THE ROLE OF TAMM-HORSFALL GLYCOPROTEIN IN CONTROLLING THE INCIDENCE OF URINARY TRACT INFECTIONS IN CATHETERIZED GERIATRIC PATIENTS

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Abstract

Cranberry juice cocktail (CJC) has been shown by numerous researchers to be a powerful inhibitor of bacterial adherence to uroepithelial cells *in vitro* and *in vivo*. In a previous study performed in this laboratory, a significant increase in antiadherence activity was seen in the urine of nursing home patients after ingestion of the cocktail. In the current study, Tamm-Horsfall glycoprotein (THP) was extracted from these urine samples and examined for antiadherence activity. A significant increase in antiadherence activity was observed in five of the eight samples. To date, neither CJC, or any of its metabolic breakdown products have been found associated with the increase in antiadherence activity that occurs after ingestion of the cocktail. This study suggests that the observed increase in antiadherence activity of the urine is due to Tamm-Horsfall glycoprotein, which is produced in the ascending loop of Henle in the kidney and secreted into the urine. Using SDS-PAGE and UV absorbance, it appears that the increased antiadherence activity of the urine is not associated with an increase in the quantity of THP but rather a qualitative change in the molecule. It is suggested that this qualitative change in THP may be due to the unfolding of the glycoprotein, exposing additional binding sites to interact with the invading bacteria.

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I. Introduction:

Urinary Tract Infection:

Urinary tract infection (UTI) can be defined as the presence of bacteriuria, which is at least 10^5 bacteria per milliliter of a fresh void midstream urine specimen.[74] Infection can be defined in two ways depending on site of infection. The first is an upper UTI, which include the ureters and kidneys.[44] This type of infection can give rise to a condition known as pyelonephritis (inflammation of kidney). The second type of UTI are those that affect the lower urinary tract including the prostate, urethra, and bladder.[44] The most prevalent condition associated with lower UTI is cystitis (inflammation of bladder). Upper UTIs are usually caused by an ascending spread of bacteria from the lower urinary tract.[27]

Bacteriuria (10^5 bacteria/ml) results in two general types of infection. One type is accompanied with symptoms (symptomatic infection), while the other type displays no symptoms even though a significant amount bacteria may be present (asymptomatic infection). Symptomatic infection of the upper urinary tract may include signs such as fever, chills, flank pain, and tenderness, while those of the lower urinary tract may include dysuria, urgency, and frequency of urination.[4] Asymptomatic infection

is a common feature in the geriatric population in which no symptoms are present even though large numbers of bacteria multiply in the urinary tract. It is believed that asymptomatic bacteriuria can result in decreased renal function [13] although others have found this conclusion unsubstantiated.[1] While the general consensus suggests that UTI is associated with increased mortality in the elderly [43] although some researchers have found no such correlation.[39] In order to clarify the dispute, Nicolle et al.[38] performed a study involving fifty institutionalized women with UTI who were either given antibiotics or no antibiotics over a one year period. After analysis, it was concluded that there was no significant differences in morbidity between the two groups.

Factors Increasing Incidence of Urinary Tract Infection:

The most important factor in increased incidence of urinary tract infection is the incomplete emptying of the urinary bladder due in part to prostatic disease in men, bladder prolapse in women, or neurogenic bladder disorder in both sexes.[57] Another important cause occurs when an individual is subjected to an atmosphere where infection is common, such as a hospital environment. Bacteriuria is thought to be the most prominent nosocomial infection in the United States of America.[32] Perineal soiling [4] and frequent catheterization [29] are also factors that are attributed to increased incidence of UTI. All of these factors are more prevalent in the elderly population,

making them the highest risk group. In addition, Glauser et al.[19] found that inflammatory agents made animals highly susceptible to UTI. The inflammatory response results in the secretion of cytokines and the release of inflammatory cells into the urine.[12] Also, there is evidence that suggests that individuals with reduced secretory IgA may be more susceptible to UTI than those with IgA at optimal levels.[68]

Urinary Tract Infection Prevalence in the Population:

The occurrence of UTI is more prevalent in the female population than the male population. This is due to two important physical differences between the female and male anatomy. Females are at a greater risk first and foremost because they have a shorter urethra and secondly, their urinary tract is in close contact with the highly bacteriolized vagina. In the younger population, the ratio of women to men affected with UTI is 30:1 whereas in the elderly population, this ratio dropss to 3:1.[6] Another factor that increases risk of UTI in the female poulation is menopause [47] whereas sexual activity poses an increased risk in both populations.[37]

Urinary Tract Infection in the Elderly Population:

Urinary tract infections are second only to respiratory infections in occurrence the elderly population.[77] In the elderly, at least 20% of women and 10% of men over the

age of 65 are bacteriuric.[4,9,27] In three surveys performed at six month intervals by Boscia et al.[7] of 184 women and 76 men over the age of 68, the percentage of individuals with UTI was 30% of women and 11% of men. In a two survey study covering a five year interval involving 101 women and 87 men over 65 years old, Sourander et al.[61] found that 38% of women and 15% of men were bacteriuric in at least one of the studies. Another group performed two surveys of 231 women and 121 men over the age of 70 and found that 44% of the women and 28% of the men were bacteriuric in at least one of the two surveys.[27]

Risk Factors Predisposing the Elderly to Urinary Tract Infection:

There is an increase in the incidence of UTI within the elderly population. The occurrence of UTI in the elderly increases with institutionalization, hospitalization, and coinciding disease. [1,62] Single survey studies indicate that at any one time, 25% of women and 20% of men in extended care facilities are bacteriuric as compared to approximately 20% of elderly women and 10% of elderly men in the general population.[62] Urinary tract infections pose a more prominent problem in the elderly than any other age group because of other factors that may mask the symptoms. UTI is more difficult to diagnose in the geriatric population because pulmonary and gastrointestinal signs dominate the clinical picture.[4] In a study performed by Gleckman

et al.[20], diagnosis was initially missed in 21% of cases of UTI. Physiological changes and medicinal procedures in the elderly population provide evidence for the increase in urinary tract infection. Powers et al.[45] identified the following factors as associated with an increase the incidence of UTI: decreased functional states, decreased mental status, bladder catheterization, and antibiotic use.

Catheterization and Incidence of Urinary Tract Infection:

Indwelling urethral catheterization is the most frequent cause of nosocomial infection.[33] It was found that once a catheter is installed, the risk of UTI increases 5-10% each day.[72] Infection that is catheter-associated is very difficult to prevent and subsequent treatment is not very effective[54,64] and increase the rate of mortality in the elderly population.[43]

A catheter serves as a frequent cause of UTI because *in situ*, it acts as a channel between the sterile bladder and the outside world.[33] It is believed that the bacteria form a thick, coherent biofilm composed of an extensive anionic network of polysaccharides on the surfaces of the catheter.[36] Bacteria within the biofilm can lead to a degree of antibiotic resistance due to ineffective penetration of the antibiotic into the biofilm. Bacterial migration along the outside of the catheter represents another method of entry. This method of infection however occurs at a much slower.[35]

Bacterial adherence to the uroepithelium near the end of the catheter must take place symptomatic infection to occur.[34] An indwelling catheter may also aid in infection by preventing the complete emptying of the bladder, always leaving a residual volume of urine for bacteria to multiply. A more serious catheter-associated infection can result if *Klebsiella* or *Proteus* species are present to split urea and form crystals that eventually lead to stone formaton. The buildup of urinary crystals can lead to extreme levels of bacteria which may also result in septicemia.[23]

Microbiology of the Urinary Tract:

Escherichia coli is the most common cause of urinary tract infection, causing up to 90% of all infections in the younger population and approximately 75% of infections in the elderly.[8,71] Other organisms that are found in the urinary tract include *Enterobacter, Klebsiella, Proteus, Pseudomonas,* and *Serratia.* The occurrence of strains other than *E.coli* in the elderly is a result of increased hospitalization and institutionalization leading to greater long-term exposure.[69] Also, increased antiboitic use and catheterization can lead to incidence of non-*E. coli* strains.[4]

Bacterial Adherence and Initiation of Urinary Tract Infection:

The most important factor in the initiation of urinary tract infection is bacterial

adherence to the uroepithelium. The adherence of bacteria to the uroepithelium allows it to overcome clearing mechanisms of the urinary tract and nutrient deprivation resulting in growth advantages and subsequent increase in toxicity.[76] Bacteria contain filamentous appendages (pili/fimbrae) that aid in the adherence to the uroepithelium. It has been suggested that type-1 fimbrae act as adhesins in the initiation of UTI.[40] Type-1 fimbrae are found on many gram negative bacteria including: *E. coli, Pseudomonas,* and *Klebsiella.* Bacteria isolated from the lower urinary tract are more likely to express type-1 pili [6] than other virulent factors such as type-P pili.[21] In a human study of 41 bacteriuric adults, it was found that 76% of the urine specimens collected contained bacteria expressing type-1 pili and 78% of the type-1 pili bacteria were associated with exfoliated cells.[29] Strains of *E. coli* expressing type-P pili have been isolated from patients suffering from upper urinary tract infection.[25]

The adherence of type-1 fimbriated bacteria to the uroepthelium has been shown to be blocked by D-mannose and hence these isolates are known as mannose-sensitive bacteria.[42] Schaefer et al. [51] have shown that pretreatment of type-1 fimbriated *E. coli* with 2.5% D-mannose blocked bacterial adherence to the huan uroepithelium whereas pretreatment of uroepithelial cells with mannose had no effect on bacterial adherence. This suggests that mannose sensitive residues occur as the receptors for type-1 fimbriated bacteria. On the otherhand, mannose appears to have no effect on bacteria

expressing the more virulent type-P pili and hence these isolates are known as mannose-resistant bacteria. Kallenius et al. [26] examined 97 children with UTI and compared with healthy controls, finding that 91% of the patients with pyelonephritis expressed type-P pili whereas only 19% of those with cystitis and 14% of those with strains causing asymptomatic bacteriuria expressed type-P pili.

There are different degrees of infection that result upon bacterial adherence. Svanborg et al. [65] reported a correlation between bacterial adherence and the severity of infection, indicating the adhesiveness of bacterial fimbrae as the prime determining factor.

Host Defense Mechanisms:

The most important factor in decreasing the occurrence of urinary tract infection is micturition with complete bladder emptying. As mentioned earlier, this feature seems to be reduced to an extent in the elderly population. Specific immunity is also considered an important host defense mechanism but its significance is questionable because patients with known cellular or humoral immunodefenciencies rarely have recurrent UTI.[66] Urine itself has antimicrobials properties such as low pH, osmolality extremes, and high urea content.[63] These defense functions seem to be decreased in the elderly also.

The Role of Cranberry Juice Cocktail in Decreasing Bacterial Adherence:

Cranberry juice cocktail (CJC) has been used for many decades as a proposed agent in prevention of urinary tract infection. It was originally thought that CJC acted by decreasing pH and increasing the concentration of organic acids in the urine to bacteriostatic levels, [19] but this view was found to be unsubstantiated by numerous investigators.[52,60] The high fructose content of CJC was also proposed as a possible mechanism for reducing UTI, but fructose rarely appears in the urine of a normal subject except after administration of abundant doses and does not undergo extensive reabsorption from the glomerular filtrate in the kidney tubules.

The first clinical testing of CJC in the reduction of UTI was performed in 1968 by Prodomas et al. [46] This study included 60 patients (44 women and 16 men) with diagnosed UTI. The patients were given 16 ounces of CJC per day for three weeks and clinical progression of their infection was analyzed. A total of 32 patients showed great improvement, 12 patients had moderate improvement, and the remaining 16 patients showed no improvement. Within six weeks after the end of CJC treatment, 27 of the patients that showed improvement had a recurrent infection. Sobota [60] demonstrated that urine collected after CJC ingestion could block adherence of *E. coli* to uroepithelial cells when this urine was used as the reaction medium for bacterial adherence assays. In addition, he also showed that CJC caused a 75% or greater

decrease in the adherence of 60% of 77 uropathogenic strains of *E. coli*. Zafriri et al.[76] demonstrated that CJC could inhibit adherence of *E. coli* to yeast, cultured cells, and mouse peritoneal macrophages.

In a study performed in this laboratory by Wollet,[75] the anti-adherence effect of urine was examined following CJC consumption by three groups of individuals consisting of: 25 individuals with no infection, 19 inflicted with chronic UTI, and 6 patients classified as chronic sufferers who had been placed on four ounces of CJC per day for two years prior to the study. The mean bacterial adherence to the uroepithelium of the 6 patients on CJC was 6.44 bacteria/cell. The 19 individuals with chronic UTI had a mean adherence of 12.69 bacteria/cell and the control group had an average of 21.22 bacteria/cell. After the addition of CJC to the urine of the control group and the untreated chronic group, an increase in anti-adherence activity was observed *in vitro* while the group previously on CJC therapy showed no observed increase.

In a recent clinical study, Avorn et al.[3] performed a well-controlled study of 153 elderly women in which half were given 300 ml of CJC per day and the other half, a placebo that tasted like cranberry juice. Measures were taken to avoid CJC intake in the placebo group. Monthly samples of urine were collected in which urinanalysis, bacterial culture, and antibiotic sensitivity testing were performed. UTI was found in

28.1% of the placebo group compared to only 15% in those on CJC therapy. Also, during the study, the placebo group was treated with antimicrobials sixteen times for UTI compared to only eight reported treatments in the CJC group, again indicating the effectiveness of CJC as a therapeutic agent.

Finally, a study was performed in this laboratory by Wilson [74] involving eight catheterized geriatric patients living in an extended care facility. The patients were asked to drink four ounces of CJC per day over a three month time interval were administered to determine the effectiveness of CJC as an anti-adherence agent in this high risk population. In this study, the patient's urine was used as the reaction medium for *in vitro* adherence assays. Over the three month time period, four of the eight patients exhibited significantly more anti-adherent urine several weeks after the beginning of CJC administration as compared to base-line controls, one patient showed moderate improvement, and the remaining three showed no improvement.

Through all of the research and subsequent conclusions, it is apparent that cranberry juice cocktail acts in a manner to reduce bacterial adherence to the uroepithelium thereby reducing the initiation of and the duration of urinary tract infections.

The Effect of Tamm-Horsfall Glycoprotein on Bacterial Adherence:

Over four decades ago, a glycoprotein of high molecular weight was prepared from human urine by Tamm and Horsfall, [17] and was subsequently called the Tamm-Horsfall glycoprotein (THP). Evidence has suggested that THP originates in the kidney and labelled antibody techniques have indicated that THP is associated with the epithelial cells of the kidney tubule.[18] Electron microscopy has confirmed that the glycoprotein possesses an unbranched fibrillar structure possibly consisting of a linear arrangement of subunits.[5] THP is classified as the most abundant protein of renal origin [31] giving rise to the exploration into its possible effects as an additional host defense mechanism against bacterial attachment to the uroepithelium.

A breakthrough in examining THP as a defense mechanism occurred in 1980 when Orskov et al. [41] demonstrated that type-1 fimbriated *E. coli* were trapped by THP in the urine. It was subsequently suggested that this entrapment prevents attachment of bacteria to uroepithelial cells, thus preventing infection. This entrapment is related to the observation that THP contains large amounts of receptors that bind to mannose-sensitive bacterial fimbrae. Electron microscopy studies have demonstrated that THP forms a pseudocapsid around type-1 fimbriated bacteria and in the absence of fimbrae, no capsule was formed.[30] In addition, Dulawa et al.[14] demonstrated that bacterial adherence mediated by mannose-sensitive type-1 fimbrae bacteria can be inhibited by

THP. Sobota et al. [59] noted that THP, at concentrations occurring in normal urine, showed anti-adherence activity for type-1 fimbriated *E. coli* and removal of THP from urine through precipitation induced a substantial increase in bacteriall adherence in *in vitro* adherence assays.

The normal concentrations of THP in urine range from 30-50*ug*/ml.[73] This concentration range plays an important role in the effectiveness of THP in decreasing bacterial adherence. At concentrations greater than 30*ug*/ml, THP has been shown to inhibit bacterial adherence.[15] At lower concentrations, the significance of THP in preventing bacterial attachment diminishes. THP concentrations in the elderly have been shown to be decreased, making bacterial adhesion more likely. In addition, Hess et al. [22] have shown that THP concentrations below that found in normal urine may inhibit calcium oxalate monohydrate crystal aggregation.

The Current Study:

Severely compromised, catheterized women ranging in age from 76 to 92 years were given four ounces of CJC per day over a twelve week period.[74] All but one of these patients had been treated for one to six symptomatic UTIs in the previous year.[74] In a previous study it had been demonstrated that upon CJC ingestion, these patients exhibited significantly more anti-adherent activity in the urine. In this study an attempt

was made to determine what component(s) was responsible for the observed increase in antiadherence activity. Since it had been previously demonstrated that THP is an active naturally occurring antiadherent agent occurring in the urine, this fraction of the urine was examined. THP was extracted fro patients urine prior to and after ingestion of cranberry juice cocktail. The THP was subjected to *in vitro* adherence testing and and an attempt was made to determine if some change in the THP occurred after ingestion of the cocktail.

II. Materials and Methods:

Patient Selection:

All of the subjects used in this study were incontinent catheterized females between the ages of 76 and 92 years old and patients of the Beeghly Oaks nursing home. All patients chosen gave written consent for their involvement in the study.

Patient Histories:

Each patient selected had a history of urinary tract infection defined as averaging three or more UTIs per year and most had suffered from recurrent infections in the year prior to the study. Eight patients were used in the study and the results described here represent four subjects, Patients 2, 6, 8, and 9. Patient 2 had no infections in the year prior to the study but had suffered from chronic UTI for several years previous to the study. Patient 6 had one infection in the year prior to the study and had a history of chronic infection. Patient 8 had seven infections in the year prior to the study and entered the study with an active infection. Patient 9 had a history of chronic infection.

Cranberry Juice Administration and Specimen Collection:

The study was performed over a 13 week period from February 8, 1992 to May

10, 1992, which included one week before the administration of cranberry juice cocktail (CJC) and 12 weeks in which the patients were given four ounces of CJC daily. In the week prior to the administration of CJC, specimens were collected from each patient on Monday, Wednesday, and Friday. The adherence data from these specimens was combined to account for day to day variances. The urine was collected by the nursing staff of Beeghly Oaks and refrigerated immediately. Administration of CJC began on the second Monday of the study. Specimens were collected 24 hours, 48 hours, and 72 hours after the beginning of CJC ingestion and then on every Monday for the remaining eleven weeks of the study. Upon collection, the specimens were taken to the laboratory and centrifuged 10 minutes at 4500*g* to remove patient cells. The urine was then stored at -20°C.

Bacterial Isolation:

Aliqouts of the specimens were plated on MacConkey's agar. Isolated colonies taken from specimens before CJC ingestion were replated on nutrient agar and genus and species were identified using API20E strips (*Fisher Scientific*, Pittsburgh, PA). After isolation and identification, 15% glycerol cultures were prepared and stored at -70°C and later used for patient adherence studies. The bacterial isolates from the respective patients were as follows: Patient 2-*Pseudomonas aeroginosa*;Patient 6-

Klebsiella pneumoniae;Patient 8-*Escherichia coli*; Patient 9-*Escherichia coli*.

Tamm-Horsfall glycoprotein (THP) Extraction:

THP was prepared from patient urine by a slight modification of Tamm and Horsfall.[67] All steps were performed at 4°C. Patient urine that was stored at -20°C was thawed and brought to 0.58M NaCl and refrigerated for 24 hours. The urine was brought to 0.58M NaCl to mimic those conditions normally found within the body. The precipitate was collected by centrifugation for 30 minutes at 4500*g*. The pellet was washed three times with 50 volumes of 0.58M NaCl and then brought up in 5ml 0.58M NaCl. This solution was resuspended and gently vortexed.

Bacterial Adherence Assay:

Patient uroepithelial cells could not be used in the study because they were too fragile and fragmented during isolation procedures.[74] Therefore, uroepithelial cells from a healthy female donor were used instead. One donor was used throughout the study to decrease variability in the receptivity of uroepithelial cells due to factors such as menstruation.[20] For each assay, 10ml of donor urine was centrifuged for 10 minutes at 4500*g* to pellet the exfoliated uroepithelial cells and the supernatant was discarded. To avoid factors such as phase variation of bacterial fimbrial expression, the bacteria

used to assay patient specimens was taken from one 48 hour static culture. Two ml of bacteria (approximately 10^9 bacteria) from static culture was used for each specimen assayed. The 2ml culture was then centrifuged for 10 minutes at 4500*g* to pellet the cells and the was supernatant discarded. Two ml of THP, previously stored in 5ml 0.58M NaCl, was then added to the bacterial pellet and gently vortexed. This suspension was then added to the donor pellet and incubated in a 37°C water bath for 30 minutes. Following incubation, the specimens were briefly vortexed to resuspend the cells and poured into disposable beakers. The samples were then removed from the beaker using a 2cc disposable syringe. A filter holder containing an 8*u*m nucleopore filter was fitted to the syringe and the contents were pushed through the filter. The sample was then washed 2 times with 100ml deionized water. The filter was then removed placed cell side down on a microscope slide and allowed to dry overnight. After the filter was dry, it was peeled off leaving the cells adhering to the glass surface. The cells were then gram stained using the Philadelphia General Hospital technique and scored.

A set of adherence assays was also performed using *E. coli* from Patient 3 against each respective patients' THP, again following the aforementioned protocol.

Scoring:

Through the use of a light microscope, 50 cells were scored on each slide for the number of gram negative rods adhering to each cell. Mean bacterial adherences were determined for each specimen. Base-line controls were produced from the data prior to the administration of CJC. The data was analyzed and compared by one-way ANOVA to determine significant changes in bacterial adherence.

Electrophoretic Procedures:

An analysis of THP was performed to determine if the observed changes in the glucoprotein were quantitative or qualitative. With the aide of Dr. Gary Walker (Associate Professor Biological Sciences, Youngstown State University), a protocol was devised using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) .

Sodium dodecyl sulfate (SDS) is a detergent that binds tenaciously to proteins causing them to assume a rod-like shape.[70] The large negative charge that SDS imparts masks the protein's inherent charge. This gives proteins an identical charge to mass ratio. They then can be separated on the basis of size by subjecting the protein to an electrical field in a polyacrylamide gel. In this study a 5% polyacrylamide gel with a 2.5% stacking gel was initially used to determine the purity of the sample.[2] A single

band of approxiomately 97.4 KDa was obtained. To increase the resolving power of the gel, a 5% stacking gel (pH 6.8) and a 12% resolving gel (pH 8.8) were used to analyze the THP extracted pre and post administration of the cocktail. Pre- and post-CJC THP previously extracted from Pateint 8 was prepared and loaded in the following manner. After washing 2 times with 0.58M NaCl, the pellet was resuspended in 1ml deionized water and transferred to 1.5ml eppendorf tube. Upon resuspension, the mixture was microcentrifuged at 4500*g* for 1 minute. The supernatant was discarded and approximately 200*u*l 4X SDS sample buffer was added to the pellet. The mixture was boiled for a few minutes to ensure proper penetration of SDS. Twenty and 30*u*l aliquots were added to the 0.75mm wells plus a molecular weight standard. The sample was then electrophoresed for 30 minutes at 100V to ensure stacking and then the current was increased to 150-200V until front reached the bottom of the gel. The gel was then stained with a quantitative stain, Coomassie blue. Any differences in band size were noted to determine any quantitative changes in the THP. To verify the identity of the THP, after electrophoresis the gel was cut vertically and one-half was stained to determine the location of the band in the unstained gel. The pre- and post-CJC THP bands were excised from the unstained gel, transferred to separate PERFECTprep™ spin columns with attached filters and released from the gel through maceration. The filtrate was then collected through microcentrifugation for one

minute.. The filtrate was then resuspended in 1ml 0.58M NaCl and a bacterial adherence assay was performed. The only difference in the adherence assay was that 1ml bacteria was used instead of 2ml to account for differences in THP concentrations from original extractions.

Ultraviolet Spectrophotometry:

Pre- and post-CJC THP from Patient 8 was subjected to UV spectrophotometry at a wavelength of 280nm to quantify the level of glycoprotein.

III. Results:

In examining the effectiveness of THP as a potent anti-adherence agent after CJC ingestion, THP was isolated from fresh void urine at various times before and after the beginning of CJC administration. THP was then used as the reaction medium for bacterial adherence assays that coincide to those conditions normally found in mammalian urinary tracts. In the initial study, the mean number of bacteria adhering to fifty uroepithelial cells was quantitated in the presence of THP. The bacterial isolate used in each assay was obtained from each respective patients' urinary tract.

THP and Original Isolates from the Patients:

Patient Two:

The original isolate from patient 2 was *Pseudomonas aeroginosa*. The initial adherence of *P. aeroginosa*, using THP isolated prior to CJC ingestion, was 15.10 bacteria per cell. The mean bacterial adherence in the ensuing three weeks following the beginning of CJC ingestion was 8.77 bacteria per cell, representing a 42% decrease in bacterial adherence. The mean bacterial adherence from week 4 to week 12 was 11.91 bacteria per cell, a decrease of 21% from that of the control. Overall, the adherence of Patient 2 *P. aeroginosa* assayed with post-CJC THP was 10.65 bacteria per cell, representing a decrease of 29.5% over the twelve week period.

Although all values throughout the twelve weeks were below that of the control, days 1, 2, 3 and 77 exemplified values significantly lower than that of the control.

Patient Six:

The original isolate from patient 6 was *Klebsiella pneumoniae*. Before CJC ingestion, the number of *K. pneumoniae* adhering per cell was 13.70, when using THP extracted from the patient's urine. The mean bacterial adherence in the following three weeks after the beginning of CJC ingestion was 3.99 bacteria per cell, representing a 70% decrease in bacterial adherence. Week 4 to week 12 had a mean bacterial adherence of 7.92, a decrease of 42% when compared to the control. Over the course of twelve weeks, the mean bacterial adherence when using post-CJC THP was 6.35 bacteria per cell, representing a 54% decrease in adherence. In summary, all values were significantly lower than the control except for the value obtained on day 70, thereby showing the effectiveness of THP after CJC ingestion in this patient.

Patient Eight:

The original isolate from patient 8 was *Escherichia coli*. Prior to CJC ingestion, the number of *E. coli* adhering per cell was 11.56. The mean bacterial adherence in the three weeks following the beginning of CJC ingestion was 9.30 bacteria per cell, a decrease of 20% in bacterial adherence. The mean bacterial adherence from week 4 to week 12 was 7.08 bacteria per cell, representing a 39% decrease in adherence

when compared to the control. When looking at the entire twelve week period, the mean bacterial adherence was 7.97 bacteria per cell, a decrease of 31% when compared to the control. In analyzing the data obtained, ten values were significantly lower than the control (days 1, 2, 3, 28, 35, 42, 49, 63, 77 and 84). Immediately following the beginning of CJC ingestion, there was a significant decrease adherence and then a subsequent increase in adherence during the following two weeks. This may have been due to a active UTI during this time.

Patient Nine:

The original isolate from patient 9 was *Escherichia coli.* Before CJC ingestion, the number of *E.coli* adhering per cell was 21.24. The mean bacterial adherence in the following three weeks after the beginning of CJC administration was 9.84 bacteria per cell, a decrease in bacterial adherence of 54%. Week 4 to week 12 had a mean bacterial adherence of 11.58 bacteria per cell, representing a 45.5% decrease when compared to the control. Overall, the post-CJC mean bacterial adherence was 10.89, representing a 49% decrease in adherence of *E. coli* to the uroepithelium. All values throughout the twelve week period were significantly lower the control.

Comparison of Original Isolate to Control:

A second study was set up to explore and compare the effects of *E. coli*, which

has been previously shown to interact specifically with THP, as a control to the effects of the original infective isolate. In this experimental approach, each respective patients' THP was assayed with *E. coli* instead of the original isolate.

Patient Two:

The mean bacterial adherence when assaying patient 2 pre-CJC THP with *E. coli* was 20.90 bacteria per cell. In the following three weeks after the beginning of CJC ingestion, the mean bacterial adherence was 10.61 bacteria per cell, a 49% decrease in adherence. After three weeks till the end of the study, the mean bacterial adherence was 14.59, representing a 30% decrease in adherence when compared to the control. Overall, there was a 38% decrease in bacterial adherence when using patient 2 post-CJC THP against *E. coli*. All values showed significant decreases in bacterial adherence except for day 77.

Patient Six:

When using THP from patient 6 assayed with *E. coli*, the mean bacterial adherence before CJC ingestion was 13.50 bacteria per cell. In the first three weeks, the mean bacterial adherence was 8.24, a decrease of 39% in bacterial adherence. Week 4 to week 12 showed a mean bacterial adherence of 14.46, representing a 7% increase in adherence when compared to the control. Overall, the mean bacterial adherence was 11.97 bacteria per cell, a decrease of 12% in bacterial adherence. During the first four

weeks, bacterial adherence was less than that of the control with day 1, 3, 7, 14 and 21 showing values significantly lower than the control. In the last eight weeks, all values were higher than the control.

Patient Eight:

In the presence of patient 8 pre-CJC THP, the mean bacterial adherence when using the control *E. coli* was 21.02 bacteria per cell. In the following three weeks, patient 8 post-CJC THP decreased the mean bacterial adherence to 8.43 bacteria per cell, a 60% decrease in adherence. In the next eight weeks, the mean bacterial adherence was 13.23, representing a 37% decrease in adherence when compared to the control. Throughout the entire twelve week period, bacterial adherence averaged 11.31 bacteria per cell, an overall 46% decrease in adherence. In analyzing the data, all values showed significant decreases in bacterial adherence except for the last two weeks.

Patient Nine:

The mean bacterial adherence when using patient 9 pre-CJC THP against the control *E. coli* was 13.76 bacteria per cell. During the course of the first three weeks following CJC ingestion, the mean bacterial adherence was 8.42 bacteria per cell, a 39% decrease in adherence. Week 4 to week 12 showed a mean bacterial adherence of 12.66, representing an 8% decrease in bacterial adherence when compared to the

base-line control. Overall, the mean bacterial adherence of *E. coli* to the uroepithelium in the presence of patient 9 post-CJC THP was 10.96, a composite decrease of 20.5% in bacterial adherence. At each time during the course of the twelve week period, the values obtained were lower than that of the control except on day 49. Days 1, 2, 3 and 7 showed values that were significantly lower than the control.

After compiling the results of the effectiveness of each patient's THP before and after CJC ingestion on the adherence of *E. coli*, each of the results from the second study were compared to those of the initial study. For patient two, CJC treatment resulted in a 29.5% decrease in *P. aeroginosa* adherence overall compared to a 38% decrease in *E. coli* adherence. The original isolate in patient six, *K. pneumoniae*, showed a 54% decrease in adherence over the twelve week period whereas only a 12% decrease in *E. coli* was seen. The original *E. coli* isolate from patient eight demonstrated a 31% decrease in adherence and the control *E. coli* isolate showed a 46% in bacterial adherence. Patient nine responded very well to CJC treatment, showing a 49% decrease in bacterial adherence compared to a 20.5% decrease in control *E. coli* adherence. After careful analysis of the results from both studies, all of which showed different degrees of decreases in bacterial adherence, it can be deduced that post-CJC THP extracted from the respective patients respond in similar

fashions to CJC treatment depending on the infective isolates.

Comparison of the Effectiveness of THP vs. Whole Urine

In comparing the effectiveness of THP to whole urine, data from Patrick Wilson's master thesis [74] was used. In his research, Wilson assessed the adherence properties of whole urine collected from patient 2, 6, and 8 before and after the administration of CJC. In determining the effectiveness of THP to whole urine, the percent change in bacterial adherence was calculated before and after CJC ingestion. For patient 2, infected with *P. aeroginosa*, THP (15.1 bacteria/cell) had a 29% increase in adherence when compared to whole urine (11.02 bacteria/cell) before CJC ingestion. After CJC ingestion, there seemed to be a shift in bacterial adherence with THP (10.65 bacteria/cell) showing a 30% decrease in adherence when compared to whole urine (15.03 bacteria/cell). Patient 6 showed somewhat of a different trend. Before CJC administration, THP (13.70 bacteria/cell) demonstrated a 35% decrease in bacterial adherence when compared to whole urine (21.21 bacteria/cell). Throughout the following twelve weeks when CJC was ingested, THP (6.35 bacteria/cell) was significantly more effective than whole urine (19.14 bacteria/cell), clearing 67% more bacteria than when assayed with whole urine. Patient 8 showed similarities between the effectiveness of THP and whole urine. Pre-CJC conditions demonstrated that THP (11.56 bacteria/cell) was 8% better at clearing bacteria than whole urine (12.66

bacteria/cell). On the otherhand, the post-CJC conditions favor whole urine (7.30 bacteria/cell) over THP (7.97 bacteria/cell) by a slim 8%. Calculation of whole urine data from patient 9 was not performed in Wilson's research, therefore no comparison can be made with this patient. In summary, it can be concluded that using THP as the reaction medium instead of whole urine is more beneficial when looking at mean bacterial adherences after CJC ingestion although whole urine may play a more prominent role in conditions before CJC ingestion.

A summary of the comparison of mean bacterial adherence using whole urine and the original isolate, THP and the original isolate, and THP and *E. coli* can be seen in table 10.

Biochemical Analysis of THP Upon CJC Ingestion:

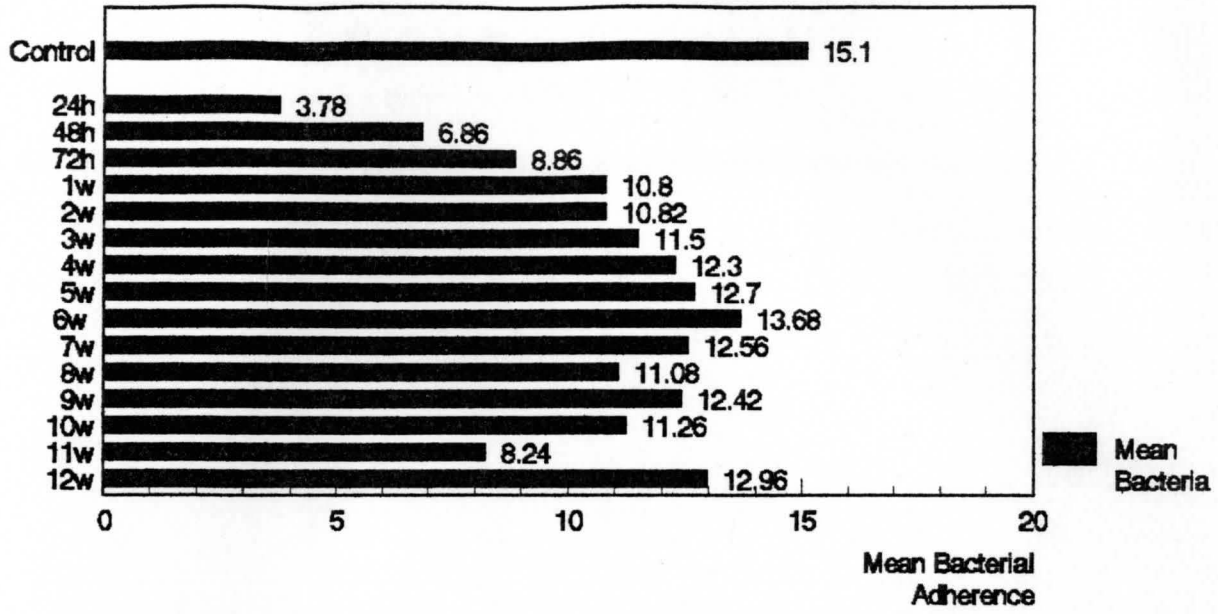
SDS-PAGE:

The mean bacterial adherence before CJC ingestion for Patient 8 was 9.30 bacteria per cell. At week 4, the mean bacterial adherence was 3.60 bacteria per cell, representing a 62% decrease in bacterial adherence from that of the control. These values are comparable to those found in the initial study (see Table 6) which showed a 57% decrease in adherence when compared to the control. In addition to this data, there was no change in the relative band size between the pre- and post-CJC THP

specimens, indicating that the effectiveness of THP must reside in conformation and not the concentration of THP upon CJC ingestion. This may be due to the unfolding of the glycoprotein upon CJC ingestion, thereby exposing additional binding sites to interact with invading bacteria. Additional support to this claim was that when subjected to UV spectrophotometry, pre-CJC THP had a slightly higher absorbance reading than the post-CJC THP sample again suggesting no increase in THP upon CJC ingestion. In summary, this biochemical analysis accomplished two things. First and foremost, it has reaffirmed our hypothesis that THP was the reason behind the effectiveness of CJC and secondly, it has provided insight into the mechanism of action that takes place within the urinary tract upon CJC ingestion.

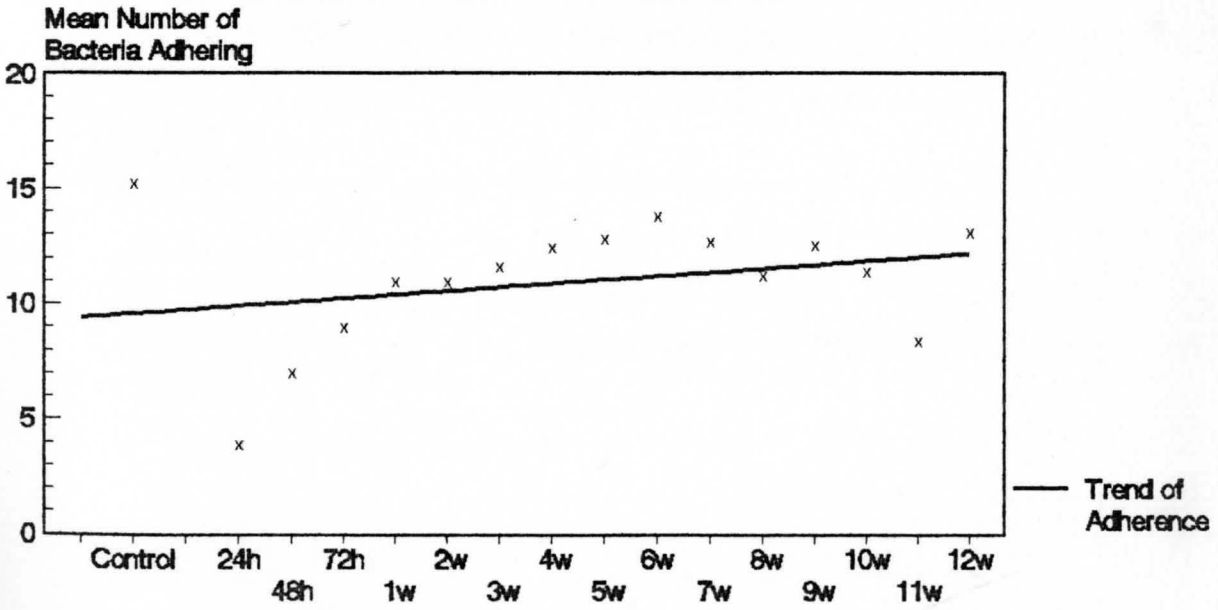
Figure 1: Bacterial adherence data for Patient 2. Bacterial isolate: *Pseudomonas aeroginosa*. A) Bar graph of mean number of bacteria adhering to 50 uroepithelial cells with THP as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelial cells including 50 cells assayed in the presence of THP extracted 3, 5, and 7 days prior to the initiation of CJC administration. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, with all other specimens collected at the indicated time following the beginning of CJC ingestion.

A) Patient 2:Mean Bacterial Adherences to Uroepithelia



KeyChart 2000

B) Patient 2: Trend of Bacterial Adherence to Uroepithelia

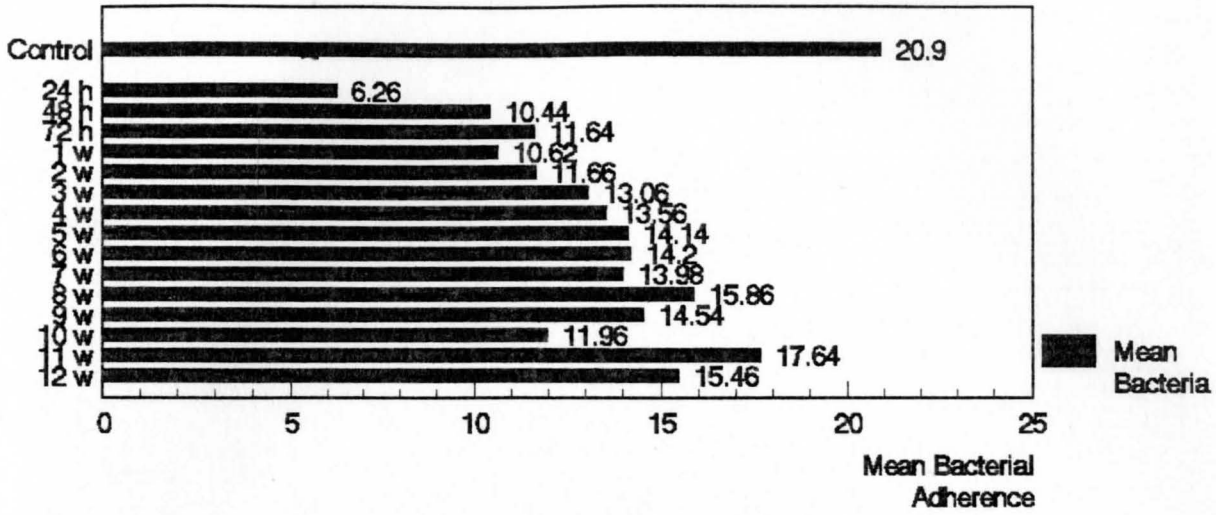


KeyChart 2000

Figure 2: Bacterial adherence data for Patient 2. Bacterial isolate: *Escherichia coli* (from Patient 3) A) Bar graph of mean number of bacteria adhering to 50 uroepithelial cells with THP as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelial cells including 50 cells assayed in the presence of THP extracted 3, 5, and 7 days prior to the initiation of CJC administration. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, with all other specimens collected at the indicated time following the beginning of CJC ingestion.

A) Patient 2: Mean Bacterial Adherences to Uroepithelia

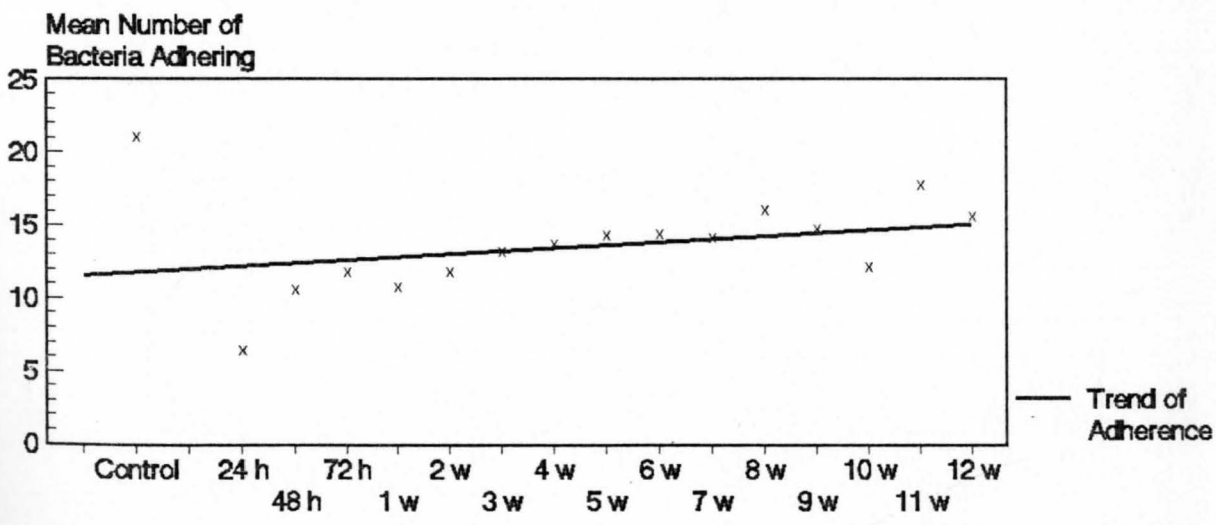
The Utilization of Patient 2 THP on Clearance of E.coli



KeyChart 2000

B) Patient 2: Trend of Bacterial Adherence to Uroepithelia

The Utilization of Patient 2 THP on Clearance of E.coli



KeyChart 2000

Figure 3: Comparison of the effects of Patient 2 THP on two different isolates. The solid line depicts the trend of *E. coli* + THP and the dashed line depicts the trend of *P. aeroginosa* + THP during the twelve week period of CJC ingestion.

Comparison of the Effects of Patient 2 THP on Two Different Isolates

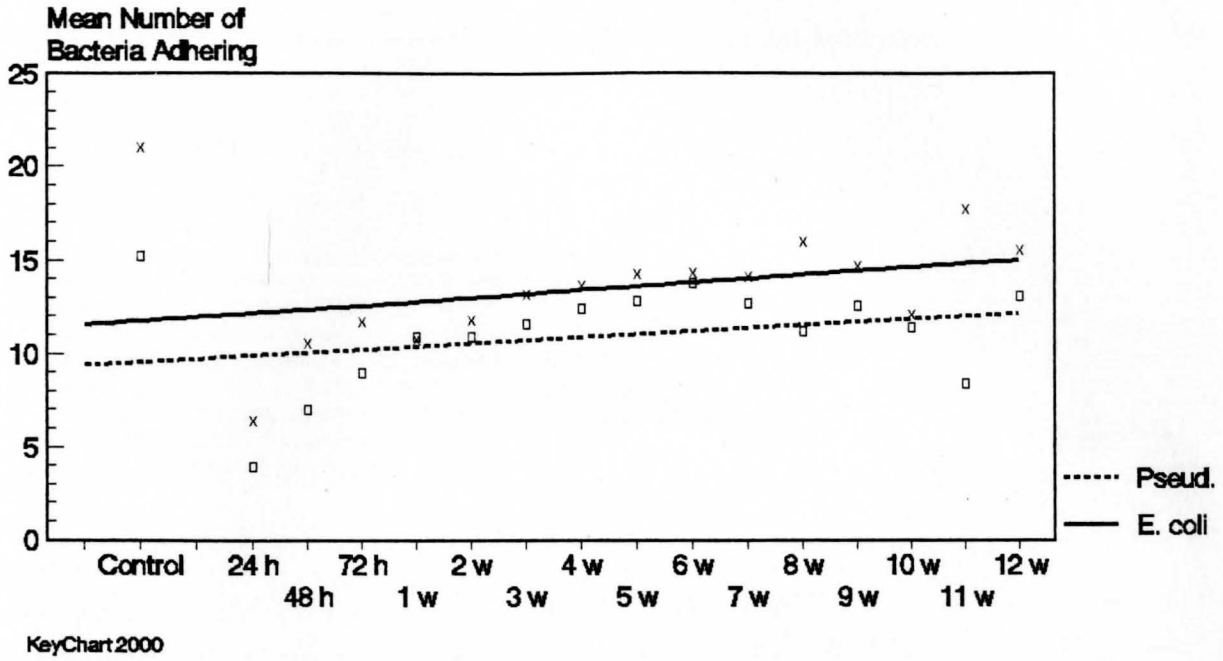
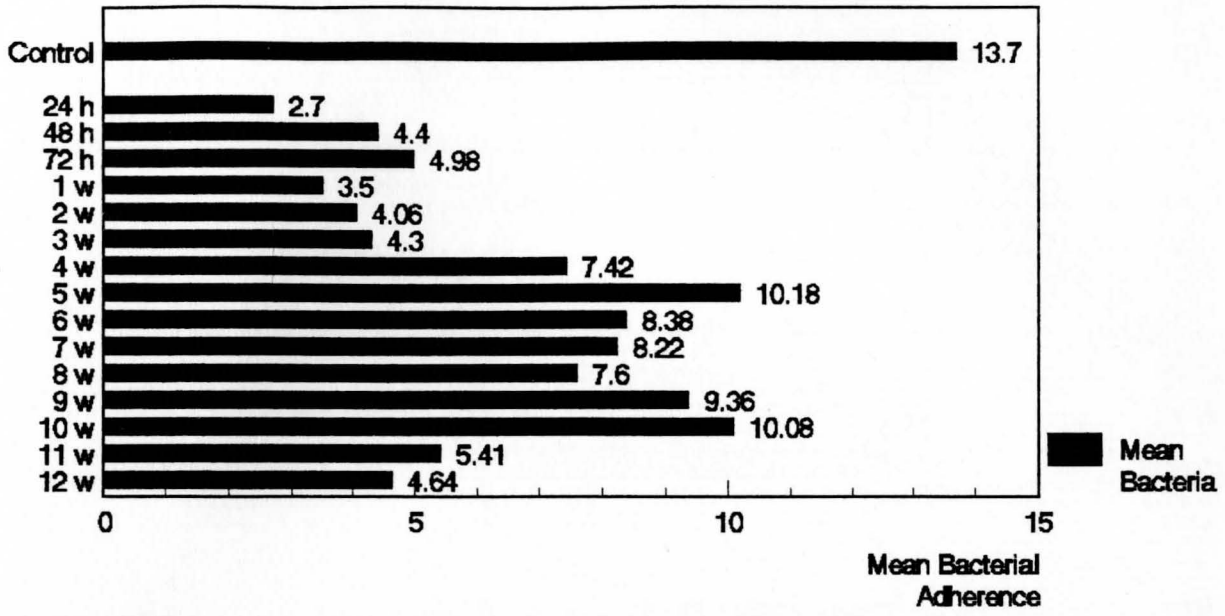


Figure 4: Bacterial adherence data for Patient 6. Bacterial isolate: *Klebsiella pneumoniae*. A) Bar graph of mean number of bacteria adhering to 50 uroepithelial cells with THP as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelial cells including 50 cells assayed in the presence of THP extracted 3, 5, and 7 days prior to the initiation of CJC administration. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, with all other specimens collected at the indicated time following the beginning of CJC ingestion.

A) Patient 6: Mean Bacterial Adherences to Uroepithelia



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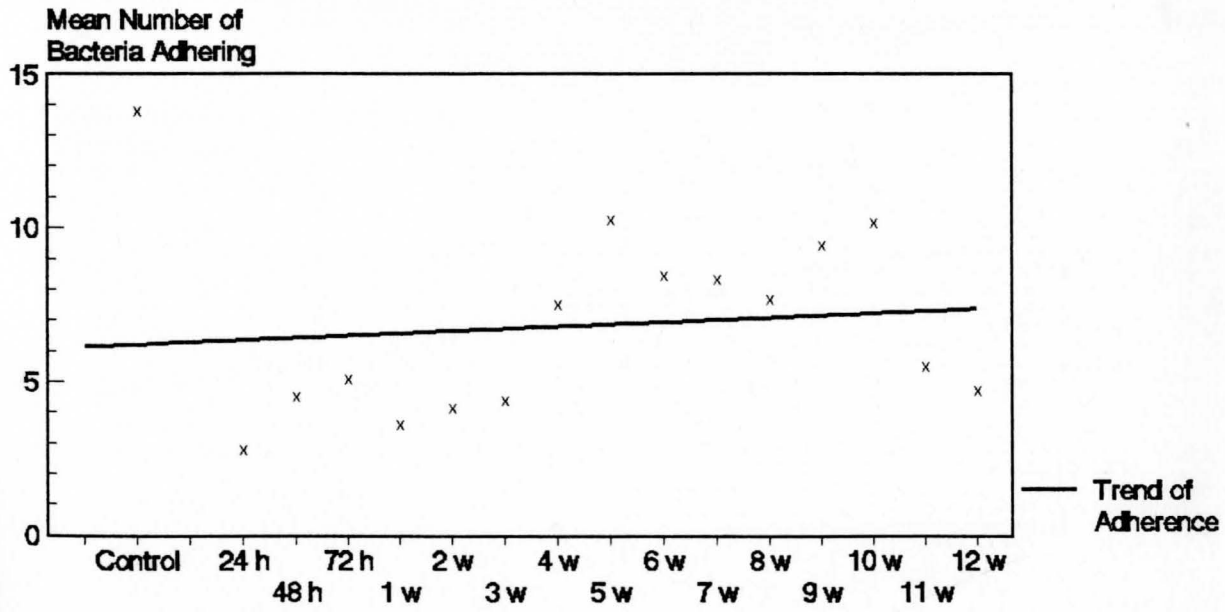
B) Patient 6: Trend of Bacterial Adherence to Uroepithelia

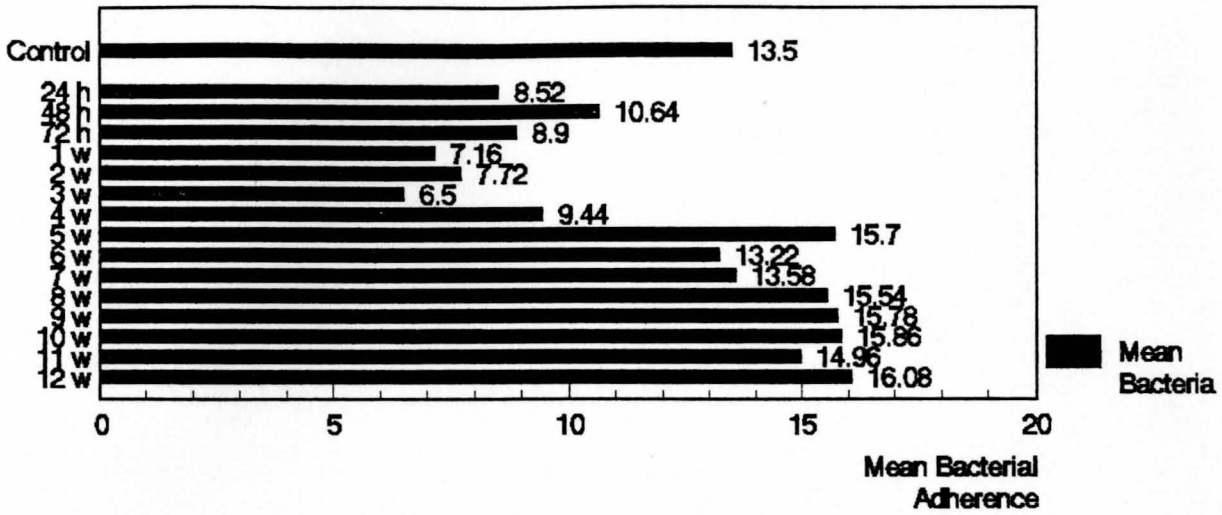


KeyChart 2000

Figure 5: Bacterial adherence data for Patient 6. Bacterial isolate: *Escherichia coli* (from Patient 3) A) Bar graph of mean number of bacteria adhering to 50 uroepithelial cells with THP as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelial cells including 50 cells assayed in the presence of THP extracted 3, 5, and 7 days prior to the initiation of CJC administration. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, with all other specimens collected at the indicated time following the beginning of CJC ingestion.

A) Patient 6: Mean Bacterial Adherences to Uroepithelia

The Utilization of Patient 6 THP on Clearance of E. coli



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B) Patient 6: Trend of Bacterial Adherence to Uroepithelia

The Utilization of Patient 6 THP on Clearance of E. coli

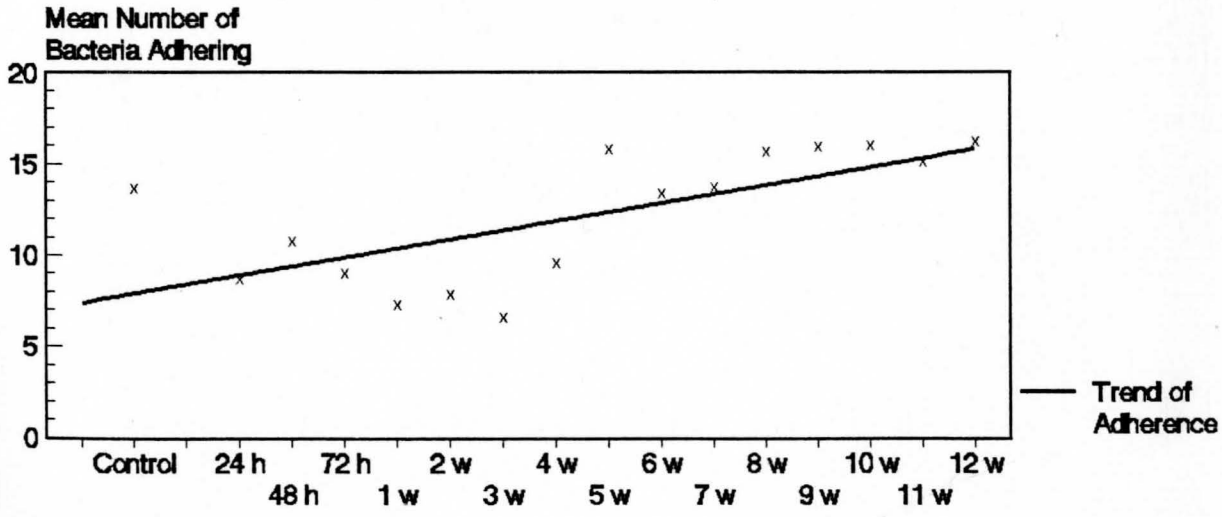

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Figure 6: Comparison of the effects of Patient 6 THP on two different isolates. The solid line depicts the trend of *E. coli* + THP and the dashed line depicts the trend of *K. pneumoniae* + THP during the twelve week period of CJC ingestion.

Comparison of the Effects of Patient 6 THP on Two Different Isolates

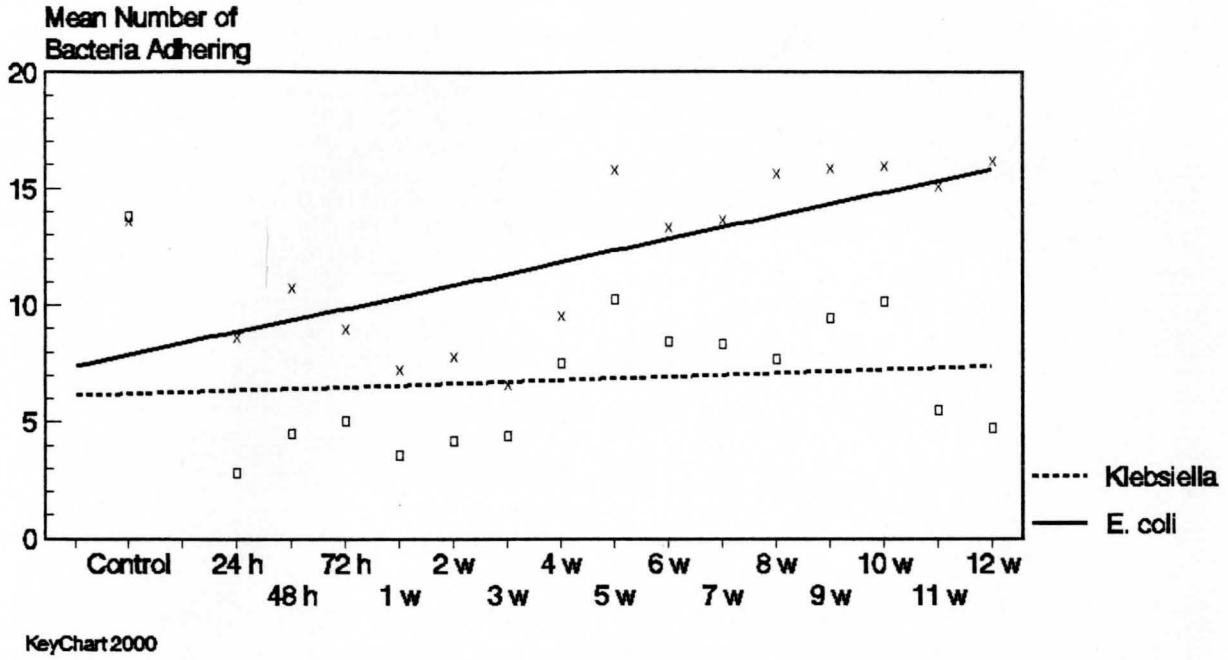
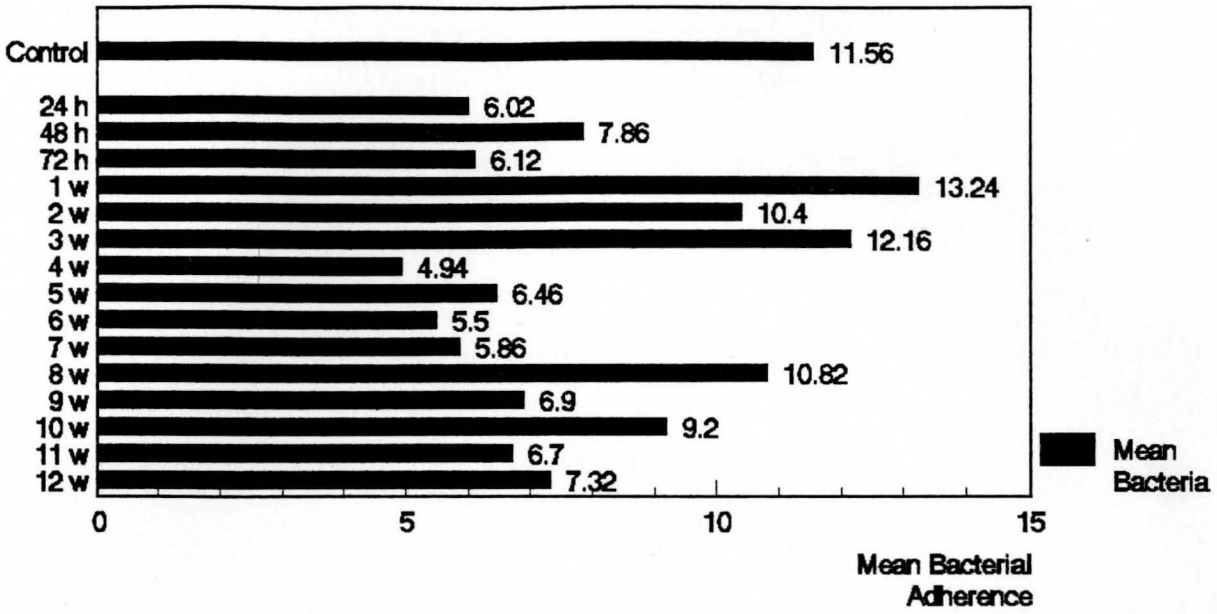


Figure 7: Bacterial adherence data for Patient 8. Bacterial isolate: *Escherichia coli.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelial cells with THP as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelial cells including 50 cells assayed in the presence of THP extracted 3, 5, and 7 days prior to the initiation of CJC administration. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, with all other specimens collected at the indicated time following the beginning of CJC ingestion.

A) Patient 8: Mean Bacterial Adherences to Uroepithelia



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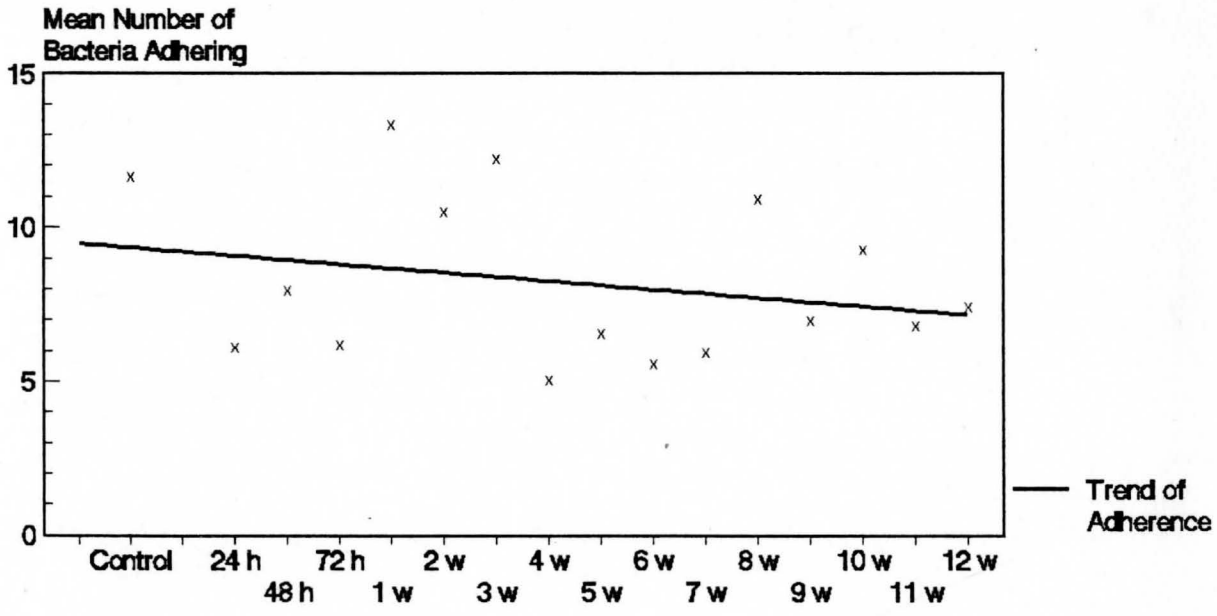
B) Patient 8: Trend of Bacterial Adherence to Uroepithelia

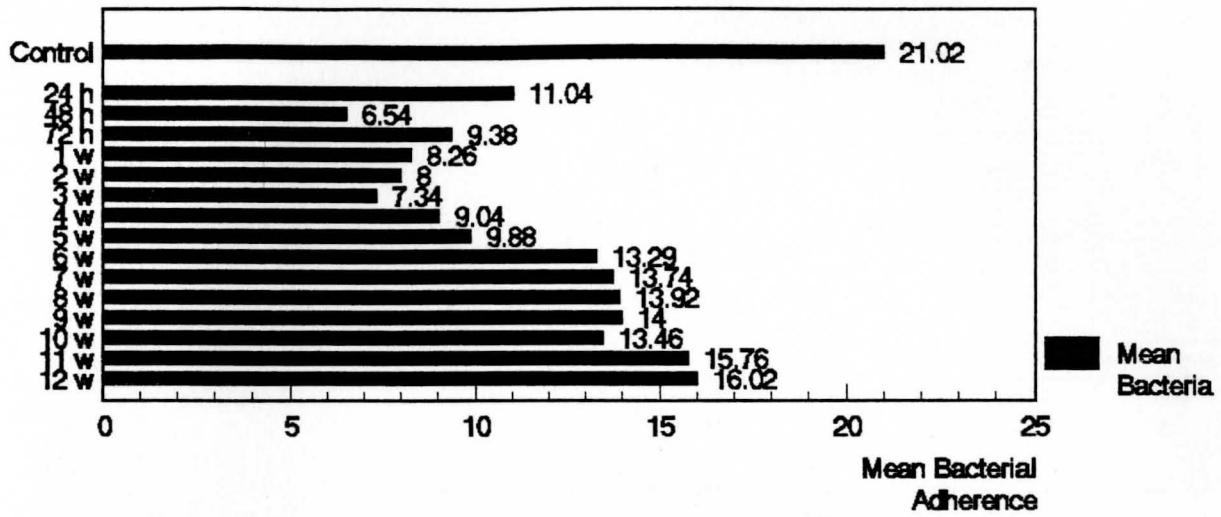


KeyChart 2000

Figure 8: Bacterial adherence data for Patient 8. Bacterial isolate: *Escherichia coli* (from Patient 3) A) Bar graph of mean number of bacteria adhering to 50 uroepithelial cells with THP as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelial cells including 50 cells assayed in the presence of THP extracted 3, 5, and 7 days prior to the initiation of CJC administration. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, with all other specimens collected at the indicated time following the beginning of CJC ingestion.

A) Patient 8: Mean Bacterial Adherences to Uroepithelia

The Utilization of Patient 8 THP on Clearance of E. coli



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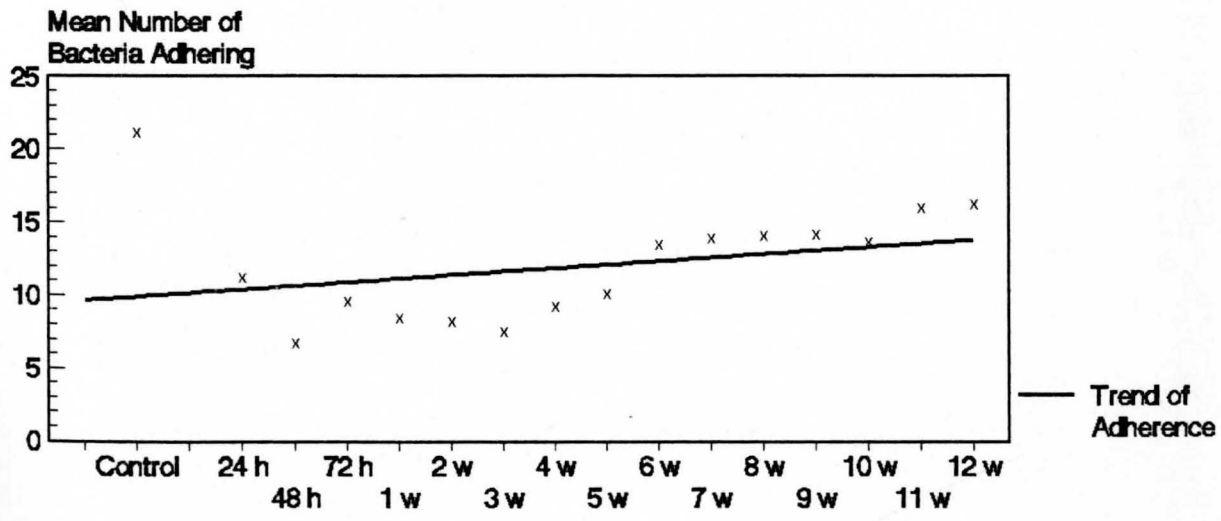
B) Patient 8: Trend of Bacterial Adherence to Uroepithelia

The Utilization of Patient 8 THP on Clearance of E. coli



KeyChart 2000

Figure 9: Comparison of the effects of Patient 8 THP on two different isolates. The solid line depicts the trend of *E. coli* (control) + THP and the dashed line depicts the trend of *E. coli* (original) + THP during the twelve week period of CJC ingestion.

Comparison of the Effects of Patient 8 THP on Two Different Isolates

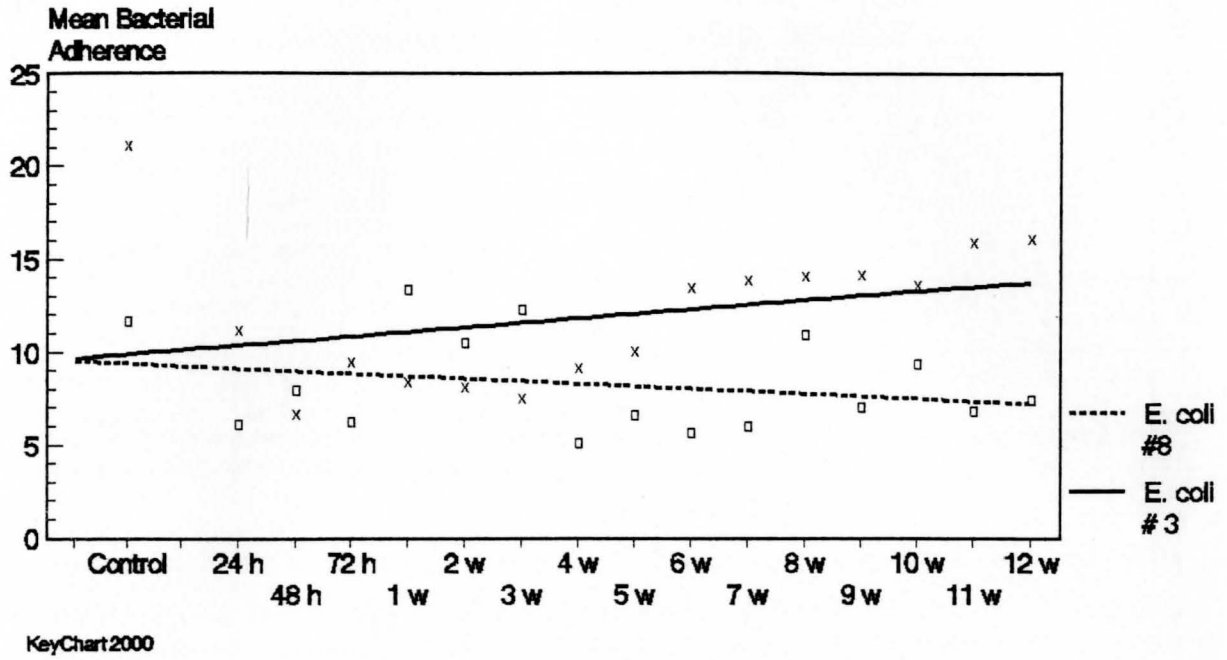
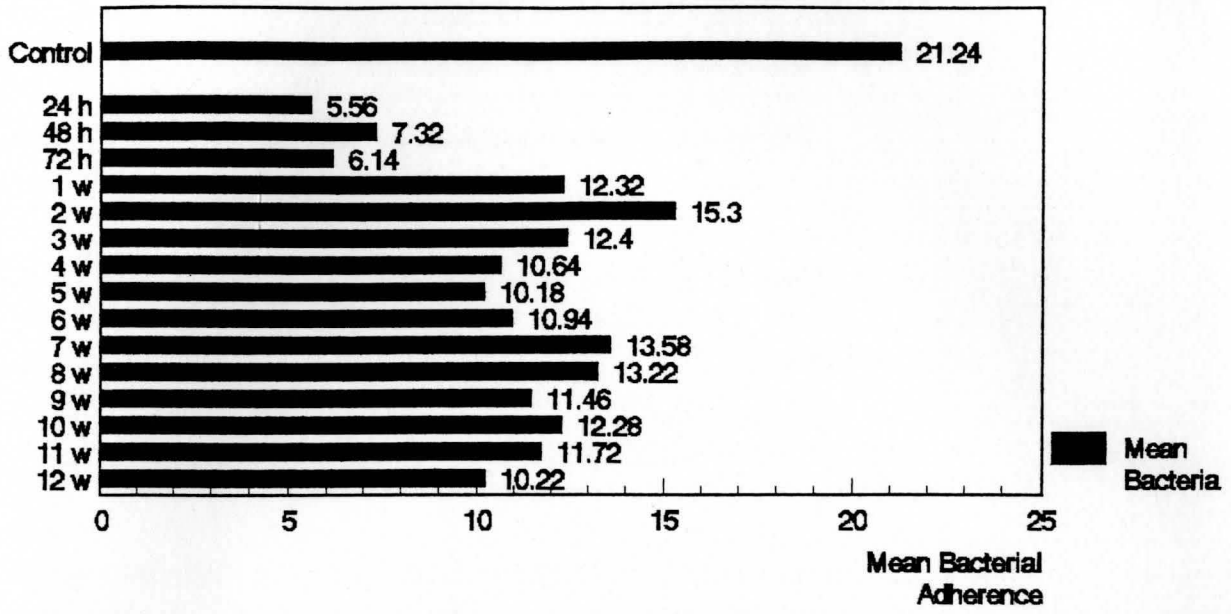


Figure 10: Bacterial adherence data for Patient 9. Bacterial isolate: *Escherichia coli*. A) Bar graph of mean number of bacteria adhering to 50 uroepithelial cells with THP as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelial cells including 50 cells assayed in the presence of THP extracted 3, 5, and 7 days prior to the initiation of CJC administration. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, with all other specimens collected at the indicated time following the beginning of CJC ingestion.

A) Patient 9: Mean Bacterial Adherences to Uroepithelia



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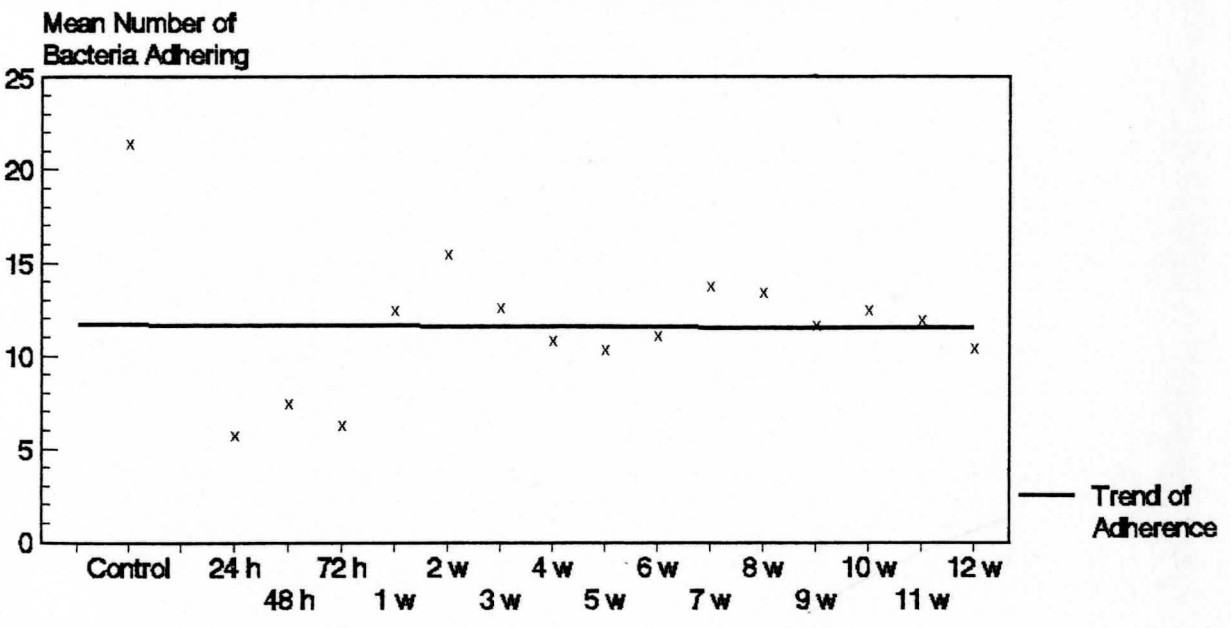
B) Patient 9: Trend of Bacterial Adherence to Uroepithelia

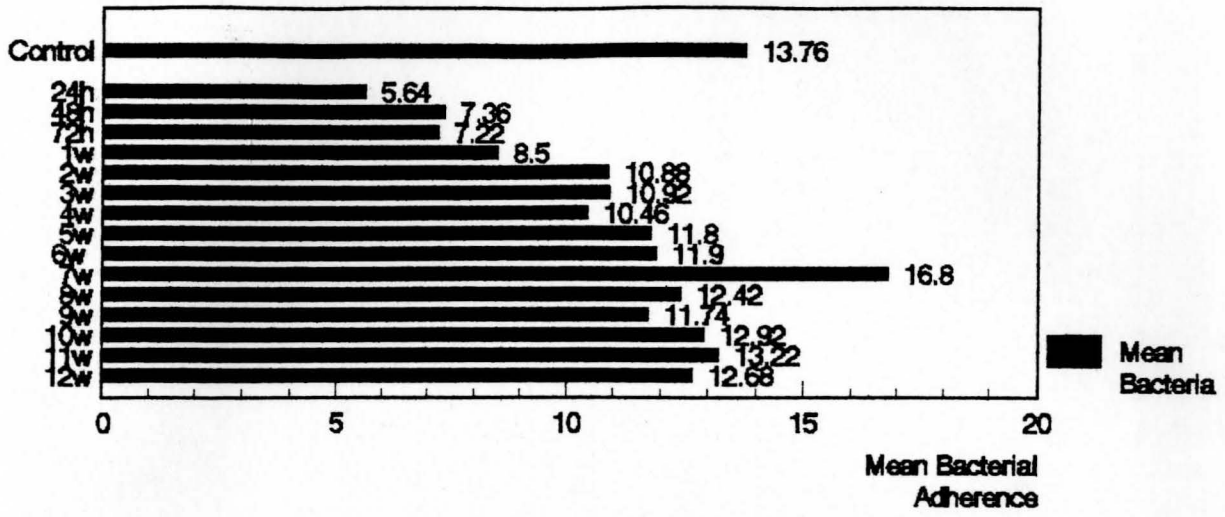


KeyChart 2000

Figure 11: Bacterial adherence data for Patient 9. Bacterial isolate: *Escherichia coli* (from Patient 3) A) Bar graph of mean number of bacteria adhering to 50 uroepithelial cells with THP as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelial cells including 50 cells assayed in the presence of THP extracted 3, 5, and 7 days prior to the initiation of CJC administration. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, with all other specimens collected at the indicated time following the beginning of CJC ingestion.

A) Patient 9: Mean Bacterial Adherences to Uroepithelia

The Utilization of Patient 9 THP on Clearance of E. coli



KeyChart 2000

B) Patient 9: Trend of Bacterial Adherence to Uroepithelia

The Utilization of Patient 9 THP on Clearance of E. coli

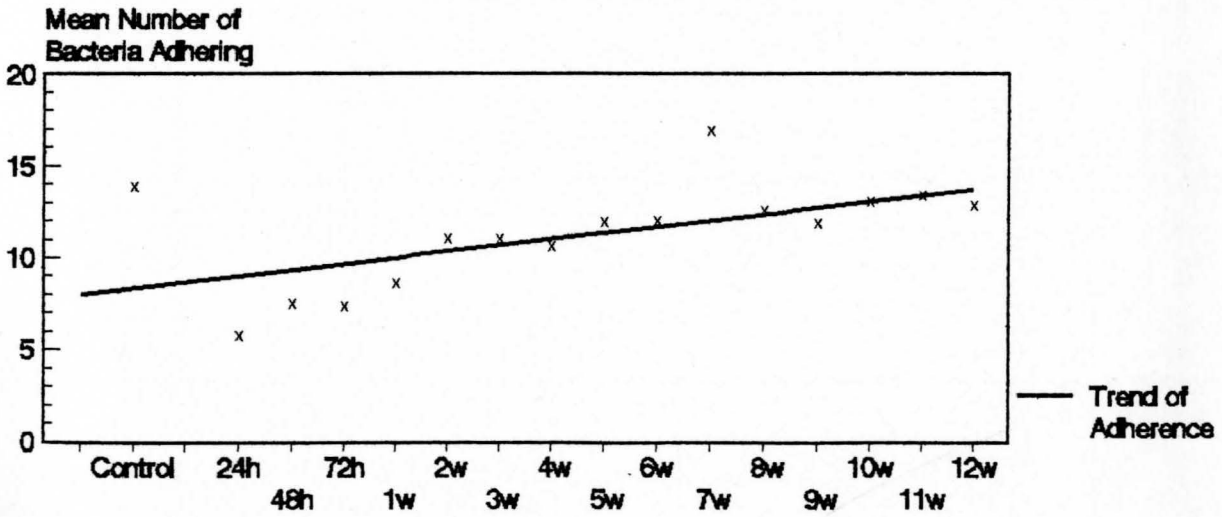


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Figure 12: Comparison of the effects of Patient 9 THP on two different isolates. The solid line depicts the trend of *E. coli* (control) + THP and the dashed line depicts the trend of *E. coli* (original) + THP during the twelve week period of CJC ingestion.

Comparison of the Effects of Patient 9 THP on Two Different Isolates

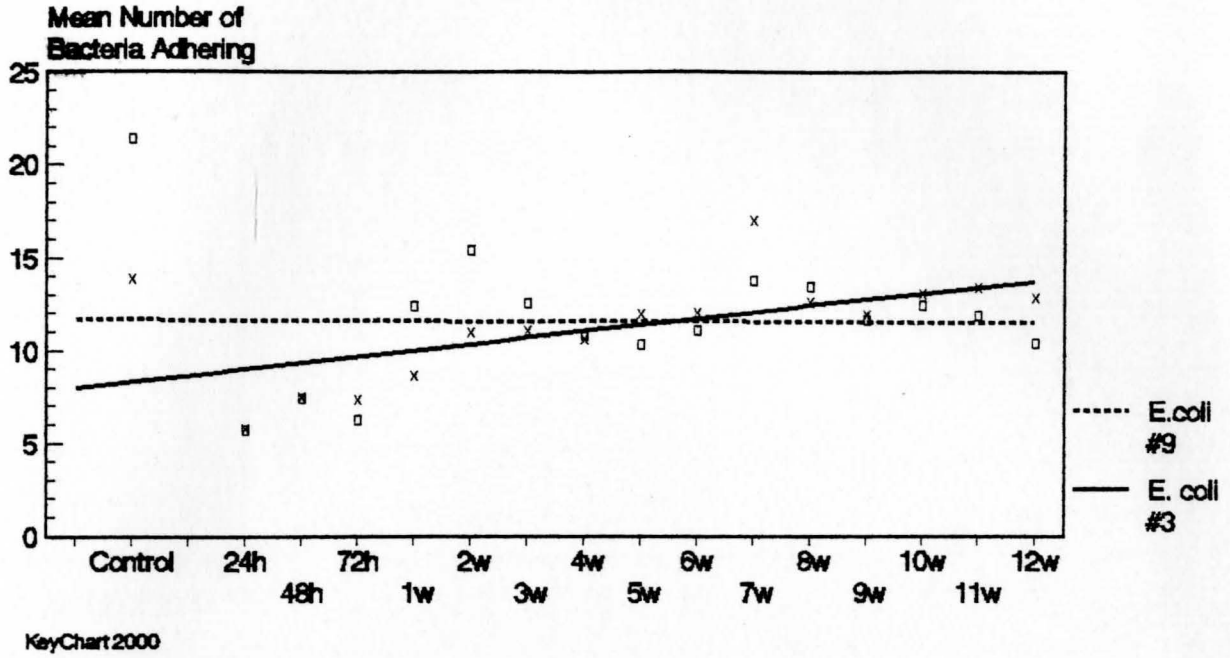

Figure 13: SDS-PAGE analysis of pre- and post-CJC THP. THP was extracted as before and washed 2X before being loaded into the gel. Pre-CJC THP was loaded into lane 1 and post-CJC was loaded in lane 2. Lane 3 contains remnants from lane 2. A molecular weight standard was loaded in lane 4 to ensure the integrity of the bands. The gel was stained with coomassie blue and any changes in band size were noted. THP is known to be a 90-100 kDa protein.

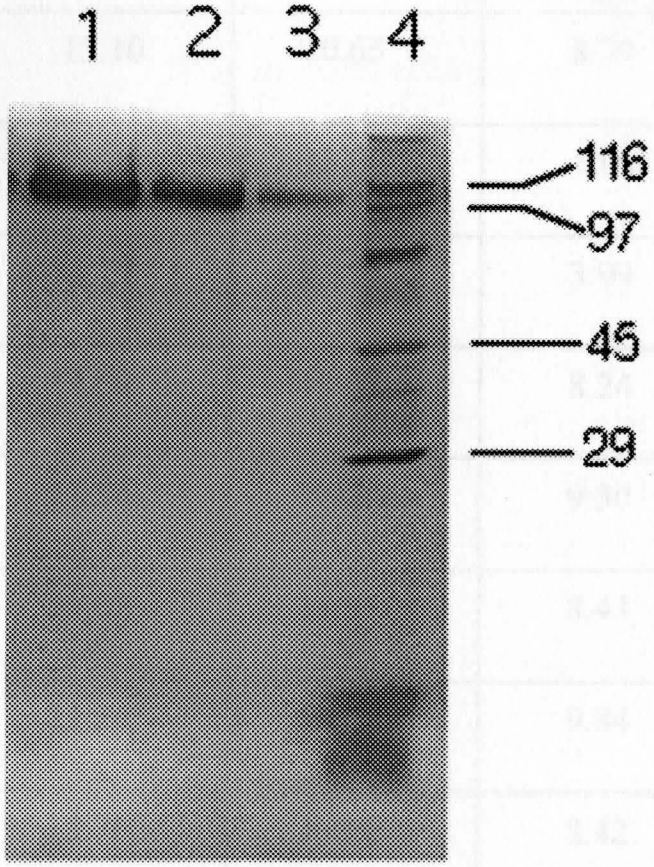
1
2
3
4
116
97
45
29

Table 1: Compiled Average Adherences for the Eight Assays Performed:

Patient Number:	**Base-line Control:**	**CJC Average:**	**First 3 Weeks Average:**	**Weeks 4-12 Average:**
2 (*P. aerogino.*)	15.10	10.65	8.77	11.91
2 ** (*E. coli)*	20.90	13.00	10.61	14.59
6 ** (*K. pneumon.*)	13.70	6.35	3.99	7.92
6 (*E. coli*)	13.50	11.97	8.24	14.46
8 ** (*E. coli*)	11.56	7.97	9.30	7.08
8** (*E. coli* {3})	21.02	11.31	8.43	13.23
9** (*E. coli*)	21.24	10.89	9.84	11.58
9 (*E. coli* {3})	13.76	10.96	8.42	12.66

**Patients with significantly more anti-adherent THP

Table 2: Mean Bacterial Adherence to 50 Uroepithelial Cells for Each Specimen of Urine from Patient Number 2 (and statistical data)

Bacterial Isolate: *Pseudomonas aeroginosa*

Sample:	Mean Bacterial Adherence:	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	15.10	
24 Hours Post-CJC	3.78*	0.0001
48 Hours	6.86*	0.0004
72 Hours	8.86*	0.0073
1 Week	10.80	0.0639
2 Weeks	10.82	0.0651
3 Weeks	11.50	0.1207
4 Weeks	12.30	0.2272
5 Weeks	12.70	0.3006
6 Weeks	13.68	0.5401
7 Weeks	12.56	0.2733
8 Weeks	11.08	0.0832
9 Weeks	12.42	0.2747
10 Weeks	11.26	0.1180
11 Weeks	8.24*	0.0055
12 Weeks	12.96	0.3829

* Significantly lower than control

Table 3: Mean Bacterial Adherence to 50 Uroepithelial Cells for Each Specimen of Urine from Patient Number 2 (and statistical data)

Bacterial Isolate: *Escherichia coli* (from Patient 3)

Sample:	Mean Bacterial Adherence:	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	20.90	
24 Hours Post-CJC	6.26*	0.0001
48 Hours	10.44*	0.0001
72 Hours	11.64*	0.0001
1 Week	10.62*	0.0001
2 Weeks	11.66*	0.0001
3 Weeks	13.06*	0.0001
4 Weeks	13.56*	0.0002
5 Weeks	14.14*	0.0007
6 Weeks	14.20*	0.0008
7 Weeks	13.48*	0.0005
8 Weeks	15.86*	0.0116
9 Weeks	14.54*	0.0063
10 Weeks	11.96*	0.0001
11 Weeks	17.64	0.1596
12 Weeks	15.46*	0.0192

* Significantly lower than control

Table 4: Mean Bacterial Adherence to 50 Uroepithelial Cells for Each Specimen of Urine from Patient Number 6 (and statistical data)

Bacterial Isolate: *Klebsiella pneumoniae*

Sample:	Mean Bacterial Adherence:	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	13.70	
24 Hours Post-CJC	2.70*	0.0001
48 Hours	4.40*	0.0001
72 Hours	4.98*	0.0001
1 Week	3.50*	0.0001
2 Weeks	4.06*	0.0001
3 Weeks	4.30*	0.0001
4 Weeks	7.42*	0.0002
5 Weeks	10.18*	0.0366
6 Weeks	8.38*	0.0016
7 Weeks	8.22*	0.0012
8 Weeks	7.60*	0.0003
9 Weeks	9.36*	0.0318
10 Weeks	10.08	0.0716
11 Weeks	5.41*	0.0001
12 Weeks	4.64*	0.0001

* Significantly lower than control

Table 5: Mean Bacterial Adherence to 50 Uroepithelial Cells for Each Specimen of Urine from Patient Number 6 (and statistical data)

Bacterial Isolate: *Escherichia coli* (from Patient 3)

Sample:	Mean Bacterial Adherence:	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	13.50	
24 Hours Post-CJC	8.52*	0.0335
48 Hours	10.64	0.2216
72 Hours	8.90*	0.0495
1 Week	7.16*	0.0069
2 Weeks	7.72*	0.0137
3 Weeks	6.50*	0.0029
4 Weeks	9.44	0.0829
5 Weeks	15.70	0.3469
6 Weeks	13.22	0.9047
7 Weeks	13.58	0.9727
8 Weeks	15.54	0.3831
9 Weeks	15.78	0.4063
10 Weeks	15.86	0.3901
11 Weeks	14.96	0.5948
12 Weeks	16.08	0.3475

* Significantly lower than control

Table 6: Mean Bacterial Adherence to 50 Uroepithelial Cells for Each Specimen of Urine from Patient Number 8 (and statistical data)

Bacterial Isolate: *Escherichia coli*

Sample:	Mean Bacterial Adherence:	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	11.56	
24 Hours Post-CJC	6.02*	0.0022
48 Hours	7.86*	0.0400
72 Hours	6.12*	0.0026
1 Week	13.24	0.3505
2 Weeks	10.40	0.5191
3 Weeks	12.16	0.7387
4 Weeks	4.94*	0.0003
5 Weeks	6.46*	0.0047
6 Weeks	5.50*	0.0008
7 Weeks	5.86*	0.0016
8 Weeks	10.82	0.6808
9 Weeks	6.90*	0.0094
10 Weeks	9.20	0.1860
11 Weeks	6.70*	0.0068
12 Weeks	7.32*	0.0179

* Significantly lower than control

Table 7: Mean Bacterial Adherence to 50 Uroepithelial Cells for Each Specimen of Urine from Patient Number 8 (and statistical data)

Bacterial Isolate: *Escherichia coli* (from Patient 3)

Sample:	Mean Bacterial Adherence:	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	21.02	
24 Hours Post-CJC	11.04*	0.0001
48 Hours	6.54*	0.0001
72 Hours	9.38*	0.0001
1 Week	8.26*	0.0001
2 Weeks	8.00*	0.0001
3 Weeks	7.34*	0.0001
4 Weeks	9.04*	0.0001
5 Weeks	9.88*	0.0001
6 Weeks	13.29*	0.0003
7 Weeks	13.74*	0.0008
8 Weeks	13.92*	0.0011
9 Weeks	14.00*	0.0128
10 Weeks	13.46*	0.0074
11 Weeks	15.76	0.0614
12 Weeks	16.02	0.0752

* Significantly lower than control

Table 8: Mean Bacterial Adherence to 50 Uroepithelial Cells for Each Specimen of Urine from Patient Number 9 (and statistical data)

Bacterial Isolate: *Escherichia coli*

Sample:	Mean Bacterial Adherence:	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	21.24	
24 Hours Post-CJC	5.56*	0.0001
48 Hours	7.32*	0.0001
72 Hours	6.14*	0.0001
1 Week	12.32*	0.0001
2 Weeks	15.30*	0.0007
3 Weeks	12.40*	0.0001
4 Weeks	10.64*	0.0001
5 Weeks	10.18*	0.0001
6 Weeks	10.94*	0.0001
7 Weeks	13.58*	0.0001
8 Weeks	13.22*	0.0001
9 Weeks	11.46*	0.0001
10 Weeks	12.28*	0.0001
11 Weeks	11.72*	0.0001
12 Weeks	10.22*	0.0001

* Significantly lower than control

Table 9: Mean Bacterial Adherence to 50 Uroepithelial Cells for Each Specimen of Urine from Patient Number 9 (and statistical data)

Bacterial Isolate: *Escherichia coli* (from Patient 3)

Sample:	Mean Bacterial Adherence:	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	13.76	
24 Hours Post-CJC	5.64*	0.0001
48 Hours	7.36*	0.0017
72 Hours	7.22*	0.0013
1 Week	8.50*	0.0098
2 Weeks	10.88	0.1562
3 Weeks	10.92	0.1620
4 Weeks	10.46	0.1043
5 Weeks	11.80	0.3344
6 Weeks	11.90	0.3596
7 Weeks	16.80	0.1345
8 Weeks	12.42	0.5092
9 Weeks	11.74	0.3684
10 Weeks	12.92	0.5454
11 Weeks	13.22	0.4483
12 Weeks	12.68	0.6300

* Significantly lower than control

Table 10: Comparison of Mean Bacterial Adherences Before and After CJC Ingestion
(Whole urine + original isolate, THP + original isolate, & *E. coli* + THP)

Patient Number:	Base-line Control:	CJC Average:	First 3 Weeks of CJC Average:	Average after Week 4:
TWO:				
Whole Urine*	11.02	15.03	11.68	17.28
THP	15.10	10.65	8.77	11.91
E. coli	20.90	13.00	10.61	14.59
SIX:				
Whole Urine*	21.21	19.14	20.00	18.51
THP	13.70	6.35	3.99	7.92
E. coli	13.50	11.97	8.24	14.46
EIGHT:				
Whole Urine*	12.66	7.30	12.33	3.95
THP	11.56	7.97	9.30	7.08
E. coli	21.02	11.31	8.43	13.23
NINE:				
Whole Urine	NA	NA	NA	NA
THP	21.24	10.89	9.84	11.58
E. coli	13.76	10.96	8.42	12.66

*Excerpted from Patrick Wilson's Research

IV. Discussion

Urinary tract infections are second only to respiratory infections as causative agents of disease in the overall population, especially the elderly. [77] It is estimated that urinary tract infections will result in an estimated eight million visits to the doctor's office in our population. Therefore, prevention and treatment of UTI is of the utmost importance in the overall population. Since antibiotic resistance seems to be on the rise, other avenues have been examined as means for control of UTI. Within this laboratory under the direction of Dr. Anthony E. Sobota, research has continued over the last twelve years pertaining to the use of cranberry juice cocktail as a possible means for the prevention and treatment of urinary tract infection. Initially, some twelve years ago, Dr. Sobota [60] first demonstrated that cranberry juice cocktail acted as a very potent bacterial antiadherence agent both *in vitro* and *in vivo* and therefore may be potentially useful for the treatment of urinary tract infection. In the *in vitro* study, bacterial adherence assays were performed using *E. coli* and uroepithelial cells that were incubated in the presence of CJC. The results showed that in the presence of CJC, adherence of *E. coli* to the uroepithelial cells decreased by 97%. In addition, by incubating CJC + *E. coli* and CJC + uroepithelial cells and then performing adherence assays, Sobota demonstrated that CJC interacts with *E. coli* but does not act on the uroepithelial cells. In the *in vivo* study, a murine model was used in which

the water source was replaced with CJC. Urine specimens were then collected and used in bacterial adherence assays. This urine exhibited significant antiadherent activity when compared to murine urine collected before CJC ingestion. In conclusion, both studies attributed the reduction of bacterial adherence to the use of CJC whether it was used *in vitro* or *in vivo*.

In addition, 22 subjects donated urine before drinking 15 ounces of CJC and again a few hours after consumption. An inherent fast acting decrease in bacterial adherence was seen in 68% of the subjects just a few hours after consumption. From these results as well as the aforementioned results, Sobota concluded that some component within the cranberry juice cocktail must be impeding the adherence of *E. coli* to the uroepithelium. From this research, it was shown that CJC decreased bacterial adherence but a plausible explanation still was not evident. This led to the exploration of a component and/or components that were responsible for the noted decrease in bacterial adherence.

The research in our laboratory centered around the Tamm-Horsfall glycoprotein as the possible unknown component responsible for decreasing adherence. In the experimental protocol, a base-line control was first established by incubating uroepithelial cells and *E. coli* with saline and then fresh void urine was used as the reaction medium in place of saline.[59] Bacterial adherence assays were then performed

that showed significant decreases in adherence of bacteria when whole urine was compared to the control. THP was then extracted from this antiadherent urine to deduce if this glycoprotein was in fact the antiadherent component. Furthermore, a sample of urine minus THP and whole urine with THP were subjected to adherence assays in the presence of *E. coli*. The results showed that THP at concentrations occurring in normal urine exemplified antiadherence activity for type-1 fimbriated *E. coli* and the removal of THP from urine induced a substantial increase in bacterial adherence. [59]

In a more recent study performed in this laboratory by Patrick Wilson, [74] instead of looking at altered tendencies in the incidence and progression of UTI after cranberry juice cocktail ingestion, a direct observation in the capacity of CJC to induce antiadherence activity in the urine of eight compromised patients in a clinical setting was made. This population group was chosen because of all the reasons mentioned in the introduction as well as the difficulties of antibiotic treatment that are present in this high risk group. In the research protocol, each respective patient was given four ounces of CJC daily for a period of twelve weeks and urine was collected on a weekly basis. This urine was then used as the reaction medium for adherence assays. Comparisons were then made between mean bacterial adherences before and after CJC ingestion. In summary, four of the eight patients displayed more antiadherent urine

several weeks after the beginning of CJC ingestion and an additional patient showed moderate improvement when compared to base-line controls. [74]

Throughout all of the research that has been performed in the last decade in this laboratory, great strides have been made in proving the worth of CJC in the prevention and treatment of UTI. In the current study, our focus turned to connecting CJC ingestion with the previously explained antiadherence effect of THP. In our protocol, THP was extracted from urine that was previously analyzed in Wilson's study and used as the reaction medium for bacterial adherence assays. The current study was divided into three parts.

In the first part of the study, the antiadherent effect of whole urine was compared to that of THP isolated from the same urine. In analyzing the whole urine samples, patients 6 and 8 showed decreases in bacterial adherence over the twelve week period while patient 2 exhibited an increase in mean bacterial adherence over the same time frame. In addition, the decrease in bacterial adherence in patient 8 was statistically significant. In contrast to those results seen with whole urine, all four patients showed decreases in adherence when THP was used instead of whole urine. Three of the four patients showed significant decreases in bacterial adherence. An interesting point that was seen pertained to the results of whole urine and THP data from patient 2. When whole urine was used as the reaction medium, there was an overall increase in

adherence whereas when THP was used as the reaction medium, a decrease in bacterial adherence was seen. These contradicting results could be due to other factors present in urine that impede the effectiveness of THP as an antiadherence agent. A somewhat similar situation was seen in patient 6, with THP by itself having a more pronounced antiadherent effect than that seen with whole urine. In summary, it can be concluded that only one of the patients benefitted from the ingestion of CJC (patient 8), even though all of the patients had significant antiadherent THP. Therefore, some change within the urine must take place before the other patients are receptive to CJC therapy.

In the second part of our research, the responsiveness of each respective patients' THP was assessed by reacting it with a control. *E. coli* was chosen as the control because it has been previously shown to be very responsive to THP. This bacteria of choice contains type-1 fimbrae that contain many mannose receptors. On the otherhand, mannose residues comprise approximately 28% of the carbohydrate concentration of THP. [17] Furthermore, THP encapsulates only type-1 fimbriated bacteria and therefore this bacteria is referred to as mannose-sensitive. Taking this factor into account, our goal was to derive a correlation between the effectiveness of each patients' THP in decreasing adherence of *E. coli* versus the original isolate. Since all four of the patients were infected with bacteria possessing mannose-sensitive

type-1 fimbriated bacteria, it was initially perceived that all results would be similar. In fact, the results showed that THP was effective in decreasing bacterial adherence in both the control and original isolates albeit to different degrees. The decreases in adherence that were seen in the control in addition to those previously seen with the original isolates when THP was used as the reaction medium further support the claim that CJC ingestion positively affects the antiadherence activity of THP.

In the final part of our study, a biochemical analysis of THP was performed in order to derive a mechanism as to how CJC causes increased antiadherence activity in THP. The glycoprotein was first analyzed through SDS-PAGE to note any changes in relative band size between the pre- and post-CJC samples The band was then extracted from the gel and used as the reaction medium for adherence assays. The results from this initial study showed that there was no change in relative band size between the pre- and post-CJC THP samples. In addition, there was a significant decrease in bacterial adherence when the post-CJC sample was compared to the control. Pre- and post-CJC THP were further analyzed through UV spectrophotometry in which no significant changes in absorbance readings were noted. In fact, THP before CJC ingestion had a slightly higher absorbance reading than THP after CJC ingestion.

These results provided insightful information into explaining the actions of CJC on

THP. Since no difference in concentration was noted between the pre- and post-CJC THP samples in both cases, it can be concluded that CJC ingestion does not decrease bacterial adherence by increasing THP concentrations. Therefore some type of qualitative change must take place that makes THP more antiadherent upon CJC ingestion. The fact that THP encapsulates only mannose-sensitive type-1 fimbriated bacteria may provide the answer to the CJC-THP process. As mentioned previously, there are many mannose residues within THP and there are mannose receptors found on type-1 fimbrae. In theory, these mannose residues must come in contact with the mannose receptors on type-1 fimbriated bacteria for binding and subsequent encapsulation to take place. Since the elderly population have been shown to have decreased levels of THP and analysis has suggested that there is no significant difference in concentration before and after CJC ingestion, some conformational change must take place that increases the affinity or more readily exposes the mannose residues in THP to type-1 fimbriated bacteria. Therefore, even though the elderly are at a disadvantage because of deviations in concentrations of THP from the norm, they serve to benefit the most from CJC therapy due in part to the proposed qualitative change that takes place in the native protein.

In an overall summary, it has undoubtedly been shown that cranberry juice cocktail elicits changes in the Tamm-Horsfall glycoprotein and these changes are responsible

for the observed decreases in bacterial adherence seen in the four catheterized, geriatric patients examined. The conclusion of THP as the "unknown component" responsible for decreases in bacterial adherence upon CJC ingestion has been strongly supported in all three portions of our research project. Finally, although preliminary findings support the hypothesis of a conformational change in native THP upon CJC ingestion, more conclusive results may be needed in order to solidify this hypothesis and thus serve as evidence that cranberry juice cocktail may be the best therapy in preventing and treating urinary tract infection in the geriatric population.

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