# THE IN VITRO EFFECT OF FRUCTOSE ON THE ADHERENCE OF *Escherichia coli* IN PATIENTS WITH CHRONIC URINARY TRACT INFECTIONS

by

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Submitted in Partial Fulfillment of the Requirement

for the Degree of

Master of Science

in the

Biological Sciences

Program

Advisor — Date: 8/5/92

Dean of the Graduate School — Date: August 28, 1992

YOUNGSTOWN STATE UNIVERSITY

AUGUST, 1992

4-10-4

THESIS APPROVAL FORM

THESIS TITLE: The in vitro effect of fructose on the adherence of Escherichia coli in patients with chronic urinary tract infections.

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## ABSTRACT

THE IN VITRO EFFECT OF FRUCTOSE ON ADHERENCE OF Escherichia coli IN PATIENTS WITH CHRONIC URINARY TRACT INFECTIONS

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Master of Science

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There have been anecdotal and published reports on the benefits of cranberry juice for the prevention and/or treatment of urinary tract infections. A previous report from our laboratory demonstrated that cranberry juice cocktail(CJC) inhibits the adherence of clinical isolates of Escherichia coli to uroepithelial and buccal cells. It was further demonstrated that the urine of individuals ingesting 15 oz. of cranberry juice showed anti-adherent activity. It has been determined that fructose, a carbohydrate present in CJC, is one of two components that is responsible for the anti-adherent properties of the cocktail. To investigate the role of fructose as an anti-adherent agent, this sugar was incubated along with uroepithelial cells and E. coli in the urine of individuals not prone to urinary tract infections and those who experience chronic infections. It was shown that 5% fructose prevents the attachment of E. coli to exfoliated epithelial cells in both groups studied. The inhibitory action of fructose on adherence of type 1 fimbriated E. coli in normal and chronic patients was 100% and 84%, respectively. A statistical comparison between normal and chronic individuals was performed and it was determined that fructose inhibits bacterial adherence

equally in both groups studied. These findings demonstrate that fructose inhibits bacterial adherence and that the activity is exhibited in the urine.

It was also demonstrated that bacterial adherence in urine of chronic patients treated with fructose is not statistically significantly different from the bacterial adherence of chronic patients ingesting CJC daily, suggesting that the anti-adherence activity of the juice may be due to fructose. In addition to fructose, the nondialyzable component of CJC was also investigated for its anti-adherent ability. It was observed that dialyzed cranberry juice inhibited bacterial adherence in 84% of normal subjects and 79% of chronic patients. It was further determined that chronic patients ingesting CJC daily are not statistically significantly different from chronic patients whose urine was treated with dialyzed CJC.

These findings demonstrate that CJC contains at least two inhibitory components that prevent attachment of E. coli expressing type 1 fimbriae and that their actions can be demonstrated in the urine. It was found that the inhibitory effects of the dialyzable component are attributed to fructose. It was observed that the anti-adherent action of fructose in the urine of chronic patients was equivalent to that of chronic patients ingesting cranberry juice. These results suggest a potential use for fructose in the prevention and management of urinary tract infections.

# DEDICATION

This work is dedicated in loving memory of my father

Walter C. Wollet

It is also dedicated to my mother, Mary F. Wollet, who instilled in me the importance of education and whose love and support made it possible.

## ACKNOWLEDGEMENTS

I would like to thank Dr. Anthony E. Sobota for the opportunity to work in his laboratory under his guidance. I am grateful for his assistance and supervision throughout my work on this project and for his suggestions and review of this thesis.

I would like to thank Dr. Robert Leipheimer and Dr. James Toepfer for their suggestions and review of this thesis.

I would also like to thank Dr. Kevin L. Scheetz of Western Reserve Care System, who arranged for collection of the specimens. I would like to thank Dr. Eugene Tereshawty, director of Beeghly Oaks Skilled Nursing Center, for his participation in this project and Mrs. Francine Maurer and Mrs. Carol Stahl and the nursing staff at Beeghly Oaks for their cooperation and providing the specimens. I would also like to thank Mrs. Richard Scheetz, nursing director of Maple Crest, Mrs. Shelby Snowden and Mrs. Lorraine Confoey of Glen View Manor, and Mrs. Margaret Commarata of Heritage Manor for providing specimens.

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# CHAPTER I

## INTRODUCTION

E. coli, part of the normal flora of the colon, is the most frequently isolated uropathogen from community and hospital-acquired urinary tract infections (Johnson,1991). The ability of the pathogen to attach to host cells is essential for the initiation of infection. Therefore, adherence can be considered a virulence factor and is prerequisite for the pathogenesis of the disease. Normally, the body's inherent defense mechanisms prevent bacterial infections from occurring; however, the combination of impairments in host defenses and bacterial virulence factors contributes to the initiation and progression of diseases. Local defenses involving mechanical, chemical and physiological mechanisms provide protection from the invasion by microorganisms (Smith, 1984). For example, cilia lining the respiratory tract prevent foreign material from entering the deeper portions of the tract. The cilia function to mechanically sweep material to the upper portion of the respiratory tract where it can be dislodged. Expelling of materials by coughing and sneezing prevents material from entering the respiratory tract. The removal of potential pathogens in the intestinal tract is accomplished by peristalsis. Micturition flushes out bacteria from the urinary tract thereby preventing colonization of the urogenital system. In addition to anatomical and mechanical host defenses, the existence of normal flora also suppresses the potential pathogens that have the

capacity to invade a tissue. The resident flora provide a means of protection from potential pathogens by competing for nutrients (Smith, 1984). Normal flora can also prevent the colonization of potential pathogens by releasing chemicals that inhibit the growth of the bacteria. For example, bacteriocins are proteins that are produced by enteric bacteria and pseudomonads that prevent colonization of the alimentary and urinary tract (Smith, 1984). Natural host defenses are an important means of protection from microorganisms and the development of infections. Thus impairment in host defenses can contribute to the pathogenesis of disease.

Both host defense mechanisms and bacterial virulence factors contribute to the development and progression of infections in the host. Expression of adhesions in strains of E. coli causing pyelonephritis and cystitis is considered a virulence factor and is necessary for the initiation of these infections (Ofek and Sharon 1990, Johnson, 1991).

Individuals within all age groups are susceptible to urinary tract infections. Infections occur in infants, children, adolescent, middle-age and the elderly. Urinary tract infections are most common among young and middle-age women, accounting for most of the 6-7 million office visits yearly in the United States (Stamm,1989). Within this group 20% of women will experience recurrent infections (Stamm, 1989). Urinary tract infections are estimated to affect 10 to 20% of women and 12% of men at some point in their lifetime (Johnson, 1991). The predominant mechanism of infection is via an ascending route (Sobel, 1987). Because the

urethra is smaller in women and is in close proximity to the anus, vaginal colonization and colonic flora contamination are possible (Bran, 1972, Sobel, 1987). This may be a reason for the increase in occurrence of urinary tract infections observed in women. Although infections are more frequent in women during younger years, the incidence of infection in men and women after age 60 are the same affecting 15% of the geriatric population (Zilkoski, 1988, Yoshikawa, 1982). Recurrent infections are common among this group giving rise to the development of resistant bacterial strains and thereby contributing to the difficulty in treating these infections with the traditional antimicrobial methods.

Urinary tract infections are among the most common bacterial infections occurring among the geriatric population and are second only to respiratory infections (Zilkoski, 1988). Various factors have been suggested for the prevalence of urinary tract infections among this group. With increasing age come debilitating mental and physical conditions that may contribute to the potential threat of infection in the elderly individual. Loss of bladder control requiring bladder catheterization, development of resistance to antimicrobial agents and obstructive uropathy have been suggested as putting these individuals at risk for urinary tract infections (Zilkoski, 1988). Some studies suggest that frequency in post-menopausal women may be related to the decrease in estrogen levels (Bladassarre and Kaye, 1991). In contrast, it has been shown that an administration of estrogen in female and male rats enhances in vitro adherence of E. coli to exfoliated bladder epithelial

cells(Sobel, 1986).The studies on the role of estrogen in the pathogenesis of urinary tract infections are contradictory and have not been conclusive. Urinary tract infections in men are usually the result of prostatitis (Baldassare and Kaye, 1991). If left untreated, urinary infection can result in gram negative bacterial sepsis, a potentially lethal infection (Johnson and Stamm, 1989). E. coli is the most common pathogen isolated from patients with diagnosed urinary tract infections (Brooks,1980). Other Gram negative bacilli of the family Enterobacteriaceae, such as Proteus mirabilis, Klebsiella pneumoniae and Enterobacter species, are isolated less often and are more frequently observed in hospital-acquired infections or in individuals with indwelling catheters (Farrar, 1983). Numerous studies have concluded that attachment of bacteria to mucosal surfaces is prerequisite in the development of urinary tract infections (Parkkinen, 1988, Firon, 1982, Ofek and Sharon, 1990). Adhesion subsequent to colonization involves the interaction of cell surface components of bacteria and the host cell surface. The adhesion process by which bacteria initiate infection is under investigation. The study of both bacterial and cell surface component interactions has provided information on their role in the pathogenesis of disease.

The glycocalyx, the outer surface of eucaryotic cells, is composed of carbohydrates in the form of oligosaccharides and polysaccharides covalently bound to membrane proteins and as oligosaccharides bound to lipids (Alberts et al., 1989). This carbohydrate moiety constitutes 2-10% of the plasma membrane in the

form of glycoproteins, glycolipids, and proteoglycans (Rosen, 1990). The exact function of the cell-surface carbohydrates is unknown. However, it is theorized they play a role in cell-cell and cell-matrix recognition (Alberts et al., 1989). In many cases carbohydrates function as ligands for the attachment of soluble proteins, microbial-host interactions and cell-cell contacts (Rosen, 1990). Often carbohydrates modify the activities of the protein to which they are bound, acting as cellular markers in many biological processes (Sharon and Lis, 1989). Cellular recognition involves the interactions of apposing cellular surfaces. This interaction is suggested to be mediated by carbohydrates and lectins.

Lectins are carbohydrate-binding proteins which recognize specific sugar residues to which they combine. Initially, lectins were isolated from seeds and vegetative portions of various plants. Concanavalin A (con A) is a plant lectin isolated from the seeds of the Jack bean and expresses sugar specificity for mannose and glucose residues (Lehninger, 1982). Studies of the action of Con A have provided knowledge of cell structures such as carbohydrate residues on the surface of eucaryotic cells. In addition to plants, lectins have been isolated in viruses, bacteria, protozoa and slime molds (Sharon and Lis, 1989). The interaction of these lectins with host cell surface carbohydrates is essential for proper functioning of cellular processes. Lectins of viruses, bacteria and protozoa bind to carbohydrates on the surface of host cells to initiate infection. Lectin-carbohydrate recognition in slime mold has been

shown to mediate cell differentiation (Sharon and Lis, 1989). The study of carbohydrate-lectin interaction has provided insight into their functions and their role in pathogenesis of diseases of protozoal, viral and bacterial origin. For example, amebiasis, a protozoal disease caused by Entamoeba histolytica, initiates infection by adhering to the colonic mucus layer prior to colonization (Ravdin,1989). In vitro studies indicate that adherence to the colonic mucosa is mediated by lectin-like proteins. Petri et al. identified the lectin for E. histolytica as Gal/GalNac (Petri et al.,1987). Antibodies that were directed against the galactose and N-acetylglucoamine specific lectin inhibited amebic adherence in vitro indicating that this structure was responsible for adherence (Sharon and Lis, 1989). Adherence of the parasite is prerequisite to the development of the disease. The virulence of the protozoa is further enhanced by the ability of the amoebae to spread through the host using lectins as a means to bind to other cells of the host (Sharon and Lis, 1989). In vitro studies by Chadee et al. (1987) on the interactions of trophozoites and colonic mucins showed that the latter are high affinity receptors for E. histolytica Gal/GalNAc adherence lectin. Amoebic adherence to rat epithelial cells was inhibited by human and colonic mucins rich in galactose and GalNac residues indicating that the use of specific carbohydrates may block adherence (Chadee et al., 1987).

Lectin-carbohydrate recognition has been studied in the pathogenesis of influenza. Studies have shown that the virus

preferentially binds to specific sialic acids on the surface of the host cells. The influenza virus recognizes N-acetylneuraminic acid residues on the surface of erythrocytes to which it binds. The binding of the virus to the specific carbohydrate residue is the initial step in the pathogenesis of this infection. Subsequently, this attachment results in the fusion of the viral and cellular membranes enabling the viral genome to enter and undergo replication in the host thereby facilitating the infection (Sharon and Lis, 1989). Several bacterial species initiate infection at the cellular level by first adhering to the epithelial cells of host mucosa via lectins extending from the bacterial surface. This recognition-attachment process is prerequisite for the pathogenesis of the disease. The pathogenesis of bacterial infections of the respiratory tract, gastrointestinal tract, urogenital tract, cardiovascular system and some infections of the central nervous system are initiated by lectin-carbohydrate interaction (Ofek and Beachey, 1980).

The ability of streptococci to adhere to epithelial cells of the oral cavity is mediated by fimbriae and directly correlates with the virulence of the bacterial infection (Beachey, 1981). Adherence of the Genus Streptococcus to human epithelial cells has been attributed to lipoteichoic acid on the surface of the bacterial cell (Beachey, 1981). The mechanism of attachment is suggested to be the result of fibrillar layer on the surface of the streptococci cell (Beachey, 1981). Gibbons and van Houte (Gibbons and van Houte, 1980) showed that treatment with trypsin removed the

fibrillar layer thereby abolishing the adherence capability of the bacteria. Ofek and Beachey (1980) note the adhesion of streptococci is mediated by lipoteichoic acid. This suggestion is based on adherence studies whereby attachment of streptococci to epithelial cells could be blocked by pre-incubating epithelial cells with lipoteichoic acid or pre-incubating the streptococci with anti-lipoteichoic acid antibody (Beachey, 1981). The mechanism of attachment and its role in infection have been studied for pathogens causing pulmonary infections, particularly Mycoplasma pneumoniae and Bordetella pertussis (Ofek and Beachey, 1980). Mycoplasma pneumoniae adhere to tracheal epithelial cells and erythrocytes (Ofek and Beachey, 1980). This attachment is blocked by glycophorin, a membrane glycoprotein that contains sialic residues (Ofek and Beachey, 1980). Bordetella pertussis, a respiratory pathogen, has the ability to adhere to epithelial cells of human lung and monkey kidney (Ofek and Beachey, 1980). Bordetella species have been shown to have an affinity for cilia of the respiratory epithelium and thus are able to gain entry into the respiratory passages via attachment (Arp, 1988). The attachment of B. pertussis to cilia is mediated by filamentous hemagglutinin, a surface protein and pertussis toxin (Arp, 1988). Strains lacking the surface protein and toxin show reduced adherence which is regained by the addition of these substances (Arp, 1988). Treatment with sodium periodate blocks the adherence of B. pertussis to ciliated cells, suggesting the receptor for this bacterial species is a polysaccharide (Arp, 1988).

The ability of enteric bacteria to adhere to the gastrointestinal mucosa has provided evidence for their role in the pathogenesis of intestinal infections. Studies by Duguid and Old (1980) have described the adhesion characteristics of bacteria expressing type 1 fimbriae including Salmonella, Shigella and E. coli. Experimental infection of laboratory rabbits with enterotoxigenic E. coli containing fimbriae caused diarrhea in these test animals. Non-fimbriated strains lacking the ability to adhere but retaining the ability to produce enterotoxin did not induce diarrhea, suggesting that attachment via the fimbriae is necessary for infection to occur (Dugid and Old, 1980). A correlation has been found between pilation and the ability to adhere to human intestinal epithelial cells (Svanborg Eden and Hansson, 1978).

The extraintestinal infections caused by the bacteria of the family Enterobacteriaceae initiate infection by adhering to epithelial cells either in the bladder or kidney prior to colonization, causing urinary tract infections. Laboratory animals experimentally infected with Proteus mirabilis showed that this bacterial species adheres to the pelvic mucosa of the kidney in rats and the bladder cells of rabbits. The ability of the bacteria to adhere was attributed to fimbriae, non-flagellar appendages extending from the bacterial surface and correlated with the capacity to promote infection. Silverbalt's study with rats suggested a role for pili in the pathogenesis of pyelonephritis by infection with Proteus species (Silverbalt, 1978). Duguid was the

first to characterize the fimbrial adhesions of E. coli in agglutination properties possessed by enteric bacteria (Duguid and Old, 1980). Based on the agglutination activity of the E. coli to erythrocytes, the bacteria were classified as mannose sensitive and mannose resistant (Dugid and Old, 1980). Those bacteria that agglutinate guinea pig erythrocytes and human erythrocytes of the A, B, O blood groups were classified as mannose sensitive. Mannose sensitive fimbriae are inhibited by mannose,alpha-mannoside, and yeast mannan(Dugid, and Old, 1980). Mannose resistant bacteria are those bacteria that agglutinate human erythrocytes and whose activity is not inhibited by mannose (Dugid and Old, 1980).

Type 1 fimbrial adherence is blocked by D-mannose, alpha-methylmannoside and by Con A, indicating that the receptor for type 1-fimbriae includes mannose residues (Johnson, 1991). The carbohydrate specificity expressed by type 1 fimbriated E. coli, Klebsiella pneumoniae, Salmonellae species have been studied via an agglutination technique using yeast cells (Johnson, 1991).

Receptors for type 1 fimbriae are located on the cell surfaces of erythrocytes, human buccal epithelial cells, intestinal cells and vaginal epithelial cells(Ofek et al., 1977, Jann, 1981). It is not clear if Type 1 fimbriae bind to human uroepithelial cells or Tamm-Horsfall glycoprotein(THP) that coats the epithelial cells. THP, a renal glycoprotein is a constituent of normal urine and considered a normal host defense against uropathogenic bacteria. In vitro studies by Orskov et al. (1980) showed that type 1 fimbriae adhere to the THP, presumably due to the mannose contained

in this protein. It has been suggested that the trapping of type 1 fimbriated E. coli by THP prevents the attachment of uroepithelial cells, thus preventing infections. Dulawa (1988) concluded that THP inhibits the adherence of type 1 fimbriated bacteria. Sobota and Apicella (1990) showed that urine from which the THP was extracted showed a decrease in the inhibitory action, indicating that THP possesses anti-adherent activity. E. coli expressing mannose sensitive fimbriae are the most commonly isolated bacteria in patients diagnosed with urinary tract infections.

The existence of more than one type of lectin in any bacterial population has been well documented (Ofek and Sharon, 1990). For example, pathogenic enterobacteria have the ability to express type 1 fimbriae, a mannose sensitive lectin, in addition to one or more additional fimbriae (Ofek and Sharon, 1990). E. coli, which typically expresses type 1 fimbriae, also has the ability to express P fimbriae which bind specifically to the disaccharide Galα4GalB residues on the epithelial cell surface(Ofek and Sharon, 1990). The agglutination of human erythrocytes by P fimbriated E. coli and adherence to epithelial cells was inhibited by substances containing this disaccharide (Ofek and Sharon, 1990). Strains of E. coli expressing P fimbriae have been isolated from patients with upper urinary tract infections (Johnson,1991). In contrast, isolates expressing type 1 fimbriae are more often recovered from the bladder and are responsible for cystitis (Hagberg, et al., 1991).

Bacteria have the ability to express fimbriae of different phenotypes (Ofek and Sharon, 1990). Strains of E. coli have the ability to express type 1 and at the same time may express a fimbria expressing a different sugar specificity (Ofek and Sharon, 1990). Expression of fimbriae in a given bacterial population may not always occur. The ability to switch between the different forms has been attributed to environmental and genetic regulation (Johnson, 1991).

The treatment of infections associated with bacterial adherence with specific carbohydrates has the potential to prevent such infections. For example, administration to mice of alpha-methyl mannoside prevented the colonization of the bladder with type 1 fimbriated E. coli (Aronson et al, 1979). In addition to the use of carbohydrates, antibodies directed against the fimbriae also offered protection. Thus, it appears that blocking receptors on the cell surface may be an effective method for preventing the development of such infections. Schaeffer et al. (1980) tested the effect of various carbohydrates on the adherence of E. coli to human urinary tract epithelial cells. A range of expression was observed. D-mannose , D-mannitol, D-lyxose, D-arabinose and D-glyceraldehyde partially inhibited adherence, while preincubation of E. coli in 2.5% D-mannose completely inhibited adherence (Schaeffer, 1980). Pre-treatment of epithelial cells with mannose or glucose showed no effect on the adherence (Schaeffer et al., 1980). Glucose was tested and showed no inhibitory or enhancing effect (Schaeffer et al., 1980). Fructose at a concentration of 1%

showed a 20% inhibition and the inhibitory effects of 1% mannose and 1% fructose were additive, resulting in 100% inhibition (Schaeffer et al., 1980). These studies suggest that attachment of E. coli to uroepithelial cells is mediated by a mannose sensitive lectin that expresses sugar specificity for mannose residues. This observation of mannose sensitivity has been confirmed for the attachment of type 1 fimbriated E. coli (Ofek and Sharon, 1990).

There is accumulating evidence that drinking cranberry juice may be useful for the prevention and treatment of urinary tract infections (Sobota, 1984, Zafriri et al., 1989). Initial reports suggested that the juice acidified the urine and thus prevented bacterial growth. However, it was demonstrated that ingestion of even large amounts of the juice resulted in only small and transient changes in urinary pH. In 1984, in a report in the Journal of Urology, Sobota demonstrated that cranberry juice and cranberry juice cocktail(CJC) are very potent anti-adherent agents. It was suggested that it was this anti-adherence activity of the juice that is important in controlling and preventing urinary tract infections. The main components of the cocktail, which include cranberry juice, fructose and vitamin C, have been tested for their anti-adherent ability. In vitro bacterial adherence studies demonstrated that fructose and cranberry juice significantly decreased adherence of E. coli to buccal and uroepithelial cells (Sobota, 1984). Zafriri et al. (1989) confirmed an inhibitory action of CJC on the adherence of E. coli to yeast, tissue culture cells, erythrocytes and mouse peritoneal macrophages. The cranberry

juice showed anti-adherent action on urinary isolates of E. coli expressing both type 1 and P fimbriae.

Since one of the active anti-adherent agent in cranberry juice cocktail is proved to be fructose, the aim of the present study was to test the in vitro effect of this carbohydrate on the adherence of urinary isolates of E. coli expressing type 1 fimbriae (Zafriri et al., 1989). Chronic urinary tract infections are a common occurrence among women, especially the geriatric population. The development of resistant strains of bacteria combined with the multitude of health problems that are common among this population contributes to the difficulty with conventional methods of treatment. In order to test the effect of fructose on adherence, urine from patients diagnosed with chronic urinary tract infections was used in this study. In addition, since a nondialyzable component of cranberry juice cocktail has been shown to possess inhibitory action on the adherence of E. coli in vitro, we also included this component in our study. To determine if there are differences in the action of the suggested anti-adherent components of CJC, fructose and dialyzed CJC were added to the urine of normal, chronic patients and chronic patients ingesting CJC daily and the three groups were compared for mean number of adhering bacteria.

# CHAPTER II

## MATERIALS AND METHODS

### Bacteria:

*E. coli* strain 1810, isolated from a patient with a diagnosed urinary tract infection, was used in this study. The identity of this isolate was confirmed via the use of 20E strips. The growth medium used in this study was tryptic soy broth (Fisher Scientific, Pittsburgh, Pa.). The organisms were grown in static culture at 37 °C for two days to enhance type 1 fimbriae formation. Expression of type 1 fimbriae was further enhanced by incubation with 1% D-mannose to the growth media. For long term storage the bacteria was kept in glycerol at -20°C (Maniatis et al., 1982).

### Exfoliated Cells:

Exfoliated uroepithelial cells were obtained from one healthy female with no history of chronic urinary tract infection. Ten milliliters of freshly voided urine was centrifuged at 4,500g for 10 minutes to pellet the cells.

### Urine Samples:

Thirty urine samples were obtained from male and female patients having a history of chronic urinary tract infections(>3/yr). This study was carried out in cooperation with the Western Reserve Care System and local nursing homes under the supervision of Dr. Kevin L. Scheetz, who contacted area physicians and obtained the clinical

specimens. Urine samples were obtained from four local nursing homes including Beeghly Oaks, Maplecrest, Heritage Manor and Glenn View manor. Samples were collected from geriatric patients with an established history of urinary tract infections who had been diagnosed as chronic. The urine samples were centrifuged at 4,500 g for 10 minutes to remove epithelial cells. The supernate was saved and stored at -20 ° C. Thirty urine samples were also obtained from male and female volunteers with no history of chronic urinary tract infections. These urine samples were centrifuged at 4,500 g for 10 minutes to pellet the cells. The cells were discarded and the supernatant saved for the bacterial adherence assay.

Bacterial Adherence:

Ten ml of urine from an individual with no history of urinary tract infections was centrifuged for 10 minutes at 4,500g to pellet the cells. Two ml samples of a 48 hr culture of *E. coli* were centrifuged for 10 minutes at 4,500 to form a pellet. To the *E. coli* pellet two milliliters of test urine was added and vortexed until the pellet was dispersed. Using a pipet, the urine containing the *E. coli* was added to the epithelial cells. These samples were incubated in a water bath at 37 ° C for 30 minutes and inverted at five minute intervals. Following incubation, a 2 ml sample was removed and filtered through a 8 um polycarbonate membrane and washed one time with one ml of deionized water to remove the non-adherent bacteria. The filters were placed on a glass slide, allowed to dry, and then removed. The adhering bacteria were gram

stained and a total of 50 epithelial cells was scored for adhering bacteria for each sample under the light microscope. Urine and epithelial cells served as the control for each sample. In addition to a urine control, a control containing epithelial cells, urine and 2ml of E. coli was also performed. For each test sample a 5% solution of fructose was added to the urine, E. coli and epithelial cells prior to incubation. In a separate test sample 2ml of dialyzed cranberry juice cocktail was added to urine, E. coli and epithelial cells before incubation.

Statistical analysis

Statistical analysis of difference between means was done by one way analysis of variance (ANOVA). Using Scheffe's multiple contrasts multiple comparisons were performed.

# CHAPTER III

## RESULTS

### Effect of E. coli adhering to exfoliated epithelial cells

For each series of experiments a control urine containing sample urine and exfoliated epithelial cells was scored for attached bacteria. This urine control served as a means to identify individuals with a pre-existing urinary tract infection by the detection of pre-attached gram negative rods.

### Effect of fructose on the adherence of E. coli to exfoliated uroepithelial cell in the urine of subjects with no history of urinary tract infections

Since fructose is one of two major constituents in cranberry juice cocktail that has been shown to elicit anti-adherent activity in vitro, E. coli was treated with 5% fructose and added to the urine of subjects with no history of urinary tract infections. The effect of fructose on bacterial adherence was tested for 25 subjects and the results are presented in table 1. The controls consisted of sample urine containing exfoliated uroepithelial cells and untreated E. coli. It can be observed from the table that when E. coli was treated with a 5% fructose solution, there was a significant decrease ($p<.0002$) in adherence as compared to control values. As indicated from the data in table 1, it was observed that fructose exerted significant anti-adherent activity in 100% of

the samples tested.

Effect of dialyzed cranberry juice cocktail on the adherence of E. coli to uroepithelial cells in the urine of normal patients

It has been demonstrated that CJC also contains, in addition to fructose, a nondialyzable component that effects bacterial adherence. E. coli was treated with dialyzed cranberry juice cocktail to examine the effect of this component. Incubation of E. coli with dialyzed cranberry juice had varying results, either inhibiting or having no effect on bacterial adherence. The results of treatment with the nondialyzable component are presented in table 2. It can be observed from the table that the dialyzed CJC exerted a significant anti-adherent activity in 64% of subjects.

Effect of fructose on the adherence of E. coli to uroepithelial cells in the urine of chronic patients

In an in vitro study, fructose at a final concentration of 5% was added to the urine of 19 patients who experience chronic urinary tract infection. The cells were scored for adhering bacteria and the results are presented in table 3. The results indicate that fructose inhibited the adherence of E. coli in 84% of the subjects.

Effect of dialyzed cranberry juice cocktail on the adherence of E. coli to uroepithelial cells in the urine of chronic patients

To the urine of the 19 chronic patients was added 2ml of dialyzed cranberry juice cocktail. As indicated from the results in table 4, the dialyzed cocktail either significantly decreased the number of adhering bacteria as compared to control values or

had no effect on adherence. It can be observed from the data in the table that dialyzed cranberry juice cocktail prevented the attachment of type 1 fimbriated E. coli in 79% of the samples tested.

Effect of fructose on adherence of E. coli to exfoliated uroepithelial cells in the urine of chronic patients who ingest cranberry juice daily

To determine if the fructose had an additive effect, the sugar was added to the urine of chronic patients who were ingesting 4oz. of cranberry juice daily. The addition of fructose to the urine of these chronic patients had varying results as indicated in table 5. Fructose either enhanced or had no effect on bacterial adherence. In two samples the mean number of adhering bacteria to each cell increased significantly over the control. The control mean bacterial per cell of 13.33 was increased upon the addition of fructose to 19.46. And in the second sample the control increased from a mean bacterial cell count of 3.04 to 5.20. In four of the six samples tested, the addition of fructose had no significant effect on adherence. These samples showed either a decrease or increase in the mean number of bacteria per cell; however, these results were not significantly different from the controls. The mean number of adhering bacteria for the control was determined to be 6.44, while the addition of fructose to the urine chronic patients ingesting CJC resulted in a group mean of 6.68. A comparison between the control and the urine treated with fructose was performed and it was determined that there is no significant

difference between these two groups. This control and fructose treated group means are presented in table 7.

Effect of dialyzed cranberry juice cocktail on the adherence of E. coli to uroepithelial cells in the urine of chronic patients who ingest cranberry juice daily

Since a nondialzyable component of cranberry juice cocktail has shown to express anti-adherent activity, dialyzed cranberry juice cocktail was added to the urine of chronic patients who ingest cranberry juice daily. It can be observed from table 6 that the addition of dialyzed cranberry juice cocktail either inhibited bacterial adherence significantly or had no effect. In half of the subjects this treatment resulted in a significant decrease in the mean number of adhering bacteria. Presented in table 7 is the mean number of adhering bacteria for chronic patients ingesting CJC and those chronic patients whose urine was treated with dialyzed CJC. It can be observed that the mean number of adhering bacteria for control and treated urine is 6.44 and 3.04, respectively. A comparison between these groups was performed and it was determined that there is no significant difference between these groups.

A comparison of the effectiveness of fructose as an anti-adherent agent.

Using Scheffe's multiple contrasts it was determined there was no significant difference in the level of bacterial adherence with the addition of fructose to the urine of normal subjects vs. chronic patients. The means for both groups were determined using one way ANOVA. The group mean for the normal population treated

with fructose was 5.39, whereas the chronic patients treated with fructose had a group mean of 4.85. It was determined using Scheffe's multiple contrasts that there is no significant difference between these two groups. Fructose showed anti-adherent activity in both groups.

Comparison of chronic patients with addition of fructose vs. chronic patients ingesting cranberry juice daily without the addition of fructose.

A statistical comparison was performed using Scheffe's multiple contrasts between chronic patients whose urine was treated in vitro with fructose and chronic patients ingesting cranberry juice daily. The group means were determined and are presented in table 7. The group mean for chronic patients treated with fructose resulted in a mean numbering of adhering bacteria of 4.85, whereas the control from the chronic patients ingesting CJC daily resulted in a group mean of 6.44. It was determined that there is no significant difference between these two groups.

Comparison of chronic patients treated with dialyzed CJC vs. chronic patients ingesting cranberry juice daily.

Using Scheffe's multiple comparisons it was determined that urine of chronic patients treated with dialyzed CJC is the same as that of chronic patients ingesting cranberry juice daily. It was determined that a group mean of 4.85 for the chronic patients treated with dialyzed CJC is no different from a group mean of 6.44 for chronic patients ingesting CJC.

TABLE 1

Effect of Fructose on bacterial adherence in the urine of normal volunteers

| Volunteer | Mean Bacteria per cell: Control | Treatment with Fructose |
|---|---|---|
| 1 | 25.48 | 2.62* |
| 2 | 28.70 | 5.56* |
| 3 | 17.76 | 8.42* |
| 4 | 26.74 | 15.56* |
| 5 | 14.92 | 5.48* |
| 6 | 23.30 | 5.44* |
| 7 | 16.06 | 3.08* |
| 8 | 26.08 | 1.44* |
| 9 | 31.02 | 2.22* |
| 10 | 21.40 | 7.32* |
| 11 | 28.02 | 5.94* |
| 12 | 17.36 | 6.82* |
| 13 | 20.98 | 3.48* |
| 14 | 20.08 | 6.84* |
| 15 | 29.18 | 6.02* |
| 16 | 18.88 | 6.18* |
| 17 | 23.80 | 10.52* |
| 18 | 21.88 | 3.16* |
| 19 | 19.37 | 3.04* |
| 20 | 18.16 | 3.92* |
| 21 | 27.14 | 2.24* |
| 22 | 20.44 | 7.22* |
| 23 | 12.88 | 4.26* |
| 24 | 10.68 | 4.26* |
| 25 | 10.26 | 3.66* |
| Group Mean: | 21.2 ± 4.24 | 5.39 ± .608 |

*Significantly different from control $p<.0002$

TABLE 2

Effect of dialyzed cranberry juice cocktail on bacterial adherence in the urine of normal volunteers

| Volunteer | Mean Bacteria per cell Control | Treatment with Dialyzed CJC |
|---|---|---|
| 1 | 25.48 | 11.54* |
| 2 | 28.70 | 9.14* |
| 3 | 17.76 | 14.28 |
| 4 | 26.74 | 12.04* |
| 5 | 14.92 | 16.72 |
| 6 | 23.30 | 21.84 |
| 7 | 16.06 | 10.58 |
| 8 | 26.08 | 4.26* |
| 9 | 31.02 | 7.22* |
| 10 | 21.40 | 3.38* |
| 11 | 28.02 | 11.58* |
| 12 | 17.36 | 8.88* |
| 13 | 20.98 | 9.26* |
| 14 | 20.08 | 12.84* |
| 15 | 29.18 | 14.66* |
| 16 | 18.88 | 13.00 |
| 17 | 23.80 | 10.04* |
| 18 | 21.88 | 16.34 |
| 19 | 19.37 | 12.54* |
| 20 | 18.16 | 10.48* |
| 21 | 27.14 | 6.80* |
| 22 | 20.44 | 8.94* |
| 23 | 12.88 | 9.90 |
| 24 | 10.68 | 8.72 |
| 25 | 10.26 | 7.50 |
| Group Mean: | 21.2 ± 4.24 | 10.90 ± .800 |

*Significantly different from control $p<.0010$

TABLE 3

Effect of fructose on the adherence of *E. coli* to uroepithelial cells in the urine of chronic patients

| Patient | Mean Bacteria per Cell: Control | Treated |
|---|---|---|
| 4 | 7.50 | 3.50* |
| 5 | 5.32 | 3.24 |
| 6 | 23.10 | 12.44* |
| 7 | 11.02 | 5.32* |
| 8 | 6.74 | 5.82 |
| 9 | 5.24 | 2.60 |
| 10 | 8.82 | 3.86* |
| 12 | 8.32 | 3.92* |
| 14 | 29.30 | 19.74* |
| 16 | 15.26 | 3.48* |
| 17 | 12.74 | 2.66* |
| 18 | 11.88 | 3.32* |
| 19 | 9.72 | 2.28* |
| 20 | 8.24 | 3.24* |
| 21 | 9.22 | 3.38* |
| 22 | 18.14 | 3.34* |
| 23 | 6.94 | 2.30* |
| 24 | 21.30 | 2.76* |
| 25 | 22.30 | 4.92* |
| Group Mean: | 12.69 ± 1.59 | 4.85 ± .975 |

*Significantly different from control $p<.010$

TABLE 4

Effect of dialyzed cranberry juice cocktail on the adherence of *E. coli* to uroepithelial cells in the urine of chronic patients

| Patient | Mean Bacteria per Cell: Control | Treated |
|---|---|---|
| 4 | 7.50 | 2.36* |
| 5 | 5.32 | 4.68 |
| 6 | 23.10 | 10.94* |
| 7 | 11.02 | 3.28* |
| 8 | 6.74 | 2.66* |
| 9 | 5.24 | 2.48 |
| 10 | 8.82 | 2.14* |
| 12 | 8.32 | 4.70* |
| 14 | 29.30 | 5.12* |
| 16 | 15.26 | 4.88* |
| 17 | 12.74 | 2.30* |
| 18 | 11.88 | 5.14* |
| 19 | 9.72 | 5.52* |
| 20 | 8.24 | 7.08 |
| 21 | 9.22 | 9.24 |
| 22 | 18.14 | 5.52* |
| 23 | 6.94 | 3.60* |
| 24 | 21.30 | 4.46* |
| 25 | 22.20 | 6.00* |
| Group Mean: | 12.69 ± 1.59 | 4.85 ± .536 |

*Significantly different from control $p<.016$

TABLE 5

Effect of Fructose on bacterial adherence in the urine of chronic patients who ingest cranberry juice cocktail daily

| | Mean Bacterial per cell | |
|---|---|---|
| Patient | Control | *E. coli* treatment with fructose |
| 1 | 3.04 | 5.20* |
| 2 | 1.84 | 2.62 |
| 3 | 6.42 | 5.74 |
| 11 | 13.33 | 19.46** |
| 13 | 4.02 | 2.54 |
| 15 | 10.00 | 4.50 |
| Group Mean: | 6.44 ± 1.81 | 6.68 ± 2.61 |

*Significantly different from control $p<.0001$
**Significantly different from control $p<.005$

TABLE 6

Effect of Dialyzed Cranberry Juice Cocktail in the urine of Chronic patients ingesting Cranberry Juice Daily

| Patient | Mean Bacteria per Cell: Control | Treatment with Dialyzed CJC |
|---|---|---|
| 1 | 3.04 | 1.64 |
| 2 | 1.84 | .80* |
| 3 | 6.42 | 7.28 |
| 11 | 13.33 | 1.36* |
| 13 | 4.02 | 3.98 |
| 15 | 10.00 | 3.20* |
| Group Mean: | 6.44 ± 1.81 | 3.04 ± .974 |

*Significantly different from control $p<.020$

TABLE 7

Group Means for Normal, Chronic and Chronic Patients Ingesting CJC

GROUP MEANS (NO. OF ADHERING BACTERIA)

| | CONTROL | FRUCTOSE | DIALYZED CJC |
|---|---|---|---|
| NORMALS: | 21.22 ± 4.24 [1] | 5.39 ± .608 [2] | 10.90 ± .800 [3] |
| CHRONICS: | 12.69 ± 1.59 [4] | 4.85 ± .975 [5] | 4.85 ± .536 [6] |
| CHRONICS ON CJC: | 6.44 ± 1.81 [7] | 6.68 ± 2.61 [8] | 3.04 ± .974 [9] |

Significant difference $p<.0013$:

1 vs 2
1 vs 3
4 vs 5
4 vs 6

# CHAPTER IV

# DISCUSSION

Previous studies have indicated that CJC contains components that inhibit the adherence of clinical isolates of E. coli expressing type 1 and P fimbriae to uroepithelial cells (Sobota, 1984, Schmidt and Sobota, 1988, Zafriri et al. 1989, Apicella and Sobota, 1990, Sobota and Apicella, 1991 ). E. coli is the most commonly isolated uropathogen responsible for the pathogenesis of urinary tract infections, responsible for causing more than 90% of infections (De Man, 1990), and E. coli expressing type 1 fimbriae are the type most often isolated from individuals with cystitis (Johnson, 1991). The ability of E. coli to express surface lectins that mediate attachment to mucosal surfaces contributes to its capacity to initiate infection. It is this attachment of lectins, in the form of fimbriae, to host cell surfaces that is necessary for the initiation and development of urinary tract infections (Ofek and Beachey, 1980, Beachey, 1981 ).

Urinary tract infections are a common bacterial infection affecting all age groups from neonates to the geriatric population (Stamm, 1989). These infections especially predominate among young and middle age women and the elderly who require catheterization (Baldassare and Kaye, 1991). Among the geriatric population recurrent infections are common and contribute to the difficulty in

treatment of such patients. Traditional methods of antibiotic therapy are less than optimal for this group because of cost, side effects and the development of resistant strains secondary to long-term treatment. Since attachment of bacteria to epithelial cells of mucosal surfaces is considered a requirement for the colonization and subsequent initiation of urinary tract infections, studies have focused on this interaction and the role of anti-adherent agents in the prevention and/or treatment of such infections. In this study we examined the potential use of anti-adherence agents for the treatment of urinary tract infections in a particularly vulnerable group, nursing home patients.

There have long been anecdotal evidence and published reports that CJC may decrease the frequency and/or severity of urinary tract infections. A clinical study performed by Podromos and co-workers involved placing 60 patients on 16 oz. of cranberry juice per day for 21 days (Podromos, 1968). The results varied following treatment, either showing improvement or having no effect(Podromos, 1968). When treatment was discontinued, at six weeks, 27 of 44 patients showing improvement had a recurring infection. It was further observed that withdrawal of the juice resulted in recurrence of infection in 61 per cent of the cases (Podromos, 1968). It is suggested by these findings that cranberry juice acts to prevent bacterial urinary tract infections and that it must be continually present to elicit its effect. In a study performed in this laboratory it was demonstrated that cranberry juice cocktail inhibited bacterial adherence by 75% in over 60% of the E. coli

clinical isolates tested. The adherence of E. coli to uroepithelial cells and buccal cells was inhibited by incubation in cranberry juice cocktail. It was further observed that urine from 15 of 22 individuals who ingested 15 ounces of cranberry cocktail significantly inhibited bacterial adherence, suggesting that a component of the cocktail is excreted or acts on products in the urine to prevent adherence (Sobota, 1984). A study performed in 1989 confirmed the results of Sobota and further demonstrated the inhibitory actions of cranberry juice on clinical isolates of E. coli (Zafriri et al., 1989). In this study it was concluded that cranberry juice contains at least two inhibitory fractions that prevent the attachment of E.coli by surface lectins to host cell surfaces. The inhibition of type 1 fimbriated E. coli in these experiments was suggested to be due to the fructose present in the cranberry juice cocktail (Zafriri et al., 1989). In addition to fructose, Zafriri and co-workers observed a nondialyzable component of the cocktail that exerted anti-adherent action on the attachment of P fimbriated E. coli.

The role of carbohydrates on bacterial cell surface components has been studied by Schaeffer et al. (1980). Various carbohydrates were tested for their effects on bacterial adherence of E. coli to exfoliated uroepithelial cells from healthy women. It was observed that D-mannose, D-mannitol, alpha-methyl-D-mannoside and yeast mannan inhibited adherence and that inhibition was dose dependent. No inhibition of bacterial adherence was observed by D-glucose, L-mannose, alpha-methyl-D-glucoside, L-

rhamnose, D-ribose, D-galactose, D-xylose, D-glyceraldehyde, and dihydroxyacetone. Fructose was also studied for anti-adherent activity. It was demonstrated that 2.5% D-fructose partially inhibited adherence of E. coli to exfoliated uroepithelial cells in vitro. These studies along with the results of the present study suggest a potential role for fructose in cell-host surface interactions. In contrast to results presented by Schaeffer et al. (1980) the present study demonstrates that fructose exerts anti-adherent activity in the urine suggesting a potential role for the utilization of fructose in vivo.

The present study was performed to examine the effects of fructose and dialyzed cranberry juice cocktail on the adherence of E. coli expressing type 1 fimbriae. Since previous studies have indicated the potential benefit of cranberry juice in the treatment of urinary tract infections, clinical samples of urine were obtained from three populations. Individuals who had no history of urinary tract infections, chronic patients and chronic patients ingesting CJC daily were included in this study. These three populations were compared to determine if the suggested inhibitors exerted anti-adherent effects in the urine. Fructose, a major carbohydrate present in cranberry juice cocktail, has been shown to inhibit bacterial adherence in vitro ( Schaeffer et al., 1980, Sobota,1984, Zafriri et al.,1989). To investigate the role of fructose as an anti-adherent agent, fructose was incubated along with E. coli and uroepithelial cells in the urine of individuals who had no history of urinary tract infections. These individuals

served as controls for those patients with chronic urinary tract infections. It was observed that upon the addition of fructose there was a significant decrease in bacterial adherence in 100% of the 25 samples tested. Thus fructose at a concentration of 5% was a potent inhibitor of E. coli expressing type 1 fimbriae in the subjects tested. This confirms our previous work that showed that fructose at concentrations occurring in the cocktail inhibits bacterial adherence (Sobota, 1984). This inhibitory action of 5% fructose was also observed by Zafriri (1989). They demonstrated that fructose exerted anti-adherent activity similar to the cocktail for three E. coli strains expressing type 1 fimbriae. They also examined the effects of fructose on yeast aggregation rate and observed that if a stock of 5% fructose and CJC was diluted equally, a comparable decrease in bacterial adherence was observed.

Since a concentration of 5% fructose had been shown to inhibit the attachment of E. coli expressing type 1 fimbriae to exfoliated epithelial cells in the urine of normal volunteers, the action of fructose as an anti-adherent agent in the urine of chronic patients was investigated. We wanted to determine if fructose would act as an anti-adherence agent in the urine of patients with chronic urinary tract infections and thus might be useful in the treatment of these patients. It was found that fructose exerts anti-adherent activity in the urine of these patients. Fructose inhibited the adherence of E. coli to exfoliated epithelial cells in the urine in 15 of 19 of the chronic patients, inhibiting the adherence in 84%

of individuals examined. Thus there appears to be no limiting factor(s) in the urine of most individuals with chronic urinary tract infections that would preclude the use of this carbohydrate in the treatment of these patients. To determine whether the anti-adherent activity of fructose was more effective among normals as compared to the chronic population, a statistical comparison was performed. Using Scheffe's multiple contrasts, no significant difference in the effectiveness of fructose as an anti-adherent agent was observed when comparing normal and chronic patients Inhibition of adherence occurred equally in both groups. In terms of anti-adherence activity, it appears that both patients with chronic urinary tract infections and normal individuals who may suffer an occasional infection may benefit from the use of fructose as a potential treatment for the control of urinary tract infections. The exact mechanism by which fructose inhibits bacterial adherence is not known; however, based on previous studies it is probable that fructose acts on bacterial fimbriae and/or eucaryotic surface components. Based on a previous study in our laboratory it was determined that cranberry juice prevents attachment of E. coli by interfering with a surface structure of the bacteria (Sobota, 1984). It was shown that preincubation of the bacteria, but not preincubation of epithelial cells in the cocktail inhibited adherence, suggesting that a component in the cocktail prevents adherence by interfering with the interaction of surface components on the bacterium (Sobota, 1984). It was also observed that washing of the E. coli resulted in restoring the

original adherence capability of the E. coli. Thus the CJC did not permanently alter the bacterial surface. Similar results were demonstrated by Zafriri and co-workers. They examined the effect of cranberry juice cocktail on yeast aggregation rate (Zafriri et al., 1989). They found that preincubation of the bacteria with a 1:2 dilution of cranberry juice cocktail inhibited yeast aggregation by the eight strains of type 1 fimbriated E. coli investigated. It was also observed that following incubation in the cocktail at a 1:2 dilution, subsequent washing of E. coli with PBS had no affect on their ability to promote yeast aggregation, suggesting that a component of the cocktail acts on a bacterial surface structure, most probably the fimbriae.

The inhibition of E. coli expressing type 1 fimbriae by the dialyzable component of the cocktail was observed in the yeast aggregation assay, adherence of E. coli to tissue cultured cells, hemagglutination inhibition and adherence of E. coli to mouse peritoneal macrophages (Zafriri et al., 1989). They concluded that the observed anti-adherence activity of the dialyzable component of the cocktail in all probability was attributable to fructose, which occurs at a concentration of 5% in the cocktail.

Schaeffer et al. (1980) suggests that carbohydrate configuration and chain length play a role in determining inhibitory activity. Inhibition of bacterial adherence was observed by the carbohydrates that consist of a hydroxyl group at carbon 2 within the same configuration of D-mannose excluding L fucose, which had no inhibitory effect (Schaeffer et al., 1980). It was

further demonstrated that any change in the carbon 2 hydroxyl group completely removed the inhibitory activity of the carbohydrate (Schaeffer et al., 1980). D-glucose differs from D-mannose only in the hydroxyl group at carbon 2 and did not have inhibitory actions on the adherence of E. coli to uroepithelial cells. This was confirmed by Sobota when he demonstrated that glucose, a major carbohydrate present in cranberry juice cocktail, exhibited no anti-adherent activity on type 1 fimbriated E. coli to epithelial cells (Sobota, 1984). Findings by Zafriri and associates also demonstrated that a 5% concentration of glucose did not yield anti-adherent activity (Zafriri et al., 1989). Schaeffer and co-workers (Schaeffer et al., 1980) also observed that carbohydrates with a hydroxyl group at carbon 2 but in the opposite configuration of D-Mannose showed no inhibitory effect. The carbohydrates showing no effect on adherence include D-glucose, L-Mannose, methyl-D-glucoside,L-rhamnose, and dihydroxyacetone (Schaeffer et al., 1980). Results of Schaeffer's study suggest that the carbohydrate binding site of E. coli ligand is directed toward the unmodified hydroxyl group at carbon 2 of D-mannose and its derivatives (Schaeffer et al., 1980). The open chain configuration of D-fructose has an aldehyde at carbon 2. However, when fructose is in solution, fructose forms a pyranose configuration, resulting in the same configuration as D-mannose at carbon 2 (Schaeffer et al., 1980). Based on these observations it is possible that the inhibitory actions of fructose are initiated by a similar mechanism as seen in mannose.

The results obtained in this study indicate that fructose inhibits the adherence of E. coli expressing type 1 fimbriae in vitro, and that this activity is exhibited in the urine. This is the first indication that a simple sugar like fructose can act directly in the urine. All previous studies on carbohydrates were performed in buffer or other aqueous solutions. In particular it has been demonstrated that there appear to be no components that are unique in the urine of patients who suffer from chronic urinary tract infections that preclude the use of this sugar as a potential treatment for chronic urinary tract infections.

To determine if the nondialyzable component of cranberry juice cocktail possessed anti-adherent activity in urine, the cocktail was dialyzed, removing small molecular weight molecules including fructose. The addition of dialyzed cranberry juice cocktail to the urine of individuals with no history of urinary tract infections either decreased or had no effect on bacterial adherence. It was found that in 16 of 25 individuals tested the nondialyzable component of the cocktail exerted anti-adherent action. These results suggest that dialyzed CJC contains a factor or factors that inhibit the adherence of E. coli expressing type 1 fimbriae in vitro. Since dialysis tubing used in this study has a cut-off point of about 8000 daltons, it is likely that a large molecule or molecules is responsible for the anti-adherent activity observed by the nondialyzable component.

The role of the nondialyzable component as an anti-adherent agent was also investigated for chronic patients. Dialyzed

cranberry juice cocktail when added to the urine of chronic patients resulted in significant inhibition in 79%.

The effects of the nondialyzable component presented in this study are in direct contrast to Zafriri and co-workers (1989). Zafriri's work on the action of the nondialyzable component concluded that this component of the cocktail inhibited the adherence of P fimbriated E. coli and not type 1 fimbriae although they did find that the dialyzed component inhibited type 1 fimbriated E. coli ranging from 12 to 22% (Zafriri et al., 1989). Here it was demonstrated that the dialyzed component has the capacity to inhibit the attachment of such bacteria. This discrepancy may be due to several factors. First the adherence test performed here using uroepithelial cells was different from the yeast agglutination test used by Zafriri, although both are purported to measure the same effect. Also the testing here was performed in urine, whereas she performed her tests in buffer. Also E. coli has the ability to express more than one type of lectin (Ofek and Sharon, 1990). Perhaps the co-expression of type 1 and P fimbriae is responsible for this discrepancy.

We have demonstrated that fructose exerts anti-adherent activity in the urine of both those individuals with no history of urinary tract infections and those who that experience chronic infections. All this work was performed in vitro, however it would appear that the same effect may occur in vivo. It has been demonstrated that an increase in anti-adherence activity is measurable in the urine of normal individuals after ingesting

cranberry juice cocktail which contains 5% fructose (Sobota,1984). We also have some indirect evidence from this study that fructose may have some value in the treatment of urinary tract infections. As part of this study the urine of six nursing home patients, who were drinking CJC each day, was examined for anti-adherence activity. The director of nursing had read our initial studies on the potential benefit of cranberry juice on the prevention and treatment of urinary tract infections and she placed the nursing home residents who had a history of chronic urinary tract infections on 4 oz. of cranberry juice daily. The patients whose urine we tested had been using the CJC for several years. Although a control study has not been performed among chronic patients, it is the impression of the nursing director that patients receiving cranberry juice daily has resulted in lower incidence of urinary tract infections among this group. In addition to a decrease in frequency of infections there is anecdotal evidence that ingestion of cranberry juice may prevent the development of urinary tract infections. It is the opinion of one chronic patient that when symptoms are experienced that cranberry juice cocktail provides relief and consequently inhibits the development of urinary tract infections.

To determine if fructose would be of clinical benefit to those individuals ingesting cranberry juice cocktail, fructose was added to the urine of individuals who ingest cranberry juice daily. The addition of fructose to the urine showed no increase in anti-adherence activity in 4 of the 6 individuals. In these 4 patients

it appears that the urine is already saturated due to ingestion of the cocktail and the addition of fructose had no additional effect. We have found that maximum anti-adherence activity occurs at about a concentration of about 3.5% fructose. Thus, if drinking CJC with 5% fructose saturated the urine it would be expected that the addition of fructose in the in vitro test would not show an increase in anti-adherence activity. We have no explanation for the increase in adherence activity observed in 2 of the 6 patients. It is probable that fructose exerts its action on the bacterial fimbriae. Ingestion of cranberry juice, thus fructose, may combine to receptor sites on these surfaces, thereby blocking potential receptor sites for the attachment of fimbriae to mucosal surfaces. It has been shown that saturation of binding sites on the bacteria by mannose prevents the adherence of bacteria to epithelial cell surface (Ofek, 1977). The blocking of type 1 fimbriae by fructose may occur by a similar mechanism; thus the addition of additional fructose would exert no enhancing effect, since the bacterial cell receptor sites for fructose would be blocked.

The addition of dialyzed CJC to the urine of these patients had no effect on the urine of 3 of 6 of these patients, possibly for the same reasons suggested above for fructose. However, the nondialyzable factor is a large molecule and thus it appears that it would be more difficult to accumulate in the urine.

The addition of fructose to the urine of chronic patients as compared to chronic patients drinking cranberry juice daily resulted in no significant difference between these groups. This

finding would suggest that one of the two anti-adherent fractions in CJC, fructose or the nondialyzable component, may account for the in vivo effect observed in those patients drinking CJC. Although the sample size of 6 is small, the adherence of E. coli to exfoliated epithelial cells in chronic patients treated with fructose is the same as chronic patients drinking cranberry juice daily. The results obtained here indicate that the in vivo effects of cranberry juice are equivalent to the in vitro effects of chronic patients treated with 5 % fructose.

Since the dialyzed component of CJC has shown anti-adherent action in the urine of chronic patients, the action of dialyzed CJC was also investigated and compared with chronic patients ingesting cranberry juice daily. It was determined that anti-adherence activity in the urine of chronic patients ingesting cranberry juice is statistically equal to the activity in chronic patients treated with dialyzed CJC. This observation suggests that the anti-adherence activity observed in the patients on CJC may also be due to the nondialyzable factor in the juice. However, of the two anti-adherence factors found in the juice, fructose seems to be a better candidate for the anti-adherence activity observed in the urine of patients on CJC since it is a smaller molecule.

Unlike previous studies this report demonstrates that fructose exerts anti-adherent activity in the urine. The in vitro action of fructose among normal subjects and chronic patients were statistically compared. It was determined that the anti-adherent action of fructose is equally effective among both groups. It was

also demonstrated that the addition of fructose to the urine of chronic patients is statistically the same as chronic patients ingesting cranberry juice.

It has been well established that bacterial adherence is required for initiation and development of urinary tract infections, which are common among the elderly population. Since side effects and the development of resistant strains contribute to the difficulty of conventional methods of treatment of urinary tract infections, studies have focused on the use of anti-adherent agents in the treatment of such infections. It has been shown that the adherence of clinical isolates of E. coli to epithelial cells is inhibited by certain carbohydrates. The present study has demonstrated that fructose exerts anti-adherent activity in the urine of normal and chronic patients in vitro. We observed that the anti-adherent action of fructose in chronic patients is the same as chronic patients ingesting CJC, indicating that fructose may be the active component that exerts anti-adherent action in urine. Normally, fructose is not excreted however; it would be a transient component of glomerular filtrate and may act on a normal constituent of the urinary tract before being absorbed. These findings suggest that fructose, a component in CJC, exerts anti-adherent activity and may have potential for use in the treatment of urinary tract infections. It was observed that the nondialyzable fraction of CJC elicits anti-adherent activity and that this component also exerts actions in the urine of both normal and chronic patients. It was further observed that the urine of

chronic patients treated with dialyzed CJC is statistically equal to the urine of chronic patients ingesting cranberry juice daily, suggesting that this component may be acting in the urine. However, as stated previously, this component is a very large molecule and it is unlikely it would be easily excreted into the urine.

The findings presented here demonstrate that fructose is one of two components of CJC that inhibits adherence of E. coli expressing type 1 fimbriae in vitro. It has been suggested, based on results of this study, that fructose exerts anti-adherent action in urine. Fructose may exert its effect either directly or indirectly by acting on urinary proteins or metabolic by-products present in the urine. Further studies are needed to determine whether ingestion of fructose would result in clinically significant decreases in bacterial adherence. Possible future research would involve, but is not limited to, a clinical study of normal and chronic patients to determine whether the in vitro effects of fructose demonstrated here are those which occur in vivo.

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