The Indirect Effect of Cranberry Juice Cocktail on Tamm-Horsfall Protein in Protection Against Urinary Tract Infection in an Elderly Population

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ABSTRACT

In vivo assays have demonstrated that cranberry juice cocktail (CJC) ingestion confers an increased anti-bacterial adherence activity to the urine. [48] This study addresses CJC's effect on the anti-adherence properties of Tamm-Horsfall Protein (THP). Four elderly females ingested 4oz. of CJC daily for 84 days. THP was then extracted from their urine samples. The THP was then used as the reaction medium for an adherence assay of bacteria to healthy donor uroepithelia cells. The patient THP was first challenged with the original infective isolate causing the urinary tract infection (UTI). Then *E. coli* was used as the control bacteria to challenge the patient THP. Mean bacteria adherent to each cell assayed in the presence of post-CJC THP was compared to the mean bacterial adherence of the pre-CJC THP control.

Significantly less bacterial adherence was found after CJC ingestion in 3 of the four patients when the THP was challenged with the original infective isolate. Average reductions in bacterial adherence were: 69%, 22% and 66% less bacteria after CJC ingestion. When patient THP was challenged with *E. coli*, all four patients showed decreased bacterial adherence after CJC treatment compared to before treatment. Average reductions in bacterial adherence were: 8%, 69%, 20% and 34% less bacteria per uroepithelial cell. Based upon these results, it was concluded that CJC acts upon THP and increases the anti-adherence activity of the THP and thus decreases the likelihood of UTI.

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DEDICATION:

This work is dedicated to my parents, Raymond and Nancy Dunn. They have always encouraged me to reach for the highest goals and have helped me to achieve them. They taught me to value education and without their support this work would not have been possible. Most of all, I thank my parents for the love and freedom that they gave me to follow my dreams wherever they led me.

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I. INTRODUCTION

Urinary Tract Infection:

Urinary tract infections represent one of the most common health problems encountered in the general population. Increased incidence of UTIs may be due to factors such as drug resistant pathogens, nosocomial infections and increased human longevity.[45] A diagnosis of urinary tract infection is made when 100,000 or more bacterial/mL can be cultured from a freshvoid midstream urine sample. Normally, the urinary tract is sterile, although the urine is an ideal medium for the growth of bacteria. The pH of normal urine ranges from 5.6 to 6.2. Alkaline urine is more suitable for bacterial growth, while urine with a pH of less than 5.5 has some bacteriostatic properties.[45] Urinary tract infections may be divided into three major clinical categories. Acute pyelonephritis is a type of upper UTI in which the kidney is infected. Acute cystitis is a symptomatic lower UTI often involving the bladder and the urethra. Asymptomatic bacteriuria occurs when there are significant numbers of bacteria present in the urine, but no clinical symptoms are present.[19]

Symptoms of UTI:

Symptoms associated with UTIs include urgency, burning sensation, painful urination, and frequency of urination. Presence of symptoms alone is not enough to make a diagnosis of UTI because only one half of patients reporting symptoms have significant

bacteriuria.[17] It is also common to find bacteriuria without the presence of any symptoms. This is known as an asymptomatic infection. In adults, the prevalence of asymptomatic UTI is six to seven times higher in women than in men. Approximately, five percent of women have an asymptomatic infection at any given time.[17]

Four major risk groups exist for UTI: elderly, males with prostatic obstruction, sexually active females, and school age females.[51] Based upon clinical observations, more than 90% of UTIs are present in the lower urinary tract. The lower urinary tract consists of the urethra and bladder. Lower UTIs are often associated with cystitis. Cystitis is the twenty seventh most frequently made diagnosis in family practice offices.[17] An ascending infection occurs when the bacteria migrate from the lower urinary tract into the upper urinary tract.[51] Upper UTIs present in the higher structures of the urinary tract usually infect the kidney. Kidney infections are associated with pyelonephritis and are often difficult to cure.[45]

Means of Transmission:

There appear to be several methods by which bacteria can be introduced into the urinary tract. In women, the bacterially colonized vagina has been implicated in the contamination of the urinary tract. The vaginal mucosa and urethra can become colonized with fecal bacteria.[45] It has been reported that women with vaginal colonization of *E. coli* tend to have more frequent UTIs than their counterparts.[51] It is also common for enteric species of bacteria

such as *E. coli* to colonize the urinary tract directly. This suggests that a significant source of bacterial contamination of the urinary tract is the bowel. Thus, the most common sources of uropathogens are the vagina and bowel.[17] The introital area and large intestine act as the major reservoirs for uropathogens.[54]

Several other risk factors exist which may predispose a patient to UTIs. Over distension of the bladder due to obstruction or avoidance of voiding has been associated with increased rates of infection. It is thought that decreased vascular circulation to the bladder wall occurs when the bladder is overfilled. This increases the bladder's susceptibility to enteric organisms.[17] Another risk factor which appears to increase the likelihood of infection is sexual activity. "Honeymoon cystitis" refers to the transient increases in bacteriuria following intercourse. Although the precise mechanism has not been determined, it is thought that urethral trauma during intercourse contributes to infection.[17] There is also evidence that suggests that the use of spermicide and diaphragm increases likelihood of UTI.[51] Host susceptibility factors also undoubtedly play a role in the initiation of a UTI. Urinary tract abnormalities such as obstructions, may cause the retention of urine and promote bacterial growth. Pregnancy in women, prostatic enlargement in men and bladder prolapse in both are predisposing factors to infection.[51] Overall, the risk of acquiring a UTI depends on: chances of encountering a virulent strain, dose, body's response to the organism, host behaviors that either increase or decrease chance of tissue invasion and innate

susceptibility to the infecting organism.[16]

Recurrent UTIs:

There exists a great deal of controversy involving why some individuals tend to develop recurrent UTIs while others do not. More than 95% of recurrent UTIs are due to reinfections from sources outside of the urinary tract.[45] Recurrent infections are often refractory to treatment. Many times, the pathogen causing the recurrent infection is different than the original one.[45] After obvious factors such as obstructions in the urinary tract have been ruled out, other considerations must be taken into account. There are two theories that attempt to explain recurrent UTIs. The host susceptibility theory states that certain hosts have special factors which make them more prone to UTIs. The random colonization theory states that changes in host resistance occur after the first infection.[54] The density and nature of receptors present on uroepithelial cells may account for *E. coli*'s ability to bind more efficiently to cells from a recurrent UTI patient than to cells from a subject without UTI.[19] The successful adherence and infectivity of an organism depends upon the properties of the host, the microorganism and the medium in which the reaction takes place. Concentrations of certain urinary factors may affect bacterial adhesion. High levels of urea and creatinine favor adhesion but high levels of potassium and immunoglobins and a low pH reduces bacterial adhesion.[18] Thus, there may be one or more factors that

increase the susceptibility of host epithelial cells to infection.[51]

Infective Agents:

The infective agents causing UTIs can vary. *E. coli* accounts for 80% to 90% of out patient UTIs. The remaining UTIs are often caused by *Proteus, Klebsiella, Enterococci, Staphlococci* and *Pseudomonas* species.[17] Eight percent of UTIs are caused by multiple organisms. Although *E. coli* is the most common uropathogen, *Pseudomonas* tends to be the most virulent one. *Pseudomonas* produces several highly toxic substances as part of the infectious process.[45] Virulence factors include: ability to attach to uroepithelial cells, production of an endotoxin, and the presence of a polysaccharide capsule.[19] Bacteria that can split urea and transform it into ammonia make the urine more alkaline and therefore promote the growth of bacteria. Examples of urea splitting bacteria are: *Proteus mirabilis, Klebsiella pneumoniae,* and *Pseudomonas* species.[45] Virulence factors appear to be more common in bacteria infecting the upper urinary tract as opposed to those bacteria infecting the lower urinary tract.[51] Three main classes of virulence factors have been found in *E. coli* causing UTI: adhesins, siderophores and toxins. Other factors, including invasiveness, the presence of a capsule and lipopolysaccharides also may be important.[16]

Treatment:

Treatment can take several different forms. Behavior

modification therapy can include: frequent voiding, post-coital voiding, adequate hydration and avoidance of stool contamination of the vagina.[17] Asymptomatic UTIs are usually not treated. First, because of the lack of symptoms, diagnosis often is not made. Second, treatment with antibiotics can result in the occurrence of resistant strains. Finally, no significant benefits have been proven to accompany antibiotic treatment of asymptomatic UTI.[17] Treatment of females with a radiologically normal urinary tract was found to be unnecessary and sometimes harmful.[19] In a symptomatic UTI, however, antibiotic drug therapy is useful. It is generally not possible to achieve a complete cure with antimicrobial drugs, but the infection can be suppressed, modified, delayed, altered or controlled to some extent.[45] The antimicrobial drugs most often used to treat symptomatic UTI are amoxicillin and trimethoprim-sulfamethoxazole. Some controversy exists concerning the effectiveness of single dose treatment versus short term treatment. Recent evidence suggests that two weeks of treatment is often superior to single dosage.[51]

UTI Virulence Factors:

The uroepithelial cells of the patient and the virulence factors present on the bacteria have both been cited as contributors to UTI. Bruce and associates performed a study to examine the effects of these factors separately. Scanning and transmission electron microscopy and *in vitro* assays were used to examine the adherence of Gram-negative bacteria to human

uroepithelial cells. The bacteria with extracellular adherence structures, such as fimbria, demonstrated a greater adherence activity than bacteria without adherence structures. Uroepithelial cells isolated from patients with recurrent UTIs had greater numbers of adherent bacteria than control cells. Control cells consisted of bladder tumor cell line (T-24) and cells from donors with no history of recurrent UTI. The increases in adherence were significant with 2 to 5 times more bacteria on recurrent UTI cells compared to controls. This evidence lends further support to the hypothesis that bacterial adherence is important for the development of UTI, and that both the extracellular adherence structures of bacteria and properties of uroepithelia are important.[10]

Although most studies have concentrated on fimbriae as the major virulence factor in *E. coli*, Mostavi and associates have recently suggested an interesting alternative. They suggested that *E. coli* produces a soluble virulence factor, a quaternary amine similar to protamine, that promotes bacterial adherence to the bladder mucosa. They used an *in vivo* bacterial infection assay in which two different substances were used. First, the supernatant from human urine in which *E. coli* was grown was injected into the experimental rabbit bladders. Secondly, human urine was injected into the control bladders. They found a significantly higher bacterial count in bladders pretreated with *E. coli* supernatant. The molecular weight of this factor is less than 3.5 kilodaltons and is cationic and therefore capable of adhering to the anionic

bladder mucus which contains glycosaminoglycans. Therefore, exposure of this mucus to a quaternary amine causes the inactivation of the mucus and increases the bacterial adherence in the bladder.[28]

***P. mirabilis* and *E. coli* Virulence Factors:**

In another study, the attachment differences between *Proteus mirabilis* and *Escherichia coli* to human uroepithelial cells *in vitro* is addressed. *P. mirabilis* is commonly found infecting young boys , elderly , catheterized patients or patients who have suffered repeated infections due to obstructions in the urinary tract. *E. coli,* on the other hand, causes most first time infections in an ambulatory population. There was no consistent relationship between the adhesive capacity of *P. mirabilis* and its clinical origin. Isolates from blood, stool and urine showed approximately the same high, adherence capabilities. This is different from *E. coli* in which symptomatic UTI isolates adhere more than stool isolates. *P. mirabilis* attached only to squamous cells, however *E. coli* attaches to both squamous and transitional epithelial cells. These differences in adherence characteristics may relate to the differences in clinical UTIs caused by these organisms.[53]

***P. mirabilis* Virulence Factors:**

P. mirabilis has several factors including a urease, fimbriae, flagellum mediated motility and hemolysin which may contribute to

its virulence. Urease hydrolyses urea and produces ammonia which acts to alkalinize the urine. Heavy fimbriation improves *P. mirabilis'* ability to adhere to renal epithelial cells. Two fimbrial types have been described for *P. mirabilis*. Mannose-resistant /Proteus-like (MR/P) fimbriae are Brinton's type IV, while mannose resistant/Klebsiella -like (MR/K) fimbriae are type III. Type III are associated with the development of acute pyelonephritis.[4] Some strains of *P. mirabilis* produce a protease capable of cleaving IgA outside of the hinge region. This protease has been shown to be active *in vivo* in UTIs.[46]

E. coli Virulence Factors:

Adherence to epithelial cells appears to be a prerequisite for the development of most UTIs. *E. coli* virulence factors include hemolysin production, iron sequestration, serum bactericidal resistance, presence of O:K:H antigens and presence of type 1 and\or type P fimbriae. The virulence of urinary isolates of *E. coli* often depends on adhesins or specific cell surface structures that mediate the adherence of bacteria to epithelial cells. Fimbriae, in particular, are thought to be important in this binding process.[21]

Fimbriae:

Based upon characteristics such as antigenic type, structure and receptor specificity, fimbriae have been classified into several different groups. Type 1, or mannose sensitive (MS) fimbriae bind to mannose-like receptors present on the surface of

epithelial cells. S fimbriae bind to sialogalactosides[21] and P fimbriae bind to α -D-Galp(1-4)- ß-Galp residues of globohexosylceramides of the P blood group antigen on uroepithelial cells.[1] Type 1 fimbriae are associated with UTIs, while type P often are present in pyelonephritis and other kidney infections. The presence of fimbriae on bacteria enable the bacteria to adhere to mucosal surfaces.[21]

An early study by Ofek demonstrated that *E. coli* attached to epithelial cells by binding to the mannose receptors on the epithelial cells. They recorded adherence as the percentage of epithelial cells having 50 or more attached bacteria. They used D-Mannose and α-D-mannopyranoside (α MM) to inhibit bacterial adherence to epithelial cells. In the presence of α -MM, *E. coli* did not adhere to epithelial cells. When the epithelial cells and the bacteria were washed, the adherence activity was restored. Thus, the inhibitory activity of the sugar is reversible. They suggested that the sugar saturates the binding sites on the bacterial surface. This prevents the bacteria from attaching to the mannose-like structure that constitutes the epithelial cell membrane receptor. When D-mannose or α MM is used to wash epithelial cells, the *E. coli* attached to the cells are removed. Therefore, the *E. coli* binding activity is mediated by a lectin-like, mannose-specific substance on the *E. coli* which binds to the epithelial cell via a mannose-like receptor. Mannose and its derivatives bind to epithelial receptors to prevent the adherence of *E. coli* to the epithelial cell. Thus, this early work by Ofek

laid the basics of *E. coli* fimbriae/epithelial cell receptor interactions and their importance in bacterial adhesion.[31] Another study used α MM to prevent the *E. coli* infection of the mouse urinary tract. They demonstrated that the presence of α MM resulted in a significant reduction in bacteriuria in mice receiving *E. coli* injections. In *Proteus mirabilis* infections, however, the α MM treatment did not prevent adherence *in vivo*. This was to be expected since α MM does not prevent the adherence of Proteus *mirabilis* to epithelium *in vitro*. The α MM treatment was highly successful in preventing *E. coli* infection.[2]

***E. coli* Fimbriae:**

A study by Hopkins and associates addressed the adherence characteristics of uropathic strains of *E. coli*. They isolated 28 strains of *E. coli* from the urine of patients with diagnosed UTIs and assayed for fimbrial type and adherence to rat bladder epithelium. They found that type 1 fimbriae were present on 61% of the isolates, both type 1 and type P fimbriae were present on 14% of the isolates and 25% of the bacterial strains did not express either type of fimbriae. They concluded that most UTIs are caused by *E. coli* with type 1 only or with a combination of type 1 and type P fimbriae. As indicated above, they also noted that the degree of fimbriation seemed to be as important in determining virulence as the presence of fimbriae themselves. They also noted that 25% of the strains did not possess type 1 or type P fimbriae but were still able to cause UTI. They suggested that this

adherence ability may be due to presence of adhesins other than type 1 or type P.[21]

Therapeutic Strategies using Receptor\ Adhesin Interactions:

Edwin Beachey addressed the role of adhesin and receptor interactions and how they may act together to mediate bacterial adherence. He suggested three main strategies to prevent the adherence of bacteria to epithelial surfaces: use adhesin or receptor analogues to competitively inhibit bacterial adherence, use sublethal levels of antibiotics to suppress bacterial adhesins and use vaccines directed against adhesins to stimulate an immune response. When mannose was injected into the urinary tract of mice, only 20% became colonized with the infective strain. The *E. coli* used in this experiment contained type 1 fimbriae on the surface of the bacteria. Thus they were able to demonstrate the practicality of using of a competitive inhibitor. Sublethal doses of streptomycin inhibited the adherence ability of *E. coli* during growing phase, but had no effect during resting phase. Thus, the time during development determines the utility of this treatment. Finally, when rats were vaccinated with type 1 fimbriae from *E. coli*, they produced antibodies and demonstrated protection against *E. coli* infections. Thus, there is good evidence to support the potential use of each of these approaches in a human population.[6]

Effect of Streptomycin Treatment:

The effects of sublethal concentrations of streptomycin on the

mannose binding and epithelial adherence activity of *E. coli* was investigated by Eisenstein in 1979. They found that sublethal doses of streptomycin, given during growing phase, would significantly reduce the epithelial cell adherence and mannose binding activity of E. coli at stationary phase. The effect of the streptomycin was most apparent in the early log phase of growth, required actively growing bacteria and was reversible. After the cultures had acquired mannose binding ability, antibiotic treatment was not effective. These results suggest that antibiotics may have another mechanism to remove bacteria from mucosal surfaces other than the simple inhibition of growth and bactericidal properties.[13] This group did a follow up study in which *E. coli* produced an aberrant type 1 fimbriae which had lost its lectin-like activity. They used sublethal concentrations of streptomycin (30 g/mL) to produce the aberrant fimbriae which demonstrated a diminished ability to adhere to epithelial cells. Although the streptomycin treated *E. coli* were heavily fimbriated, their fimbriae were longer and had lost mannose binding activity. Thus, streptomycin treatment may lead to the production of an aberrant fimbrial protein possibly by misreading messenger RNA. Therefore, streptomycin treatment may be beneficial in preventing the adherence of drug resistant strains of bacteria.[14]

Antibody Treatment:

Abraham and associates produced antibodies which were directed against either the type 1 fimbriae, located on the surface of bacteria, or the D-mannose receptors which are present on the

host epithelial cells. A quaternary structure specific antibody to the type 1 fimbriae of *E. coli* prevented the attachment of type 1 fimbriae and related *E. coli* to mannose containing eukaryotic cells. An antibody directed against D-mannose, the sugar determinate of the host cell receptor, also prevented the attachment of *E. coli* to epithelial cells. When the antibodies were administered intraperitoneally, the mice were protected against the mannose sensitive *E. coli* injected into their bladders. They suggest that the antibodies may work by blocking the initial bacterial attachment to epithelial tissue and that the adhesin and receptor specific antibodies protect the urinary tract after they pass into the urinary space. Thus, antibodies against type 1 fimbriae and/or D-mannose can help prevent bacterial colonization of *E. coli*.[1]

In a study by Orskov, the F7 fimbrial antigen of *E. coli* is compared to the type 1 fimbriae. Using the microscope, they detected large amounts of mucus present in the urine and observed that bacteria with type 1 fimbriae adhered strongly to the threads of mucus. The mucus prevented the bacteria with type 1 fimbriae from adhering to the epithelial cells. They noted that different individuals produce different quantities of mucus in their urine. The type 1 fimbriae adhered to the mucus and not the cells, but the F7 fimbriae adhered to the cells and not the mucus.[33] They suggest that the urinary mucus traps bacteria with type 1 fimbriae and prevents their adherence.[34] Another study investigated the factors present in normal human urine which can inhibit the binding ability

of adhesins on *E. coli*. It was found that type 1 fimbriae will bind to immobilized Tamm-Horsfall glycoprotein.[35] Hanson et al. suggested the possibility of a cross-reaction occurring between Tamm-Horsfall protein and Gram-negative bacteria. This would account for the presence of autoantibodies against THP in all humans. THP may act as a nonspecific defense against Gram -negative bacteria by preventing the attachment of the bacteria to the walls of the urinary tract.[19]

Elderly Population:

An elderly population presents a unique combination of host, bacterial and environmental factors that can effect the pathogenesis and management of a UTI. The elderly tend to have a high mortality associated with UTI, especially in patients that are hospitalized, institutionalized or catheterized. Increased susceptibility to UTI in the elderly may be due to acquired abnormalities of the urinary tract, physiologic changes associated with aging and increased exposure to therapeutic and environmental risk factors. Unusual pathogens are frequently encountered in an elderly population, making treatment more complicated. More than 20% of women and 10% of men over the age of 65 have bacteriuria. Prevalence of bacteriuria increases with age, concurrent disease, institutionalization and hospitalization.[43] Risk factors for UTI include: cerebrovascular accident, decreased functional capacity, decreased mental status , antibiotic prophylaxis and urinary catheterization.[39] The likelihood of bacteriuria in a functionally

impaired nursing home resident is approximately twice that of a healthy subject living at home. Age related changes in an elderly population include: obstructive uropathy, neurogenic dysfunction, reduction in cell mediated immunity and increased bacterial receptivity of epithelial cells. Contamination risks increase because of urinary and fecal incontinence, decreased prostatic and vaginal antibacterial factors, and urethral instrumentation and catheterization. In addition to *E. coli*, other pathogens such as *Proteus, Klebsiella* and *Pseudomonas* often cause UTI in the elderly population. This difference in uropathogens, increased frequency of multi-organism infections and antibiotic resistant strains of uropathogens is probably due to increased hospitalization/ institutionalization, catheterization and antibiotic use in the elderly. Prompt treatment of symptomatic UTI in the elderly is strongly recommended and usually involves the use of antimicrobial agents. However, it should be noted that the elderly are more susceptible to the toxic effects of antibiotics. Because the metabolism and excretion of antibiotics may be impaired in the elderly, drug levels in the blood stream may rise to dangerous levels and cause renal damage. It is also possible that adverse drug interactions may occur in patients taking many medications. Thus, antibiotic treatment of UTI in the elderly must be carefully monitored.[43]

A large clinical study performed by Boscia and associates addressed bacteriuria in an ambulatory, elderly population. Bacteriuria was more common (18.2%) in the 373 women studied than

in the 150 men who demonstrated bacteriuria only 6% of the time. Functionally impaired nursing home residents had bacteriuria rates of 23.5% while those living in apartments had rates of 12.1%. Thus, functional status appears to be an important factor in bacteriuria. A longitudinal study found that a high turnover of infected and noninfected individuals was common and that persistence of the same infective organism was rare. Thus, the bacteriuria was transient in most cases. Due to the transient nature of bacteriuria, investigators have advised against the treatment of asymptomatic bacteriuria in the elderly.[9] The higher rates of bacteriuria in nursing home residents may be due to the more debilitated state of the patients including: incomplete bladder emptying, perineal soiling, and urethral catheterization.[5]

Another clinical trial, performed by Nicolle and associates, assigned fifty elderly, institutionalized women with asymptomatic bacteriuria to either a treatment or a nontreatment group. Over the course of a year, the treatment group had a 31% lower prevalence of bacteriuria than the nontreatment group. The nontreatment group had a 71% persistence of infection with the same organism. The antibiotic treatment was associated with increased risk of reinfection, adverse effects to the antimicrobial drugs and the appearance of drug resistant organisms. There were no differences in morbidity or mortality observed between the two groups. Thus, no benefits resulted from treatment and some harmful effects were associated with antibiotic treatment of asymptomatic bacteriuria. Thus, Nicolle concludes that treatment of asymptomatic bacteriuria

is contraindicated in an elderly population.[30]

Effect of Catheterization:

The insertion of a Foley catheter is the most common cause of nosocomial infection. Catheter caused infections can be difficult to prevent and treat and result in significant morbidity and mortality. Bacteria can be introduced into the bladder along with the catheter during instrumentation. Once the catheter is in place, the bacteria can grow in a film and ascend the interior surface of the catheter into the bladder. It is also possible for bacteria to migrate from outside the body along the exterior surface of the catheter into the bladder. This mode of entry is often the slowest. Thus, the catheter acts as a bridge to allow the bacteria access to the normally sterile bladder. The catheter circumvents the bladder's defense mechanisms and promotes bacteriuria. The Foley catheter does not drain the bladder completely, a small quantity of urine remains and provides a medium for the bacteria to grow. The use of antibiotics in short term catheterization may reduce infections and serious complications. In longterm catheterization cases, however, antibiotic treatment is counterproductive. Bacteriuria will occur eventually and the bacteria will be more virulent and possibly even resistant to the antimicrobial drugs. It is best to avoid indwelling urethral catheterization if possible or to reduce the amount of time that the catheter is in place. The bacteriuria accompanying Foley catheterization remains a challenge

to treatment.[29]

Elderly Host Defense Mechanisms:

The elderly population may lack host defense mechanisms normally present in a younger population. Urinary defenses can include low pH, high urea, extremes of osmolality, high organic acid content and bacteriocidal prostatic secretions. Aging often leads to a decline in renal function resulting in less urine acidity, less urea in the urine and fewer extremes in osmolality. Elderly men may also produce less prostatic secretions. Incomplete bladder emptying may be one of the most important mechanisms for increased bacterial infection. Impaired bladder emptying in the elderly may result from bladder prolapse in women, prostatic disease in men and a neurogenic bladder in either sex.[5] Tamm-Horsfall protein, an important host defense against type 1 fimbriated bacteria, is excreted in lesser amounts in the elderly.[47] These type 1 fimbriae allow bacteria to persist and colonize catheters.[27] Thus, the elderly have several factors leading to increased susceptibility to UTI and more complications involved in treating UTI.

Cranberry Juice Treatment of UTI:

Cranberry juice has been used as a common homeopathic remedy for the prevention and possible treatment of urinary tract infections for many years. Old wive's tales have advised drinking cranberry juice for centuries. Many people have reported

improvement in urinary health and have attributed this to use of the juice. The reported benefits that patients gain from drinking cranberry juice have sparked interest in the mode of action of cranberry juice cocktail (CJC) and how it may protect against the occurrence of urinary tract infections. Initially, CJC was thought to acidify the urine and therefore increase its bacteriostatic properties. The administration of cranberry juice in varying amounts was used to determine its role in urine acidification. By controlling dietary conditions, the effect of CJC ingestion on urine acidification and calcium excretion was quantitated. Initially, subjects responded to CJC treatment with a lowered pH or increased acidification. However, this effect was found to be transient and the effect was lost after continued treatment.[23] Thus, acidification of the urine does not appear to account for the observed benefits attributed to use of the juice to control urinary tract infections.

It has been observed that prunes, plums and cranberries all increase urine acidity by producing hippuric acid. At the time of this report, the exact concentrations of beta-hydroxybutyric acid, citric acid, malic, glucuronic and quinic acids were not known in cranberry juice.[23] Additional investigations noted that although cranberry juice has a high acidity, the ingestion of the juice is often an unreliable means of increasing the acidity of the urine. The bacteriostatic properties of hippuric acid, however, are well documented.[52] This led to the investigation of the potential of the cranberry juice to increase the concentration of hippuric acid in

the urine as a possible mode of action.[8]

Many organic acids have anti-bacterial properties. In order to determine the concentrations of the major organic acids in cranberry juice, high pressure liquid chromatography (HPLC) was used. Single strength, undiluted, cranberry juice was used to separate and determine the quantities of quinic, malic, and citric acids present. It was demonstrated that quinic acid accounted for 1.32%, while malic and citric acids accounted for 0.92% and 1.08% respectively.[11] Benzoic and quinic acids are thought to be the precursors of the hippuric acid secreted by the kidneys. When ingested, cranberry juice alone rarely will raise the hippuric acid concentration of the urine to the 0.02 to 0.04M level at pH 5.0 necessary to retard growth of common urinary tract pathogens.[8] When the pH of the urine is raised to 5.6, closer to the normal pH of the urine, five times more hippuric acid is necessary to achieve the same effect as at ph 5.0. Thus, hippuric acid was ruled out as a mode of action of cranberry juice. Therefore, the decreased bacteriuria found in subjects ingesting CJC must be related to the bacteriostatic properties of the cranberry juice and not dependent upon urine pH or hippuric acid formation.[7,50]

Clinical trials of CJC Treatment:

One of the first clinical trials addressing the effectiveness of cranberry juice for the treatment of UTIs was performed by Prodromos and associates. Sixty human subjects, consisting of 44 females and 16 males, drank 16 ounces of cranberry juice per day

for twenty one days. Thirty two subjects showed significant improvement, 12 were moderately improved and 16 showed no improvement. Six weeks after CJC ingestion stopped, 61% of the subjects experienced a recurrent infection. Thus, this study demonstrated that CJC ingestion can be useful for the treatment of urinary tract infections.[40]

CJC Inhibits Bacterial Adherence:

In 1984, Sobota was the first to suggest that cranberry juice works by inhibiting the ability of bacteria to adhere to the urinary cells. He demonstrated that fresh cranberry juice, as well as the cranberry juice cocktail (CJC), would inhibit the ability of *E. coli* to bind to the surface of uroepithelial cells. He also tested the urine of human subjects and mice and found that the urine of subjects drinking CJC showed significant antiadherence activity. A total of 77 isolates of *E. coli* were tested. Adherence was inhibited by CJC by 75% or more in over 60% of the isolates. The urine from the mice drinking CJC decreased the adherence of *E. coli* to uroepithelial cells by approximately 80%. Fifteen of the 22 human subjects who drank 15 ounces of CJC produced significantly antiadherent urine 1 to 3 hours after ingestion of CJC. The site of action of CJC appeared to be associated with an interference of an adherence mechanism or surface component of *E. coli*. When *E. coli* was preincubated with CJC, attachment was strongly inhibited. However, when the epithelial cells were preincubated with CJC, no difference in attachment was observed. When *E. coli* cells were

washed, the bacteria returned to their normal adherence pattern. Based upon these observations, it was suggested that cranberry juice may inhibit bacterial adherence to epithelial cells by interfering with a surface component of *E. coli*.[48]

A follow up study in 1988 by Schmidt and Sobota, extended the antiadherence studies to include Gram-negative rods isolated from urine, sputum, wounds and stool. These clinical isolates included species of *E. coli, Proteus, Klebsiella, Enterobacter* and *Pseudomonas*. Urinary isolates demonstrated a mean adherence of 13.7 bacteria per cell, while non-urinary isolates had a mean adherence of 5.73 bacteria per cell. Treatment with CJC inhibited the adherence activities of other Gram negative rods in addition to *E. coli*. It was suggested that there may be some factor in CJC that binds to a surface component present on Gram negative rods that inhibits bacterial adherence. It was also reported that in most cases the juice never totally cleared all bacteria from the uroepithelial cells and there is always some residual population of bacteria on the cells.[44]

In vivo, anti adherence activity was detected both in the urine and on urinary epithelial cells. They concluded that CJC treatment should provide some protection against urinary pathogens responsible for UTIs.[44]

A study performed by Zafriri investigated the adherence properties of *E. coli* that express surface lectins of defined sugar specificity. They examined the effects of CJC and cranberry juice on bacterial adherence *in vitro* to yeasts, tissue culture cells,

erythrocytes and mouse peritoneal macrophages. They found that CJC inhibited the adherence of urinary isolates of *E. coli* with type 1 fimbriae and with P fimbriae but not CFA/1 adhesin. Type 1 fimbriae are mannose sensitive and P fimbriae are specific for α-D-Gal(1-4)-ß-D-Gal. They attributed the inhibitory activity of CJC on Type 1 fimbriated *E. coli* to the presence of fructose in CJC. Fructose, however, is only 10% as active as methyl α-D-mannoside in inhibitory activity. The inhibitory factor for P fimbriae was nondialyzable and detected only after the bacteria were preincubated with CJC. They concluded that CJC contains two or more inhibitors of lectin mediated adherence of urinary pathogens *in vitro*. They did not test these results *in vivo*. They suggested that the consumption of food or beverages containing certain lectins or carbohydrates may affect a lectin sugar mediated adherence. They also suggested that the drinking of CJC may influence urinary concentrations of THP and that this may inhibit the adherence activity of *E. coli* with type 1 fimbriae.[57] A follow up to that study demonstrated that juices from the plants of the genus Vaccinium, in particular, cranberries and blueberries, were able to inhibit adherence of bacterial isolates to epithelial cells. They also suggested that these antiadherence agents may act in the gut or the bladder to prevent bacterial growth in these sites.[32]

In 1994, a large scale clinical trial was initiated by Avorn and associates to examine, *in vivo*, the reported potential of cranberry juice to influence the outcome of infections of the

urinary tract. The study consisted of 153 elderly women whose average age was 78.5 years. The control group consumed 300mL per day of a placebo drink while the experimental group drank 300mL per day of CJC. The experimental group drinking CJC had a 58% decrease in bacteriuria compared to the control group. This effect did not appear within the first month after randomization, but became strikingly apparent after month 1. This time delay may be consistent with the modification of gut flora. These observations suggest that cranberry beverage has some bacteriostatic mode of action in the bladder. This large scale study provided significant statistical evidence to support the original suggestion of Sobota [48] that CJC may be useful for the treatment of UTI.[3]

In a recent study in our laboratory, Patrick Wilson [56] addressed the use of CJC as an antiadherence agent to control chronic UTI in an elderly population. Eight elderly female nursing home patients ingested 4 ounces of CJC each day for twelve weeks. Their urine was then tested for anti-adherence activity. Four of the eight patients demonstrated significant increase in antiadherence activity in their urine. Reductions in bacterial adherence ranged from 62% to 26%. This reduction in bacterial adherence became prevalent after 4 weeks during CJC ingestion. [56]

Role of Fructose:

To address the effect of fructose, a component of CJC, and its role as an antiadherence agent for type 1 fimbriated *E. coli in*

vivo, HPLC was used to detect the passage of ingested fructose to the urine. There was no detectable fructose accumulation in the urine 4 hours after five subjects drank 5%-15% fructose solutions. Therefore, he suggested that fructose cannot be the primary antiadherence agent of CJC that is observed in the urine acting on type 1 fimbriae. Additional observations suggested that CJC may act indirectly upon Tamm Horsfall protein to increase the antiadherence effect of the urine.[56]

THP:

In 1951, Igor Tamm and Frank Horsfall isolated a mucoprotein in a high degree of purity from normal human urine. The mucoprotein was isolated by precipitation with 0.58 Molar sodium chloride. The fraction was shown to consist of a single homogenous substance using electrophoretic and ultracentrifugal criteria. They demonstrated that the glycoprotein was responsible for all the hemagglutination inhibiting activity of such urine. They also characterized several biological, chemical and physicochemical properties of the isolated material. The material proved to be a polymer of high molecular weight with a high degree of molecular assymetry and biological activity . This product was called Tamm-Horsfall glycoprotein and was later referred to as Tamm-Horsfall Protein or THP.[55]

Uromodulin:

In 1985, a glycoprotein called uromodulin was extracted from

the urine of pregnant women with lectin adherence columns. It was found to be a specific ligand for cytokines such as interleukin-1 , interleukin-2 and tumor necrosis factor. The cytokines bound to specific carbohydrate sequences. Using cDNA and amino acid sequencing, Kumar and Muchmore found that the protein backbone of THP and uromodulin are identical . They determined that the differences in bioactivity are probably due to glycosylation differences between THP and uromodulin and are not due to amino acid sequence differences. For this reason, THP refers to the glycoprotein isolated by salt precipitation and uromodulin is that glycoprotein isolated from pregnancy urine by lectin adherence.[24]

Abundance of THP:

Tamm-Horsfall Protein (THP) is the most abundant protein found in normal human urine . The excretion rates range from 20 g to 200 g per day in humans.[24] THP is produced in the cells of the ascending limb of the loop of Henle and the adjacent segment of the convoluted tubule.[41] The kidneys of all placental mammals produce THP . In the body, the distribution of THP is primarily limited to the kidney.[24] The molecular weight of THP is approximately 90 kilodaltons. In an aqueous solution, however, hydrophobic aggregates of several million daltons form and are called uromucoid.[15] This illustrates THP's tendency to gel readily in solution . This property appears to be dependent upon intact glycosylation. Various other factors such as calcium and sodium ions, albumin and radio contrast media have also been shown to

increase gel formation.[24]

The amount of THP excreted appears to be independent of sex, salt load or diuresis . Day and night THP excretion rates were observed to be similar. Haugen did not find much fluctuation in THP excretion rates for a single individual. He concluded that one night's urine sample would yield an accurate expression of uromucoid excretion rate.[20] Lynn et al. found that THP excretion rates may change as much as 18-fold in the same individual in the course of a day . The half-life of THP varies considerably from a minimum of 3 hours to a maximum of 168 hours. They demonstrated a positive correlation between amount of THP excreted and the volume of urine sample collected.[26] In another study, Reinhart also found THP excretion rates varied widely when the same individual supplies repeat samples.[42] Thus, intraindividual fluctuations in THP excretion are common and may depend on factors such as time of day and amount of urine voided.

Role of THP:

The role that surface mucins, such as THP, play in the bladder was investigated by Parsons. They hypothesized that the mucin may be an antibacterial defense to prevent binding of bacteria. Initially, the mucosa lining the bladder can resist bacterial colonization . After the bladder was treated with acid, bacterial attachment could occur. The acid treatment damaged the mucin lining and allowed the bacteria to adhere to the bladder wall. When the mucopolysaccharide was resynthesized, microbial attachment was

again inhibited. This indicated that the mucopolysaccharide or one of the proteins in it, was responsible for the antiadherence effect.[38] Thus, the importance of surface mucin in the bladder was recognized. It is an antibacterial defense mechanism that prevents bacteria from adhering to the walls of the bladder.[36,37]

THP's role in the urinary tract has undergone extensive investigation in recent years. Urine has been shown to exhibit anti-bacterial properties and urine which has had the THP removed from it shows a marked loss of anti-adherence activity. Therefore, THP in concentrations found in normal urine has an anti-adherence activity for *Escherichia coli* with type 1 fimbriae.[49] Strains of *E. coli* that express type 1 fimbriae have been shown to specifically adhere to urinary mucus . In the abundant amounts present in normal urine, urinary slime has been shown to trap *E. coli* with type 1 fimbriae.[33] The free mucus captures the bacteria in the urinary tract and removes them during the process of micturition.[47] This bacterial adherence to suspended THP would inhibit epithelial attachment and promote removal of bacteria during micturition.[15] Therefore, THP was proposed to be a natural host defense mechanism against UTI by preventing bacterial adherence to the urinary epithelium . Depending upon the concentration of THP in the urine, THP may either promote or prevent the adherence of *E. coli* to uroepithelial cells. Above concentrations of 35 g/mL, THP has anti-adherence activity. Below concentrations of 17 g/mL ,THP has been shown to actually promote bacterial adhesion.[49] This data concurs with findings that state

that low levels of THP may be responsible for a predisposition to UTI.[25] Normally, however, the range of THP concentrations in the urine is from 30 to 50 g/mL and this would be sufficient to inhibit the attachment of bacteria with type 1 fimbriae . The anti-adherence activity of THP can be reversed by calcium levels that are higher than those found in normal urine. This may have important implications for persons taking calcium supplements.[49] Since low levels of THP excretion was correlated with a predisposition to UTI, Reinhart compared the THP excretion rates of women with recurrent UTI to controls . They found no significant decrease in THP excretion in those subjects with recurrent UTIs. They did notice that diabetics in general had a lower urinary THP level.[41] Thus, the anti-adherence effects of THP in the urinary tract are well documented but questions still remain concerning the importance of quantity of THP and the mechanism of action.

Over the years, studies have been performed to elucidate the mode of action of THP on bacteria. *E. coli* is the most common uropathogen found in lower UTIs and many strains contain MS and/or P fimbriae . These MS fimbriae appear to play an important role in the establishment of lower urinary tract infection.[42] Bacterial colonization and clinical UTI are dependent upon the binding of the pili on the bacterial cell to the specific receptors on the uroepithelial cell.[22] Mannose-sensitive or MS pili are also referred to as type 1 fimbriae . These type 1 fimbriae are proteinaceous appendages which are present on the cell wall of many bacteria including most members of the Enterobacteriaceae family.

Type 1 fimbriae mediate attachment of the bacteria to mammalian host cell receptors. The host cell receptor is thought to be a glycoprotein containing an exposed mannose residue. Attachment of pili to this receptor can be blocked by mannose and α-methyl mannoside. Electron micrographs have shown the formation of a pseudocapsule around bacteria with type 1 fimbriae. Dissolved THP adheres to the fimbriae and forms the capsule.[25] The mannose present on the carbohydrate chain of THP appears to be responsible for THP's ability to bind to mannose sensitive fimbriae.[34] Sugar inhibition studies show that THP binds to fimbriae via its mannose side chains and that the THP receptor site on fimbriae has lectin like properties . This suggests that THP acts as a receptor analog for bacteria expressing type 1 fimbriae .[42] Dulawa found that THP could inhibit bacterial adherence in a specific manner that was reversible. When the THP was washed off of the cultured cells, the anti-adherence effect was lost. No other glycoproteins studied exhibited a similar anti-adherence effect. Bacterial adhesion was reduced dose dependently by THP. Although diabetics display an altered carbohydrate composition of THP, there was no significant difference in anti-adherence activity of THP in diabetics compared to controls. He also noted that at low concentrations of THP, some individuals display an increased bacterial adherence. The immune system, through production of endogenous urinary products such as THP, can inhibit bacterial adhesion mediated by type 1 fimbriae.[12] THP is thought to trap type 1 fimbriated *E. coli* and this facilitates bacterial clearing.[34] The range of concentrations of

THP that promote bacterial clearing are well within normal human urine conditions.[25] Thus, THP may be a primary host defense mechanism aimed at preventing the colonization of the urinary tract by bacteria containing the type 1 fimbriae.

The Current Study:

The current study addresses the effect of cranberry juice cocktail treatment on THP in the prevention of urinary tract infections in an elderly population. Four female nursing home residents ranging in age from 76 to 92 years consumed 4 oz of CJC each day for a total of twelve weeks. All four patients were prone to UTI due to their decreased functional capacity and indwelling urethral catheters. Prolonged urethral catheterization virtually guarantees the development of a UTI. Thus, this is a very difficult population to treat in which traditional treatment, such as antimicrobial drugs, are contraindicated. CJC has no ill effects and is usually tolerated very well by patients. Thus, any benefit derived from CJC treatment is of value to these patients. A previous study of these patients indicated an increase in the antiadherence activity of the urine after ingestion of CJC.[56] In this study, the THP is extracted from the urine. The THP is then used as the reaction medium of a bacterial adherence assay. In this way, the antiadherence activity of the THP before and after CJC ingestion can be compared. To control for the effect of bacterial isolate on development of UTI, the patient's THP was challenged with both the original infective isolate and the

control, E. coli. Thus, the effect of CJC ingestion upon the anti-adherence activity of THP was investigated.

II. MATERIALS AND METHODS

PATIENT SELECTION:

The four patients used in this study were residents of Beeghly Oaks nursing home who gave written consent for their involvement in the study. The subjects were incontinent and had indwelling urethral catheters. They ranged in age from 76 to 92 years and had a history of urinary tract infections as defined as three or more UTIs per year. Most had recurrent infections in the year prior to the study. Patient one and three each had four infections in the previous year. Patient five had five infections in the previous year and entered the study with an infection. Patient seven had three infections the previous year and entered the study bacteriuric.

Cranberry Juice Treatment and Urine Collection Procedures:

The treatment spanned a thirteen week period from February 8, 1992 to May 10, 1992. The first week, specimens were collected prior to cranberry juice cocktail (CJC) treatment. For twelve weeks, patients consumed four ounces of CJC each morning. *Ocean Spray* cranberry juice cocktail was given since it is the most commonly consumed form of cranberry juice. CJC contains about

33% cranberry juice and other ingredients such as high fructose corn syrup and vitamin C. The week before CJC ingestion, samples of urine were collected from each patient on Monday, Wednesday, and Friday. The adherence data from these three samples were averaged to form the baseline control. This was done to account for slight day to day differences in anti- adherence activity. CJC ingestion began on the second Monday. Samples were collected 24 hours, 48 hours and 72 hours after CJC ingestion began. Urine samples were also collected every Monday for the following eleven weeks of the study. All urine samples were collected by the Beeghly Oaks nursing staff and were refrigerated immediately. The samples were centrifuged for 10 minutes at 4,500g to remove patient cells and the urine was stored at -20C.

BACTERIAL ISOLATION:

Aliquots of the specimens were plated on MacConkey's agar the day of collection. Colonies isolated from pre-CJC urine were replated on nutrient agar and the genus and species was identified using API 20E strips (*Fisher Scientific*, Pittsburgh, Pa.\US). After identification and isolation, 15% glycerol cultures were stored at -70C for later use in adherence assays. The infective isolates from each of the following patients were: Patient 1, *Proteus mirabilis*; Patient 3, *Escherichia coli*; Patient 5, *Pseudomonas aeruginosa*; and Patient 7, *Pseudomonas aeruginosa*.

The arrangements for urine sample collection, administration of the cocktail to the patients, urine preparation and bacterial

isolation were performed by Patrick Wilson[56]

TAMM-HORSFALL PROTEIN EXTRACTION:

The THP extraction procedure used in this study is an adaptation of the one initially used by Tamm and Horsfall to define the glycoprotein known as THP.[55] The saline precipitation procedure is specific for THP while other methods such as lectin adherence columns have isolated similar substances such as uromodulin. The classical saline precipitation procedure was used in this study. The frozen urine samples were thawed and 10mL of patient urine was placed in a test tube. Then, 0.336g of NaCl was added to the urine and the sample was vortexed to assured sufficient mixing. The sample was then refrigerated overnight to aid in the precipitation of THP. The next day, the samples were centrifuged at 4C for thirty minutes at 4960 g. The supernatant containing urine was aspirated and the pellet containing the THP is washed with 10mL of 0.58M NaCl and mixed using a vortex. The centrifugation and wash step is repeated two more times. Following the fourth centrifugation, the pellet containing the THP was resuspended, by vortexing, in 5 mL of saline solution and either used directly in the adherence assay or placed in cold storage.

ADHERENCE ASSAY:

The purpose of the adherence assay is to create an *in vitro* model of the human urinary tract. Uroepithelial cells were obtained from the urine of a single healthy female. Urine was collected and the cells were harvested and used for the adherence assay the same day. Ten mL of donor urine was centrifuged at 4,500rpm for 10 minutes. This caused the exfoliated uroepithelial cells to pellet out of solution and the supernatant was discarded. The bacterial infective isolate from the patient urine was incubated at 37C for 48 hours in a static culture. Two mL of bacterial culture (approx. 10 9 bacteria) were centrifuged at 4,500rpm for 10 minutes to pellet out the bacteria. The supernatant was then discarded. The pelleted bacteria were then mixed with 2mL of THP solution using a vortex mixer. This solution was then added to the donor uroepithelial cells and mixed gently. The specimens were incubated for thirty minutes in a water bath at 37C. Following incubation, the specimens were vortexed briefly to resuspend the cells. The samples were poured into disposable beakers and a 2mL aliquot was collected using a disposable syringe. The syringe was then fitted to a filter holder containing an 8 m nucleopore filter. The specimen was then gently pushed through the filter. The filters were then washed with 100mL of deionized water to remove any unattached bacteria. The filters were then placed cell side down on a glass microscope slide and allowed to dry overnight. The next day, the filter was pealed off the slide . The cells were left adherent to the slide surface. The slide could now be Gram stained. Negative controls for each experimental run

were prepared using donor cells and saline to rule out the possibility of bacteria present on the donor uroepithelial cells. Baseline or pre-CJC controls were prepared using donor cells, bacteria and pre-CJC THP to get a baseline adherence rate of the patient's infective isolate to uroepithelial cells in the presence of original THP. The same healthy, female donor was used to supply uroepithelial cells throughout the study.

GRAM STAINING PROCEDURE:

The Gram stain procedure used in this study is the one used by Philadelphia General Hospital. The slide with the adherent cells was covered with crystal violet. This is a primary stain that will stain Gram+ bacteria a blue or purple color. Five drops of sodium bicarbonate are then added to each slide and the slides were permitted to incubated for 1 minute. This step acts as a buffer to adjust pH. The slide was then covered with an excess of Gram's iodine. The iodine acts as a mordant and helps the Gram+ bacteria retain the crystal violet stain. The slide was then decolorized by washing with acetone. The acetone decolorizes the Gram- organisms. After drying, the slide was then flooded with safranin for three minutes. The safranin counter stains the Gram- bacteria a red or pink color. Each slide was then rinsed liberally with water to wash off any excess dye. All of the bacteria used in this study were Gram- rods and would appear pink or red under the microscope in the scoring procedure. The uroepithelial cells also stained pink

to red.

SCORING PROCEDURE:

Using an oil immersion lens of a light microscope with a total magnification of 1000X, 50 uroepithelial cells were observed on each slide. For each cell, the number of adherent Gram - rods was counted. For each slide, the mean number of adherent bacteria was calculated. The data from each slide was compared by one-way ANOVA to the controls to determine significant changes in bacterial adherence.

III. RESULTS

In this study, THP is isolated from whole urine at various times after the commencement of CJC ingestion and is used as the reaction medium for a bacterial adherence assay that mimics the conditions found in a human urinary tract. In the first study, the mean number of bacteria adhering to each of fifty uroepithelial cells, assayed in the presence of THP, is quantitated. The original infective isolate, obtained from the patients urine, represents the source of the bacteria used in each study.

Patient One:

The original infective isolate from patient 1 was *Proteus mirabilis*. The initial *P. mirabilis* adherence, using THP isolated from urine prior to CJC ingestion, was 6.5 bacteria per cell. The mean bacterial adherence in the three weeks following the commencement of CJC ingestion was 12.56 bacteria per cell, an increase of 93%. The mean bacterial adherence from week 4 to week 12 was 20.9 bacteria per cell, an increase of 223%. Overall, patient 1's *P. mirabilis* adherence, assayed using THP, increased 157% following CJC treatment. Day 1 to day 21, and day 56 ,after ingestion of CJC, were not significantly different from control values. However, day 28 through 84, with the exception of day 56, had values significantly higher than the controls.

Patient Three:

The original infective isolate from patient 3 was *E. coli*. Prior to CJC ingestion, the number of *E. coli* per cell was 17.62

when assayed in the presence of THP extracted from the urine. The first three weeks following commencement of CJC ingestion, the mean bacterial adherence was 4.98 *E. coli* per cell. This is a 72% decrease. Week 4 to week 12 had a mean adherence rate of 5.78 bacteria per cell, a decrease of 67% when compared to control. Thus, the overall effect of CJC treatment on patient 3 was a decrease of 69% of *E. coli* adherent to uroepithelial cells. From day 1 to day 84 following CJC ingestion, there were significantly less bacteria adhering to the uroepithelial cells. At no time were there more adherent bacteria after CJC ingestion than before CJC ingestion.

Patient Five:

Pseudomonas aeruginosa was the original infective isolate from patient 5. Prior to CJC treatment, the control adherence rate was 5.26. For the first three weeks of treatment, the average adherence was 4.61 *P. aeruginosa* per cell, indicating a decrease of 12% bacterial adherence. After the first three weeks of treatment, the bacterial adherence fell to 3.79 *P. aeruginosa* per cell, a decrease of 28% when compared to controls. Overall, Patient 5 demonstrated a 22% decrease in bacterial adherence after treatment with CJC. Days 21, 28, 35, 49 and 56 all had significantly less adherent bacteria than the control. All other days were not significantly different than the control.

Patient Seven:

Patient 7 was also originally infected with *P. aeruginosa*. The control rate of bacterial adherence was 14.8 *P. aeruginosa* per

cell. Within three weeks of CJC treatment, the bacterial adherence rate fell to an average of 5.75 bacteria per cell, an improvement of 61%. After three weeks of treatment, the adherence rate was 4.5 bacteria per cell, a decrease of 70% compared to controls. Overall, CJC treatment resulted in a decrease of 66% in adherence of *P. aeruginosa* to uroepithelial cells. Day 1 all the way through day 84 demonstrated bacterial adherence values significantly less than the control. At no time were there any bacterial adherence greater then the control.

Compare Original Isolate to Control:

A comparative study was initiated to investigate the effects of *E. coli,* which is known to specifically interact with THP, as a control to be compared to the original bacterial isolate. When Patient 1's pre-CJC THP was assayed using *E. coli* as the bacterial isolate, the control bacterial adherence was 7.64 *E. coli* per cell. From 1 to 21 days after CJC ingestion, the average bacterial adherence was 6.47 *E. coli* per cell, a decrease of 15% in bacterial adherence over this period. After three weeks of CJC ingestion, an average of 7.34 *E. coli* were adherent to each cell. This indicated a decrease of 4%. Overall, CJC ingestion in patient 1 corresponded to an 8% decrease in *E. coli* adherence when assayed in the presence of the patient's THP. When the individual samples were tested for significance, day 1,3 and 7 showed significant decreases in bacterial adherence while all other days were not significantly different from controls.

When patient 3's THP was assayed in the presence of *E. coli,*

the pre-CJC adherence rate was 17.62 *E. coli* per cell. For the first three weeks of CJC ingestion, the mean *E. coli* adherence was 4.98, a decrease of 72%. After three weeks of CJC ingestion, the average adherence rate was 5.78 *E. coli* per cell, a decrease of 67% compared to control. Overall, patient 3's THP responded to CJC treatment with a 69% decrease in bacterial adherence. Every post CJC value showed significantly less bacterial adherence when compared to the pre CJC control.

When patient 5's pre-CJC THP was assayed in the presence of *E. coli,* the control adherence rate was 13.76 bacteria per cell. Within three weeks of CJC ingestion, the mean bacterial adherence was 13.87 *E. coli* per cell. This value is not significantly different from the control. From day 28 to day 77, however, the bacterial adherence dropped to an average of 9.2 *E. coli* per cell. This indicates a 33% decrease in bacterial adherence from week 4 to week 12 of CJC ingestion. Using a probability alpha value of 0.05, days 3, 35, 42, 49, 63, 70 and 77 showed significant decreases in bacterial adherence while the other days showed no significant difference.

Patient 7's control rate of *E. coli* adherence to uroepithelial cells assayed in the presence of the patient's THP before CJC ingestion was 9.22 bacteria per cell. Within three weeks of CJC ingestion, the mean bacterial adherence fell to 6.13 *E. coli* per cell, a decrease of 33% in bacterial adherence. After 3 weeks of CJC ingestion, average bacterial adherence fell to 6.08 *E. coli* per cell. This demonstrates a 34% decrease in bacterial adherence in

post CJC THP compared to pre CJC THP. All days after CJC ingestion had significantly less adherent bacteria compared to controls except for day 42 which showed no significant difference.

For each patient, the original infective isolate's bacterial adherence was compared to the adherence of *E. coli*. For patient 1, CJC treatment resulted in 157% increase in *P. mirabilis* adherence and an 8% decrease in *E. coli* adherence. *E. coli* was the original infective isolate for patient 3 and CJC treatment resulted in a 69% decrease in *E. coli* adherence. Patient 5's original *P. aeruginosa* isolate responded to CJC treatment with a 22% decrease in adherence while *E. coli* adherence decreased 20% after CJC ingestion. Patient 7's *P. aeruginosa* also responded favorably to CJC treatment with a 66% decrease in bacterial adherence. Patient 7's *E. coli* adherence decreased 33% after CJC treatment. Thus, there are differences in individual patients' THP and how that THP responds to CJC treatment.

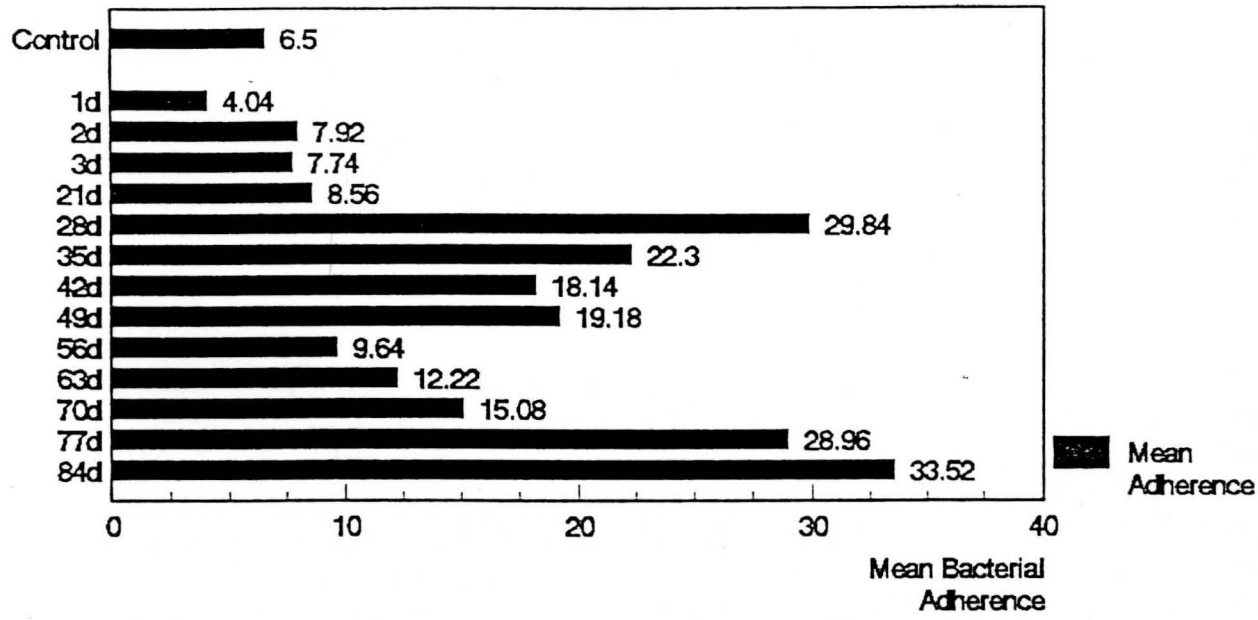
To compare the effectiveness of THP vs whole urine, data from Patrick Wilson's masters thesis [56] was used. Wilson determined the bacterial adherence properties of the whole urine of patients 1,3,5 and 7 both before and after CJC ingestion. To determine the effectiveness of THP versus whole urine, the percent change in bacterial adherence for THP compared to whole urine was calculated both before and after CJC ingestion. For patient 1, infected with *P. mirabilis,* THP had 61% of the bacterial adherence found with whole urine before CJC ingestion, but THP had 121% bacterial adherence compared to whole urine after CJC treatment. Patient 3,

on the other hand, infected with *E. coli*, THP had a higher (134%) bacterial adherence before CJC treatment than whole urine and a lower bacterial adherence after CJC ingestion. Patients 5 and 7, both infected with *P. aeruginosa*, show a similar pattern. The THP from patients 5 and showed a lower adherence (25% and 57% respectively)than whole urine bacterial adherence prior to CJC treatment. Their post CJC bacterial adherence for THP is also lower (51%and 14%) than that of whole urine.

When comparing the original bacterial isolate's bacterial adherence to *E. coli*'s bacterial adherence using THP as the reaction medium, CJC treatment results in a decrease in 3 of the 4 patients' original isolates' bacterial adherence. CJC treatment results in a decrease in 4 of the 4 patients' *E. coli* bacterial adherence. CJC treatment of patient 1 results in a 157% increase in *P. mirabilis* adherence and a 8% decrease in *E. coli* adherence. CJC treatment of patient 3's THP results in a 69% decrease in *E. coli* adherence. CJC treatment of patient 5 caused a 22% decrease in *P. aeruginosa* adherence and a 20% decrease in *E. coli* adherence. Patient 7 also benefitted from CJC treatment, demonstrating a 66% decrease in adherence of *P. aeruginosa* and a 33% decrease in *E. coli* adherence. A summary of the comparison of mean bacterial adherence using whole urine and original isolate, THP and original isolate and THP and *E. coli* can be seen in the final table entitles "Mean Bacterial Adherence After CJC Ingestion Compared to Before CJC Ingestion".

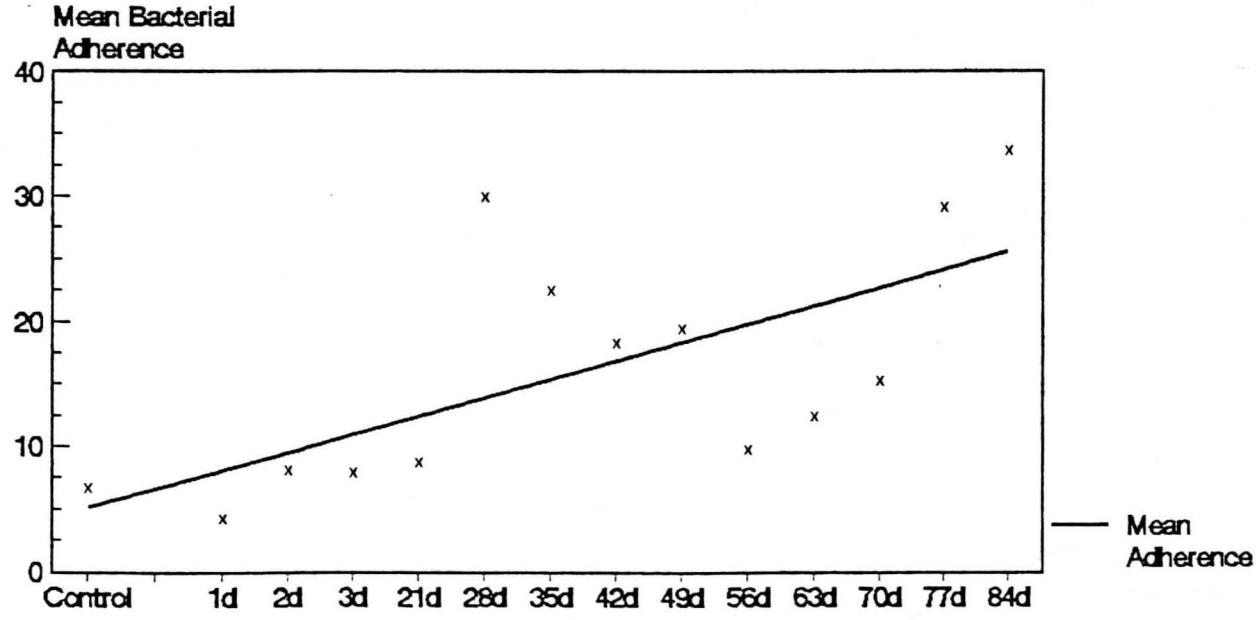
Bacterial adherence data for patient 1. The bacterial isolate is *Proteus mirabilis.* The bar graph presents the mean number of adherent bacteria to each of fifty epithelial cells assayed in the presence of THP extracted from urine at various times after the commencement of cranberry juice ingestion. The horizontal axis refers to the mean number of bacteria adhering to each of fifty uroepithelial cells. The vertical axis refers to the duration of time after commencement of CJC ingestion that the THP was extracted fron the urine. The control represents the mean bacterial adherence to 50 uroepithelial cells assayed in the presence of THP extracted from urine 1 week prior to cranberry juice ingestion. Other THP specimens were collected at the amount of time indicated following the commencement of CJC ingestion. The second plot demonstrates the bacterial adherence trend throughout the study. The control represents THP collected prior to CJC ingestion . All other specimens were collected at the indicated time after commencement of CJC ingestion.

Patient 1: Mean Proteus mirabilis Adherence to Uroepithelia



KeyChart 2000

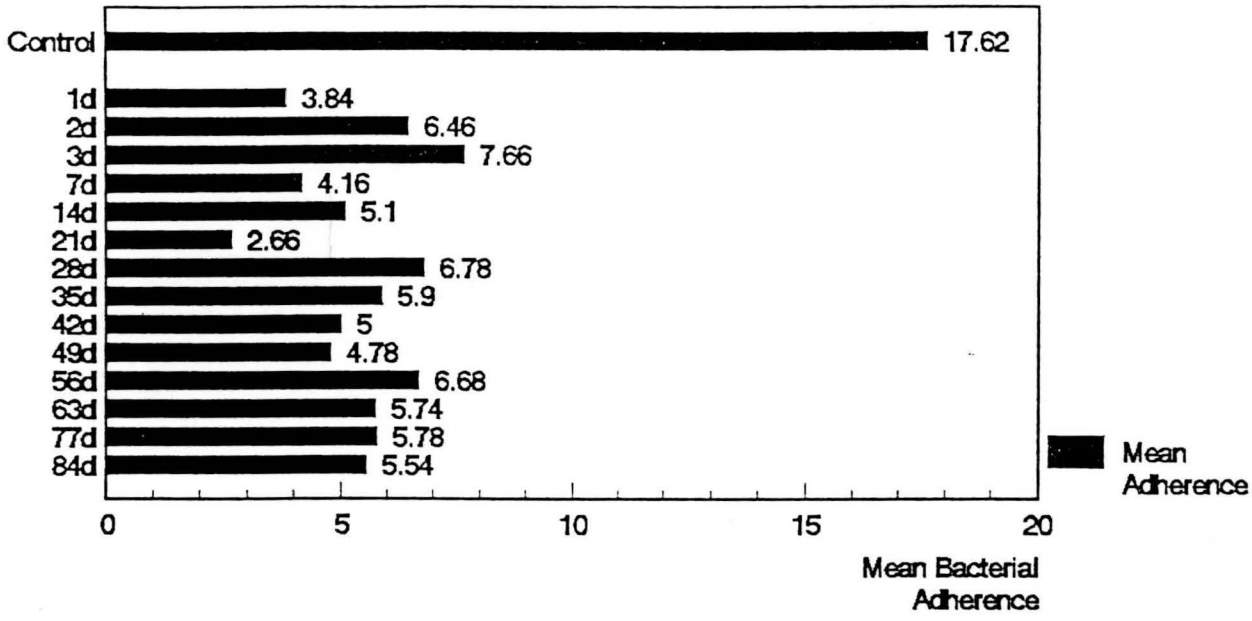
Patient 1: Mean Proteus mirabilis Adherence to Uroepithelia



KeyChart 2000

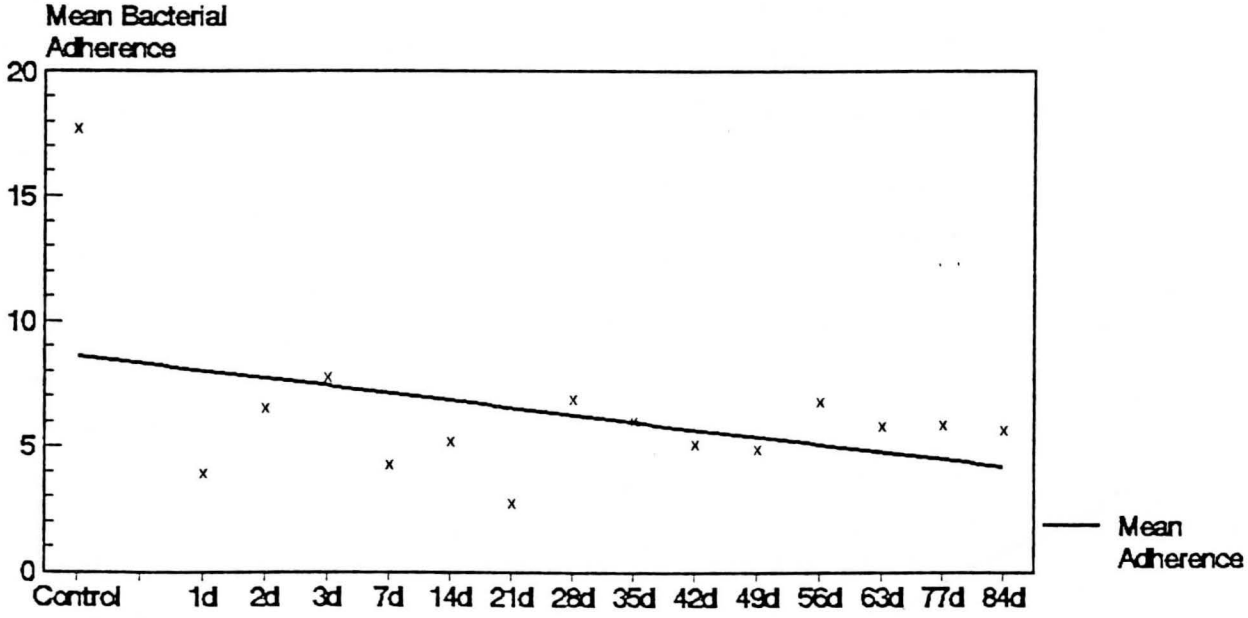
Bacterial adherence data for patient 3. The bacterial isolate is *Escherichia coli.* The bar graph presents the mean number of adherent bacteria to each of fifty epithelial cells assayed in the presence of THP extracted from urine at various times after the commencement of cranberry juice ingestion. The horizontal axis refers to the mean number of bacteria adhering to each of fifty uroepithelial cells. The vertical axis refers to the duration of time after commencement of CJC ingestion that the THP was extracted fron the urine. The control represents the mean bacterial adherence to 50 uroepithelial cells assayed in the presence of THP extracted from urine 1 week prior to cranberry juice ingestion. Other THP specimens were collected at the amount of time indicated following the commencement of CJC ingestion. The second plot demonstrates the bacterial adherence trend throughout the study. The control represents THP collected prior to CJC ingestion . All other specimens were collected at the indicated time after commencement of CJC ingestion.

Patient 3: Mean Escherichia coli Adherence to Uroepithelia



KeyChart 2000

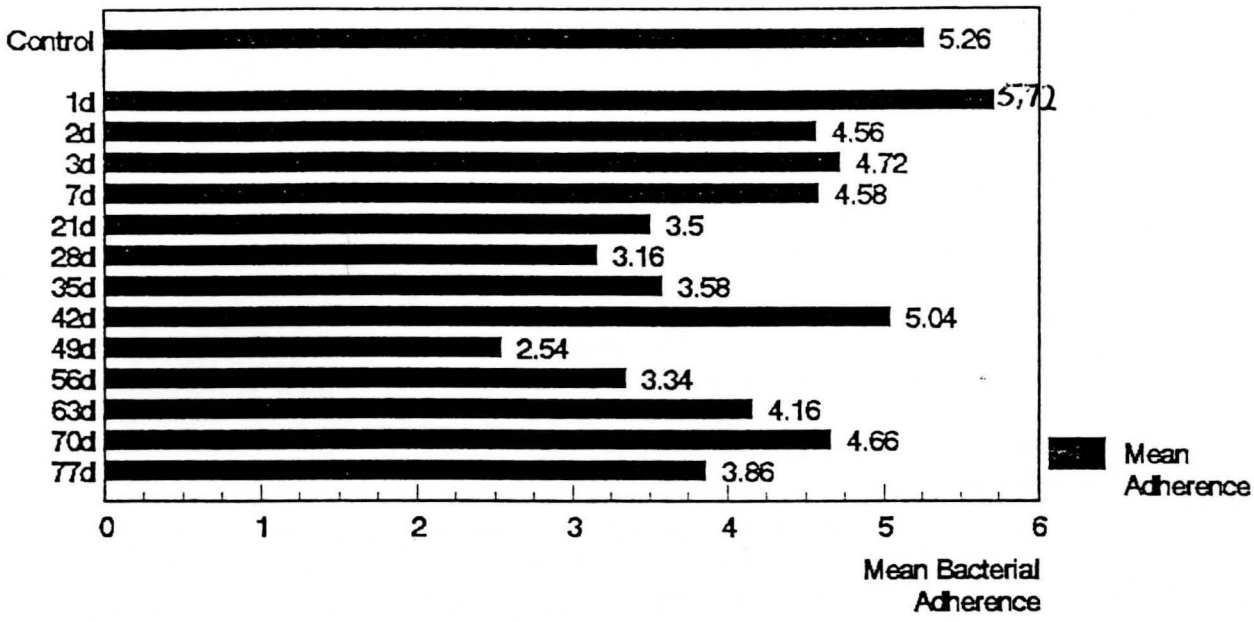
Patient 3: Mean Escherichia coli Adherence to Uroepithelia



KeyChart 2000

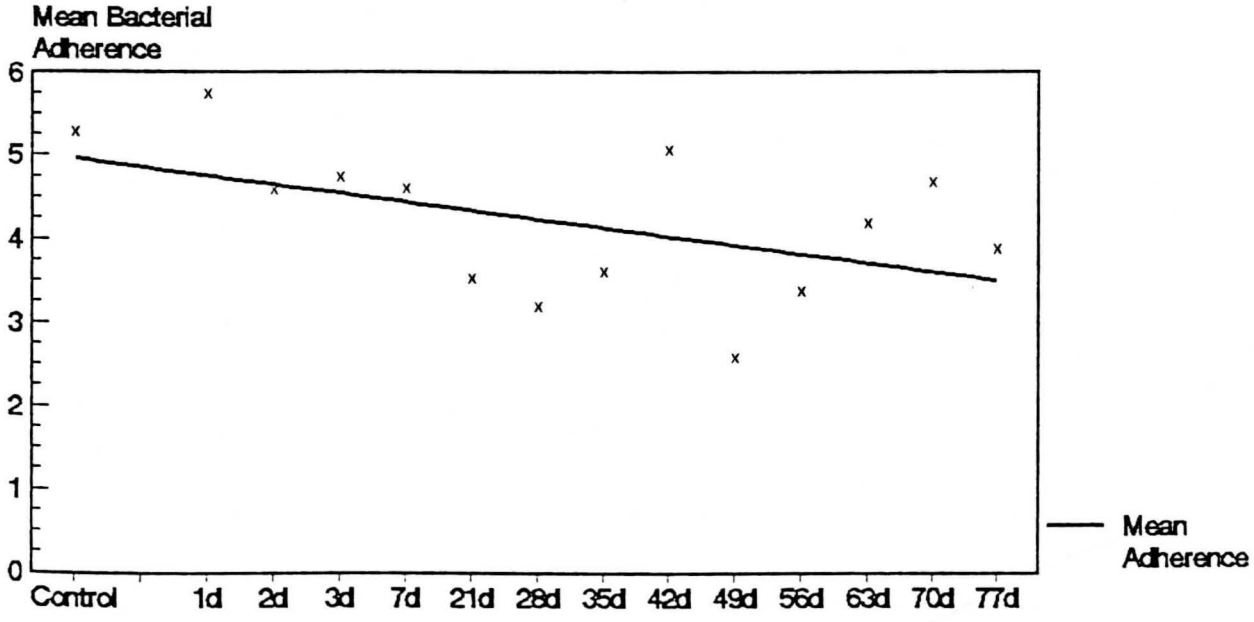
Bacterial adherence data for patient 5. The bacterial isolate is *Pseudomonas aeruginosa*. The bar graph presents the mean number of adherent bacteria to each of fifty epithelial cells assayed in the presence of THP extracted from urine at various times after the commencement of cranberry juice ingestion. The horizontal axis refers to the mean number of bacteria adhering to each of fifty uroepithelial cells. The vertical axis refers to the duration of time after commencement of CJC ingestion that the THP was extracted fron the urine. The control represents the mean bacterial adherence to 50 uroepithelial cells assayed in the presence of THP extracted from urine 1 week prior to cranberry juice ingestion. Other THP specimens were collected at the amount of time indicated following the commencement of CJC ingestion. The second plot demonstrates the bacterial adherence trend throughout the study. The control represents THP collected prior to CJC ingestion . All other specimens were collected at the indicated time after commencement of CJC ingestion.

Patient 5: Mean Pseudomonas aeruginosa Adherence to Uroepithelia



KeyChart 2000

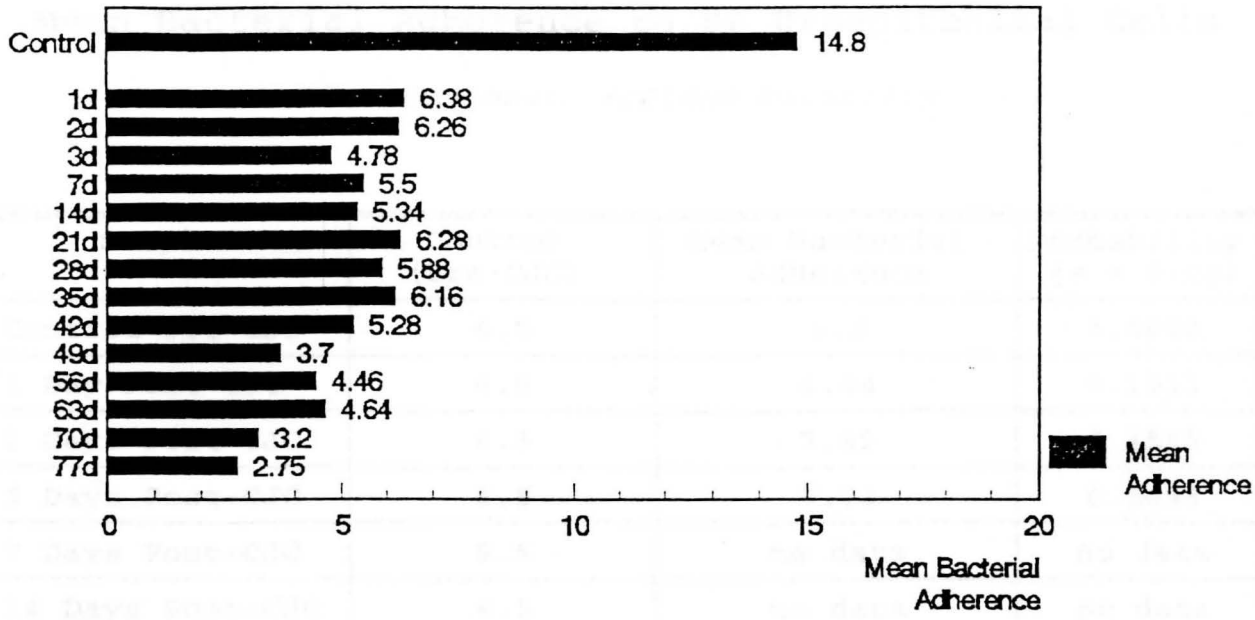
Patient 5: Mean Pseudomonas aeruginosa Adherence to Uroepithelia



KeyChart 2000

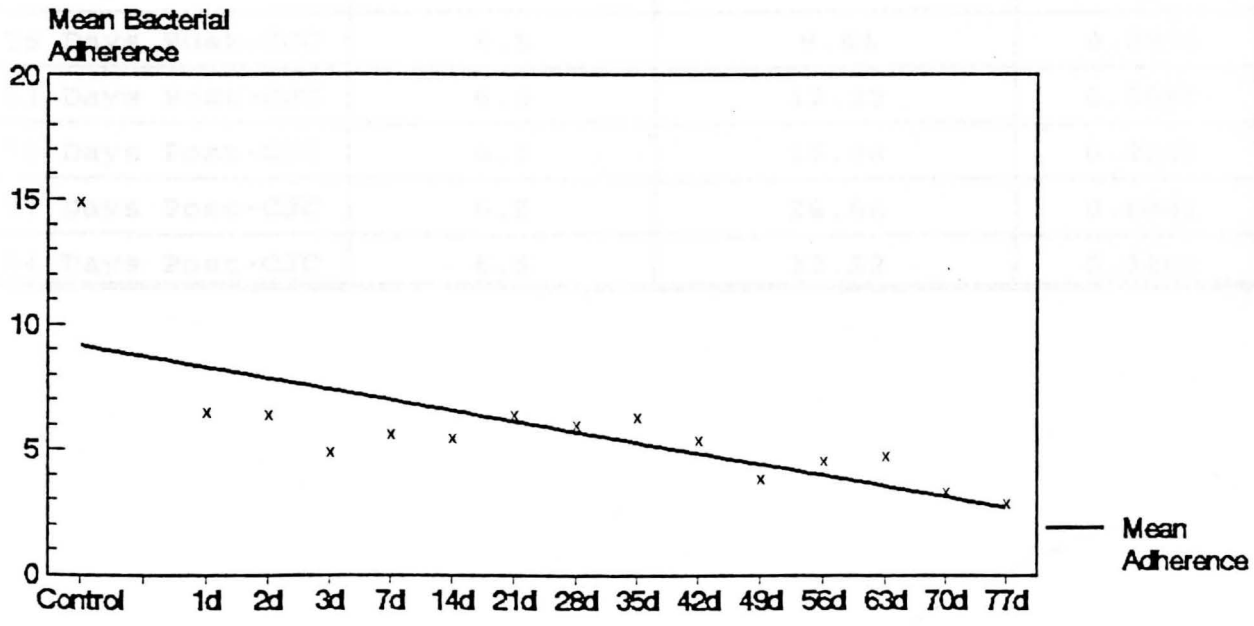
Bacterial adherence data for patient 7. The bacterial isolate is *Pseudomonas aeruginosa.* The bar graph presents the mean number of adherent bacteria to each of fifty epithelial cells assayed in the presence of THP extracted from urine at various times after the commencement of cranberry juice ingestion. The horizontal axis refers to the mean number of bacteria adhering to each of fifty uroepithelial cells. The vertical axis refers to the duration of time after commencement of CJC ingestion that the THP was extracted fron the urine. The control represents the mean bacterial adherence to 50 uroepithelial cells assayed in the presence of THP extracted from urine 1 week prior to cranberry juice ingestion. Other THP specimens were collected at the amount of time indicated following the commencement of CJC ingestion. The second plot demonstrates the bacterial adherence trend throughout the study. The control represents THP collected prior to CJC ingestion . All other specimens were collected at the indicated time after commencement of CJC ingestion.

Patient 7: Mean Pseudomonas aeruginosa Adherence to Uroepithelia



KeyChart 2000

Patient 7: Mean Pseudomonas aeruginosa Adherence to Uroepithelia



KeyChart 2000

Mean Bacterial Adherence to 50 Uroepithelial Cells

Patient one: *Proteus mirabilis*

Sample:	Control (Pre-CJC)	Mean Bacterial Adherence	Probability ($\propto$ = 0.05)
Control Pre-CJC	6.5	6.5	1.0000
1 Day Post-CJC	6.5	4.04	0.1913
2 Days Post-CJC	6.5	7.92	0.4505
3 Days Post-CJC	6.5	7.74	0.5099
7 Days Post-CJC	6.5	no data	no data
14 Days Post-CJC	6.5	no data	no data
21 Days Post-CJC	6.5	8.56	0.2738
28 Days Post-CJC	6.5	29.84	0.0001
35 Days Post-CJC	6.5	22.3	0.0001
42 Days Post-CJC	6.5	18.14	0.0001
49 Days Post-CJC	6.5	19.18	0.0001
56 Days Post-CJC	6.5	9.64	0.0955
63 Days Post-CJC	6.5	12.22	0.0085
70 Days Post-CJC	6.5	15.08	0.0001
77 Days Post-CJC	6.5	28.96	0.0001
84 Days Post-CJC	6.5	33.52	0.0001

Mean Bacterial Adherence to 50 Uroepithelial Cells

Patient three: *Escherichia coli*

Sample:	*Control (Pre-CJC)*	*Mean Bacterial Adherence*	*Probability (α = 0.05)*
Control Pre-CJC	*17.62*	*17.62*	*1.0000*
1 Day Post-CJC	*17.62*	*3.84*	*0.0001*
2 Days Post-CJC	*17.62*	*6.46*	*0.0001*
3 Days Post-CJC	*17.62*	*7.66*	*0.0001*
7 Days Post-CJC	*17.62*	*4.16*	*0.0001*
14 Days Post-CJC	*17.62*	*5.1*	*0.0001*
21 Days Post-CJC	*17.62*	*2.66*	*0.0001*
28 Days Post-CJC	*17.62*	*6.78*	*0.0001*
35 Days Post-CJC	*17.62*	*5.9*	*0.0001*
42 Days Post-CJC	*17.62*	*5.00*	*0.0001*
49 Days Post-CJC	*17.62*	*4.78*	*0.0001*
56 Days Post-CJC	*17.62*	*6.68*	*0.0001*
63 Days Post-CJC	*17.62*	*5.74*	*0.0001*
70 Days Post-CJC	*17.62*	*no data*	*no data*
77 Days Post-CJC	*17.62*	*5.78*	*0.0001*
84 Days Post-CJC	*17.62*	*5.54*	*0.0001*

Mean Bacterial Adherence to 50 Uroepithelial Cells

Patient five: *Pseudomonas aeruginosa*

Sample:	Control (Pre-CJC)	Mean Bacterial Adherence	Probability ($\propto$ = 0.05)
Control Pre-CJC	5.26	5.26	1.0000
1 Day Post-CJC	5.26	5.72	0.4829
2 Days Post-CJC	5.26	4.56	0.2858
3 Days Post-CJC	5.26	4.72	0.4102
7 Days Post-CJC	5.26	4.58	0.2998
14 Days Post-CJC	5.26	no data	no data
21 Days Post-CJC	5.26	3.5	0.0074
28 Days Post-CJC	5.26	3.16	0.0014
35 Days Post-CJC	5.26	3.58	0.0106
42 Days Post-CJC	5.26	5.04	0.7372
49 Days Post-CJC	5.26	2.54	0.0001
56 Days Post-CJC	5.26	3.34	0.0130
63 Days Post-CJC	5.26	4.16	0.1411
70 Days Post-CJC	5.26	4.66	0.3957
77 Days Post-CJC	5.26	3.86	0.0652
84 Days Post-CJC	5.26	no data	no data

Mean Bacterial Adherence to 50 Uroepithelial Cells

Patient seven: *Pseudomonas aeruginosa*

Sample:	Control (Pre-CJC)	Mean Bacterial Adherence	Probability ($\propto$ = 0.05)
Control Pre-CJC	14.8	14.8	1.0000
1 Day Post-CJC	14.8	6.38	0.0001
2 Days Post-CJC	14.8	6.26	0.0001
3 Days Post-CJC	14.8	4.78	0.0001
7 Days Post-CJC	14.8	5.5	0.0001
14 Days Post-CJC	14.8	5.34	0.0001
21 Days Post-CJC	14.8	6.28	0.0001
28 Days Post-CJC	14.8	5.88	0.0001
35 Days Post-CJC	14.8	6.16	0.0001
42 Days Post-CJC	14.8	5.28	0.0001
49 Days Post-CJC	14.8	3.7	0.0001
56 Days Post-CJC	14.8	4.46	0.0001
63 Days Post-CJC	14.8	4.64	0.0001
70 Days Post-CJC	14.8	3.2	0.0001
77 Days Post-CJC	14.8	2.75	0.0001
84 Days Post-CJC	14.8	no data	no data

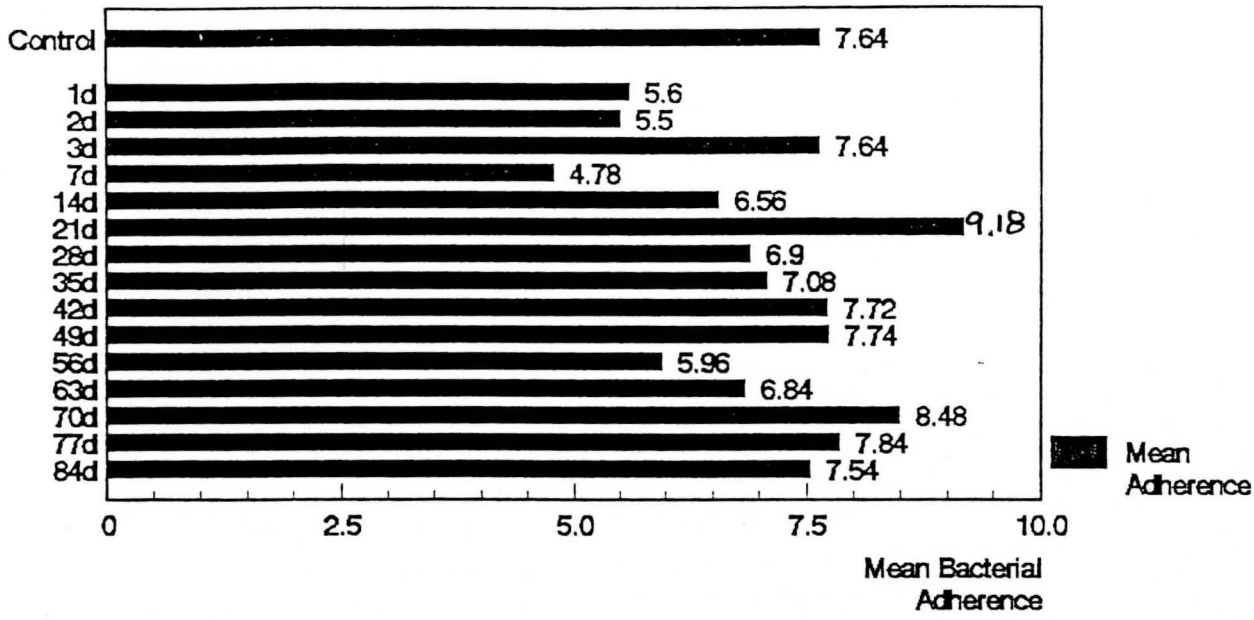
Bacterial Adherence of Original Isolates

Assayed with Patient THP

Patient THP	Bacterial Isolate	Pre CJC Adherence	1-21d Post CJC	27-84d Post CJC
Patient 1	*P.mirabilis*	6.5	12.56	20.9
Patient 3	*E. coli*	17.62	4.98	5.78
Patient 5	*P.aeruginosa*	5.26	4.61	3.79
Patient 7	*P.aeruginosa*	14.8	5.75	4.5

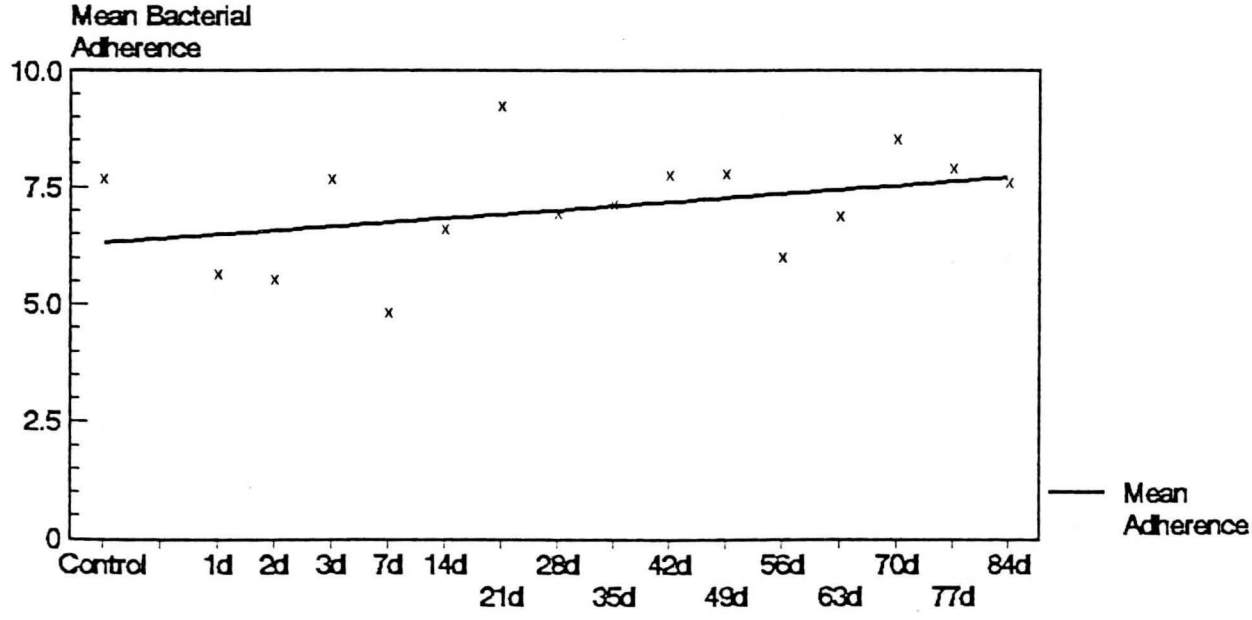
Bacterial adherence data for patient 1. The bacterial isolate is *Escherichia coli.* The bar graph presents the mean number of adherent bacteria to each of fifty epithelial cells assayed in the presence of THP extracted from urine at various times after the commencement of cranberry juice ingestion. The horizontal axis refers to the mean number of bacteria adhering to each of fifty uroepithelial cells. The vertical axis refers to the duration of time after commencement of CJC ingestion that the THP was extracted fron the urine. The control represents the mean bacterial adherence to 50 uroepithelial cells assayed in the presence of THP extracted from urine 1 week prior to cranberry juice ingestion. Other THP specimens were collected at the amount of time indicated following the commencement of CJC ingestion. The second plot demonstrates the bacterial adherence trend throughout the study. The control represents THP collected prior to CJC ingestion . All other specimens were collected at the indicated time after commencement of CJC ingestion.

Patient 1: Mean Escherichia coli Adherence to Uroepithelia



KeyChart 2000

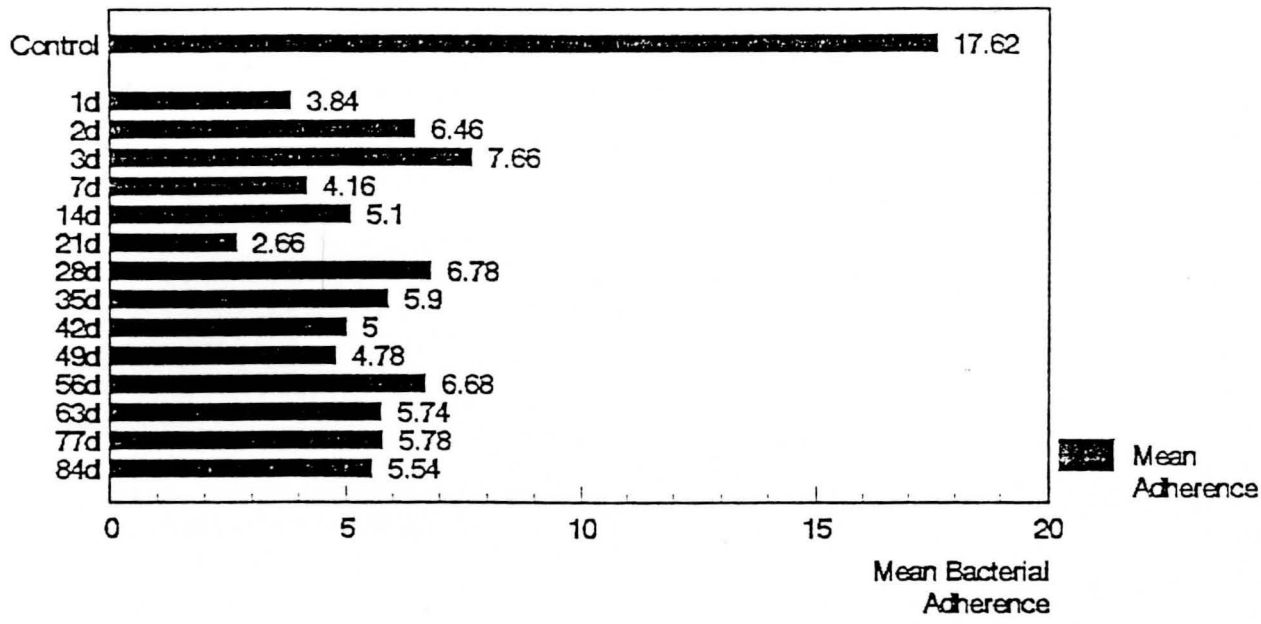
Patient 1: Mean Escherichia coli Adherence to Uroepithelia



KeyChart 2000

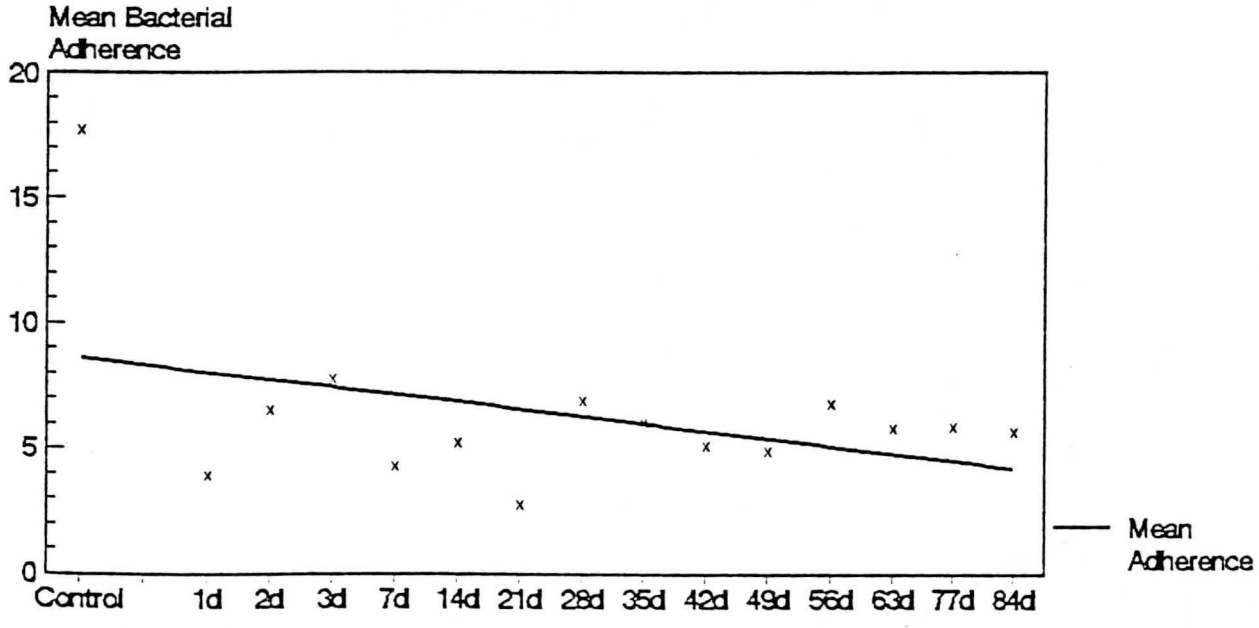
Bacterial adherence data for patient 3. The bacterial isolate is *Escherichia coli.* The bar graph presents the mean number of adherent bacteria to each of fifty epithelial cells assayed in the presence of THP extracted from urine at various times after the commencement of cranberry juice ingestion. The horizontal axis refers to the mean number of bacteria adhering to each of fifty uroepithelial cells. The vertical axis refers to the duration of time after commencement of CJC ingestion that the THP was extracted fron the urine. The control represents the mean bacterial adherence to 50 uroepithelial cells assayed in the presence of THP extracted from urine 1 week prior to cranberry juice ingestion. Other THP specimens were collected at the amount of time indicated following the commencement of CJC ingestion. The second plot demonstrates the bacterial adherence trend throughout the study. The control represents THP collected prior to CJC ingestion . All other specimens were collected at the indicated time after commencement of CJC ingestion.

Patient 3: Mean Escherichia coli Adherence to Uroepithelia



KeyChart 2000

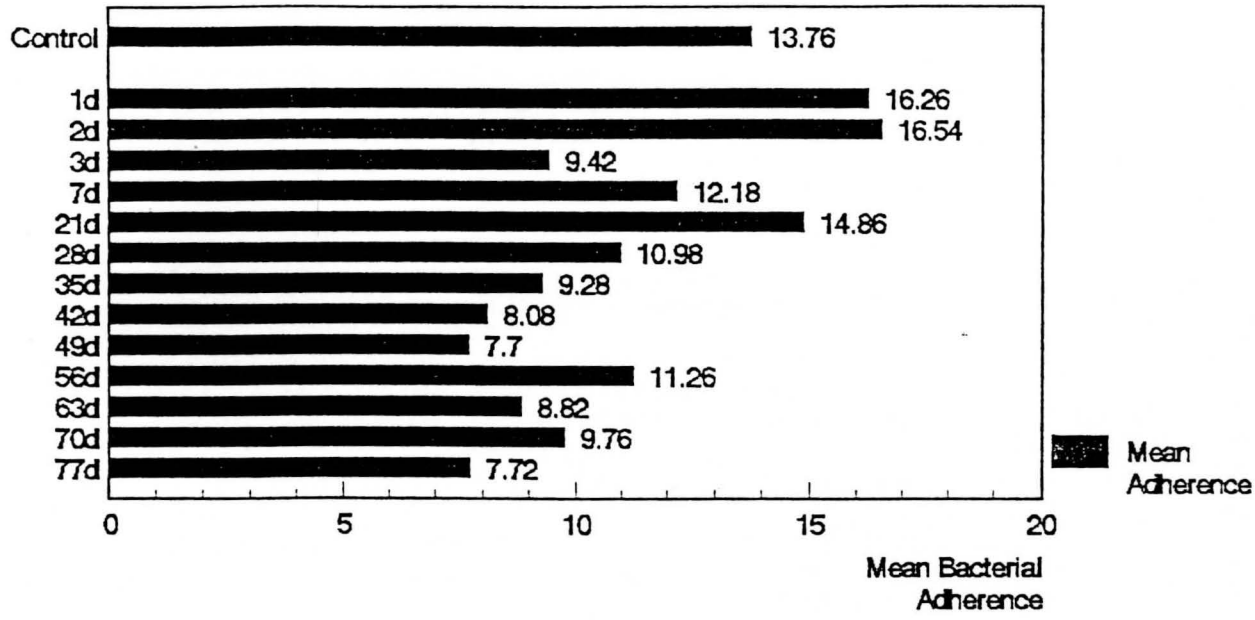
Patient 3: Mean Escherichia coli Adherence to Uroepithelia



KeyChart 2000

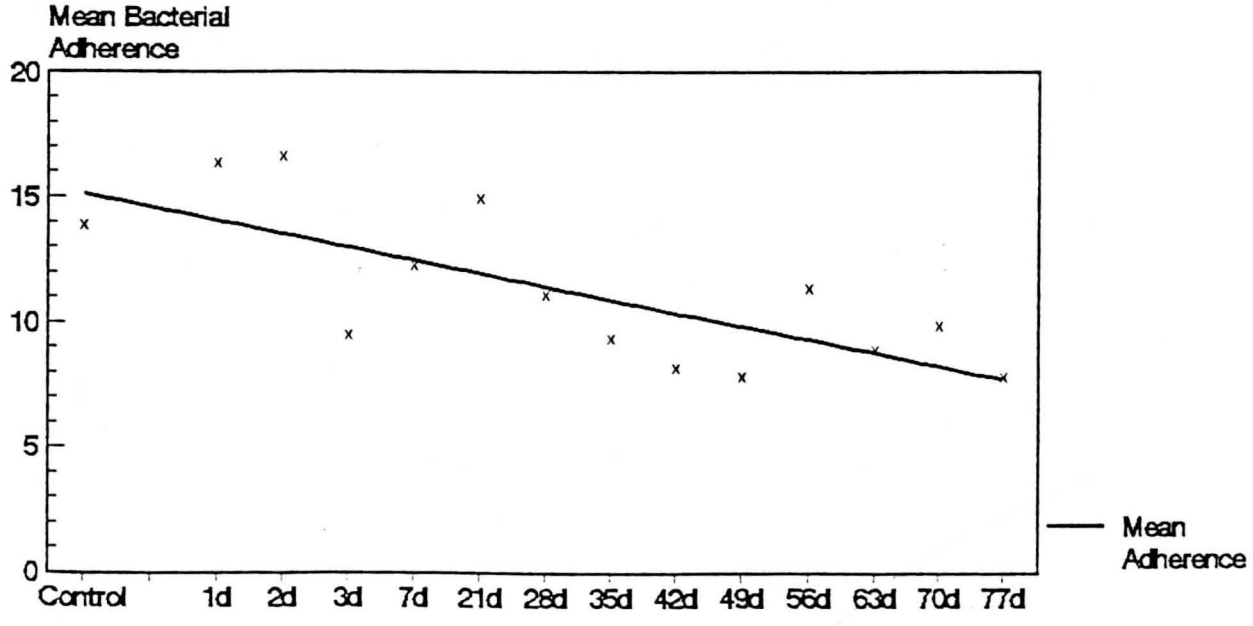
Bacterial adherence data for patient 5. The bacterial isolate is *Escherichia coli.* The bar graph presents the mean number of adherent bacteria to each of fifty epithelial cells assayed in the presence of THP extracted from urine at various times after the commencement of cranberry juice ingestion. The horizontal axis refers to the mean number of bacteria adhering to each of fifty uroepithelial cells. The vertical axis refers to the duration of time after commencement of CJC ingestion that the THP was extracted fron the urine. The control represents the mean bacterial adherence to 50 uroepithelial cells assayed in the presence of THP extracted from urine 1 week prior to cranberry juice ingestion. Other THP specimens were collected at the amount of time indicated following the commencement of CJC ingestion. The second plot demonstrates the bacterial adherence trend throughout the study. The control represents THP collected prior to CJC ingestion . All other specimens were collected at the indicated time after commencement of CJC ingestion.

Patient 5: Mean Escherichia coli Adherence to Uroepithelia



KeyChart 2000

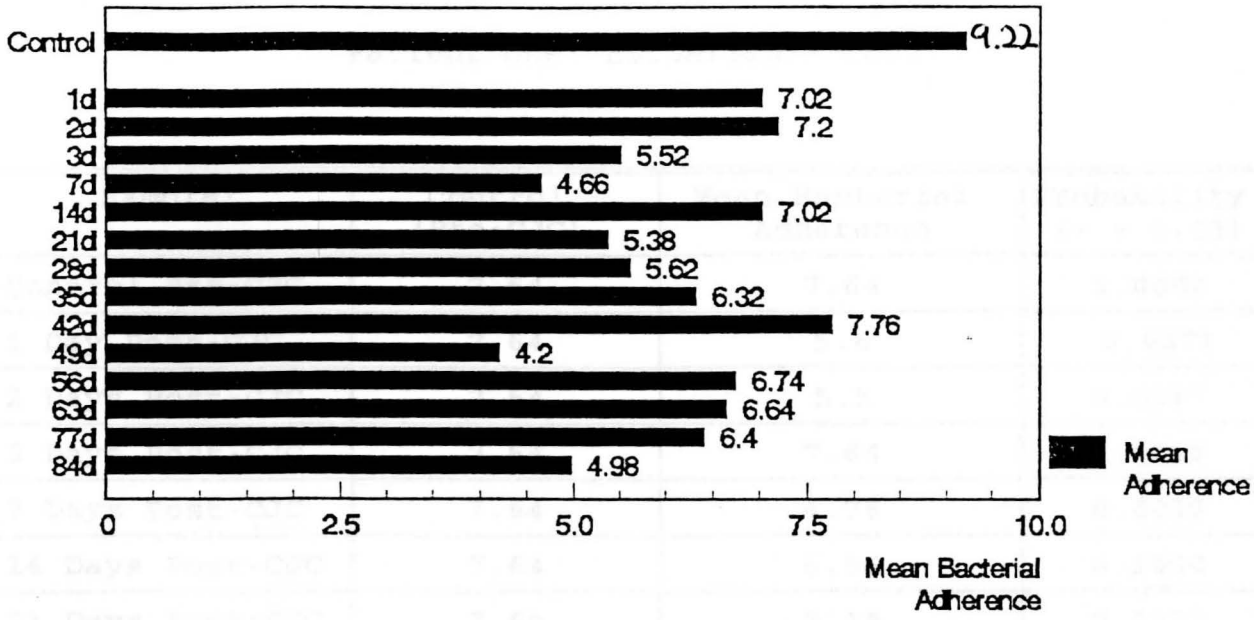
Patient 5: Mean Escherichia coli Adherence to Uroepithelia



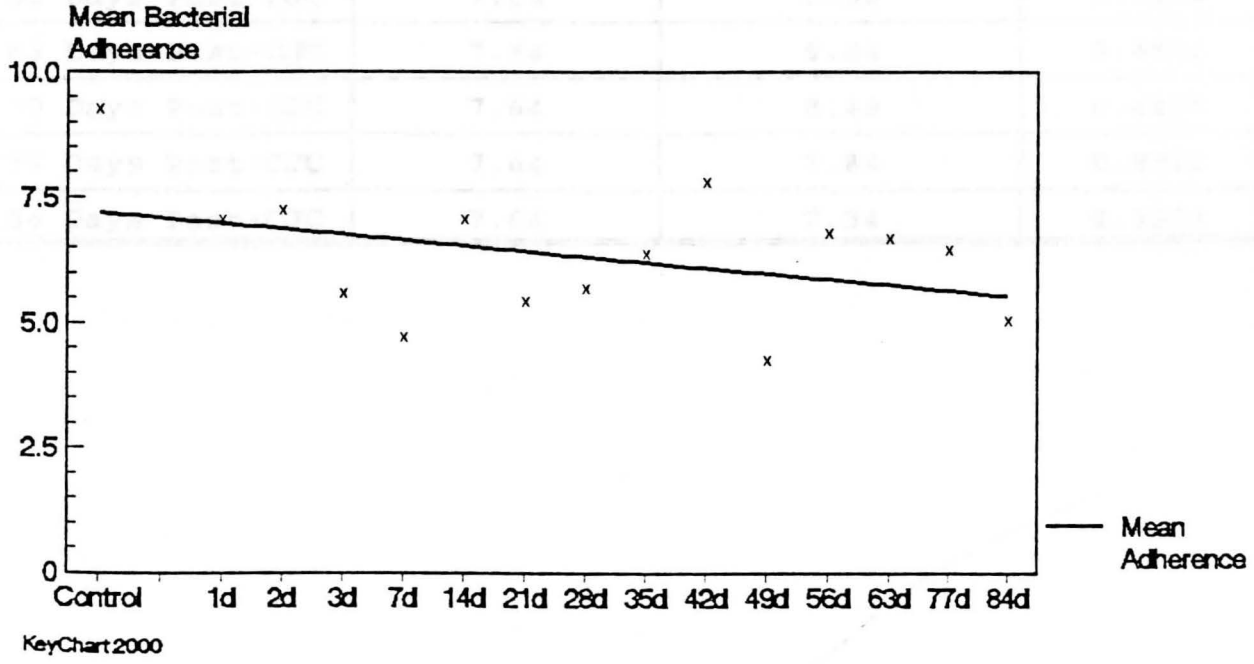
KeyChart 2000

Bacterial adherence data for patient 7. The bacterial isolate is *Escherichia coli.* The bar graph presents the mean number of adherent bacteria to each of fifty epithelial cells assayed in the presence of THP extracted from urine at various times after the commencement of cranberry juice ingestion. The horizontal axis refers to the mean number of bacteria adhering to each of fifty uroepithelial cells. The vertical axis refers to the duration of time after commencement of CJC ingestion that the THP was extracted fron the urine. The control represents the mean bacterial adherence to 50 uroepithelial cells assayed in the presence of THP extracted from urine 1 week prior to cranberry juice ingestion. Other THP specimens were collected at the amount of time indicated following the commencement of CJC ingestion. The second plot demonstrates the bacterial adherence trend throughout the study. The control represents THP collected prior to CJC ingestion . All other specimens were collected at the indicated time after commencement of CJC ingestion.

Patient 7: Mean Escherichia coli Adherence to Uroepithelia



Patient 7: Mean Escherichia coli Adherence to Uroepithelia



Mean Bacterial Adherence to 50 Uroepithelial Cells

Patient one: *Escherichia coli*

Sample:	Control (Pre-CJC)	Mean Bacterial Adherence	Probability ($\propto$ = 0.05)
Control Pre-CJC	7.64	7.64	1.0000
1 Day Post-CJC	7.64	5.6	0.0371
2 Days Post-CJC	7.64	5.5	0.0287
3 Days Post-CJC	7.64	7.64	1.0000
7 Days Post-CJC	7.64	4.78	0.0035
14 Days Post-CJC	7.64	6.56	0.2690
21 Days Post-CJC	7.64	9.18	0.1152
28 Days Post-CJC	7.64	6.9	0.4487
35 Days Post-CJC	7.64	7.08	0.5664
42 Days Post-CJC	7.64	7.72	0.9347
49 Days Post-CJC	7.64	7.74	0.9275
56 Days Post-CJC	7.64	5.96	0.1292
63 Days Post-CJC	7.64	6.84	0.4694
70 Days Post-CJC	7.64	8.48	0.4475
77 Days Post-CJC	7.64	7.84	0.8564
84 Days Post-CJC	7.64	7.54	0.9279

Mean Bacterial Adherence to 50 Uroepithelial Cells

Patient three: *Escherichia coli*

Sample:	*Control (Pre-CJC)*	*Mean Bacterial Adherence*	*Probability (α = 0.05)*
Control Pre-CJC	*17.62*	*17.62*	*1.0000*
1 Day Post-CJC	*17.62*	*3.84*	*0.0001*
2 Days Post-CJC	*17.62*	*6.46*	*0.0001*
3 Days Post-CJC	*17.62*	*7.66*	*0.0001*
7 Days Post-CJC	*17.62*	*4.16*	*0.0001*
14 Days Post-CJC	*17.62*	*5.1*	*0.0001*
21 Days Post-CJC	*17.62*	*2.66*	*0.0001*
28 Days Post-CJC	*17.62*	*6.78*	*0.0001*
35 Days Post-CJC	*17.62*	*5.9*	*0.0001*
42 Days Post-CJC	*17.62*	*5.00*	*0.0001*
49 Days Post-CJC	*17.62*	*4.78*	*0.0001*
56 Days Post-CJC	*17.62*	*6.68*	*0.0001*
63 Days Post-CJC	*17.62*	*5.74*	*0.0001*
70 Days Post-CJC	*17.62*	*no data*	*no data*
77 Days Post-CJC	*17.62*	*5.78*	*0.0001*
84 Days Post-CJC	*17.62*	*5.54*	*0.0001*

Mean Bacterial Adherence to 50 Uroepithelial Cells

Patient five: *Escherichia coli*

Sample:	Control (Pre-CJC)	Mean Bacterial Adherence	Probability ($\propto$ = 0.05)
Control Pre-CJC	13.76	13.76	1.0000
1 Day Post-CJC	13.76	16.26	0.2216
2 Days Post-CJC	13.76	16.54	0.1741
3 Days Post-CJC	13.76	9.42	0.0341
7 Days Post-CJC	13.76	12.18	0.4396
14 Days Post-CJC	13.76	no data	no data
21 Days Post-CJC	13.76	14.86	0.5905
28 Days Post-CJC	13.76	10.98	0.1741
35 Days Post-CJC	13.76	9.28	0.0287
42 Days Post-CJC	13.76	8.08	0.0056
49 Days Post-CJC	13.76	7.7	0.0031
56 Days Post-CJC	13.76	11.26	0.2020
63 Days Post-CJC	13.76	8.82	0.0111
70 Days Post-CJC	13.76	9.76	0.0399
77 Days Post-CJC	13.76	7.72	0.0019
84 Days Post-CJC	13.76	no data	no data

Mean Bacterial Adherence to 50 Uroepithelial Cells

Patient seven: *Escherichia coli*

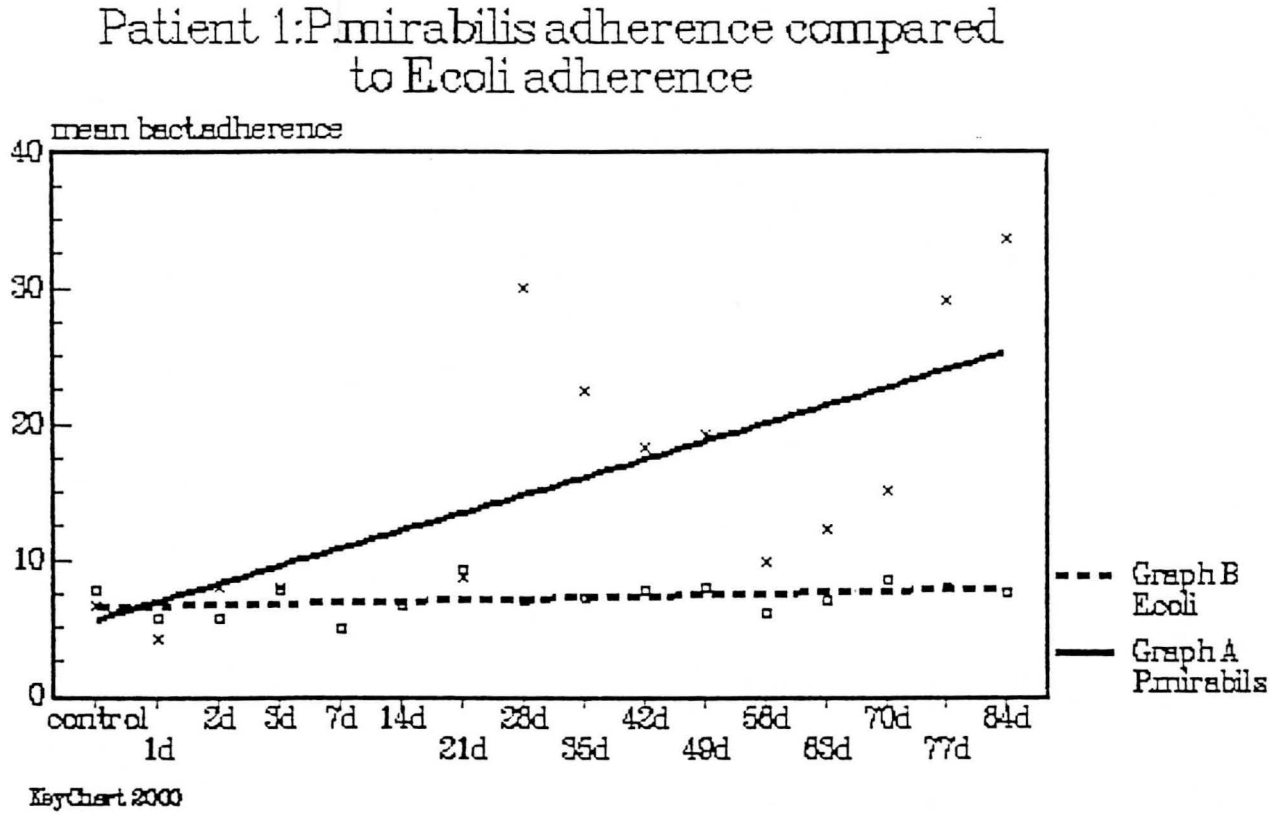
Sample:	Control (Pre-CJC)	Mean Bacterial Adherence	Probability ($\propto$ = 0.05)
Control Pre-CJC	9.22	9.22	1.0000
1 Day Post-CJC	9.22	7.02	0.0175
2 Days Post-CJC	9.22	7.2	0.0291
3 Days Post-CJC	9.22	5.52	0.0001
7 Days Post-CJC	9.22	4.66	0.0001
14 Days Post-CJC	9.22	7.02	0.0175
21 Days Post-CJC	9.22	5.38	0.0001
28 Days Post-CJC	9.22	5.62	0.0001
35 Days Post-CJC	9.22	6.32	0.0018
42 Days Post-CJC	9.22	7.76	0.1143
49 Days Post-CJC	9.22	4.2	0.0001
56 Days Post-CJC	9.22	6.74	0.0009
63 Days Post-CJC	9.22	6.64	0.0006
70 Days Post-CJC	9.22	no data	no data
77 Days Post-CJC	9.22	6.4	0.0002
84 Days Post-CJC	9.22	4.98	0.0001

Bacterial Adherence of E.coli Isolates

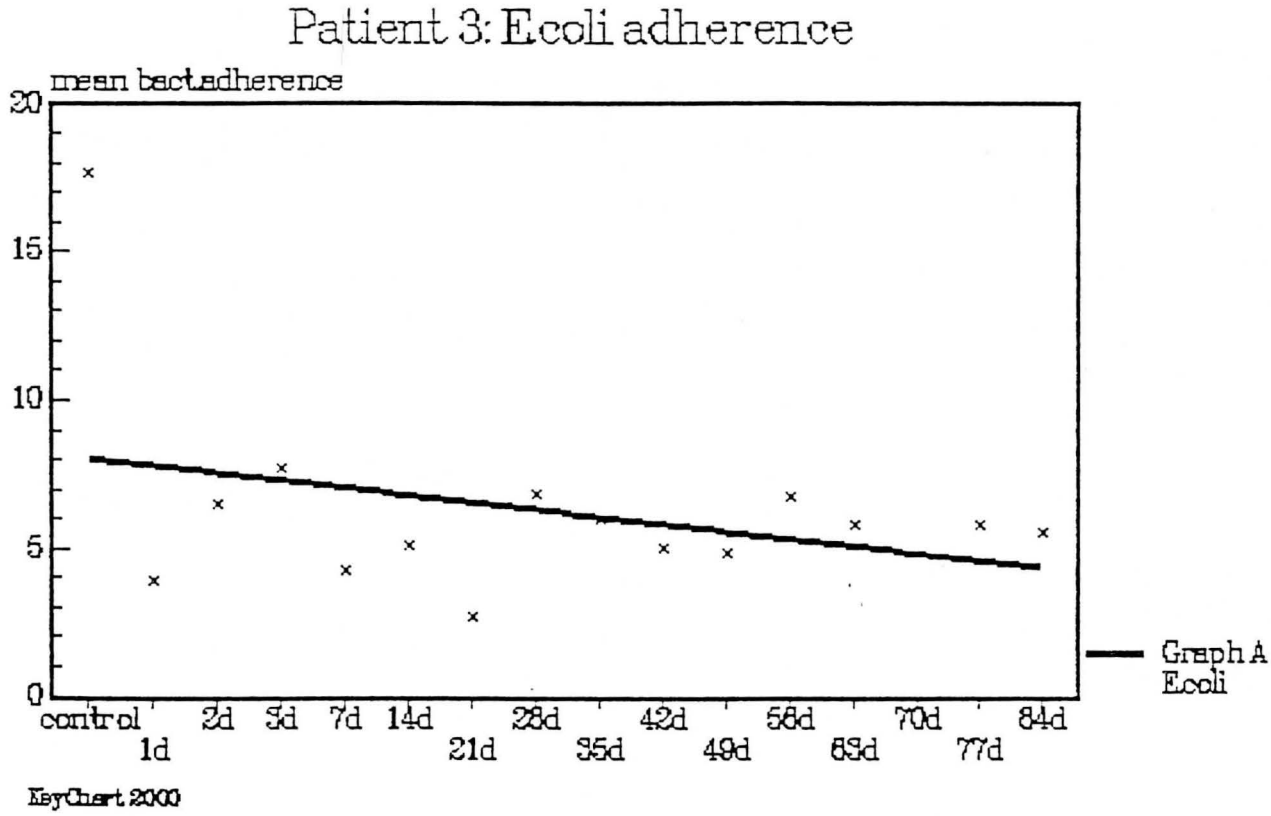
Assayed with Patient THP

Patient THP	Bacterial Isolate	Pre CJC Adherence	1-21d Post CJC	27-84d Post CJC
Patient 1	*E.coli*	7.64	6.47	7.34
Patient 3	*E. coli*	17.62	4.98	5.78
Patient 5	*E.coli*	13.76	13.87	9.20
Patient 7	*E.coli*	9.22	6.13	6.08

Bacterial adherence data for patient 1: The original infective isolate was *Proteus mirabilis*. The comparative bacterial isolate used was *Escherichia coli*. This graph compares the trend of bacterial adherence of the original infective isolate to the bacterial adherence of the comparative isolate. The vertical axis represents the mean number of bacteria adhering to each of fifty uroepithelial cells. The horizontal axis represents the time duration following the commencement of CJC ingestion at which the urine sample was collected. The reaction medium for this assay was the THP extracted from the urine sample.

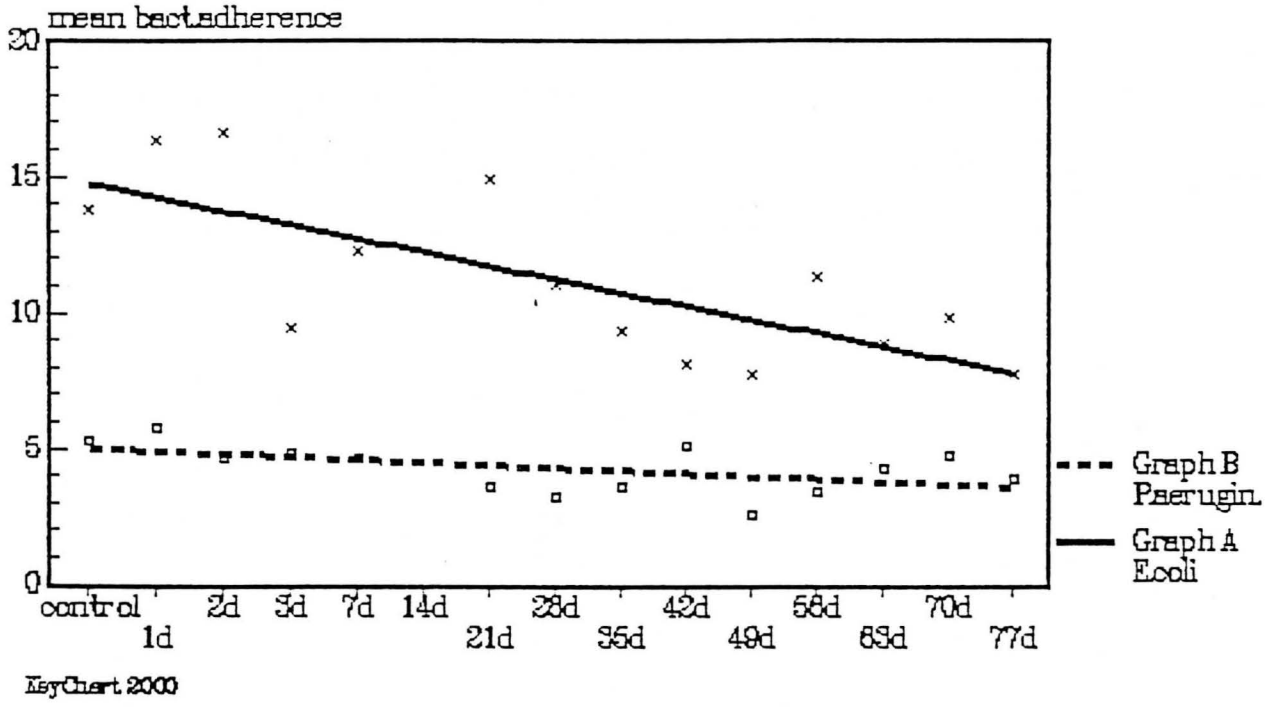

Patient 1:P.mirabilis adherence compared to E.coli adherence
mean bactadherence
40
30
20
10
0
control
1d
2d
3d
7d
14d
21d
28d
35d
42d
49d
56d
63d
70d
77d
84d
Graph B
Ecoli
Graph A
P.mirabils
KeyChart 2000

Bacterial adherence data for patient 3: The original infective isolate was *Escherichia coli.* This graph demonstrates the trend of bacterial adherence of the original infective isolate. The vertical axis represents the mean number of bacteria adhering to each of fifty uroepithelial cells. The horizontal axis represents the time duration following the commencement of CJC ingestion at which the urine sample was collected. The reaction medium for this assay was the THP extracted from the urine sample.

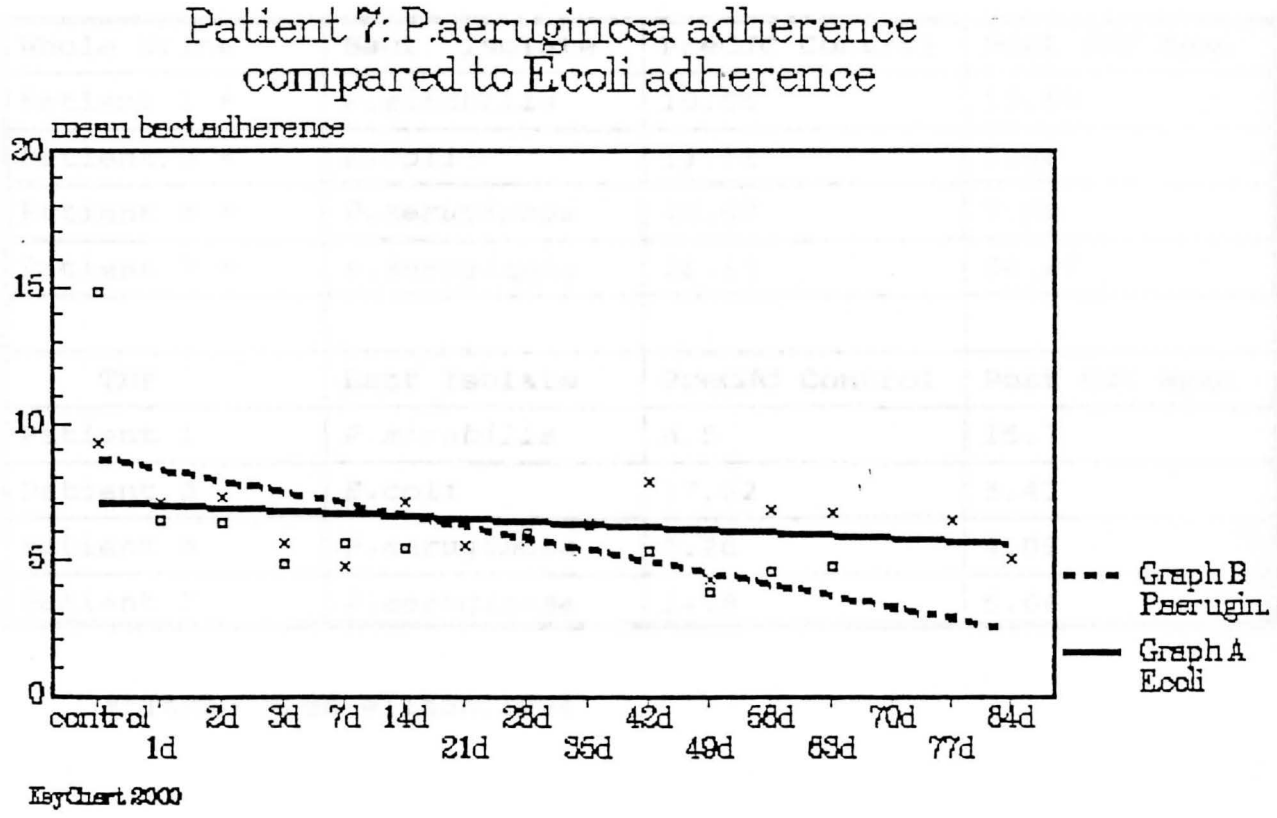

Patient 3: Ecoli adherence
mean bactadherence
20
15
10
5
0
control
1d
2d
3d
7d
14d
21d
28d
35d
42d
49d
56d
63d
70d
77d
84d
Graph A
Ecoli
KeyChart 2000

Bacterial adherence data for patient 5: The original infective isolate was *Pseudomonas aeruginosa.* The comparative bacterial isolate used was *Escherichia coli.* This graph compares the trend of bacterial adherence of the original infective isolate to the bacterial adherence of the comparative isolate. The vertical axis represents the mean number of bacteria adhering to each of fifty uroepithelial cells. The horizontal axis represents the time duration following the commencement of CJC ingestion at which the urine sample was collected. The reaction medium for this assay was the THP extracted from the urine sample.

Patient 5: P.aeruginosa adherence compared to Ecoli adherence



Bacterial adherence data for patient 7: The original infective isolate was *Pseudomonas aeruginosa*. The comparative bacterial isolate used was *Escherichia coli*. This graph compares the trend of bacterial adherence of the original infective isolate to the bacterial adherence of the comparative isolate. The vertical axis represents the mean number of bacteria adhering to each of fifty uroepithelial cells. The horizontal axis represents the time duration following the commencement of CJC ingestion at which the urine sample was collected. The reaction medium for this assay was the THP extracted from the urine sample.


Patient 7: P.aeruginosa adherence compared to Ecoli adherence
mean bact.adherence
20
15
10
5
0
control
1d
2d
3d
7d
14d
21d
28d
35d
42d
49d
56d
63d
70d
77d
84d
Graph B
P.aerugin.
Graph A
Ecoli
EasyChart 2000

Comparison of Whole Urine Bacterial Adherence to THP Bacterial Adherence

Whole Urine	Bact Isolate	PreCJC Control	Post CJC Mean
Patient 1 *	*P.mirabilis*	10.56	13.85
Patient 3 *	*E.coli*	13.11	7.08
Patient 5 *	*P.aeruginosa*	20.69	7.95
Patient 7 *	*P.aeruginosa*	26.13	36.47
THP	Bact Isolate	PreCJC Control	Post CJC Mean
Patient 1	*P.mirabilis*	6.5	16.7
Patient 3	*E.coli*	17.62	5.43
Patient 5	*P.aeruginosa*	5.26	4.09
Patient 7	*P.aeruginosa*	14.8	5.04

* Taken From Wilson,1994

Comparison of Original Bacterial Isolate Adherence to the Bacterial Adherence of *E.coli* Using THP as the Reaction Medium

THP	Bact Isolate	PreCJC Control	Post CJC Mean
Patient 1	*P.mirabilis*	6.5	16.7
Patient 3	*E.coli*	17.62	5.43
Patient 5	*P.aeruginosa*	5.26	4.09
Patient 7	*P.aeruginosa*	14.8	5.04
THP	Bact Isolate	PreCJC Control	Post CJC Mean
Patient 1	*E.coli*	7.64	7.01
Patient 3	*E.coli*	17.62	5.43
Patient 5	*E.coli*	13.76	10.98
Patient 7	*E.coli*	9.22	6.10

Mean Bacterial Adherence After CJC Ingestion Compared to Before CJC Ingestion

A comparison of whole urine (original isolate) to THP(original isolate) to THP (*E. Coli*)

	Whole Urine* (orig.isolate)	THP (orig.isolate)	THP *E.coli*
Patient 1	131%	257%	92%
Patient 3	54%	31%	31%
Patient 5	38%	79%	80%
Patient 7	139%	33%	66%

*Taken From Patrick Wilson ,1994

IV. DISCUSSION

Research in our laboratory has focused on the role of cranberry juice ingestion and the effectiveness of CJC as a possible treatment for UTI. In 1984, Dr. Anthony E. Sobota [48] was the first to suggest that cranberry juice inhibited bacterial adherence. To determine if CJC had the ability to block bacterial adherence, he performed *in vitro* and *in vivo* studies. The *in vitro* study consisted of assaying bacterial adherence of *E. coli* to uroepithelial cells in the presence of CJC. Undiluted cranberry juice resulted in a greater than 97% inhibition of bacterial adherence. By incubating either the cells with CJC or the *E. coli* with CJC, washing and then performing the adherence assay, he determined that CJC acted upon the bacteria and not the epithelial cells. Although these results seemed to show the mechanism of action of CJC *in vitro, in vivo* studies were needed to elucidate the physiological action of CJC. The first *in vivo* study used a murine model in which the CJC replaced the water source. When the murine urine was used in the place of the CJC in the adherence assay, the urine significantly inhibited bacterial adherence when compared to control urines. When the mouse urine from the *in vivo* trial was used in place of CJC from the *in vitro* trial in the adherence assay, the results were not significantly different. Thus, the effectiveness of CJC in mice was proven to decrease bacterial adherence, but a human study was still needed. Twenty-two human subjects volunteered to donate a urine sample, drink 15 oz. of CJC and then donate another urine sample 1-3 hours later.

Fifteen of the twenty-two subjects demonstrated a significant decrease in *E. coli* adherence to uroepithelial cells after ingesting CJC. Sobota concluded that some factor in the juice inhibits bacterial adherence by interfering with a surface component on the *E. coli*.[48] This paper was the first to demonstrate the effectiveness of CJC treatment both *in vitro* and *in vivo* in inhibiting bacterial adherence. At this time, however, there were still many unanswered questions regarding the delivery of the factor to the urinary tract.

In another study in our lab, the antiadherence activity of Tamm-Horsfall Protein (THP) was investigated.[49] When *E. coli*, epithelial cells and saline were incubated, a baseline level of bacterial adherence was achieved. When urine was used as the reaction medium instead of saline, a significant decrease in bacterial adherence was observed. To determine if THP was the antiadherence agent in the urine, the THP was removed from the urine by precipitation with NaCl. When urine without THP was used in the adherence assay, there was a significant increase in bacterial adherence compared to whole urine, but no significant difference compared to saline controls. The addition of THP to the adherence assay at concentrations greater than 35 ug/mL significantly reduced the number of adherent *E. coli*. Thus, Sobota and Apicella concluded that at the concentrations present in normal urine, THP acts as an anti-adherence agent and may help prevent UTI caused by type 1 fimbriated *E. coli*.[49]

A third study addressed the effectiveness of CJC treatment

for UTI in an elderly population. Patrick Wilson [56] initiated a study in which 4 oz of CJC was administered daily to 8 members of a high UTI risk population for a period of 12 weeks. The 8 elderly females were nursing home residents with indwelling urethral catheters and decreased functional capacity and were at a high risk for the development of a UTI. These patients were selected since this population is normally difficult to treat with antibiotics and it was thought that the cocktail would be useful in controlling the initiation of infections. Whole urine samples, from these patients, were used as the reaction medium for the adherence assay consisting of healthy donor epithelial cells and the original infective isolate causing the UTI. Comparisons of mean bacterial adherence before and after CJC ingestion revealed that 4 of the 8 patients had a significantly more antiadherent urine after CJC ingestion. The average reduction in bacterial adherence in the four patients showing a positive response after CJC treatment ranged from 28% to 62%. This is a dramatic improvement given the difficulties in treating an elderly, catheterized population for infections of the urinary tract.[56]

The current study was initiated to further clarify the protective role of CJC by examining the effect of the cocktail on the THP present in the urine. As previously noted, Sobota and Apicella[49] demonstrated that THP appears to be the dominant antiadherence factor in normal urine. THP was extracted from the urine samples previously analyzed for antiadherence activity by Wilson[56] and used as the reaction medium for the adherence assay.

In part one of this investigation, the original infective isolates from the patients' UTI were used as the source of bacteria. This permits a comparison of the antiadherence activity of whole urine and THP extracted from this urine.

The whole urine of patients 3 and 5 showed a significant decrease in bacterial adherence after treatment with CJC. In patients 1 and 7 no decrease was observed.[56] When THP, extracted from the urine of these patients was tested, significant decreases in bacterial adherence were observed in patients 3,5 and 7. Several conclusions can be drawn from these observations. This study reaffirms that THP is a major natural antiadherence factor in the urine.[49] Also it appears that in at least two and possibly three of the patients in this study that ingestion of CJC results in a significant increase in the antiadherence activity of this molecule. This was evident in patients 3 and 5 in which both the whole urine[56] and THP extracted from this urine showed significant increases in antiadherence activity. In patient 7, while no increase in antiadherence activity was observed with whole urine[56], the THP extracted from this urine showed increased activity. There appear to be two possibilities here, first, the increased antiadherence activity of the THP in the urine of this patient was masked by other factors in the urine or second, there was a specific inhibition of the antiadherence properties of the THP. In either case, it was evident that removal of the THP from this environment demonstrated that the THP retained its antiadherence

properties and perhaps more important for this study was able to respond to CJC with an increase in antiadherence activity.

It thus appears that for at least half of the patients in this study the the regular use of CJC increases the antiadherence properties of the urine and specifically enhances the antiadhenence activity of the THP in the urine and thus may be useful for the prevention and/or treatment of urinary tract infections in these patients. In the one patient in which a positive effect was observed on the THP but not on whole urine, it may be possible in other ways to modify the urine by diet or other treatments to take advantage of the increased antiadherence activity of the THP.

In part two of this study the antiadherence activity of the THP was tested against *E. coli* containing type 1 fimbriae. THP is a glycoprotein which contains numerous mannose residues as part of the carbohydrate moiety. As noted above, it has been previously demonstrated that THP significantly inhibits the adherence of *E. coli* with type 1 fimbriae to epithelial cells.[49]

Since pathogens other than *E. coli* were isolated from this study population, we wanted to determine if the patients treated with CJC were possibly unresponsive to the treatment because their THP was unaffected by the cocktail. In particular, we wanted to test the THP from patient 1. In this patient *P. mirabilis* was the isolate and contains type III and type IV fimbriae both of which are mannose resistant [4] and would not be expected to respond to THP. In this patient it was found that the THP was also not responsive to *E. coli*. It is thus evident that not all patients will benefit

from the use of the cocktail. In some, as represented by patients 3 and 5, a positive antiadherence response can be detected in the urine[56] and THP and positively impacts on the pathogen isolated from this patients urine. Thus the use of CJC would be beneficial for these patients. In patient 7, antiadherence activity was not evident in the urine[56] but was positive for THP isolated from this urine. As indicated above, there may be other ways to modify the urine to permit the antiadherence activity of the THP to be realized. It is also possible that this patient's urine would show a positive response to other challenging pathogens, such as E. coli, and thus the cocktail would be a useful treatment for this patient. For patient 1, it appears the the use of the cocktail would offer no benefit since no positive response was observed in the urine or THP isolated from this urine.

It was also observed in this study and in previous studies in this laboratory that CJC or urine or THP extracted from urine from patients treated with the cocktail rarely completely prevented bacteria from adhering to uroepithelial cells. Even after treatment, an average of two to three bacteria remain attached to the epithelial cells. This appeared to be the case if large numbers, >50, or small numbers <10, of bacteria adhered to the epithelial cells in the absence of treatment. Thus it would follow that *in vivo* a similar situation would exist and treatment with the cocktail would not completely prevent all bacterial adherence. While this is not ideal, the reduction in bacterial load should permit the immune system to deal with these lower numbers of

bacteria. And perhaps in some cases a combination of treatments with antibiotics and CJC would be most beneficial.

The observation that the THP of three of four of the patients showed a positive response to the cocktail strongly suggests that this may be the manner in which the cocktail increases the antiadherence activity of the urine. Other ongoing investigations in this laboratory also support this suggestion. HPLC studies have not been able to demonstrate any fraction from the cocktail in urine showing increased antiadherence activity after ingestion of the cocktail.(Sobota-unpublished data) SDS Page electrophoresis, has shown that there is no quantitative change in the THP after drinking the cocktail.(Entler-unpublished data) It thus appears that there is a qualitative change in the THP in response to the cocktail. At this time we are hypothesizing that the cocktail promotes an unfolding of the carbohydrate moiety of the molecule that exposes more mannose residues and resulting in an increase in the antiadherence activity of the molecule and the urine.

In summary we have demonstrated that the regular use of CJC promotes an increase in antiadherence activity in the urine[56] of nursing home patients and this increase in activity appears to be primarily due to an increase in the antiadherent activity of the THP. Coupled with the clinical studies of Avorn, which demonstrated that CJC reduces the incidence of urinary infections in hospitalized patients,[3] this would suggest that any treatment that increases the antiadherence activity of the THP should be beneficial for the prevention and treatment of urinary tract

infections. Since the cocktail is readily available, well tolerated and cost effective it would appear to be the best choice of prevention of UTI currently available.

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