effect on bacterial adherence. Any decrease noted after ingestion would be due to some factor(s) or metabolite of the cranberry juice.

Therefore cranberry juice appears to contain an active factor(s) that can survive normal metabolism in man and can accumulate in the urine, or possibly a byproduct of cranberry juice metabolism accumulates in the urine. Similar suggestions were made by Sobota (1984). In his study fifteen mice were given cranberry cocktail in replacement of their water for a period of 14 days. Urine samples collected from the mice were found to significantly inhibit adherence of bacteria to epithelial cells.

Previous results along with results from this study indicate that there is a factor present in the cranberry juice which appears to alter the way bacteria adhere to mucosal surfaces. This active factor, which is the potent inhibitor of bacterial adherence, is not known at this time, nor is it known exactly how it interferes with bacterial adherence. From both the in vivo and in vitro studies performed here, it appears that it alters the uroepithelial cell surface components as well as the surface properties of the bacteria. The active factor present in cranberry juice appears to bind

to bacterial isolates in a very specific manner. This was demonstrated earlier when washing of the cranberry treated bacteria did not remove the majority of the active factor. The slight decrease in adherence seen with cranberry treated epithelial cells may be due to non-specific binding of the factor to the epithelial cells. In this case, the factor can then be carried over to the bacteria during their interaction resulting in decreased adherence.

Results from the time study indicate that this inhibitor is also quite stable. The active factor or some metabolite of it first appeared in the urine approximately one hour after ingestion, Urine samples collected 4 hours after ingestion of 12 ounces of juice still continued to show a significant decrease in adherence. Sobota (1984) performed a similar experiment in which human subjects ingested 15 ounces of cranberry cocktail and urine samples were then collected 1 to 3 hours after ingestion, Fifteen of 22 subjects showed significant antiadherence activity 1 to 3 hours after drinking the juice. Difference in anti-adherence activity in the urine collected from each subject could be explained by the difference in uroepithelial cell populations and the rate of metabolism for each subject. It was already noted by several researchers that variation in uroepithelial cell receptivity between individuals exists (Schaeffer, et al.,19'79) (Schaeffer, et al., 1981) (Parson and Schmidt, 1980). This factor present in the urine after ingestion of the cranberry cocktail juice was found in high

enough concentrations allowing it to bind to or affect the receptor sites on the uroepithelial cell and still be present in high enough concentrations in the urine to inhibit adherence.

There have been several reports on the beneficial side effects of cranberry juice in the treatment of urinary tract infections. Steinlieb (1963) recommended an 8 ounce glass of cranberry juice four times a day in patients with certain urinary tract infections. A study by Frodomas, et al. (1966) showed the inhibiting effect of cranberry juice on bacteria. Sixty patients with known acute urinary tract infections were treated with 16 ounces of cranberry juice per day for three weeks. At the end of the time, 53% of the patients had a positive clinical response and 20% had moderate improvement. After the cranberry juice therapy had been discontinued, 61% showing improvement had recurring urinary tract infections.

Evidence has accumulated to show that in order for adherence to occur between bacteria and epithelial cells, the epithelial cell must contain specific receptor sites that are recognized by specific molecules on the bacteria. A compound is needed to mimic the structure of the membrane receptor and bacterial adhesin so as to act as a competitive inhibitor of bacterial adherence. The use of nontoxic, nonantibiotic bacteriostatic agents such as cranberry juice in the treatment of urinary tract infections may be very beneficial, Cranberry juice is found to be well tolerated

for long periods of time by patients, with no clinical side effects (Prodomas, et al., 1966). It may be especially useful for long term prophylaxis in patients with recurrent urinary tract infections, in which long term antibiotics would present problems.

BIBLIOGRAPHY

Aronson, M.O.,O. Medalia, L. Schori, D. Mirelman, N. Sharon, and I. Ofek, 1979. Prevention of colonization of the urinary tract of mice with Escherichia coli by blocking of bacterial adherence with methyl-D-manno-pyranoside. J. Infect. Dis. 139: 329 - 333.

Beachey, E.H. 1981. Bacterial adherence: Adhesion-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. of Infect. Dis. 143: 325 - 345.

Bodel, P.T., R. Cotran, and E.H. Kass. 1959. Cranberry juice and the antibacterial action of hippuric acid. J. Lab. Clin. Med. 54: 881 - 888.

Brumfitt, W., J. Cooper and J.M.T. Hamilton-Miller. 1981. Prevention of recurrent urinary infections in women: A comparative trial between nitrofurantoin and methenamine hippurate. J. Urol. 126: 71 - 74.

Chick, S., M.J. Harber, R. Mackenzie, and A.W. Asscher. 1981. Modified method for studying bacterial adhesion to isolated uroepithelial cells and uromucoid. Infect. Immun. 34: 256 -261.

Durtz, D.J. and Mills, J. "Immunity and Infection," Basic and Clinical Immunology. Edited by D.P. Sites, et al. California: Lange Medical Publications, 1982.

Eisenstein, B.I., E.H. Beachey, and I. Ofek, 1980. Influence of sublethal concentrations of antibiotics on the expression of the mannose - specific ligand of Escherichia coli. Infect. Immun. 28: 154 - 159.

Eisenstein, B.I., I. Ofek, E.H. Beachey. 1979. Interference with the mannose binding and epithelial cell adherence of Escherichia coli by sublethal concentrations of of streptomycin. J. Clin. Invest. 63: 1219 - 1228.

Fellers, C.R., B.C. Redman and E.M. Parrott. 1933. Effect of cranberries on urinary acidity and blood alkali reserve. J. Nutrition 6: 455 - 463.

Harber, M.J., S. Chick, R. Mackenzie, and A.W. Asscher. 1982. Lack of adherence to epithelial cells by freshly isolated urinary pathogens. Lancet. i: 586 - 588.

Hirschberger M., D. Mirelman, M.M. Thaler. 1977. The effects of mannose and its derivatives on interactions of pathogenic E.coli with newborn intestinal mucosa. Gastroenterology. 72: 1069.

Kahn, H.D., V.A. Panariello, J. Saeli, J.R. Sampson, and E. Schwartz. 1967. Effect of cranberry juice on urine. J. of Am. Dietetic Assoc. 51: 251 - 254.

Kunin, C. "Microbiological Aspects of Urinary Tract Infections." Infections of the Urinary Tract. Edited by Edward Kass and William Brumfitt. Chicago: University of Chicago Press.. 1975.

Moen, D.V. 1962. Observations on the effectiveness of cranberry juice in urinary infections. The Wisconsin Med. J. 61: 282 - 283.

Ofek, I., A. Mosek, and N. Sharon. 1981. Mannose-specific adherence of Escherichia coli freshly excreted in the urine of patients with urinary tract infections, and of isolates subcultured from the infected urine. Infect. Immun. 34: 708 - 711.

Ofek, I. D. Mirelman, and N. Sharon, 1977. Adherence of Escherichia coli to human mucosal cells mediated by mannose-receptors. Nature 265: 623 - 625.

Parsons, C.L. and J.D. Schmidt. 1980. In vitro bacterial adherence to vaginal cells of normal and cystitis prone women. J. of Urology 123: 184 - 187.

Prodromos, P.N., C.A. Brusch, and G.C. Ceresia. 1966. Cranberry juice in the treatment of urinary tract infections. Southwest Med. 47: 17 - 20.

Schaeffer, A.J., S.K. Amundsen, and L.N. Schmidt. 1979. Adherence of Escherichia coli to human urinary epithelial cells. Infect. Immun. 24: 753 - 759.

Schaeffer, A.J., J.M. Jones, and J.K. Dunn. 1981. Association of in vitro Escherichia coli adherence to vaginal and buccal epithelial cells with suspectibility of women to recurrent urinary tract infections, New Eng. J. of Med. 304: 1062 - 1066.

Schaeffer, A.J., S.K. Amundsen, J.M. Jones. 1980. Effect of carbohydrates on adherence of Escherichia coli to human urinary tract epithelial cells. Infect. Immun. 30: 531 - 537.

Sharon, N., et al. "Bacterial adherence to cell surface sugars." Adhesion and Microorganism Pathogenicity: Ciba Foundation Symposium, 1980. Edited by K. Elliott New York: Pittman, 1981.

Sugarman, B., S.T. Donta. 1979. Adherence of Enterobacteria

Silverblatt, F.J. and Ofek, I. "Effect of pili on susceptibility of Proteus mirabilis to phagocytosis and on adherence to bladder cells." Infections of the Urinary Tract. Edited by Edward Kass and William Brumfitt. Chicago: University of Chicago Press. 1975.

Smith, D.R. General Urology. Los Altos, California: Lange Medical Publishing. 1984,

Sobota, A. 1984. Inhibition of bacterial adherence by cranberry juice: Potential use for the treatment of urinary tract infections. J. of Urology. 131: 1013 - 1016,

Stamey, T. "Urinary Tract Infections in Women." Campbell's Urology. Edited by J.H. Harrison, et al. Philadelphia: W.B. Saunders Co., 1978.

Sternlieb, P. 1963. Cranberry juice in renal disease. N. Eng. J. Med. 268: 57.

Sugarman, B., S.T. Donta. 1979. Adherence of Enterobacteriaceae to human buccal cells, J. Med Microbiol. 12: 373 - 378.

Svanborg Eden, C., B. Eriksson and L.A. Hanson. 1977. Adhesion of Escherichia coli to human uroepithelial cells in vitro. Infect. Immun. 18: 767 - 774.

Svanborg Eden, C., U. Jodal, L.A. Hanson, U.Lindberg. 1976. Variable adherence to normal human urinary tract epithelial cells of Escherichia coli strains associated with various froms of urinary tract infection. Lancet 490 - 492.

Svanborg Eden, C. and H.A. Hanson. 1978. Escherichia coli pili as possible mediators to attachment to human urinary tract epithelial cells. Am. Soc. Micro. 21" 229 - 237.

Svanborg Eden, C. G.L.Janson, and U. Lindberg. 1979. Adhesiveness to urinary tract epithelial cells of fecal and urinary Escherichia coli isolates from patients with symptomatic urinary tract infections or asymptomatic bacteriuria of varying duration, J. of Urology 122: 185 - 188.

Svanborg Eden, C. and U. Jodal. 1979. Attachment of Escherichia coli to urinary sediment epithelial cells from urinary tract infection prone and healthy children. Infect. Immun. 26: 837 - 840.

Svanborg Eden, C.,.P. Larsson, and H.Lomberg. 1980. Attachment of Proteus mirabilis to human urinary sediment epithelial cells in vitro is different from that of Escherichia coli. Infect. Immun. 27: 804 - 807.

Svanborg Eden, C., et al. "Adhesion of Escherichia coli in urinary tract infection," Adhesion and Microorganism Pathogenicity: Ciba Foundation Symposium, 1980. Edited by K. Elliott New York: Pittman, 1981.

Wright, D.N. and J.M. Matsen. 1984. Diagnosing urinary tract infections. Diag. Med. 7: 44 - 53.

Zar, J.H. Biostatistical Analysis. Englewood Cliffs, N.J.: Prentice-Hall. 1974.