# FACTORS INFLUENCING THE PREVENTION OF URINARY TRACT INFECTION BY TAMM-HORSFALL PROTEIN FOLLOWING THE INGESTION OF CRANBERRY JUICE COCKTAIL

by

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

Youngstown State University

July, 1996

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## ABSTRACT

Cranberry Juice Cocktail (CJC) has been shown to be a potent inhibitor of bacterial adherence to uroepithelial cells. As a result, the use of this juice has been investigated as a possible alternative to antibiotics for the prevention and treatment of urinary tract infection. Through other studies done in this laboratory, the urinary glycoprotein Tamm-Horsfall protein (THP) which functions to reduce adherence of bacteria to uroepithelial cells, has been shown to be altered or activated by consumption of CJC. Previous research has focused on the benefits of CJC treatment in primarily elderly populations. This study was designed to examine a larger population as well as several factors including sex, age, regular use of CJC and antibiotic use to determine which factors may influence the ability of CJC to activate THP.

A total of 83% of participants showed reduced adherence of *Escherichia coli* to uroepithelial cells after ingesting CJC illustrating the strong antiadherence capabilities of the juice. This is in agreement with other research. The factors that seem to effect on CJC's ability to activate THP are sex of the individual, previous CJC consumption and antibiotic use. Age does not seem to play a critical role in a younger population. Three subgroups were also seen in the population after one dose of CJC: those who respond well, those who respond moderately, and those who do not respond. It may be possible to predict how well CJC treatment would work for an individual based on these profiles. Due to a lack of serious side effects and good acceptance by patients, treatment with CJC provides an alternative to antibiotic use.

## ACKNOWLEDGEMENTS

I would first of all like to thank Dr. Anthony E. Sobota, my graduate advisor, for all of his encouragement and patience throughout this project. Dr. Sobota instilled a desire to learn and an enthusiasm for science in his graduate students that I will always carry with me. I also thank him for all his advice and direction that made this manuscript possible.

I would also like to acknowledge Dr. Diane Fagan and Dr. David Asch for all of the valuable suggestions and revisions they provided. Both Dr. Fagan and Dr. Asch were invaluable resources as part of my thesis committee.

Finally, I would like to thank Susan Dunn who provided me with support and encouragement for the entirety of this project. Susan instructed me when I first entered the lab and was never too busy to answer my questions. She also stood beside me in the difficult times and gave me the needed push to continue. To her I owe much of this manuscript and the drive to succeed.

## DEDICATION

This manuscript is dedicated to my parents, Thomas and E. LaVonne Slaven, who taught me the value of an education and the desire to pursue knowledge. From an early age my parents encouraged me to reach for the highest goals and provided the opportunities to achieve them. My parents also provided much needed support throughout my graduate years for which I am grateful.

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### Urinary Tract Infection:

Urinary tract infections (UTI), defined as $10^5$ bacteria or greater per milliliter of fresh void midstream urine, represent a major health problem. UTIs are one of the most commonly seen disorders by family physicians.[17] These infections may be characterized by frequent need to urinate, pain during urination and the presence of blood in the urine.[16] The appearance of these symptoms classify the infection as a symptomatic UTI. This type of infection can lead to serious complications including formation of infectious stones, bacteremia and septicemia.[22] Eventually severe renal damage may occur if symptomatic infections are not treated.[48] An asymptomatic infection is an infection in which the appearance of symptoms does not occur. This type of infection had been associated with mortality in the elderly along with symptomatic UTIs[40] but treatment of asymptomatic infections has been questioned. Nicolle et. al. found that a group of elderly patients receiving treatment for asymptomatic infections had a reduced survival due to adverse reactions to antimicrobials.[31] Therefore the treatment of asymptomatic infection often poses greater risks for the individual than no treatment at least until a suitable alternative to antibiotics is found.

The normal urinary tract is usually sterile and urine with a pH below 5.5 has been shown to have bacteriostatic properties.[52] The normal range of pH for urine is from 5.6 to 6.2 with alkaline urine being more conducive to bacterial colonization.

**Microbiology:**

UTIs can infect the upper urinary tract or the lower urinary tract. Infection of the upper urinary tract involves structures superior to the bladder (ie. the kidneys and ureters) and often results in inflammation of the kidneys or pyelonephritis. Lower urinary tract infections involve the urethra and bladder leading to urethritis and cystitis. Progression of UTIs begins with infection of the lower urinary tract which may ascend into the upper urinary tract. Foxman and associates report that most of the organisms responsible for urinary tract infection are normal bowel flora.[16] The most common organism of infection is *Escherischia coli* representing the cause of approximately 90% of urinary tract infections in ambulatory patients. In individuals over 50, *E. coli* is responsible for approximately 73% of UTIs. Other organisms reported to be pathogenic in the urinary tract include *Proteus mirabilis, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, and Neisseria gonorrhoeae.*[52] *P. mirabilis* does not normally cause UTI in the normal urinary tract thus infection by this organism may use virulence factors

different than those employed by *E. coli* and similar organisms.[19] Gram-negative bacilli are the major microbiological group involved in the pathogenesis of UTI with only 5 to 10 percent of infections caused by gram-positive cocci.[52] The major gram-positive organisms involved in UTIs are *Staphlococcus aureaus,* Group D *Streptococcus* and *Staphlococcus epidermidis.* Finally, the most common fungus involved in UTIs is *Candida albicans.*

**Risk Factors:**

There are several risk factors that may predispose an individual to UTIs. Among these factors, four groups are considered major risk groups. These are: the elderly, sexually active females, school age females and males with prostatic obstruction.[58] It has been reported that about half of all women have experienced a UTI by their late twenties and that infection may make these individuals susceptible to subsequent reinfection.[16] It has also been observed that females have a six to seven times higher incidence of UTI than males and that 5% of women have an asymptomatic UTI at any given time.[17] This group has several different sources of entry for infecting bacteria. The vaginal mucosa may become infected from fecal bacteria[52] as the introital area and large intestine harbor many organisms that can act as uropathogens.[59] The vagina itself acts as a reservoir for numerous bacterial strains that may infect the urinary tract.

Other risk factors may also be involved in the development of UTI. There may be different host susceptibility factors that make an individual more or less likely to develop an infection Pregnancy, bladder prolapse, prostatic enlargement and other urinary tract abnormalities may promote bacterial colonization.[58] Also sexual activity has been linked to risk of UTI. Bacteriuria is seen to increase after intercourse and has been termed "Honeymoon cystitis".[17] Urethral trauma, spermicide use and diaphragm use may all increase the chances of developing a UTI.[17,58] Finally, over distension of the bladder may play a role in predisposing to UTI. This overfilling can be due to obstruction or avoidance of urination and may decrease circulation in the bladder. This increases the possibility that infecting organisms will colonize the bladder.[17] An individuals risk of infection then depends on behavior of the host, the response of the host's body to the infecting organism, strain of infecting bacteria, host susceptability to the organism, dose and the chance of coming in contact with a virulent strain of bacteria.[16]

The elderly present a unique population in regard to risk of UTI. This group has multiple factors that contribute to an increased likelihood of developing infection. The major risk factor in this group is incomplete emptying of the bladder upon urination.[4] This condition occurs due to neurogenic bladder,

prostatic disease in men, bladder prolapse in women. Other disorders contributing to increased risk of UTI include prior cerebrovascular accident, decreased mental status, catheterization of the bladder and decreased functional status.[41] Another important factor in the elderly is a decrease in the production of Tamm-Horsfall protein, the urinary glycoprotein that acts as a defense mechanism against bacteria expressing type 1 pili.[54] Studies have also shown that the elderly have increased adherence rates of pathogenic bacteria to uroepithelial cells.[43] All of these factors contribute to the fact that in regard to bacterial infections urinary tract infections are second only to respiratory infections in the elderly.[64]

**Importance of Adherence:**

Adherence of bacteria to the epithelia of the urinary tract represents the initial step in the pathogenesis of UTI.[8] Adherence of bacteria to the uroepithelial cells is important to their virulence.[30] This adherence is believed to be due to extracellular structures on the bacteria (ie. pili or fimbriae). Bruce et. al. showed that bacteria containing these external structures showed better adherence than those organisms lacking in these structures.[8] This group also found that adherence depended on the source of the uroepithelial cells. Patients with a previous UTI showed increased adherence to their uroepithelial

cells in contrast to those reporting no history of UTI. The increases in adherence showed 2 to 5 times more bacteria adhering to cells from recurrent UTI patients when compared to controls. Mostafavi et al. proposed that adherence of *E. coli* involves the production of a soluble virulence factor by the bacteria.[30] The proposed virulence factor is a quaternary amine similar to protamine. In an in vivo assay, the substance was shown to promote bacterial adherence and virulence suggesting a role for this soluble factor in initiating adherence to bladder mucous. It was suggested that the factor promotes virulence by inactivating urinary mucus, the initial defense against UTI.

The body has developed numerous mechanisms to block the adherence of bacteria to the uroepithelia. The main defense is the lining of the urinary tract with a mucous layer.[39] This mucous layer is composed of proteoglycans, glycoproteins and glycosaminioglycans. The urinary glycoprotein Tamm-Horsfall protein is the major glycoprotein acting against Type 1 fimbriated bacteria. The bacteria adhere to the mucous and are then flushed from the urinary tract providing the body with an important mechanism for removing the pathogens. A second mechanism, as previously mentioned, is the pH of urine. The often acidic urine provides an environment that may not be suitable for the growth of many organism. Many uropathogens grow best under alkaline conditions thus making acidic urine

advantageous in preventing UTI.[52] Also the high urea content, extremes in osmolality, organic acid content and prostatic secretions in the urine act as defense mechanisms. Finally, complete bladder emptying reduces the risk bladder colonization.

**Treatment of UTI:**

There are several methods of treatment that can be employed upon diagnosis of a UTI. For symptomatic infection, antibiotics are commonly used to reduce the symptoms associated with this type of infection. Amoxicillin and trimethoprimsulfamethoxazole are the two most commonly used antibiotics for the treatment of symptomatic UTIs. It is reported that the duration of antibiotic therapy is arbitrary, however, leading to problems in determining correct dosage and length of treatment with these agents.[17] There is no apparent advantage for treatment of asymptomatic UTI with antibiotics. Use of antibiotics in females with radiologically normal urinary tracts was occasionally harmful and found to be unnecessary.[19] Furthermore, in a study comparing antibiotic treated women with untreated women, it was shown that there was no significant difference in the occurrence of bacteriuria in the two groups.[17] In fact, the treated group was more likely to have symptoms appear with a new infection suggesting a tolerance to the organism by the treated women was reduced by treatment with antibiotics. Other therapeutic

options for symptomatic and asymptomatic infections include behavior modification strategies including frequent voiding, post-coital voiding, avoidance of contamination of the vagina from feces and use of lubricants during coitus.[17] It is also suggested that avoiding overfilling of the bladder could provide protection against UTI.

**Mechanisms of Adherence:**

As described above, bacterial adherence to the cells of the urinary tract is the initiating event of a urinary tract infection. This adherence is accomplished by the presence of structures on the outer surface of bacteria known as fimbriae or pili. These structures contribute to the virulence of a bacterial strain along with the production of endotoxins and the presence of a polysaccharide capsule.[19] Virulence factors may be more common in bacteria infecting the upper urinary tract than in those infecting the lower urinary tract.[58] *E. coli*, the most common uropathogen of the lower urinary tract, has virulence factors including adhesins, siderophores and toxins that are used to increase the infective ability of this organism. Other factors such as invasiveness and the presence of lipopolysaccarides may also lead to increased virulence in a bacterial strain.[16]

Adherence of bacteria is also subject to environmental

conditions making adherence dependant on the properties of the microorganism, the host and the environment in which adherence is to take place. In a study of 100 urine specimens from normal donors, increased concentrations of urea and creatinine have been shown to increase adherence of *E. coli* to uroepithelial cells.[18] In contrast, increases in potassium, the presence of immunoglobins and high pH were all seen to decrease adherence of the bacteria. Edwin Beachy examined adhesin-receptor interactions and found that there were multiple factors involved in the adherence of bacteria to the surface of cells.[5] Attachment of the bacteria was seen to be subject to type of host tissue, bacterial species, genetic susceptibility of the host and other nonspecific factors. Many bacterial strains preferentially adhere to specific tissue types and are less adherent to others. For example, Beachy reports that *Streptococcus salivarius* is found abundantly on dental plaque but did not adhere to epithelial cells of the tongue. This phenomena is known as "tissue tropism". A specific strain of bacteria may also be needed for effective attachment to occur. For example, Beachy states that *E. coli* CFA/I and CFA/II infections are only seen in humans while *E. coli* K88 infections only occur in pigs. Genetic factors influencing attachment were also examined using *E. coli* K88. Pigs resistant to this infection were cross-bred with susceptible pigs and the trait was seen to be inherited as an

autosomally dominant trait lending evidence to genetic factors playing a role in virulence. Finally, nonspecific factors including antibacterial enzymes of mucous and antibodies play a role in blocking adherence of bacteria to cells. Desquamation of epithelial cells may also be a mechanism blocking attachment.

The outer surface of eukaryotic cells contains carbohydrates that are covalently bound to other membrane components.[2] These carbohydrates on the cell surface are in the form of oligosaccharides and polysaccharides that bind to proteins and lipids in the membrane and make up 2 to 10% of the plasma membrane.[47] These carbohydrates are believed to play a role in microbial adherence as well as cell to cell interactions. Many of the carbohydrates on the cell surface have be studied using a lectin Concanavalin A which is isolated from the Jack bean.[27] Lectins are proteins that recognize specific carbohydrates and have the ability to bind to these sugar residues. Concanavalin A is specific for mannose and glucose residues. By using this lectin, studies of cell surface carbohydrates successfully determined the carbohydrates present on various cell types. Lectins have also been found in bacteria, viruses and protozoa and allow these organisms to interact with the cell which they infects.[53] Thus, the carbohydrate moieties on the surface of the cell play an important role in bacterial attachment.

Filamentous nonflagellar structures of bacteria known as

fimbriae or pili were first described as bacterial organelles by Houwink and Van Iterson.[34] They were also the first to suggest that fimbriae functioned as organs of adhesion in bacteria. The name fimbriae is the Latin term for "fibers" and was first given to these structures. Later the term pili meaning "hairs" was given to the structures by Brinton. Initially, scientists recognized six types of fimbriae that could be expressed by bacteria and also discovered a correlation between hemagglutination and fimbriation. Briton also named six distinct types of fimbriae. Type 1 or mannose sensitive (MS) was the classification given to the most commonly expressed fimbriae and is the type found in most strains of *E. coli*. The presence of these fimbriae on bacterial cells give the bacteria the ability to attach to mucosal surfaces.[21] Type 1 fimbriae from different *E. coli* strains are serologically closely related and their role in adherence is similar in the different strains.[35]

Early studies have shown that the attachment of *E. coli* to epithelial cells occurs via the binding of these cells to mannose residues on the cells.[32] This adherence was later attributed to the presence of type 1 fimbriae when it was seen that bacteria expressing these fimbriae agglutinated yeast cells and guinea pig erythrocytes containing mannose residues.[1] The fimbriae were demonstrated to be the organs of adherence by the development of an antibody against the fimbriae and the use of mannose residues

to block binding by the fimbriae. Abraham and associates developed two sugar-specific antibodies, AO7 which was D-mannose specific and F12 which was N-acetylgalactosamine specific as well as an antibody against the fimbriae.[1] These antibodies blocked the adherence of bacteria expressing type 1 fimbriae. The antibodies against the fimbriae and the mannose residues on the host cells were anti-adherent in situ and both antibodies prevented bacterial attachment to renal cells. It was hypothesized that the bladder contains N-acetylgalactosamine residues and that the second antibody reacted with these residues in turn blocking adherence of the type 1 fimbriae expressing bacteria.[1] Further evidence supporting the role of type 1 fimbriae was presented when sublethal concentrations of streptomycin reduced the mannose binding activity of *E. coli*.[12] This also provided evidence that antibiotics may work to reduce adherence instead of only being bacteriostatic or bacteriocidal. This effect of streptomycin was later attributed to the fact that the antibiotic causes bacteria to produce aberrant fimbrial proteins once again indicating the fimbriae as organs of adherence.[13] Finally, studies in which mannose was added to a mixture of bacteria and epithelial cells showed blocking of adherence. [32] The binding sites on the bacterial surfaces were believed to be saturated by the addition of mannose and thus the bacteria were unable to attach to the uroepithelial cells.

Type 1 fimbriae are one of a number of different adhesive structures that can be expressed by *E. coli*. The fimbriae are classified by the specific receptor for adhesin proteins that are found at the tip of the filaments.[51] As previously mentioned, type 1 fimbriae are specific for mannose residues on the cell surface or in substances like Tamm-Horsfall uromucoids. A second type of fimbriae commonly expressed by *E. coli* are P fimbriae. These fimbriae are also known as Gal-Gal fimbriae due to their specificity for α-galactosyl-1,4-β-galactose residues. These fimbriae are specific for three antigens in the human P blood group. The P type fimbriae are also classified as mannose resistant and are not dependant on the presence of mannose for binding to occur. These fimbriae are commonly seen in pyelonephritis and other kidney infections while type 1 fimbriae are associated with UTI.[21] Hopkins and associates examined the expression of fimbriae by *E. coli* by isolating 28 strains of the bacteria from UTI patients.[21] Type 1 fimbriae were expressed by 61% of the isolates while type 1 and type P were both found on 14% of the isolates. Neither type was expressed by 25% of the isolates. These results demonstrated that the majority of UTIs are caused by bacteria expressing type 1 fimbriae or a combination of type P and type 1. The group not expressing either fimbriae was believed to be expressing a different type of adhesin that was not assayed for. This group also found evidence

that the degree of fimbriation may be a determinant of virulence along with other *E. coli* virulence factors such as hemolysin production, iron sequestration, resistance to serum bacteriocides and the presence of O:K:H antigens. Finally, S type fimbriae bind sialogalactosides and can also be expressed by *E. coli*.

**Role of Urinary Tract Mucous:**

Several studies have drawn a link between bacterial adherence and urinary tract mucous. Orskov and associates found in a comparison of fimbrial antigen F7 and type 1 fimbriae that bacteria with type 1 fimbriae attached strongly to mucous and rarely to epithelial cells.[34] Mannose resistant fimbriae were seen to adhere strongly to the cells but rarely to mucous. It was suggested that the urinary mucous trapped the type 1 expressing bacteria and blocked the adherence of these bacteria to the epithelial cells. Also it was demonstrated that normal urine contains considerable amounts of mucous and type 1 fimbriae were observed to adhere specifically to this urinary mucous. The presence of mucous was further demonstrated when a rabbit model showed a layer of the mucous on the bladder epithelium.[39] Parsons and associates suggested that this mucous layer acts as a barrier keeping *E. coli* from adhering to the cells.[39] It was also found that different individuals may produce varying amounts of mucous.[35] It was concluded that the urinary mucous may

function as a trap for Enterobacteriaceae expressing type 1 fimbriae by providing a site of adherence for the bacteria and acts as a nonimmune resistance mechanism against these bacteria. A further attempt to classify the urinary mucous was accomplished when it was shown that type 1 fimbriae will bind to immobilized Tamm-Horsfall protein (THP), the most abundant uromucoid glycoprotein found in urine.[36] Hanson and associates suggested that Tamm-Horsfall protein may interact with gram negative bacteria and this may also represent a nonspecific defense mechanism preventing the adherence of bacteria to uroepithelial cells.[19]

**Tamm-Horsfall Protein:**

Tamm-Horsfall protein was first characterized by Igor Tamm and Frank Horsfall in 1951.[60] These researchers developed a method for purifying the protein by precipitation with sodium chloride. The material extracted was seen to be homogeneous when tested electrophoretically and via ultracentrifugation. Tamm and Horsfall also described many of the biological, chemical and physicochemical properties of the isolated protein. The protein was shown to be of a high molecular weight and to be made up of thread-like molecules. Based on their studies of the mucoprotein, the substance later was given the name Tamm-Horsfall protein or THP.

THP is the most abundant protein found in normal human urine.[56] Based on gel filtration in the presence of sodium dodecyl sulphate, this protein was estimated to have a molecular weight of approximately 76000 - 82000 daltons.[14] The production site for THP appears to be the cells of the ascending loop of Henle and the segment of the convoluted tubule adjacent to the loop of Henle.[44] The excretion rate for the protein ranges from 20µg to 200µg per day.[25] The distribution of the protein appears to be limited to the kidney and substances such as calcium, sodium, albumin and radio contrast media have been seen to cause THP to gel. This gel formation occurs due to glycosylation and hydrophobic aggregates of the protein leading to the formation of uromucoid.[15]

In a study by Haugen, it was demonstrated that in individuals the THP excretion rates were seen not to fluctuate very much.[20] Excretion rates for day and night were similar and these rates were not influenced by sex, salt load or diuresis. Lynn and associates had conflicting results. They found that THP excretion rates could change 18-fold in an individual during a single day.[29] They also noted that the amount of urine collected seemed to influence THP secretion values and the half-life of the glycoprotein was seen to vary from 3 hours to 168 hours. Additional studies by Reinhart and associates also found the THP excretion rates to vary in the same individual over the course of

a day.[45]

A second glycoprotein called uromodulin was isolated from urine samples obtained from pregnant women.[25] This protein was isolated through the use of lectin adherence columns and was analyzed using cDNA and amino acid sequencing. Researchers found that the protein backbone of uromodulin and THP were identical. Uromodulin was also shown to be a ligand for interleukin-1, interleukin-2, and tumor necrosis factor making its bioactivity different than that of THP. The difference in the bioactivity of the two glycoproteins is due to glycosylation differences and not differences in the amino acid sequences. To distinguish the two proteins THP refers to the glycoprotein that is isolated by sodium chloride precipitation and uromodulin is isolated from pregnant donors by lectin adherence columns.

**Bioactivity of THP:**

Parsons and associates investigated the role of mucoproteins as an antibacterial defense in the bladder.[37,38] It was suggested that the primary antibacterial defense mechanism in the bladder appears to be secretory. This was shown by disrupting the secretion of mucin and observing that the resistance to bacterial adherence returns in less than 24 hours. It was hypothesized that resistance to bacterial infection was due to the need of a reciprocally charged protein on the surface of the bladder for

bacteria to be able to attach. The mucin covers these proteins blocking the needed attachment sites for the bacteria.

The role of Tamm-Horsfall protein as an anti-adherence agent was examined in more detail by several investigators. It was shown that in normal urine, mucoproteins can trap *E. coli* expressing type 1 fimbriae.[35] Other studies demonstrated that the urinary mucous captures bacteria and that urination removes these organisms from the urinary tract.[54] This led to the suggestion that THP is an antibacterial defense mechanism effective in guarding against urinary tract infection by blocking of adherence of bacteria to the uroepithelia.[15] Research by Sobota and Apicella later confirmed this suggestion.[56] They found that removal of THP from the urine significantly decreased the anti-adherence properties of urine when tested against *E. coli* expressing type 1 fimbriae. This is significant because strains of *E. coli* with these fimbriae have been shown to attach to urinary mucous. THP concentrations above 35 μg/ml were shown to have the anti-adherence activity. If the concentration of THP is too low, an individual may be predisposed to a UTI.[26] Calcium levels also seem to have an effect on the activity of THP.[56] If calcium levels exceed normal values in the urine the anti-adherence activity of the THP is decreased making high levels of calcium conducive to the development of UTIs.

**Mechanism of THPs anti-adherence activity:**

The manner in which THP influences infections in the bladder is not clear. As previously discussed, there is some evidence that the concentration of THP plays a role in susceptibility to UTI.[26] Results obtained by Reinhart and associates do not support this observation.[45] These researchers found no significant differences in the levels of THP excretion in recurrent UTI sufferers and normal controls suggesting that quantity is not a critical factor. Also, the range of THP needed for bacterial clearing was seen to be within normal urine conditions lending support to the hypothesis that it is quality and not quantity that is important for THPs activity.[26]

The adherence of *E. coli* to uroepithelial cells occurs via type 1 fimbriae or P fimbriae found on the outer surface of the bacteria. Type 1 fimbriae specifically seem to play a role in the development of lower UTIs. The type 1 fimbriae attach to host cells by binding with a host cell receptor that is specific for the bacterial adhesin.[23] The host receptor is believed to be a glycoprotein on the host cell containing mannose residues with which the type 1 mannose-sensitive fimbriae react. This is supported by research that shows blocking of fimbrial attachment

to cells by the addition of mannose.[49] The effects of THP seem to be the formation of a pseudocapsule around bacteria with type 1 fimbriae. THP has been shown to adhere to the fimbriae and form the capsule which has been seen on electron micrographs.[26] THP contains a mannose residue on its carbohydrate chain that may act as a receptor for the type 1 fimbriae expressed by bacteria such as *E. coli*.[35] Reinhart and associates have conducted sugar inhibition studies and found that THP possibly acts as a receptor analog for bacteria expressing mannose-sensitive fimbriae.[44] It was also seen that the fimbrial receptor site appears to have lectin-like properties and that the binding of fimbriae to THP is due to the mannose side chains of the protein. Therefore, THP is an important defense mechanism in the lower urinary tract since *E. coli* and other gram negative rods containing type 1 fimbriae, cause 80 to 90% of non-nosocomial urinary tract infections.[44]

Additional studies with THP have further characterized the anti-adherence effects of the protein. Dulawa found the effects of THP to be reversible, providing further evidence of the importance of this protein in protection against UTI.[10] When THP was washed off of cultured cells anti-adherence activity was lost by these cells. Other glycoproteins used in the study did not show the same effect as the THP. Thus it was concluded that the immune system produces substances such as THP to inhibit bacterial attachment to the uroepithelial cells. This makes THP

a critical defense mechanism against the development of a urinary tract infection.

**Cranberry Juice Cocktail (CJC):**

For over 100 years cranberry juice has been used as a folk remedy to treat urinary tract infections. Within the past 10 years evidence has started to accumulate suggesting a specific role for the juice in controlling and reducing the incidence of infections. Several investigators have found evidence that cranberry juice may work as an effective prevention measure and treatment for UTI. Cranberry juice and Cranberry juice cocktail (CJC) were both found to be very potent inhibitors of bacterial adherence.[55] This finding led to the attempt to isolate the mechanism of CJCs preventive activity.

The first attempts to explain the action of CJC suggested that the juice was responsible for increasing the acidity of urine making the environment unsuitable for bacterial colonization[57] which was shown to be incorrect. Ingestion of cranberry juice results in only slight changes in urinary pH.[24,55] Avorn and associates administered 330 ml CJC per day for 6 months to one study group and a placebo to a second group and found the placebo groups urine to have a pH of 5.5 compared to the cranberry consuming group urine which had a more alkaline pH of 6.0.[3] Thus urine acidification does not appear to be the

mechanism of action of CJC. It was also hypothesized that CJC consumption increased levels of organic acids in the urine such as hippuric acid.[7] It was seen that prunes, plums and cranberries all increased hippuric acid concentrations in the urine and it was known that hippuric functions as a bacteriostatic agent. Cranberry juice alone, however, does not increase hippuric acid to levels that would allow the urine to exhibit bacteriostatic properties. This showed that cranberry juice was not a reliable acidifier of urine and that the accumulation of hippuric acid did not explain the mechanism of action of cranberry juice.

To further examine the possibility that acids were the active agents in cranberry juice high pressure liquid chromatography studies were done on the juice.[9] It was known that a number of organic acids exhibit antibacterial characteristics. Amounts of quinic, malic and citric acids were tested in undiluted cranberry juice. Quinic acid made up 1.32% of the acid in the juice, malic acid made up 0.92%, and citric acids made up 1.09%. Benzoic acid and quinic acids play a role in the formation of hippuric acid in the kidneys. As previously mentioned, however, hippuric acid was discounted as the mechanism of action of cranberry juice and the other acids are in low percentages leading to the conclusion that acidification is not the primary mechanism of CJCs activity. The decreased

bacteriuria after CJC consumption must therefore be due to the juice itself and not changes in urine pH or hippuric acid.[6,57]

**Anti-adherence properties of CJC:**

After concluding that CJC harbored antibacterial activity, attempts were made to isolate the active component in the juice. In 1984, a study looked at individual components of CJC to isolate the active factor or factors present.[55] The four components examined were the cranberry juice, the fructose in the juice, Vitamin C, and the pH of the CJC which is 2.6. The pH was found not to be a significant factor as adherence was not effected below a pH of 2.0. Fructose had been suggested as the active component based on evidence that the addition of fructose to urine blocked adherence of bacteria to uroepithelial cells.[62,63] Fructose was not tested in vivo, however. Sobota showed that no additive effect was seen when fructose and CJC was used in combination suggesting that fructose was only a minimal factor effecting adherence in the CJC.[55] Also, High Performance liquid chromatography studies were unable to detect any fructose accumulation in the urine after consumption of 5%, 10% or 15% fructose solutions providing further evidence that fructose is not the active component in CJC.[61] Testing of Vitamin C showed that the vitamin had no anti-adherence activity.[55] Both pure cranberry juice from concentrate and freshly squeezed juice

displayed strong anti-adherent activity pointing to the juice as the active component of CJC.

The anti-adherent ability of CJC has been well established. The first clinical trial examining the effectiveness of CJC for treatment of UTIs was done by Prodromos and associates in 1968.[42] A study group of 44 females and 16 males were given 16 ounces of cranberry juice per day over a period of 21 days. Significant improvement was observed in 32 participants while only 16 showed no improvement. Recurrent infection occurred in 61% of the participants after the consumption of CJC was terminated showing that CJC can be an effective treatment for UTI. The suggestion that CJC acts by blocking adherence of bacteria to uroepithelial cells was first proposed by Sobota in 1984.[55] He found that adherence of *E. coli* to uroepithelial cells was decreased after treatment with CJC in 60% of the 77 isolates tested. In mice in which CJC was substituted for their normal water supply, the anti-adherence activity of the urine was increased by 80%. Testing on human subjects showed that in 15 of 22 participants that ingested 15 ounces of CJC the urine showed significant anti-adherence activity within 1 to 3 hours after consumption.

Preincubation of *E. coli* with CJC dramatically reduced the adherence of the bacteria to uroepithelial cells. Preincubation of the uroepithelial cells with CJC showed no effect. This led to the conclusion that the activity of CJC was to interfere with

adherence of the bacteria via the surface components on the bacteria. This was further established when washing of the bacteria resulted in a return to the normal adherence pattern.

Zafriri and associates confirmed that CJC exhibits anti-adherent properties.[63] These researchers tested the effects of CJC against the mannose-specific lectins associated with type 1 fimbriae and the α-gal(1→4)β-gal specific lectins associated with P fimbriae. It was shown that cranberry juice was inhibitory to both expressed fimbriae though it appeared that two separate inhibitors were responsible. This led to the conclusion that a possible nondialyzable component existed in the cranberry juice and exhibits anti-adherent properties. This nondialyzable portion was later found to be present only in blueberry and cranberry products.[33] Interestingly, the blueberry and the cranberry are members of the same genus, *Vaccinium*.

A second study on the anti-adherence ability of CJC was conducted by Sobota and Schmidt in 1988.[50] This study examined the effects of CJC on Gram negative rods isolated from various sources. The organisms examined included *E. coli, Proteus, Klebsiella, Enterobacter,* and *Pseudomonas*. In the study, urinary isolates showed an increased mean adherence over non-urinary isolates. CJC showed anti-adherence effects against the other gram negative rods besides *E. coli* that was tested in the first study. Complete clearing of cells by CJC does not occur,

however. Between 2 to 5 bacteria are usually left adhering to the cells, but CJC does provide some protection against UTI.

A large clinical trial was recently conducted by Avorn and associates.[3] In this study 153 elderly women with a mean age of 78.5 years were divided into two groups. One group was given 300 ml of CJC each day for 6 months while the other group receive a placebo mixture that looked and tasted like cranberry juice but contained no juice. Anti-adherent activity appeared after 4 to 8 weeks and lasted from 4 to 8 weeks after consumption of CJC was terminated. The group given CJC had less bacteriuria than the placebo group and were more likely to make the transition from bacteriuric urine to nonbacteriuric urine. The researchers suggested that these results are due to interference with bacterial adherence and that adherence may be a good target for the treatment of UTIs. The researchers also suggested that further studies on the effects of antibiotics and CJC in conjunction need to be done. In their study group, only modest reduction in adherence were seen in those using antibiotics.

Recent studies in this laboratory by Patrick Wilson and Mary Beth Wollet examined the anti-adherent effects of urine after the ingestion of CJC. In a test group consisting of 25 individuals with no infection (Group 1), 19 chronic UTI sufferers (Group 2) and 6 chronic UTI patients that were receiving 4 ounces of CJC per day for 2 years prior to the study (Group 3), CJC was shown

to increase the anti-adherence activity of urine in both groups 1 and 2.[62] Group 3 showed no change in anti-adherence activity in the urine. It was suggested that their urine was saturated with the anti-adherent component of the CJC so they showed little change in the study. This group did have the lowest mean bacterial adherence initially at 6.44 ± 1.81 bacteria per cell compared to the control mean of 21.22 ± 4.24 bacteria per cell. Another study involved eight elderly female nursing home patients that were given 4 ounces of CJC each day for twelve weeks.[61] Anti-adherent urine was demonstrated in 4 of the 8 patients showing a decrease in adherence ranging from 26% to 62%. This effect was first observed at about 4 weeks following the initiation of CJC consumption.

**The Current Study:**

The goal of the current study was to identify factors influencing the effectiveness of cranberry juice cocktail as a treatment for urinary tract infection. Five factors were examined to have a possible effect on the antiadherence activity after the consumption of cranberry juice cocktail. These factors consisted of age, sex of the participant, previous CJC consumption within a month of the study, antibiotic use within 6 months of the study and history of UTI within 6 months of the study. Urine samples were collected at 3 intervals with the

first sample representing the pre-CJC ingestion control, the second sample being collected at 2 days after CJC ingestion and the third sample being collected at 21 after CJC ingestion. Tamm-Horsfall protein was then extracted from the samples and tested for anti-adherence activity. The activity of the THP of different individuals was then compared based on each of the four factors discussed above. The purpose of this comparison was to isolate those factors that influenced the effectiveness of CJC in influencing the THP of the participants in this study.

Little information has been collected concerning UTIs in males.[28] This means that the effectiveness of the natural defense mechanisms against UTI have not been extensively compared between men and women. UTI has mainly been considered a female disease due to the large number of women that suffer from these infections.[28] At young ages, UTI is much more common in women and girls, but in the elderly, numbers of men and women sufferers are nearly equal. In infants, males have a higher rate of UTI than females and it is in this period of life that males have their highest incidence of UTI.[46] The high rate of UTI seems to be reduced by circumcision. UTI in older boys and young men are rare and usually caused by operative procedures such as catheterization.[28] By age 65, the percentage of men suffering UTI is nearly equal to that of women. The events leading up to an infection in men have not been well characterized. Most cases of

UTI involve enlargement of the prostate gland. In males, the urethra is longer than that of the female making ascension of bacteria more difficult. Interestingly, THP excretion rates between men and women seem to be the same.[20]

This study is an attempt to test if sex and age of the participants are important factors in predicting the effectiveness of CJC in preventing UTI.

Another factor examined in this study is the combined effects of antibiotics and CJC. It was mentioned in previous studies[3] that a study of antibiotics and CJC had not been done but would provide information regarding the benefits of combined therapy. One of the factors examined in the current study is the use of antibiotics and its effect on THP's activity after CJC ingestion. Finally, the effects of previous CJC use are examined.

## II. MATERIALS AND METHODS

### Collection procedure:

The participants in this study were university students. The collection procedure took place in a time period of 21 days. The selection of 21 days for the trial period was based on a baseline study that was run previous to the larger study. In this baseline trial the effects of cranberry juice cocktail were seen to last approximately 21 days. After this point an increase in adherence was seen. Three collections of urine were obtained from each of the participants as described below. The first collection occurred prior to the ingestion of the cocktail. At this time a urine sample was obtained and each of the participants were given 4 ounces of Ocean Spray Cranberry Juice Cocktail. CJC was used because it is the most commonly consumed cranberry beverage. At the time of the first collection the students were also asked to fill out a questionnaire. The questionnaire asked the individual to report age, sex, date of last Cranberry product consumption (if within one month of the study), date of antibiotic use (if within 6 months of the study) and date of latest UTI (if within 6 months of the study). The first collection involved 81 participants. The second collection took place on April 27, 1995, two days after ingestion of the

CJC. A total of 75 donors participated on this date. The final collection was taken on May 16, 1995, twenty-one days after the ingestion of CJC. All of the participants from the second collection returned for the third collection. During the course of the study the urine donors were asked not to consume any cranberry products other than the original 4 ounces of cocktail. Once the samples were collected, the urine was placed in test tubes and frozen for later extraction of THP. The study group consisted of individuals with ages ranging from 18 to 40. A total of 30 males and 30 females were randomly selected for the study.

**Extraction Procedure:**

The extraction of Tamm-Horsfall protein from the donated urine samples followed a protocol developed by Igor Tamm and Frank Horsfall.[60] Ten mL of donor urine was placed in a centrifuge tube and 0.336 grams of NaCl was added. The sample was vortexed and then placed in the cold at $4^0$ C overnight to precipitate the THP. The following day the samples were centrifuged at 4°C for 30 minutes at 4,960 x g in a refrigerated centrifuge. A series of washings were then done to purify the precipitated THP. The samples were washed with 10 mL of 0.58 M sodium chloride and recentrifuged at 4°C for 30 minutes. The supernatant was then discarded and the precipitate was subjected

to a second washing again using the 0.58 M sodium chloride. The samples were recentrifuged and washed a final time before resuspension of the precipitate in 5 mL of 0.58 M sodium chloride. The THP samples were then frozen to be used in an adherence assay.

**Adherence Assay:**

An adherence assay was done using uroepithelial cells from one healthy female donor and THP from the urine donors. Ten mL of urine from the uroepithelial cell donor was centrifuged at 4,500 x g for 10 minutes to pellet the cells. The supernatant was then discarded. *E. coli*, containing type 1 fimbriae, was grown for 24 hours at 37°C and used in this assay. The bacterial stock was isolated from an elderly patient at Beeghly Oakes Nursing Home by Patrick Wilson.[61] For the adherence assay, 2 mL of *E. coli* was placed in a test tube and centrifuged for 10 minutes at 4,500 x g to pellet the bacteria. The supernatant was discarded and 2 mL of the extracted THP was added to the *E. coli* pellet. The mixture was vortexed and the resulting suspension was added to the uroepithelial cell pellet. This mixture was vortexed and then incubated at 37°C for 30 minutes in a water bath. After the incubation period, the 2 mL of the sample was pushed through an 8μm nucleopore filter and then washed twice with 50 mL of deionized water to remove bacteria not

adhering to the cells. Finally, the filters were removed from the filter holders and placed on microscope slides to be allowed to dry overnight. Once dry, the filters were removed leaving the cells adhering to the glass slides. The slides were finally gram stained and scored.

**Gram Staining:**

The gram staining technique used in this study was the Philadelphia General Hospital method. The microscope slides were covered with crystal violet and 5 drops of sodium bicarbonate were added. This was allowed to sit for 1 minute at which time the slides were rinsed with Gram's iodine and allowed to sit for 10 seconds. Next, the slides were rinsed with acetone to decolorize. The slide was allowed to dry and then counterstained with Gram's safranin for 3 minutes. Finally, the slides were rinsed with deionized water and allowed to dry at which time they would be scored. In the scoring only the gram negative organisms were counted as this study examined the adherence of *E. coli* which is a gram negative rod.

**Scoring:**

The slides were scored using a light microscope. Fifty cells on each slide were scored by counting the number of gram negative rods adhering to each cell. A mean adherence was

calculated for each THP sample. The data was compared by using a one-way ANOVA to determine if significant changes in adherence had occurred. The $\alpha$ value used was 0.05. Means were then calculated for each of the factors examined.

**Consent:**

Dr. Sobota made arrangements for subject participation and had received approval from the Human Subjects Research Committee.

## III. RESULTS:

### Donor Profiles:

Table 1 shows the profiles of the 59 individuals for which data was collected. One male sample was lost when no results were observed from the initial adherence assay. Only a very small volume of urine was obtained from this donor and the procedure could not be repeated thus excluding this individual from the study. The study group consisted of 29 males and 30 females ranging in age from 18 years to 35 years. The mean age of the group was 20.3 years. A total of 31 participants (52.5%) were 20 years of age or less with the majority of this group being female (58.1%). The remaining 47.5% of participants were 21 or older and a majority of this group consisted of males (57.1%). Only one individual, Donor #50, reported suffering a previous urinary tract infection within 6 months of the study. Prior cranberry juice ingestion or consumption of other cranberry products was reported in 27.1% (16 of 59) of the individuals . Of this group, 62.5% (10 of 16) were female users and 37.5% were male users. A total of 20.7% of males and 33.3% of females reported regular use cranberry products. Use of antibiotics was reported by 23 individuals (38.9%), 56.5% of this population were female and 43.4% male.

### Effects of CJC ingestion on Adherence:

An increase in the antiadherence activity of THP was observed in a majority of samples after the consumption of CJC (Figure 1). The mean bacterial adherence for the study population at 0 days, 2 days and 21 days is presented in Figure 1. It can be observed that 10 of the 59 individuals (17.0%) showed no significant increase in antiadherence activity at either 2 days or 21 days after ingestion of the cocktail. The remaining 83.0% of the donors showed significant anti-adherence activity at either 2 or 21 days or at both 2 and 21 days. Within this group, a significant increase in antiadherence activity was observed only after 21 days in 28.6% of the donors. One individual showed a significant increase in antiadherence activity at 2 days but was non-significant at 21 days. A total of 69.4% of the participants showed a significant increase in antiadherence activity at both 2 and 21 days after using cranberry juice cocktail.

**Comparative response of males and females:**

A comparison of the response of males and females to the cocktail can be seen in Figure 2. Male participants had a much greater percentage of individuals showing a positive response. A total of 28/29 males (96.6%) and 21/30 (70.0%) females showed an increase in the antiadherence activity of the THP after drinking the cocktail. When the different time periods are examined a

positive response was observed for 1 male(3.4%) and 0 females at 2 days only, 5 males (17.3%) and 9 females (30.0%)at 21 days only and 22 males (75.9%) and 21 females at both 2 and 21 days.

**The effects of age:**

The results for the two different age groups examined in this study show similar activity (See figure 3). An increase in antiadherence activity was seen in 80.6% of participants 20 years or younger and in 85.7% of the participants 21 years or older. A positive response was observed at the following time periods: at 2 days only, 32.3% individuals <21 and 14.2% of individuals >21, at 21 days only, 48.3% for those <21 and 67.9% for those >21, and at both 2 and 21 days 48.3% for those <21 and 67.9% for those individuals >21 years (Figure 3).

**CJC use and anti-adherence:**

A total of 11/16 (68.8%) of individuals who had been using CJC prior to the study showed an increase in antiadherence activity and 38/43 (88.4%) of individuals who had not been using the CJC prior to the study showed an increase in antiadherence activity. A positive response was observed at the following time periods: at 2 days only, 1 of the non-users (2.3%) and none of the users, for 21 days only, 11 (25.6%) non-users and 3 (18.8%) users and for both 2 and 21, 26 nonusers (60.5%) and 8

users (50.0%) (Figure 4).

**The effects of antibiotics and CJC:**

Non-antibiotic users showed a greater percentage of individuals, 31/36 (86.1%) responding to CJC ingestion than those using antibiotics, 20/23 (78.2%). In those using antibiotics none of participants showed significant decreases at 2 days only, 39.1% at 21 days only and 39.1% on both days. For those not using antibiotics these percentages are 3.2% at 2 days only, 13.9% at 21 days only and 69.4% on both days (Figure 5).

Table 1. DONOR INFORMATION

| SAMPLE | AGE | SEX | UTI | DATE OF UTI | CJC USE | DATE OF USE | ANTIB. USE |
|---|---|---|---|---|---|---|---|
| 1 | 21 | F | NO | - | YES | 4/21 | YES |
| 2 | 20 | F | NO | - | NO | - | YES |
| 3 | 20 | F | NO | - | YES | 4/18 | NO |
| 4 | 18 | M | NO | - | NO | - | YES |
| 5 | 20 | F | NO | - | NO | - | YES |
| 6 | 19 | F | NO | - | NO | - | YES |
| 7 | 20 | F | NO | - | NO | - | YES |
| 8 | 26 | F | NO | - | YES | 4/23 | YES |
| 9 | 19 | F | NO | - | NO | - | NO |
| 11 | 22 | M | NO | - | NO | - | NO |
| 12 | 24 | F | NO | - | NO | - | YES |
| 13 | 21 | M | NO | - | NO | - | NO |
| 14 | 23 | F | NO | - | NO | - | NO |
| 15 | 21 | M | NO | - | NO | - | YES |
| 16 | 20 | M | NO | - | NO | - | YES |
| 19 | 19 | M | NO | - | YES | 3/4 | YES |
| 21 | 20 | M | NO | - | NO | - | NO |
| 22 | 20 | M | NO | - | YES | 4/11 | NO |
| 23 | 19 | F | NO | - | YES | 4/16 | YES |
| 24 | 19 | M | NO | - | NO | - | NO |

Table 1. (continued)

| SAMPLE | AGE | SEX | UTI | DATE OF UTI | CJC USE | DATE OF USE | ANTIB. USE |
|---|---|---|---|---|---|---|---|
| 25 | 35 | M | NO | - | NO | - | NO |
| 27 | 22 | F | NO | - | YES | 4/21 | NO |
| 28 | 20 | F | NO | - | YES | 4/15 | YES |
| 30 | 20 | M | NO | - | NO | - | NO |
| 31 | 20 | M | NO | - | NO | - | NO |
| 32 | 20 | M | NO | - | NO | - | NO |
| 35 | 23 | M | NO | - | YES | 7/21 | NO |
| 36 | 32 | F | NO | - | NO | - | NO |
| 39 | 23 | F | NO | - | YES | 4/21 | NO |
| 40 | 18 | F | NO | - | NO | - | NO |
| 41 | 18 | F | NO | - | NO | - | NO |
| 43 | 20 | F | NO | - | NO | - | NO |
| 45 | 20 | F | NO | - | NO | - | NO |
| 46 | 21 | M | NO | - | NO | - | YES |
| 47 | 21 | M | NO | - | NO | - | NO |
| 48 | 24 | M | NO | - | NO | - | YES |
| 50 | 23 | F | YES | 1/95 | YES | 3/23 | YES |
| 51 | 22 | F | NO | - | NO | - | YES |
| 52 | 25 | M | NO | - | NO | - | YES |
| 53 | 21 | M | NO | - | NO | - | YES |

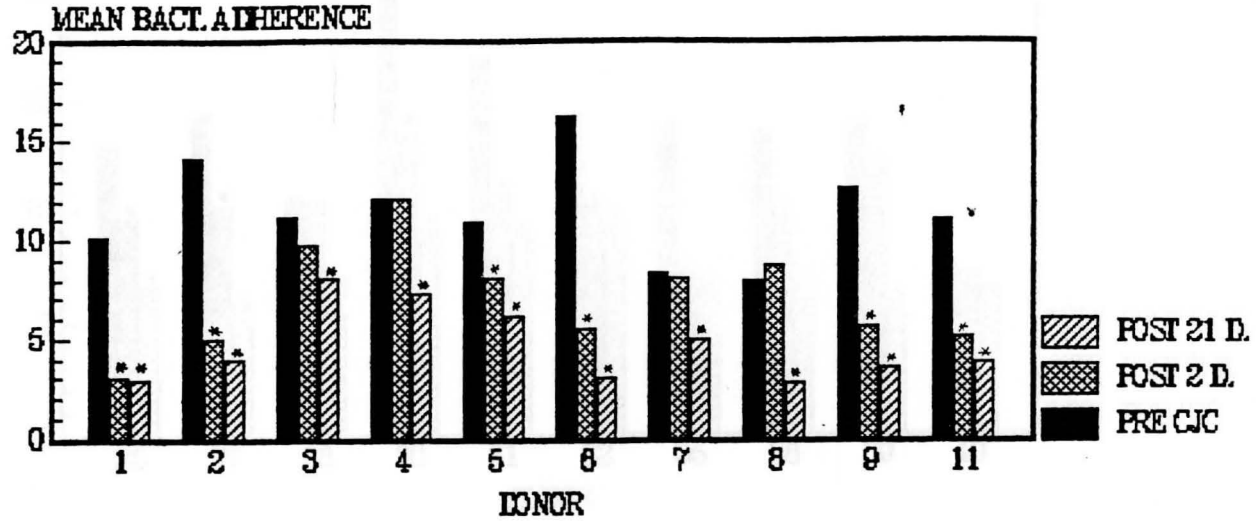
Table 1. (continued)

| SAMPLE | AGE | SEX | UTI | DATE OF UTI | CJC USE | DATE OF USE | ANTIB. USE |
|---|---|---|---|---|---|---|---|
| 54 | 20 | F | NO | - | NO | - | YES |
| 55 | 20 | M | NO | - | NO | - | YES |
| 56 | 21 | F | NO | - | YES | 4/1 | NO |
| 57 | 20 | F | NO | - | NO | - | NO |
| 58 | 19 | F | NO | - | NO | - | NO |
| 60 | 20 | F | NO | - | NO | - | NO |
| 61 | 20 | M | NO | - | NO | - | NO |
| 62 | 21 | M | NO | - | YES | 3/16 | NO |
| 64 | 19 | M | NO | - | NO | - | NO |
| 67 | 21 | M | NO | - | YES | 4/11 | NO |
| 68 | 20 | F | NO | - | NO | - | NO |
| 69 | 19 | F | NO | - | YES | 3/25 | YES |
| 70 | 30 | M | NO | - | NO | - | NO |
| 71 | 25 | M | NO | - | YES | 3/20 | NO |
| 72 | 21 | F | NO | - | NO | - | NO |
| 73 | 20 | M | NO | - | NO | - | YES |
| 74 | 21 | M | NO | - | NO | - | NO |
| 77 | 24 | F | NO | - | NO | - | NO |
| 82 | 21 | M | NO | - | NO | - | NO |

**Figure 1.** Bacterial adherence at pre-CJC, 2 days post-CJC and 21 days post-CJC time periods for all participants in the study. The number assigned to each participant is indicated on the x-axis of each graph. The bars in the graph represent mean bacterial adherence of *Escherischia coli* to 50 uroepithelial cells. The pre-CJC bar represents the control mean for 50 cells prior to ingestion of 4 ounces of CJC. An * identifies means that show a statistically significant decrease compared to control means ($p < 0.05$).

# THE ANTIADHERENCE EFFECTS OF THP FOLLOWING CJC INGESTION

## MEAN BACTERIAL ADHERENCE TO UROEPITHELIA



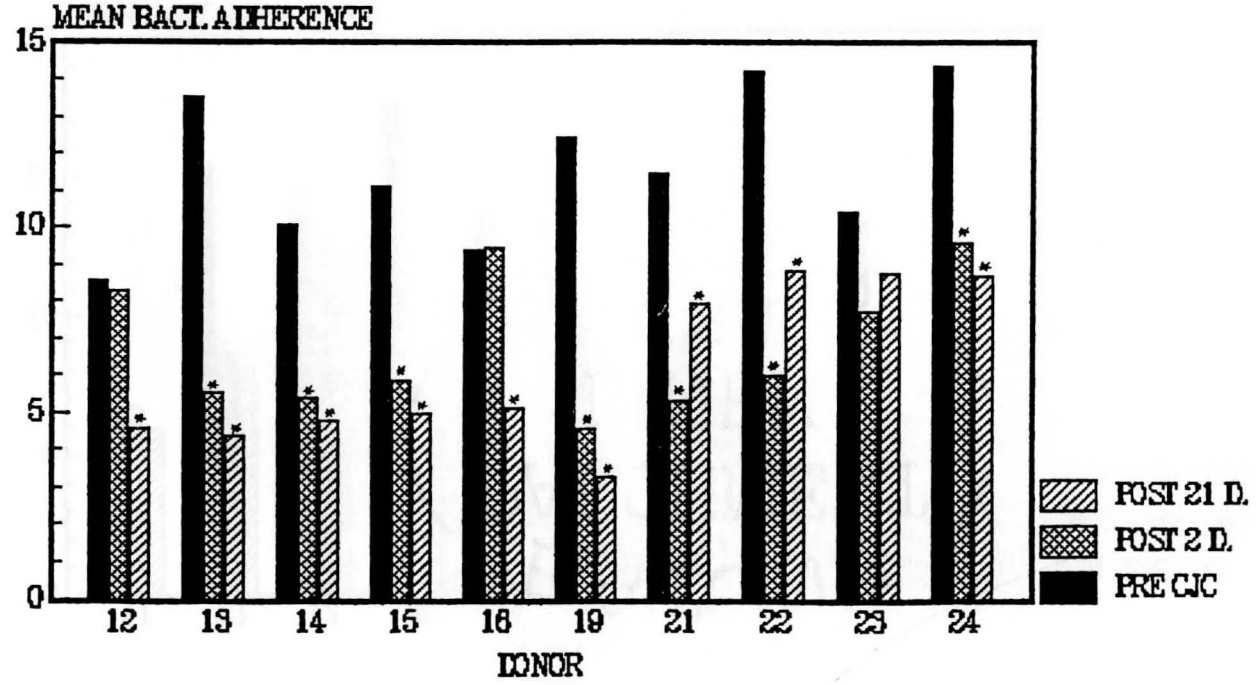
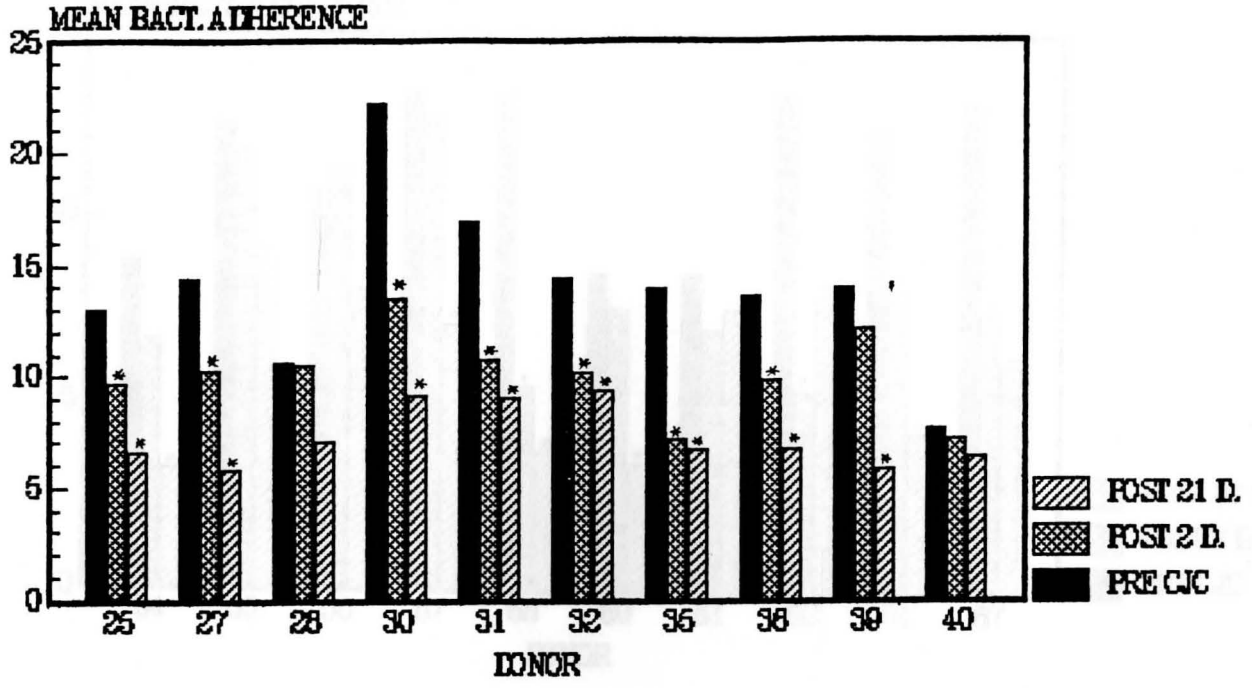



Figure 1 (continued)



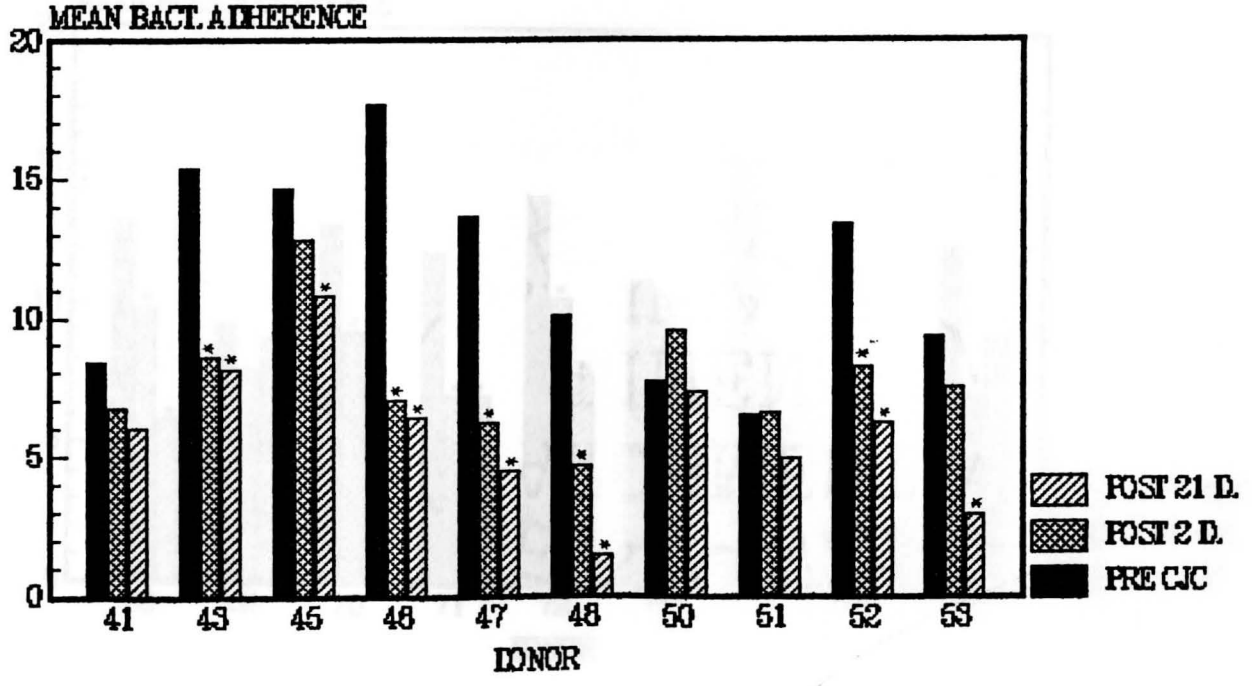
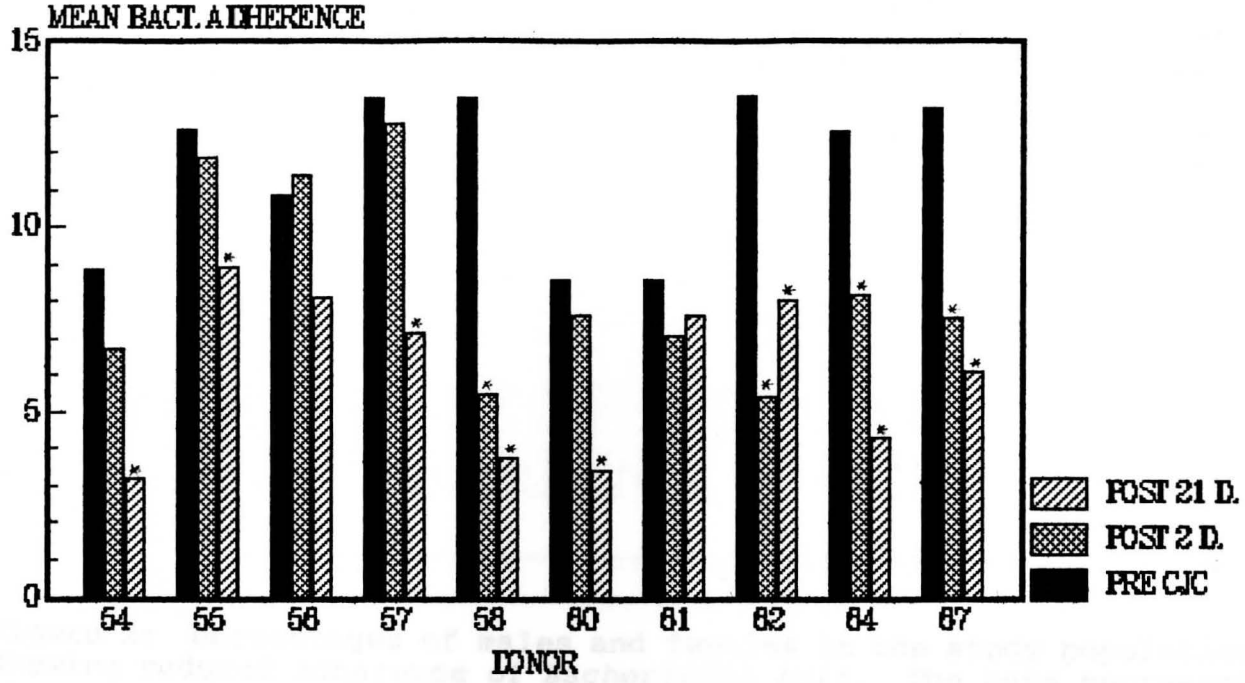
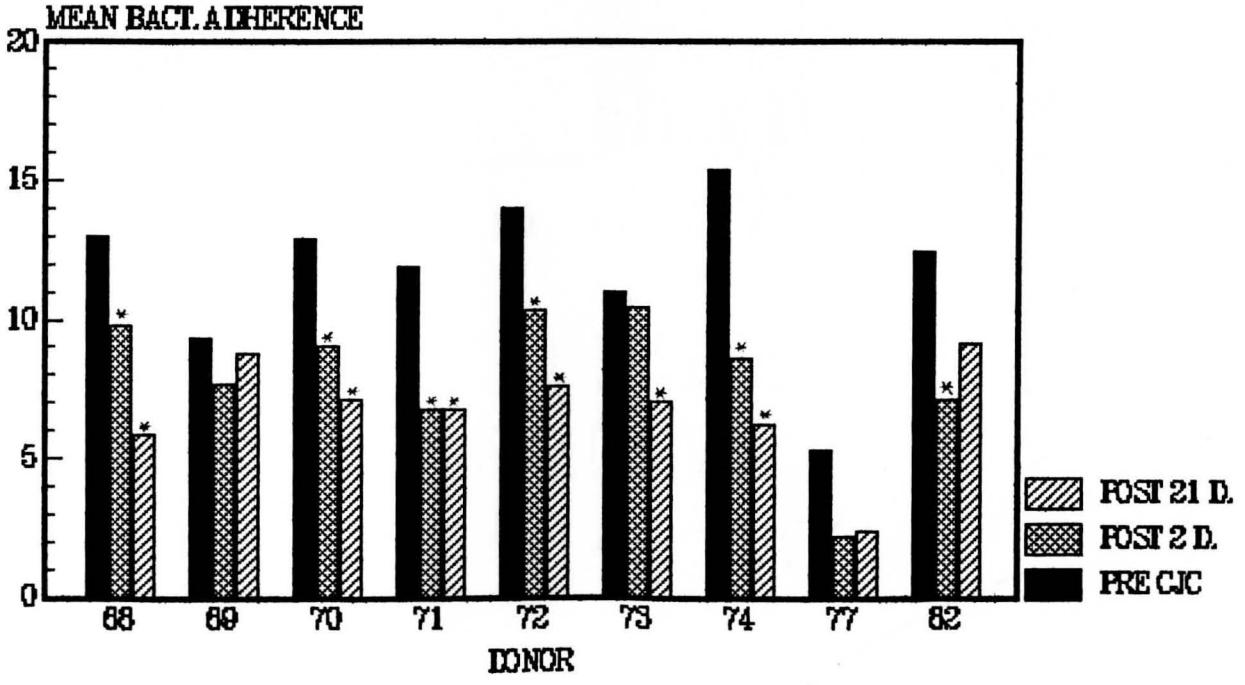



Figure 1 (continued)



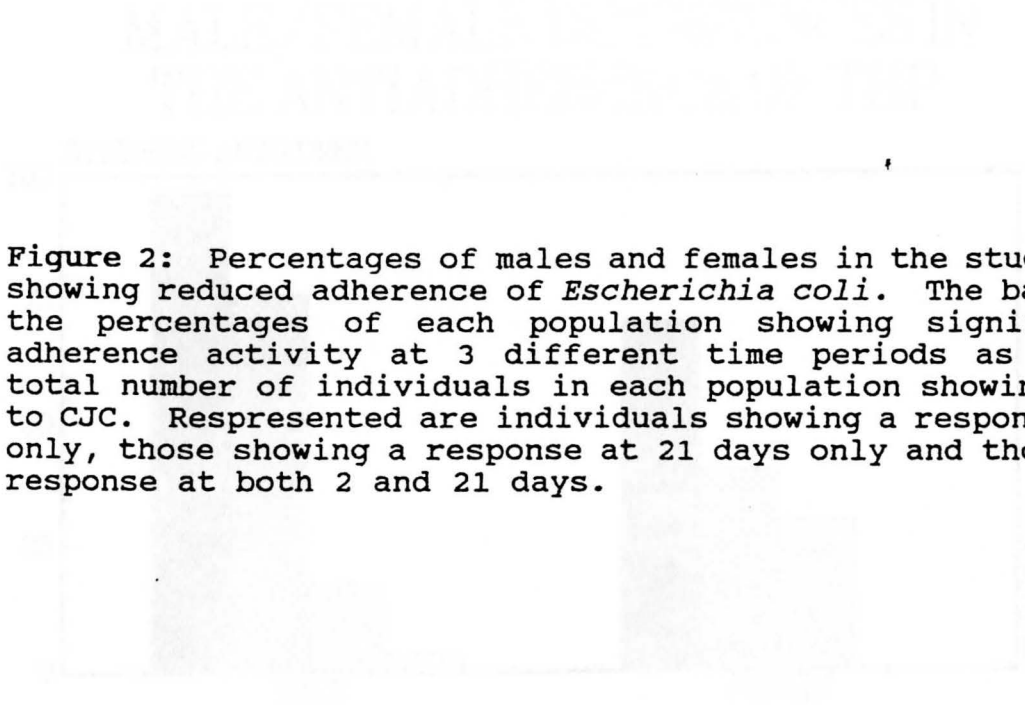


**Figure 2:** Percentages of males and females in the study population showing reduced adherence of *Escherichia coli*. The bars represent the percentages of each population showing significant anti-adherence activity at 3 different time periods as well as the total number of individuals in each population showing a response to CJC. Respresented are individuals showing a response at 2 days only, those showing a response at 21 days only and those showing a response at both 2 and 21 days.

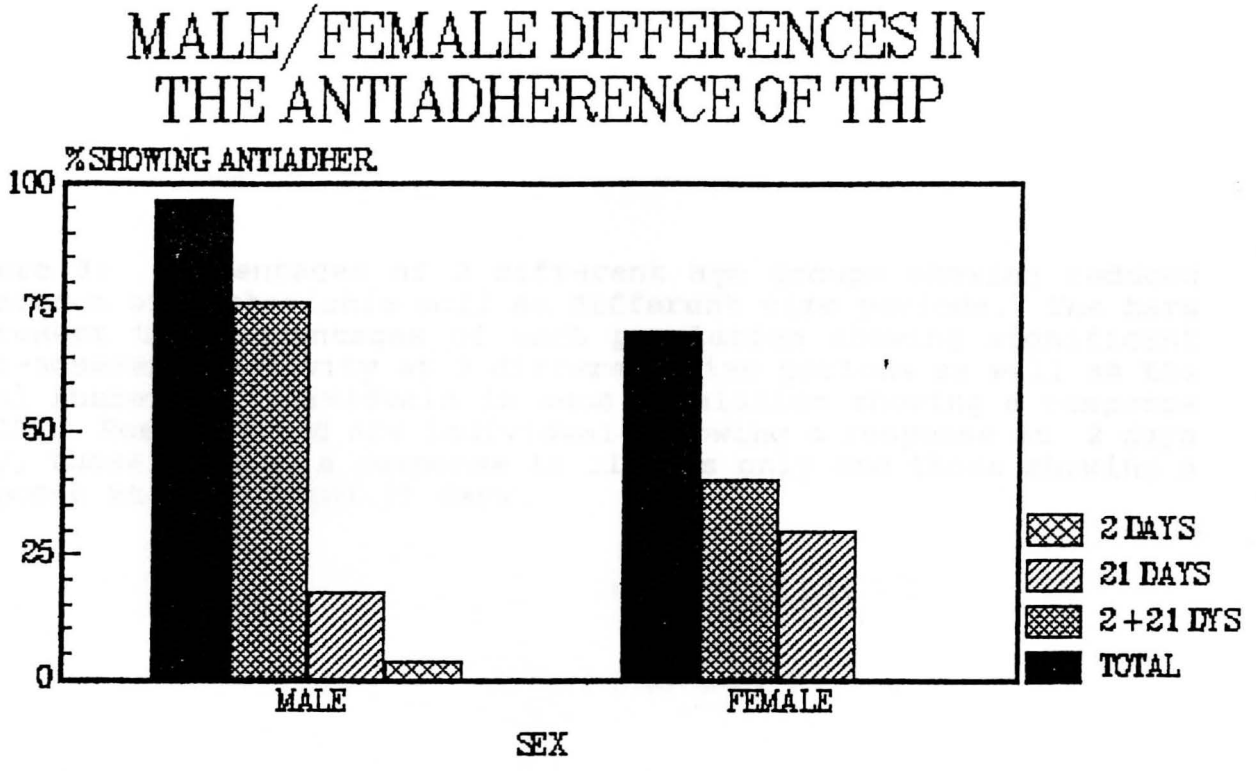

MALE/FEMALE DIFFERENCES IN
THE ANTIADHERENCE OF THP
% SHOWING ANTIADHER.
100
75
50
25
0
MALE
FEMALE
SEX
2 DAYS
21 DAYS
2+21 DYS
TOTAL

**Figure 3:** Percentages of 2 different age groups showing reduced adherence of *Escherichia coli* at different time periods. The bars represent the percentages of each population showing significant anti-adherence activity at 3 different time periods as well as the total number of individuals in each population showing a response to CJC. Respresented are individuals showing a response at 2 days only, those showing a response at 21 days only and those showing a response at both 2 and 21 days.

# AGE DIFFERENCES IN THE ANTI–ADHERENCE ACTIVITY OF THP

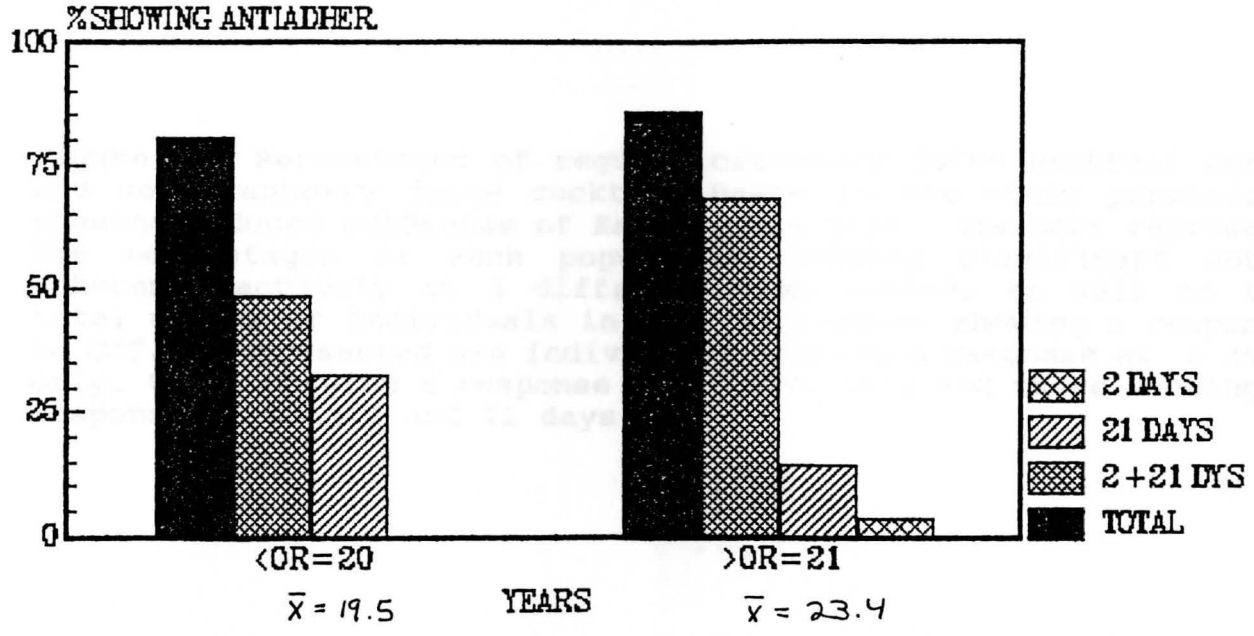


**Figure 4:** Percentages of regular cranberry juice cocktail users and non cranberry juice cocktail users in the study population showing reduced adherence of *Escherichia coli*. The bars represent the percentages of each population showing significant anti-adherence activity at 3 different time periods as well as the total number of individuals in each population showing a response to CJC. Respresented are individuals showing a response at 2 days only, those showing a response at 21 days only and those showing a response at both 2 and 21 days.

# THE EFFECTS OF PREVIOUS CJC USE ON THE ACTIVITY OF THP

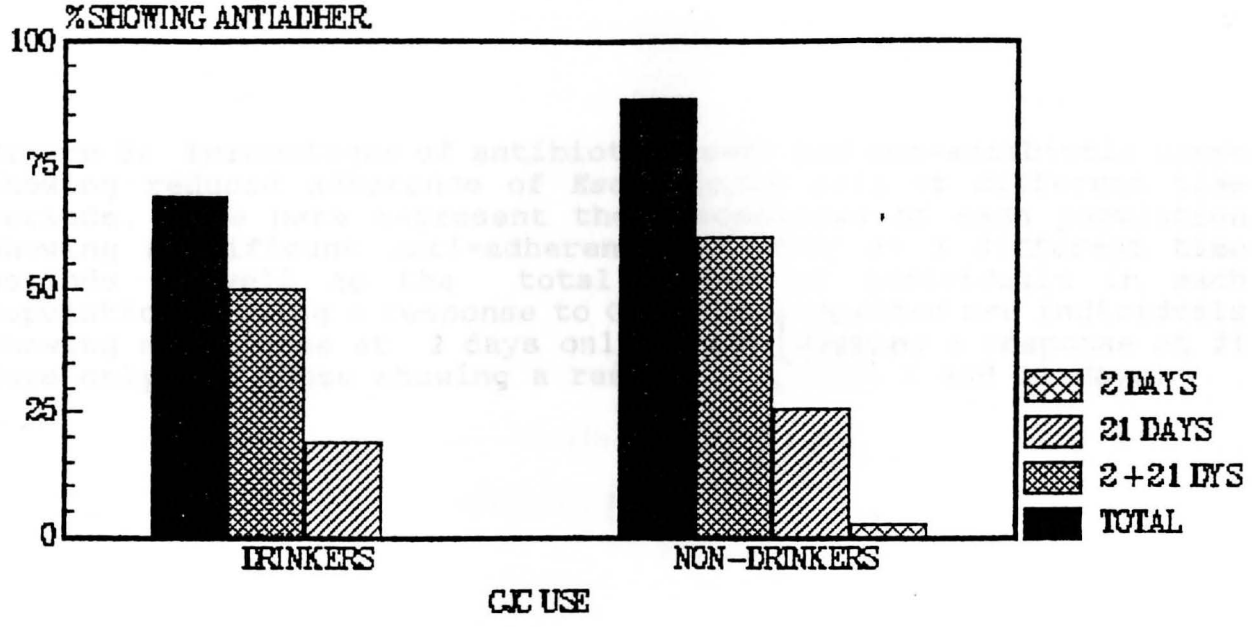
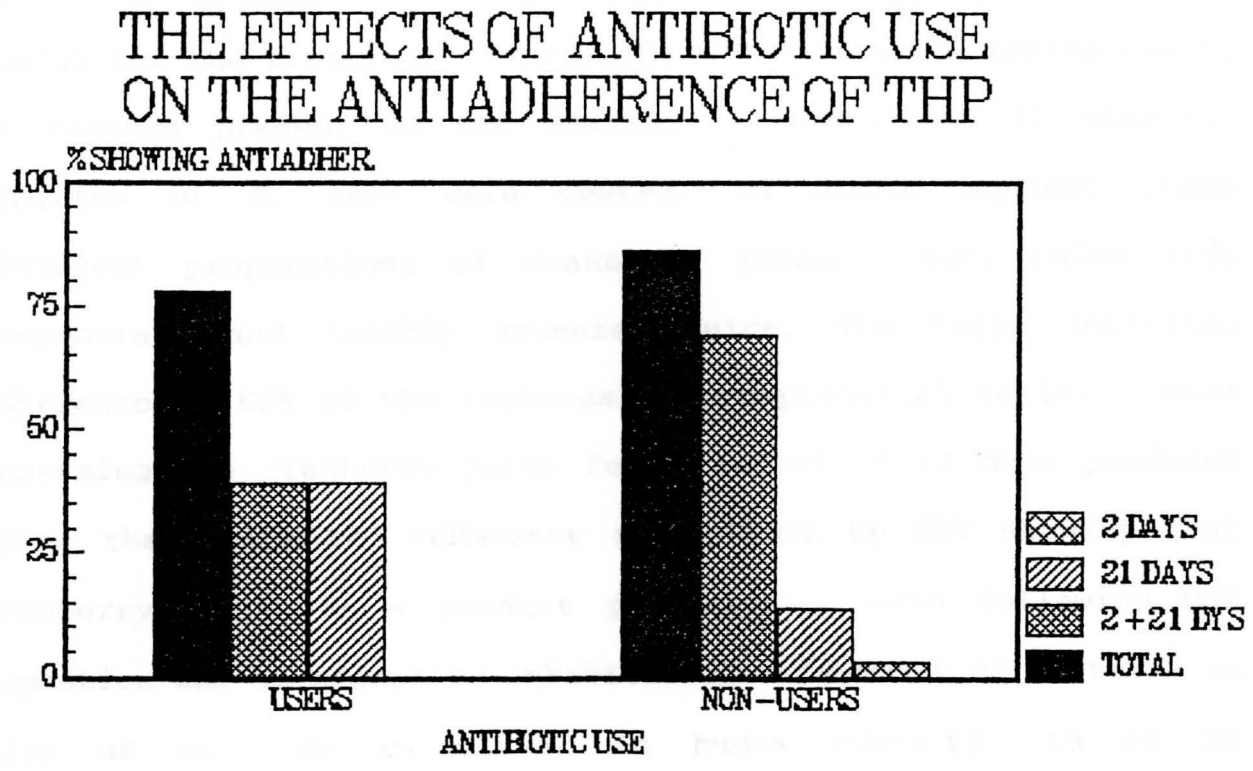


**Figure 5:** Percentages of antibiotic users and non-antibiotic users showing reduced adherence of *Escherichia coli* at different time periods. The bars represent the percentages of each population showing significant anti-adherence activity at 3 different time periods as well as the total number of individuals in each population showing a response to CJC. Respresented are individuals showing a response at 2 days only, those showing a response at 21 days only and those showing a response at both 2 and 21 days.


THE EFFECTS OF ANTIBIOTIC USE
ON THE ANTIADHERENCE OF THP
%SHOWING ANTIADHER.
100
75
50
25
0
USERS
NON-USERS
ANTIBIOTIC USE
2 DAYS
21 DAYS
2+21 DYS
TOTAL

## IV. DISCUSSION

The first in-depth examination of the in vitro and in vivo effects of cranberry juice cocktail was initiated in this laboratory by Dr. Anthony E. Sobota.[55] In his preliminary work, an attempt was made to determine how the cocktail might be useful for the treatment of UTIs and to isolate the active factor or factors present in the cocktail. A total of 77 clinical isolates of *E. coli* were tested, *in vitro,* against three different preparations of cranberry juice: CJC, juice from concentrate and freshly squeezed juice. The juice inhibited adherence of 60% of the isolates to uroepithelial cells. Mice consuming the cranberry juice for a period of 14 days produced urine that inhibited adherence of *E. coli* by 80% showing that cranberry juice or a product produced *in vivo* following CJC ingestion was an effective inhibitor of attachment of bacteria *in vivo* as well as in vitro. In human subjects, 15 of 22 participants showed significant antiadherence activity in the urine within 1 to 3 hours after consuming 15 ounces of CJC showing that the juice was also effective in a human population. The next step was to try and isolate the factor in the juice responsible. Different components of the cocktail were tested for antiadherence activity. Cranberry juice, fructose, vitamin C and the pH of the CJC were tested for ability to produce urine that was inhibitory to bacterial attachment. The results of

these tests showed that the active factor was present in the cocktail was not the vitamin C, the pH of the juice or the fructose added to the cocktail. Finally, inhibition of bacterial attachment was determined to be the mechanism by which CJC decreased bacteriuria. Several observations led to this conclusion. It was observed that pre-incubation of the bacteria with CJC inhibited attachment and washing of bacteria led to a return of normal adherence patterns. Also the addition of CJC to epithelial cells with adhering bacteria led to release of these bacteria.

In 1988, CJC was tested against different urinary and non-urinary bacterial isolates by Schmidt and Sobota.[50] The results of this study showed that CJC was primarily effective against gram-negative rods that are the causative agents of UTI. It was concluded that the antiadherent activity was not specific for a certain bacterial strain but that the activity of the CJC was due to interference with a general adherence mechanism common to the different bacterial isolates. Extended antiadherence activity for 1 to 3 hours after drinking the CJC was hypothesized to be due to a low molecular weight factor (< 1,000) in the juice that may move from the GI tract to the urine. This low weight factor was isolated through dialysis studies. Only a small amount of this factor may be needed to inhibit bacterial attachment.

In 1991, significant progress was made in the search for an active factor in CJC causing antiadherence activity to increase in urine after drinking the juice. Orskov and associates suggested that Tamm-Horsfall protein (THP) acted as a natural defense mechanism preventing the adherence of bacteria to epithelial cells.[35] A potential link between THP and CJC was made when Sobota and Apicella observed a significant decrease in the anti-adherent properties of urine once THP was removed from the urine.[56] *E. coli* expressing type 1 fimbriae showed increased attachment after removal of THP. This suggested that THP was the active anti-adherence factor in urine and that it may be THP that is acted upon by CJC or some component of CJC. This was followed by a forth study which examined the in vitro effect of fructose on the adherence of *E. coli*.[62] Fructose is a component of CJC and was thus tested for anti-adherence activity. Wollet found that fructose was inhibitory in vitro for cells from both normal individuals and individuals with demonstrated chronic urinary tract infections.[62] It was known that fructose was active against bacteria expressing type 1 fimbriae.[63] This fact and the results of Wollet's study suggest that CJC's activity may be mediated, in part by fructose through interference with the type 1 fimbriae of *E. coli*.[62] It was also demonstrated that in addition to fructose, CJC contains another nondialyzable active factor. This was also suggested by Zafriri and associates.[63]

Patrick Wilson continued the work on CJC in this laboratory by initiating a study in which he examined the use of CJC to control UTIs in elderly female patients confined to a nursing home.[61] Six of eight patients showed decreased adherence levels after consuming CJC daily with four of the six showing significant reductions in bacterial attachment. It was also observed that antiadherent activity did not become significant until about four weeks after the initiation of the study. A similar observation was reported by Avorn.[3] Wilson concluded that CJC may be a useful agent to reduce UTIs in high risk population. More recent investigations in this laboratory have centered on examining the anti-adherence activity of THP after CJC ingestion. Susan Dunn continued the work of Patrick Wilson by testing the antiadherence activity of THP extracted from the urine which had been tested by Wilson. Dunn demonstrated that this THP showed significant inhibitory activity against *E. coli* as well as other bacterial isolates.[11] This observation further implicated THP in the in vivo activity of CJC. It was suggested that THP is somehow altered or activated by the presence of some component of CJC resulting in an increased antiadherent activity for this molecule and associated increased protection against urinary infection.

The current study was developed to examine a larger population to determine if, and to what extent, CJC might

increase the natural antiadherence activity of THP in vivo. In addition this study, focused on a number of factors, including sex, age, regular use of CJC and antibiotic use, that may influence the ability of CJC to activate THP. In contrast to previous studies in which CJC was given daily, only a single dose (14 ounces) of CJC was given to each participant. The purpose of this single dose was to observe how long one serving of CJC could maintain the anti-adherence activity of THP.

When examining the entire study population of 59 individuals, the effectiveness of CJC in preventing adherence of *E. coli* is clearly illustrated. In this study, 83% of the entire population showed a significant decrease in adherence over a 21 day period following ingestion of cranberry juice. CJC seems to be an effective anti-adherence agent in this population regardless of donor profile. In the segment of the population showing a positive response, 49 of 59 participants, 69.4% showed a significant increase in anti-adherence activity at both 2 and at 21 days post-CJC ingestion. This shows that CJC activity appeared fairly quickly and had a tendency to last at least until 21 days after ingestion.

Haugen and associates report no differences in the THP excretion rates between males and females.[20] We have been unable to demonstrate any quantitative change in THP after ingestion of CJC in either males or females. Based on this information, and

the the results obtained in this study comparing males to females, it appears that there is a qualitative difference in the THP of males and females. A total of 28 of 29 (96.6%) males tested showed significant decreases in adherence of bacteria after drinking CJC as compared to the female population that had only 21 of 30 or 70.0% of participants showing reduced adherence. Interestingly, 42.9% of the females showing a positive response had no significant decrease in bacterial adherence until 21 days after drinking CJC compared to 17.9% of the males. Thus, males showed a more rapid response and greater number of individuals responding to CJC treatment. It has been suggested that males suffer less UTIs than females due to the longer urethra.[52] The data from this study suggest that activity of THP may also play a role in reducing the incidence of UTI in males. It may be possible that males produce a more active form or have THP that is activated more quickly than females leading to a greater number of responders to CJC treatment. Even though the quantity may be similar, the quality of THP between males and females may differ enough to offer greater protection to males. Therefore, sex of the individual does appear to play a role in the effects of CJC on THP.

Age, at least within the age-group studied, seems to have little influence on the effectiveness of THP after CJC consumption. The data from this study shows little difference in

anti-adherence activity between the group that was 20 years or younger and the group that was 21 or older. The 20 or younger group had 80.6% (25 of 31) of participants with reduced adherence while 85.7% (24 of 28) of the 21 or older group showed the same reduction. The time periods in which the increase in antiadherence activity appeared are also similar between the two groups. Even though the elderly are a high risk group for UTI[58], the age range in this study, from 18 to 35, seems to show little effect on THP activity after use of CJC.

A difference in antiadherence activity was seen when comparing regular CJC drinkers to those not normally consuming CJC. Only 11 of 16 (68.8%) of regular CJC drinkers showed decreased adherence during the study compared to 38 of 43 (88.4%) of the non-CJC drinkers. A total of 31.2% of regular CJC users showed little decrease in adherence within 21 days of drinking CJC. This suggests that the anti-adherence mechanism acted upon by CJC may be saturated in regular CJC drinkers as compared to non-CJC drinkers making subsequent treatment with the juice relatively less effective. This possible saturation was also suggested by Wollet.[62]

The effects of antibiotic use on CJC treatment was also examined. Avorn and associates mention a lack of information regarding the combination of antibiotics and CJC to treat UTI.[3] The results of this study show that antibiotic users show a

lesser response to CJC treatment than non-antibiotic users. A total of 31 of 36 (86.1%) of non-antibiotic users showed a positive response to CJC ingestion. The antibiotic users had only 18 of 23 (78.2%) of individuals responding to CJC. Antibiotic use appears to interfere with the activity of CJC. Antiadherence activity was seen at 21 days only for 50.0% of the antibiotic users showing a response to CJC. This shows this group to be slower responders than the non-antibiotic using group who had 80.6% of CJC responders showing decreases at both 2 days post-ingestion and 21 post-ingestion. It is possible that the group using antibiotics responded less to CJC due to lower initial infection rates than the non-antibiotic group. Other research has reported the presence of residual bacteria that remain after CJC treatment.[50] It may be possible that antibiotics also leave a residual population of bacteria that are less likely to respond to CJC or other forms of treatment than populations of bacteria found in non-antibiotic users. As a result, antibiotic use does seem to be a factor influencing the effectiveness of CJC in treating UTI.

Finally, although the studies performed by Dunn and Entler in this laboratory differ in format from the current study some comparisons can be made. Dunn and Entler examined the effect of CJC on the antiadherence activity of THP on a cohort of elderly female nursing home patients, with diagnosed chronic urinary

tract infections, who were asked to drink the juice daily for a period of 8 weeks. In the present study a younger population (18 to 35 years) was requested to drink the cocktail one time and their THP was analyzed for antiadherence activity at 2 and 21 days. In the first group, it took on average 4 weeks to respond to continuous use of the cocktail. In the second group, a majority of individuals showed a positive response within 2 days after drinking the cocktail. Thus it would appear age and/or chronic infection would tend to decrease the effectiveness of the cocktail. And it may follow that the increased tendency for urinary tract infections in older patients, and in particular in female patients, may be related to a decrease in the responsiveness and activity of the THP.

To summarize the results of this study, the effects of CJC on THP seem to be influenced by the sex of the individual, previous CJC consumption and antibiotic use. Age does not seem to play a critical role in a population ranging from age 18 to age 35. It can also be seen from the data presented that most of the study population did respond favorably to CJC treatment. This shows CJC to be an effective alternative to antibiotics for the prevention and possible treatment of UTI and is in agreement with the other research conducted in this and other laboratories. Regardless of profile, most individuals responded well to CJC, which lacks the potentially harmful side effects associated with

the use of antibiotics. It can also be seen from this data that there is large variability in the type of response seen in individuals. Viewing the data for each individual participant in the study suggests three possible subgroups in the study population: 1) those who respond well, 2) those responding moderately, and 3) those who do not respond. By examining this data it may be possible to predict which individuals would respond well to CJC treatment and those who would reap no benefit from this type of treatment. For example, the 21 day procedure used in this study could be conducted on an individual and a profile seen for response to a single dose of CJC. If the individual's profile shows them to be an individual who responds well to the cranberry juice, this person is a good candidate for treatment of UTI with CJC. Thus the protocol followed in this study may provide a 21 day test to determine the effectiveness of CJC as a treatment alternative and whether or not an individual should continue using CJC.

One additional question is posed by this study, how one dose of CJC could show effects on THP up to 21 days after ingestion. At this time, there is no clear explanation for this observation. THP has been reported to have a half-life with a minimum of 3 hours and a maximum of 168 hours. This means that any CJC-activated THP should be cleared from the urinary tract long before the 21 day period. One possible explanation for the

prolonged effect may be a retaining of the CJC factor responsible for activating THP at the production site of THP in the loop of Henle. If this CJC factor can be stabilized and remain in the loop of Henle, it may be possible that the newly synthesized THP is activated immediately following production. This would explain how THP could be cleared from the urinary tract and more active THP would be produced up to 21 days.

The use of CJC as a treatment for UTI was proposed by Sobota in 1984. Since then, additional evidence has accumulated suggesting that this treatment is a viable alternative for the treatment of urinary infections in a variety of different populations. Although the mechanism of CJCs activity has still not been elucidated, CJC is well accepted by patients and lacks serious side effects making this a very good alternative to antibiotics. Isolation of this mechanism will provide valuable information regarding the body's natural defense mechanisms as well as important targets for therapeutic development.

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Appendix

93-2 70

YSU

Youngstown State University / Youngstown, Ohio 44555-3601
Department of Biological Sciences
(216) 742-3601

TO: Dean Gordon Mapley

FROM: Tony Sobota

RE: Response to HSRC

DATE: April 20, 1993

This is in response to three issues of concern regarding the proposal, "Potential Use of Cranberry Juice Cocktail to Treat Urinary Track Infections." If a pateint is no longer catheterized, he/she will be removed from the study. The next of kin have been asked to sign the release form for those patients who are not competent to sign. The patients will be limited to 6 oz of cranberry juice cocktail daily, however, no other limits will be placed on the diet.

93-271

YSU

Youngstown State University / Youngstown, Ohio 44555-3409
Office of the Dean
College of Arts and Sciences
(216) 742-3409

TO: Tony Sobota

FROM: Gordon Mapley Gordon

SUBJECT: *HSRC Response to Research Proposal*

DATE: March 17, 1993

The Human Subjects Research Committee has recommended the endorsement of your proposal entitled "Potential Use of Cranberry Juice Cocktail to Treat Urinary Tract Infections". In addition to the proofreaders comments (see attachment), there was concern about three issues, two of which we previously discussed. For the official file, would you please comment on the following: (1) should a patient cease to be catheterized, will he/she be dropped from the study or will an alternate method of urine collection be implemented? (2) are all patients competent to sign a release form, and if this is not the case, who provides the release -- next of kin? (3) will the patients be limited to 6 oz. of cranberry juice daily with no other juices, to at least 6 oz. with additional cranberry juice possible, or to 6 oz. of cranberry juice with unlimited additional juices? We assume that this last issue was discussed with the patients or with their families.

Thank you for your cooperation.

I assume we are not proofreaders of these proposals so should not make note of the choice of the word "resistance" (Perhaps intending "resistant"?) as a modifier for "populations" at item four (4) on page 2 of the Review Form, or the use of the word "and" instead of "an" in the first line of the "Description of Proposal" even though this will apparently accompany the printed "Informed Consent Form" which also contains a mechanical error in the word "outweight" instead of "outweigh." Nevertheless, I feel that anything bearing our University name should be as good as we can make it. I would hope that my own mechanical mistakes would be picked up by friendly eyes also.

93-2

YSU

Youngstown State University / Youngstown, Ohio 44555-3091
Dean of Graduate Studies
(216) 742-3091

March 29, 1993

Dr. Anthony Sobota
Department of Biological Sciences
UNIVERSITY

Dear Dr. Sobota:

I am pleased to notify you that based upon the recommendation of the Human Subjects Research Committee your project, "Potential Use of Cranberry Juice Cocktail to Treat Urinary Tract Infections" (Human Subjects Research Committee file #93-2), has been approved.

Sincerely,

Barbara Brothers

Barbara Brothers
Acting Dean of Graduate Studies

BB:ksg

cc: Dr. Mapley, Chair, HSRC ✓

DISPOSITION REPORT FORM

Youngstown State University Human Subjects Research Committee

To: Dean of Graduate Studies

From: Human Subjects Research Committee

Project Title "Potential Use of Cranberry Juice Cocktail to Treat Urinary Tract Infections"

Principal Investigator(s) Anthony E. Sobota HSRC File Number 93-2

Duration of Research 3 months

Source of Funding None

On March 17, 1993, the Human Subjects Research Committee voted to make the following recommendation to you regarding the above-named research proposal:

X Approval

____ Approval with the restrictions attached hereto

____ Disapproval for the reasons attached hereto

Projected dated of annual review _____

The Committee was composed of the following people:

1. Dr. Gordon Mapley
2. Dr. Steve Ellyson
3. Mr. James Granito
4. Dr. George Letchworth
5. Dr. D. James Reagan
6. _____
7. _____
8. _____
9. _____

Dr. Gordon E. Mapley

Chairman, HSRC

Youngstown State University
Human Subjects Research Committee

## REVIEW FORM

☑ Initial ☐ Modification

Date Submitted 1/26/93 File Number 93-2 (entered by HSRC)

Proposal Title Potential Use of Cranberry Juice Cocktail to Treat Urinary Tract Infections

Prinicipal Investigator(s) Anthony E. Sobota

Department(s) Biology

Anticipated Funding Source None

Projected Duration of Research 3 mos. Projected Starting Date 2/18/93

Other organizations and/or agencies, if any, involved in study
Western Reserve Care Centers and Beeghly Oaks of the Western Reserve Care System

## AGREEMENT

A. I (We) hereby state that I (we) will follow and conform to the University policy and to the regulations and procedures established by the Youngstown State University Human Subjects Research Committee and published in the "Human Subject Research: Regulations and Procedures" handbook.

B. I (We) also agree to allow the Human Subjects Research Committee or its delegate(s) access to any pertinent records of research connected with this project, and further to supply the Committee with documentation of both (my) (our) selection procedures and informed consent procedures.

C. I (We) further agree to inform the Committee promptly, through the Office of Graduate Studies, of any changes I (we) wish to make that would involve human subjects, and to supply the Committee with such progress reports as it may require.

1. **Description of Proposal** (Nature of study in abstract form)

See attached sheet.

2. **Subject(s) Risks** (see DHHS guidelines)

Cranberry juice cocktail is classified as a food material. There is no published information indicating that there is any risk in ingesting six ounces of cranberry juice cocktail.

3. **Safeguards**

Not applicable

4. **Potential Benefits of Research to Subjects**

This treatment may result in the control and/or prevention of chronic urinary tract infections without the longterm use of antibiotics which can result in toxicity, side effects, and the emergence of resistance populations.

5. **Subject Selection Process**

Patients will be selected based on past history. Those patients with a history of urinary tract infections will be eligible for participation in the program. Beeghly Oaks nursing home has already agreed to supply patients for the study. Final selection of the patients will be made in consultation with Eugene F. Tareshawty M.D. and Kevin Sheets M.D.

Signature

Signature

Description of Proposal:

In 1984 we published and article in the Journal of Urology which demonstrated that cranberry juice cocktail actively inhibits the attachment of urinary pathogens to uroepithelial cells. In subsequent articles we demonstrated that the active factor in the cocktail can be demonstrated in the urine after ingestion of the cocktail. We have a manuscript in review that demonstrates that the addition of the cocktail to urine from patients with chronic urinary tract infections significantly inhibits the attachment of bacteria to urinary cells. We have thus suggested that the ingestion of the cocktail, or some factor in the cocktail, may be beneficial in the treatment of chronic urinary tract infections. We propose to undertake a limited in vivo pilot study on geriatric patients with chronic urinary tract infections. The patients would be asked to ingest six ounces of the cocktail each day and their urine would be collected and monitored for pathogens and analyzed for antiadherence activity.

This proposal has been presented by Dr. Sheets to all appropriate committees at Western Reserve Care Systems and has been accepted.

Youngstown State University
Human Subjects Research Committee

# DESCRIPTION OF STUDY

From Anthony E. Sobota, Ph.D.
Investigator

To _____
Subject

Your voluntary participation as a research subject in the study described below is greatly appreciated. The investigation will be conducted from _____ to _____.
date date

**Please read carefully the information below and sign this form where indicated.**

Purpose of Study:

To determine if cranberry juice cocktail may be useful in the treatment of chronic urinary tract infections.

Procedures to be Used:

Patients will ingest six ounces of cranberry juice cocktail each day and urine will be collected for analysis.

Your Responsibilities are:

Drink six ounces of cranberry juice cocktail each day.

Possible Risks Inherent in the Study are:

None documented

Alternative Procedures:

None

Potential Benefits Resulting from this Study:

Better treatment for chronic urinary tract infections

_____
Investigator