THE ROLE OF BACTERIAL ADHERENCE IN THE PATHOGENESIS OF RECURRENT URINARY TRACT INFECTIONS

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THE ROLE OF BACTERIAL ADHERENCE IN THE PATHOGENESIS OF RECURRENT URINARY TRACT INFECTIONS

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ABSTRACT

To determine the role of bacterial adherence in the pathogenesis of recurrent urinary tract infections (UTI) we investigated *in vitro* bacterial adhesion to buccal, vaginal epithelial and uroepithelial cells, from 21 patients (both colonized and non-colonized) with recurrent UTI, and 10 normal healthy controls.

The mean number of adherent *Escherichia coli* per cell in the colonized patient group was: 35.8 $\pm$ 1.9, 32.8 $\pm$ 1.9, and 35.6 $\pm$ 1.9), in the non-colonized patient group (31.7 $\pm$ 2.6, 32.7 $\pm$ 1.5, and 33.4 $\pm$ 1.4), and the control group, (15.5 $\pm$ 2.7, 15.9 $\pm$ 3.1, and 15.3 $\pm$ 4.0) for vaginal, buccal and uroepithelial cells, respectively. The mean number of adherent bacteria per cell in the patient group (colonized and non-colonized) was significantly higher than that of controls ($\rho < .005$).

In vitro adhesion of the patient's own *E. coli* to buccal, vaginal, and uroepithelial cells did not vary significantly ($\rho = .30$) between the physiological age groups (10 pre- and 11 post-menopausal). Also, there was no significant difference ($\rho = .30$) in the level of adherence during peak estrogen level (week 2 of the menstrual cycle, as compared to declining estrogen levels).

In evaluating the overall patient group (colonized and non-colonized), 8 patients receiving antimicrobial prophylaxis had a significantly ($\rho < .005$) lower level of adherence than 10 patients not receiving prophylaxis (26.7 $\pm$ 1.3 vs. 35.1 $\pm$ 1.3 and 30.4 $\pm$ .9 vs. 34.6 $\pm$ .8), for vaginal and uroepithelial cells, respectively.

A similar trend was observed when examining only the same 5 patients when receiving antimicrobial prophylaxis comparing them to when they were off prophylaxis (at least one month following antimicrobial prophylaxis). The mean number of adherent bacteria per vaginal epithelial cell was observed to be 28.4 ± 1.6 vs. 34.6 ± 1.3 and the mean number of bacteria per uroepithelial cell was 29.9 ± 2.2 vs. 33.2 ± 2.5, for patients receiving and no longer receiving antimicrobials, respectively ($\rho > .05$).

The mean number of adherent bacteria per buccal epithelial cell did not differ significantly ($\rho = .35$) from patients receiving antimicrobial prophylaxis compared to those not receiving prophylaxis in either the overall patient group or the same patients.

With the addition of 2.5% wt./vol. of D-mannose to the bacterial suspension prior to addition of epithelial cells, bacterial adherence was inhibited completely.

Increased receptivity of epithelial cells for bacteria plays a role in the increased frequency of vaginal colonization in susceptible patients. This may be mediated by mannose-containing receptors.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Urinary tract infections (UTI) are regarded as a group of infectious diseases of varying clinical severity. Urinary tract infections may be manifested as: acute pyelonephritis (kidney infection), acute cystitis (bladder infection), and bacteruria (restricted to urine). (Lindberg et al, 1975).

Recurrent urinary infection implies that the original infection is still present during or after therapy despite an attempt at eradication, or that infection has recurred following therapy. The infection may be temporarily eradicated with relapse or reinfection subsequent to therapy. Bacterial relapse is the re-emergence of the same bacterial strain in the urine within a short time following an apparently successful course of therapy. Bacterial reinfections result from new organisms gaining entrance into the urinary tract from the fecal-vaginal reservoir. (Harding and Ronald, 1974).

Recurrent urinary infection is a significant problem in approximately 10% of the adult female population. (Waters, 1969). Kunin et al (1964) has shown that 80% of infections recur within five years following therapy in schoolgirls. He noted that 72% were due to E. coli, and that over 80% of the recurrences in this population were reinfections.

Stamey et al, 1971 has shown that the colon is a reservoir for bacteria that cause these infections, and that colonization of the vaginal introitus occurs before the organism gains access to the urinary tract. Women with recurrent infections are

assumed to have a biologic defect that permits heavy colonization of the mucosal perineum with Enterobacteriaceae as compared to those resistant to recurrent infection (Fowler and Stamey, 1977).

There are a number of nonspecific and specific factors influencing a bacterial species' ability to colonize the vagina or any mucosal surface. Nonspecific factors include the net charge on the surface of both the bacterial and host cells, which may be overcome by the attractive forces between hydrophobic molecules present in varying numbers on the two cell surfaces (Bell, 1978). Specific factors include membrane receptors and complementary bacterial components required for adherence (Schaeffer et al, 1980, 1981).

Adhesion of microorganisms to epithelial cells may serve as an essential first step in the pathogenesis of recurrent UTI. The concept that adherence of bacteria to vaginal epithelial cells plays a part in the pathogenesis of vaginal colonization has been supported (Fowler and Stamey, 1977; Källenius and Winberg, 1978; Svanborg-Edén et al, 1979), all of whom employed the visual technique, and questioned by Parsons (1979), who employed the ^{14}C-radiolabelling technique, by in vitro studies comparing adherence in controls and patients, usually on the basis of a single determination in each subject.

While our project was in progress, Schaeffer et al (1981) compared in vitro adherence of one strain of Escherichia coli

to 497 samples of vaginal cells obtained frequently from healthy women and patients with recurrent urinary tract infection using ^{3}H-uridine-labelled E. coli (018).

Our study involved the evaluation of in vitro adherence of E. coli (patients' own organism) to buccal, vaginal, and uro-epithelial cells from patients with recurrent UTI and normal healthy controls in an attempt to determine the role of bacterial adherence in the pathogenesis of recurrent UTI. The method used in our study involved radiolabelling of E. coli with ^{125}I prior to incubation with epithelial cells. The ^{125}I-radiolabelling method (Swanson et al, 1975) was compared to the viability adherence assay (Slack and Wheldon, 1978) in terms of sensitivity for detection of in vitro bacterial adherence to epithelial cells. The ^{125}I-radiolabelling method eliminates the problems of attempting to remove the indigenous flora from the epithelial cells, and the subjectivity associated with visual counting of colony forming units to determine adherence which is required in the viability adherence assay. The use of radioactively labelled bacteria in place of the viable counting system increases the validity and sensitivity of the in vitro adherence assey (Schaeffer et al, 1981).

Unlike Parsons et al (1979) who used ^{14}C-labelled E. coli and Schaeffer (1981) who used ^{3}H-uridine labelled E. coli, our study utilized radiolabelling of E. coli with ^{125}I. The advantage to ^{125}I-labelling compared to that of ^{14}C and ^{3}H is

that ^{125}I allows for higher counts per unit of time, therefore allowing for the use of smaller amounts, and resulting in greater sensitivity. Even though the measurement of adherence is determined on a ratio basis, the greater energy emission of ^{125}I (gamma emittor) as opposed to ^{14}C or ^{3}H (beta emittors), allows for a greater accuracy of measurement. Also, the use of ^{125}I eliminates the problem of long-term waste storage. Both ^{14}C and ^{3}H are beta emittors, therefore requiring the use of a scintillation counter and toluene, resulting in radioactive organic wastes (Vaeth, 1972).

^{14}C has a half-life of 5,738 days and ^{3}H has a half-life of 12.62 years, while ^{125}I radioactively decays in 60 days, therefore dispensing with the problem of long-term storage of radioactive waste (Vaeth, 1972).

The disadvantage of working with radioisotopes whether ^{14}C, ^{3}H, or ^{125}I involves their potential as carcinogens. A further disadvantage of ^{125}I, not found with ^{14}C or ^{3}H, is that it may be absorbed by the thyroid, and could result in thyroid tumours (Mclean, 1964).

As well, both ^{14}C and ^{3}H-uridine labelling of bacteria both require culturing of the E. coli with the radioactive component allowing for incorporation into the cell, whereas the ^{125}I method allows for direct labelling of the E. coli to the external surface. Culturing of the bacteria requires a much more cumbersome and time-extended protocol than that of the ^{125}I method, therefore allowing

less room for technical error with the ^{125}I method (David and Reisfeld, 1974).

The second part of the study involved the pre-incubation of E. coli (018) with a 2.5% D-mannose prior to incubation with epithelial cells in an attempt to corroborate with the finding by Schaeffer et al (1980) that a receptor analogue (i.e., D-mannose interferes with microbial adherence and colonization in vivo (Aronseon et al, 1979).

In summary, the objective of our study was to determine the role of bacterial adherence in the pathogenesis of recurrent urinary tract infections by employing a ^{125}I-radioisotope method. The specific aim was to compare the adherence of E. coli (subject's own organism) to buccal, vaginal, and uro-epithelial cells from non-colonized and colonized patients with a history of recurrent urinary tract infection and normal healthy controls with no history of UTI. In this investigation, such parameters as hormonal status, antimicrobial prophylaxis, physiological age and the presenceof carbohydrate were evaluated for their effect on bacterial-epithelial cell adhesion.

LITERATURE REVIEW

A. ROLE OF ADHERENCE IN THE PATHOGENESIS OF UTI

The ability of a microorganism to adhere to a mucosal surface is considered a prerequisite to the colonization of some mucosal surfaces. This interaction between the bacterial and mucosal surfaces, which promotes adhesion, has been shown to be mediated by surface characteristics of the bacteria and of the mucosal surface, and by the presence of antibody, particularly secretory IgA (Fubura and Freter, 1973; Savage, 1972; Ellen and Gibbons, 1972; Liljemark and Gibbons, 1971; Bertschinger *et al*, 1972; Gibbons and van Houte, 1971).

Studies on the adherence of *E. coli* to human epithelial cells have supported the concept that bacterial-epithelial cell specificity may be involved in the adherence process.

Fowler and Stamey (1977) examined the role of bacterial adherence to human vaginal epithelial cells by the visual technique. Cell preparations from 20 patients and 20 controls were placed in gluteraldehyde immediately after the vaginal cells with adherent *E. coli* were lifted from the dermalon filter and examined with scanning electron microscopy. They found that the mean number of adherent 06 *E. coli* per cell for patients and controls was (42.5 ± 25.5 and 19.4 ± 9.4, respectively) $\rho < .001$. They showed that *E. coli* adhered more readily to vaginal cells from women with recurrent urinary infection than to similar cells from control women resistant to urinary infection, suggesting that biologic susceptibility to recurrent urinary infections in women

is related to a defect at the cellular level that encourages or favours bacterial adherence.

In a study done by Mardh and Westrom (1976), vaginal cells were obtained from healthy, non-pregnant, regularly menstruating women, 18 to 35 yrs old. The number of bacteria that adhered to the epithelial cells was counted under a light microscope (X 765). Lactobacilli showed a uniform adherence pattern to the vaginal epithelial cells, with mean values ranging from 7 to 17 bacteria per cell. Also, 4 to 20 times more gonococci than lactobacilli were found to adhere per vaginal epithelial cell. Basically, the experiments showed a differential ability among bacteria that occur in the female genital tract in their adherence rate to vaginal epithelial cells. The study indicated that the attachment of bacteria to exfoliated vaginal epithelial cells is a selective phenomenon.

In a similar study, Källenius and Winberg (1978) examined bacterial adherence to periurethral epithelial cells in girls prone to urinary tract infections. The bacterial-epithelial cell preparation was examined by oil-immersion light microscopy (X 1000). Fifty epithelial cells were scanned and the mean number of attached *E. coli* (075) per cell was calculated. The mean number of bacteria per epithelial cell was registered for patients (some infected and others non-infected at the time of examination) and for controls. The median value for the mean number of bacteria per cell was 0.2 (range of means 0-73) for healthy controls and 2.0 (range 0-41) and 1.2

(range 0-47) for non-infected and infected patients, respectively. ($\rho < 0.02$). In summary, Källenius and Winberg (1978) found that infection-prone girls had a significantly higher mean number of adhering bacteria to epithelial cells from the periurethral region than the healthy controls. One factor facilitating colonization may be an increased receptivity of the mucosal cells of these areas to adherence of bacteria.

A number of studies have examined adhesion to human buccal epithelial cells. Strains of Neisseria gonorrhoeae adhered strongly to human buccal epithelial cells (Punsalang and Sawyer, 1973). With Proteus mirabilis, the ability of these microorganisms to adhere to oral and bladder mucosal cells is associated with their capacity to initiate retrograde pyelonephritis in experimental animals (Silverblatt, 1974). Liljemark and Gibbons (1973) observed attachment of Candida albicans to rat tongue and cheek cells and found that C. albicans adhered in lower numbers to epithelial cells from conventional rats than to those from germ-free rats, suggesting that indigenous flora may interfere with attachment and colonization by Candida.

Schaeffer et al (1981) noted an increased level of adherence of E. coli (018) to buccal epithelial cells from patients with a history of recurrent urinary tract infections, as opposed to normal, healthy controls. Eleven healthy women (age range, 22 to 32 years) with no history of urinary tract or vaginal infection were compared to 24 patients (age range, 22 to 69) with at least three culture-

documented urinary tract infections in the preceding year. The *E. coli* (018) were labelled with ^{3}H-uridine and then combined with the epithelial cells in the adherence assay. When compared with control cells, buccal cells from patients were significantly more receptive (7.1 $\pm$ .4 vs. 11.7 $\pm$ 1.2 bacteria per cell, $\rho = 0.002$), as was adherence to vaginal cells in patients and controls (10.1 $\pm$ 0.9 vs. 3.8 $\pm$ 0.4 bacteria per cell, $\rho < .001$). This increased level of adherence of *E. coli* (018) to buccal epithelial cells from patients may be attributed to a possible generalized change in mucosal adhesive characteristics.

Although Schaeffer's study was well done and resulted in a valid determination of bacterial adherence, his study was limited since he used only *E. coli* (018) for all experimental trials. The use of the patients' own organisms would parallel the *in vivo* situation more closely than the use of a standard serogroup of *E. coli* in the determination of *in vitro* adherence.

Fowler and Stamey (1977) have reported data suggesting that *in vitro* adherence of uropathogens to vaginal epithelial cells is greater in cystitis-prone females than in controls using a visual technique involving direct counting of adherent *E. coli* (06) using light microscopy with oil immersion (X 1000). These data are compatible with the observation that a cystitis-prone female colonizes her periurethral area (Cox and Hinman, 1968) and her vaginal introitus (Stamey and Sexton, 1975) with uropathogens before developing a lower urinary tract infection. Alternatively, Parsons *et al* (1979) found no statistically significant differences among values

for adherence of E. coli type 04 to the vaginal cells of control and cystitis-prone females. Six female patients were studied (aged 19 to 36 years) with a minimum of three culture-documented urinary tract infections in the preceding six months. Control subjects were six pre-menopausal women (aged 21 to 38 years) who had never had a urinary tract or vaginal infection. Adherence was determined by radiolabelling E. coli (04) with ^{14}C prior to incubation with epithelial cells. The values for mean bacterial adherence to vaginal cells of control females at pH 4.0 and 6.4 were (mean $\pm$ S.D.), 76 $\pm$ 57 and 65 $\pm$ 46, respectively. Values for mean bacterial adherence to vaginal cells of cystitis-prone females were 74 $\pm$ 64 at pH 4.0 and 80 $\pm$ 62 at pH 6.4. Parsons et al (1979) found no statistically significant difference among values for adherence of E. coli type 04 to the vaginal cells of control and cystitis-prone women at either pH 6.4 or 4.0.

Schaeffer and Stamey (1979), in evaluating the adherence of E. coli to human urinary tract epithelial cells, observed that adherence tended to be higher during the estrogen-dependent phase of the menstrual cycle and diminished shortly after the time of expected ovulation. The impact of hormones on bacterial colonization of rat vaginal epithelium has been studied by Larsen et al (1976, 1977). Colonization varied cyclically and peaked during the pre-estrus and estrus phases. Additional studies demonstrated that a peak in estrogen secretion was reflected by maximum colonization (Larsen et al, 1977). These observations may suggest

that receptor sites on uroepithelial cells are more available during certain stages of cell development or hormonal influence (Schaeffer et al, 1979). The ability of bacteria to adhere to uroepithelium is probably influenced in part by the surface characteristics of the epithelial cell, that is, by the number of receptors, density and availability of receptors or, most likely, by all (Schaeffer et al, 1981; Svanborg-Edén et al, 1980).

B. BLADDER AND SECRETORY ANTIBODY DEFENCE MECHANISMS IN URINARY TRACT INFECTIONS (UTI)

Several factors seem to determine whether bacteria adhere to mucosal surfaces of the urinary tract. Some bacterial types adhere preferentially to urinary mucosal surfaces as evidenced by the predominance of UTI's being caused by a small group of *E. coli* serotypes (01, 02, 04, 06, 07, 016, 018 and 075) (Lindberg *et al*, 1975). A probable reason for the predominance of a small group of *E. coli* serotypes in UTI relates to the differing surface antigen content (Uehling and Balish, 1978).

UTI in humans is followed by increased urinary IgA, and IgA secretion is characteristic of local immune protection (Uehling and Steihm, 1971); Uehling and Hong, 1977). In the oral cavity, local immunization of the rat increases salivary IgA and decreases dental infections (Michalek *et al*, 1976). In the human, salivary secretory IgA inhibits bacterial adherence to buccal epithelial cells (Williams and Gibbons, 1972). *In vitro*, attachment of bacteria to human voided urinary epithelial cells can be prevented by urine containing specific antibodies (Svanborg-Edén *et al*, 1976). However, Uehling and Balish (1978) were not able to correlate decreased adherence with urinary IgA levels.

Plasma cells in the lamina propria of cervical glands secrete IgA and these secretions continually bathe the vaginal vestibule (Tomasi and Grey, 1972; Hulka and Omsan, 1969). The joining of two locally produced IgA molecules with a glycoprotein (secretory piece) synthesized by epithelial cells produces secretory IgA (Tomasi and Grey, 1972).

Secretory IgA is the predominant class of antibody in secretions bathing mucous membranes (Tomasi and Grey, 1972). S-IgA has been demonstrated to serve a protective function against viral infections, but the mechanism by which S-IgA antibodies function in the disposal of bacterial antigens is not understood. Although S-IgA has been reported to bind to and agglutinate bacteria to which it is specifically directed, S-IgA is not generally considered to be bactericidal, to mediate complement dependent bacterial lysis, to bind to macrophages, or to enhance phagocytosis (Hanson *et al*, 1977).

It is possible that by merely binding to, and aggregating bacteria, S-IgA may exert an immune protective action. Bacteria colonizing an exposed mucous surface must attach to the surface to prevent being washed away by bathing secretions. Bacterial species exhibit surprising specificity in the degrees to which they can attach to surfaces, and this property directly influences their colonization (Gibbons and van Houte, 1971; Liljemark and Gibbons, 1971; Ellen and Gibbons, 1972).

The recognized ability of S-IgA to specifically bind to bacterial cells and affect their aggregation could influence their adherence and, hence, their colonization of a mucosal surface. Williams and Gibbons (1972) reported that human parotid S-IgA does, in fact, interfere with bacterial adherence to epithelial cells *in vitro*. This function *per se* would limit the colonization of indigenous organisms as well as exogenous

pathogens on mucosal surfaces, and could be the major mechanism of immunity mediated by secretory immunoglobulin (Hanson et al, 1977).

In urine, from patients with urinary tract infections, specific antibodies of the IgA class dominate and IgG are commonly found, but IgM antibodies are rare (Sohl-Akerland et al, 1979). The higher proportion of IgA antibodies in urine than in serum and the parallelism between the levels of IgA and secretory component containing antibodies in urine (Sohl-Akerland et al, 1979) suggest a predominance of locally formed IgA antibodies in urine. Svanborg-Edén and Svennerholm (1978) have shown that antibodies of IgG and and S-IgA types were efficient in blocking attachment in vitro. The mechanism underlying the antiadhesive effect of antibodies is at present not known. Agglutination of bacteria, steric hindrance due to antibodies binding to any bacterial surface structure or interaction with specific receptors on bacteria or epithelial cells may be of importance in explaining how secretory IgA and IgG antibodies may prevent the adhesion of E. coli to human urinary tract epithelial cells (Svanborg-Edén and Svennerholm, 1978).

Stamey and associates have shown that the vaginal introitus was colonized infrequently with pathogenic organisms in female subjects who had never experienced urinary tract infections (Stamey et al, 1971) whereas, in women with frequent urinary tract infections, pathogenic organisms were not only cultured from the vaginal introitus in high concentrations but also

persisted between periods of bacteriuria. It follows that female subjects with a mechanism to retard or prevent colonization of the vaginal introitus and distal urethra with Enterobacteriaceae would have a distinct advantage in breaking the chain of events necessary to infect the bladder by an ascending route (Tuttle et al, 1978).

Direct killing of bacteria by the bladder vesical mucosa has been suggested by some investigators (Cox and Hinman, 1969; Uwaldi et al, 1965; Hand et al, 1971).

But the bladder has also been shown to utilize a secretory defence mechanism to resist infection (Uehling et al, 1969). The bladder may rely on two classes of antibodies for its primary defence; IgA, an antibody of low specificity, which would function in the uninfected bladder, reacting to a host of bacterial strains but controlling only small quantities of microbes; and IgG, an antibody of high specificity which would operate in the bladder infected with a single bacterial strain, and because of its high specificity would be able to neutralize far greater quantities of bacteria than IgA (Parsons et al, 1975).

The bladder may not be the only urinary tract tissue that utilizes a secretory defence mechanism to resist infection. The urethra, andperhaps even the vaginal mucosa could rely on it.

Stamey et al (1971) proposed that there is a biologic difference at the level of the vaginal introitus and urethra between women who develop and those who do not develop UTI's that predisposes to colonization with enteric bacteria. Stamey

hypothesized that there is a deficient production of vaginal IgA antibody in bacteriuric women.

Alternatively, Kurdydyk et al (1980), initially employing an indirect immunofluorescence method, showed no significant difference in specific local antibody in cervicovaginal washings from 22 patients with a history of recurrent UTI and 29 normal controls with no history of UTI.

Consequently, a sensitive radioimmunoassay technique was developed to detect cervicovaginal antibody. IgG and IgA levels were measured in washings from 10 colonized patients, 13 non-colonized patients and 12 controls. There were no significant differences in IgG and IgA levels in cervicovaginal washings among the three groups.

Stamey's (1971) findings were not confirmed by Kurdydyk et al (1980) in a study using a more sensitive and specific technique to that of Stamey and his group in measuring cervicovaginal IgA. Kurdydyk's supportive data appears to be valid, and although their findings are not congruent with the proposed hypothesis of the present study, they cannot be considered invalid since it is likely that there is more than one factor contributing to the increased epithelial cell adherence in patients with UTI promoting colonization.

Kurdydyk et al (1980) were unable to demonstrate a significant decrease of cervicovaginal antibody in patients with recurrent infections which may allow the adherence of E. coli to periurethral cells

and thus promote colonization. The present study proposes that the increased receptivity of epithelial cells for bacteria plays a role in the increased frequency of vaginal colonization in susceptible patients.

The increased receptivity is most likely multifactorial, that is, there may be a deficient production of vaginal IgA antibody in bacteriuric women (Stamey, 1971), along with an increased level of mannose-containing protein receptor present in secretions (Schaeffer et al, 1980), as well as a host of bacterial and non-specific factors affecting adherence (Lindberg et al, 1975).

C. BACTERIAL FACTORS MEDIATING ADHERENCE

The adhesion between bacteria and epithelial surfaces may be specific and selective, involving bacterial cell-surface components and specific surface components of the host mucosal cells (Varian and Cooke, 1980).

The bacterial surface components may be fimbriae. These are nonflagellar filamentous bacterial surface appendages composed of hydrophobic proteins (Brinton, 1965; Duguid *et al*, 1955; Howink and van Itersons, 1950). Piliated bacteria stick to surfaces, both inorganic latex particles and organic animal or plant tissue (Duguid, 1968). Besides bacterial binding due to this general "stickiness" (Brinton, 1965), specific attachment to certain hosts and tissues occurs (Duguid, 1968; Gibbons, 1977), and is thought to be a virulence factor for bacteria colonizing or causing infection of mucous surfaces (Gibbons, 1977).

Bacterial adhesions have been classified according to the agglutination patterns resulting when bacteria bind to erythrocytes from various species. Bacteria which cause agglutination of guinea pig erythrocytes inhibited in the presence of D-mannose are defined as carriers of type 1 pili (Duguid, 1968). Such pili are found on most enterobacteria without any obvious relation to the virulence of the strains (Brinton, 1965; Duguid *et al*, 1955, 1968, 1979).

The adhesions of another group of *E. coli* cause agglutination

of human erythrocytes which is unaffected by D-mannose. These mannose-resistant adhesions may or may not be of a pilus morphology (Duguid et al, 1979) and have been associated with human diarrheal disease and urinary tract infection (Evans et al, 1975, 1977).

In subsequent studies, Brooks et al (1980, 1981) have observed that E. coli causing urinary tract infections were found to possess the following properties significantly more frequently than normal faecal strains: i) K-antigen titre of > 1/32 ii) haemolysin iii) type 1 pili iv) O serotype 2,6 and 75 v) fermentation of both dulcitol and salicin vi) H serotype 1. In addition, mannose-resistant haemagglutination was more common in the urinary tract group, but only the strains from patients with antibody-coated bacteria in the urine were significantly different from the faecal E. coli. Some of these properties appear to endow E. coli with ability to resist host defence mechanisms and to produce disease in the urinary tract.

Strains rich in K-antigen are more resistant to phagocytosis, antibody-binding and killing by complement (Glynn and Howard, 1970), while strains producing type 1 pili are probably able to resist hydrokinetic clearance by adhering to uroepithelium (Svanborg-Edén and Hanson, 1978; Schaeffer et al, 1979).

Mannose-resistant haemagglutination has been shown to be mediated by pili other than type 1 in some strains and by undefined components in others (Duguid et al, 1979). Possibly this latter

property plays a role in adhesion to the uroepithelium. Alternatively, the mannose-resistant haemagglutinating factor may be connected with invasiveness; Minshen et al (1978) found a close correlation between haemagglutinating activity and virulence for chicken embryos. The haemolysins produced by E. coli may damage the uroepithelium (Brooks et al, 1980).

The relationship between O serotype and pathogenicity for the urinary tract remains unclear, although virulence of E. coli for experimental animals appears to depend partly on the O-antigen (Brumfitt and Heptinstall, 1960; van der Bosch et al, 1979).

Hanson et al (1977) reported that most of the unobstructive cases of UTI are caused by E. coli, and patients are usually infected by the O-serotypes which predominate in their bowel.

There is no obvious connection between ability to ferment salicin, dulcitol and possession of H1 antigen and uropathogenicity (Brooks et al, 1980). Also, the strains causing pyelonephritis are much more resistant to the bactericidal activity of normal human serum than the strains in patients with asymptomatic bacteriuria (Lindberg et al, 1975).

Furthermore, capsular bacterial antigens are virulence factors. Hanson et al (1977) report that 50-70% of urinary isolates of E. coli had only one of 10 K antigens. Among these antigens the K1 antigen was the most common.

Another possible factor mediating bacterial adherence is non-specific physicochemical interactions (Friedman and Moon, 1977, 1980).

Earlier studies of the physicochemical properties of certain *E. coli* strains have shown that negative surface charges and liability to hydrophobic interaction are correlated with enhanced association to granulocytes, HeLa cells (Allison *et al*, 1966), and small intestinal epithelium. Consistent with previous studies, Öhman *et al* (1981) demonstrated smooth-type lipopolysaccharide, elevated negative charge and liability to hydrophobic interaction in *E. coli* strains isolated from patients with acute pyelonephritis, whereas strains isolated from acute cystitis and asymptomatic bacteriuria showed a more heterogenous pattern.

Studies of the surface structures of *E. coli* strains causing urinary tract infections indicated that the virulence is limited to quantitative and qualitative differences in the O-antigen, K-antigen, fimbria structures and nonspecific physicochemical interactions (Friedman and Moon, 1977, 1980; Öhman *et al*, 1981; Glynn and Howard, 1970; van der Bosch *et al*, 1979).

D. CARBOHYDRATE INHIBITION

Specific attachment of bacteria to cell surfaces of a susceptible host is a prerequisite for colonization and infection (Jones and Freter, 1977; Ofek *et al*, 1978). It is now well established that *E. coli* bind to human epithelial cells and that this binding in most cases can be specifically inhibited by mannose and its derivatives (Duguid and Gillies, 1957; Salit and Gotschlich, 1977; Ofek *et al*, 1978; Schaeffer *et al*, 1980; Ofek *et al*, 1981).

Eshdat *et al* (1978) described the isolation and partial characterization of a mannose-specific lectin consisting of protein subunits with identical M.W. of $\cong$ 36,500. The protein was isolated from a pathogenic strain of *E. coli* (7343) and appears to be located on the bacterial surface. It may be responsible for the adherence of the bacteria to various cells by binding to mannose residues on the outer surface of the epithelial cell.

Other studies have also shown that sugar residues on the surface of epithelial cells may serve as receptors for the binding of some *E. coli* strains (Ofek *et al*, 1978). D-mannose has been shown to inhibit the adherence of intact bacteria to epithelial cells (Ofek *et al*, 1977, 1978, 1981; Aronson *et al*, 1979; Schaeffer *et al*, 1980). The possibility that the mannose-binding ligand on *E. coli* is associated with pili is supported by the observation that the binding of purified pili to monkey kidney cells is specifically inhibited by D-mannose (Salit and Gotschlich, 1977).

In addition, further studies have shown that the fraction of organisms which adhered to epithelial cells and exhibited a high degree of mannose-binding activity were heavily piliated. The non-adherent fraction of organisms, on the other hand, lacked detectable mannose-binding activity and were nonpiliated (Ofek and Beachey, 1978). The presence of pili on E. coli isolated from the urine of patients with urinary infection has been correlated with the ability of the bacteria to bind to human uroepithelial cells, but adherence was not inhibited by D-mannose (Svanborg-Edén and Hanson, 1978). Also, Schaeffer et al (1979) have shown that adherence of E. coli to uroepithelial cells can be completely inhibited by α-D-mannose, suggesting that a mannose-containing carbohydrate on the host epithelial cell is involved in adherence. This is in accordance with other studies on mannose-sensitive adherence of E. coli (Ofek et al, 1978; Schaeffer et al, 1980; Duguid and Gillies, 1957; Old, 1972; Ofek and Beachey, 1978). These observations, along with studies on concanavalin A (Schaeffer et al, 1980), a mannose and glucose specific ligand indicate that mannose residues are widely distributed among animal and human cells and probably serve as receptors for the attachment of E. coli which possess mannose-binding activity (Ofek et al, 1978; Schaeffer et al, 1980).

Nevertheless, it is possible that receptors other than mannose may serve for the attachment of E. coli to epithelial cells. Duguid (1964) noted that haemagglutination of certain

human isolates of E. coli could not be inhibited by D-mannose. Some of these mannose-resistant, enterotoxigenic E. coli have been shown to possess a so-called colonization factor (Evans et al, 1977). Also, it has been reported that β-galactosyl residues on epithelial cells may serve as receptors for the adherence of certain E. coli strains of porcine origin possessing K88 antigen (Gibbons and van Houte, 1975). Other studies indicate that the adhesive properties of epithelial cells for E. coli may vary from tissue to tissue (Ellen and Gibbons, 1972; Frost, 1975) or from subject to subject (Fowler and Stamey, 1978). Conversely, the adhesiveness of a particular epithelial cell may vary from one clinical isolate to another (Svanborg-Edén et al, 1976).

Leffler and Svanborg-Edén (1980) were the first to demonstrate the ability of a specific glycolipid which acts as a receptor for bacterial adherence and haemagglutination. Glycolipids act as receptors for bacterial toxins (Yamakawa and Nagari, 1978) and a role of glycolipids as receptors for adhering bacteria has been indicated in other systems (Kearns and Gibbons, 1979). The glycolipid pattern of various tissues may thus be an important determinant in host-parasite interactions.

Individual variations in the glycolipid composition of the urogenital epithelium may contribute to the susceptibility of patients with recurrent urinary tract infections. Globotetraosylceramide may be recognized as a receptor for most E. coli attaching to human urogenital cells (Leffler and Svanborg-Edén, 1980).

The P blood group consists of three antigens, all of which have recently been described as glycosphingolipids (Race and Sanger, 1975; Naiki and Marcus, 1974). The p^k antigen (ceramide trihexoside) and the P antigen (globoside) are two major components on the human erythrocyte surface, whereas the P_1 antigen is a very minor component (Naiki and Marcus, 1975; Naiki *et al*, 1975). There are two common phenotypes, P_1 and P. Individuals of the P_1 phenotype, (3/4 of the population), synthesize both the P_1 and P antigens, whereas P_2 individuals synthesize the P antigen but not the P_1 antigen.

Fletcher *et al* (1979) has shown that erythrocytes from P_1 individuals (P antigen of the human blood group P system, suggested to be a glycolipid containing the sugar sequence Gal α1-4 Gal) also contain increased amounts of the p^k antigen (i.e., globotriaosylceramide acting as receptors for uropathogenic *E. coli*).

E. EFFECTS OF SUB-MINIMUM INHIBITORY CONCENTRATIONS (MIC's) OF ANTIBIOTICS ON ADHESION

E. coli exposed *in vitro* to sub-MIC's of antibacterial agents undergo a spectrum of morphologic changes resulting in abnormal forms, such as elongated filaments (Lorian and Popoola, 1972, 1975; Rolinson *et al*, 1977). These changes are related to the concentration of each drug used and the length of the period of exposure (Rolinson *et al*, 1977; Klainer and Perkins, 1970). The majority of the bacteria that retained their adhesive capacity after treatment with ampicillin were not elongated. It is not known whether changes in bacterial structure after treatment with ampicillin account for the decreased rate of attachment. The elongated bacterial forms may have a lower statistical chance of finding epithelial cells in solution because of clumsy movement. They may also fit less well into the convexities or concavities of the epithelial cell surface, and may not bind with sufficient force (Sandberg *et al*, 1979). Ampicillin may also interfere specifically with the formation of the bacterial structure (possibly pili) responsible for adhesive capacity. Pili may be present to a lesser extent (Svanborg-Edén and Hanson, 1978), or may be inserted in a less functional way into the changed cell wall.

However, neither chloramphenicol nor nitrofurantoin affected attachment by *E. coli* (Sandberg *et al*, 1979), although chloramphenicol and possible nitrofurantoin inhibited protein synthesis (Lorian and Popoola, 1972; Brinton, 1965).

Brinton (1965) indicated that E. coli "pilus subunits" were preformed and that the transformation of pili could continue in the presence of growth inhibitory concentrations of chloramphenicol. Although Sandberg et al (1979) were not able to demonstrate this, the treatment with bacteriostatic substances like nitrofurantoin or chloramphenicol still may decrease the adhesive capacity of E. coli.

By increasing the bacterial generation time (Garrett and Wright, 1967) and decreasing the proportion of the bacterial population that reaches the stationary growth phase, these substances may also affect attachment.

Vosbeck et al (1979) reported that the adhesion of bacteria grown in the presence of subminimal inhibitory concentrations of tetracycline, clindamycin, or trimethoprim-sulfametrole was reduced in a manner that was dose-dependent. This effect may be based on differential inhibition of the synthesis of bacterial surface components that participate in the recognition and adhesion process (Hirashima et al, 1973).

Eisenstein et al (1979) have reported inhibition of the adhesion of E. coli to human buccal epithelial cells by sub-MIC's of streptomycin. Antibiotics, at concentrations below the minimum inhibitory concentration, may have profound effects on surface properties of bacteria, and may modify epithelial cell receptors, that may be pertinent for their ability to

colonize and infect human mucosal surfaces (Eisenstein *et al*, 1980; Schaeffer *et al*, 1981).

Penicillin causes an enhanced loss of lipotechoic acid, the ligand that binds group A streptococci to host cells. Concomitantly, the treated organisms lose their adhesive properties (Alkan *et al*, 1978). The same drug inhibits the expression of the mannose-specific ligands in *E. coli* by distorting cell wall biosynthesis (Eisenstein *et al*, 1980). Streptomycin suppresses the formation and expression of the mannose-specific ligand in *E. coli*, probably by acting on the bacterial ribosome to induce misreading of MRNA, which often leads to abnormal protein synthesis.

In summary, sublethal concentrations of antibiotics can alter the ability of certain bacteria to adhere to epithelial cells (Ofek and Beachey, 1980; Schaeffer *et al*, 1981).

MATERIALS AND METHODS

A. Study Population

Ten healthy women (age range, 22 to 45 years) with no history of a urinary tract or vaginal infection were sampled once. All were pre-menopausal. Twenty-one patients (age range, 22 to 70 years) with a history of at least three culture-documented urinary tract infections in the preceding year were followed for 2 to 48 weeks. Ten were pre-menopausal, and 11 post-menopausal. The number of determinations in each control and patient was based on the subject's compliance. Quantitative cultures of the vaginal introitus and urine were performed at each visit. In the colonized patient group, single cell samples were obtained from 20 subjects and two samples from one subject. In the non-colonized patient group, single samples were obtained from five subjects, two samples from five subjects, three samples from two subjects, four samples from one subject, five samples from four subjects, six samples from two subjects, and seven samples from two subjects.

B. Collection of Specimens

a) Collection of Swabs

Periurethral Swabs

Each participant was placed in the dorsal lithotomy position on the examining table. The nurse spread the labia apart exposing the urethral meatus and with a sterile cotton-tipped applicator, a specimen was collected by a circular motion around the entire urethra. The swab was placed in a sterile screw-capped glass tube containing 1 ml of PBS, pH 7.2 (Appendix A).

Rectal Swab

A cotton-tipped applicator moistened with PBS was inserted 1 cm into the rectum and rotated 360°. The applicator was placed into 1 ml of PBS.

Vaginal Swab

A cotton-tipped applicator was inserted into the vagina at the level of the hymenal ring, and the specimen was collected by a circular motion around the vagina. Each swab was placed into 4.5 ml of PBS.

Buccal Swab

A cotton-tipped applicator was inserted into the mouth, and the specimen was collected by rubbing each side of the buccal mucosa. Each swab was placed into 4.5 ml of PBS.

b) Collection of Epithelial Cells

Cells were obtained from the vaginal introitus or buccal mucosa with a sterile cotton-tipped swab, which was placed in 4.5 ml of PBS, subjected to vortexing, and discarded. Uroepithelial cells were obtained from freshly voided midstream urine specimens, followed by centrifugation at 300 x g for 10 min and subsequent washing with 5 ml of PBS.

C. Laboratory Procedures

a) Processing of Periurethral and Rectal Swabs

The tubes containing the swabs were vortexed at high speed for one minute to suspend organisms and the applicator stick was rotated in the tube and removed. 0.01 and 0.001 aliquots of the fluid were inoculated onto split-MacConkey blood agar plates. The plates were incubated at 37°C for 18-24 hours. Organisms were identified by standard bacteriological methods and all potential urinary pathogens were isolated and stocked.

b) Processing of Epithelial Cells

Cells obtained with a sterile cotton-tipped swab were placed in 4.5 ml of PBS, subjected to vortexing and the swab was discarded. Uroepithelial cells were obtained from freshly voided midstream urine specimens, followed by centrifugation at 300 x g for 10 min, and subsequent washing with 5 ml of PBS. The cells were then harvested from the solution by centrifugation for 10 min at 300 x g, suspended in minimal essential medium and dimethyl sulfoxide (final concentration 5% vol./vol.), frozen in an acetone dry ice bath, and stored at -20°C. The cells were thawed at 37°C and diluted with PBS to a concentration of 10^{6}cells per ml.

c) Viable Count Assay

E. coli was grown for 24 hours at 37°C on Mueller-Hinton agar. Colonies were transferred via a swab into PBS warmed to 37°C and the suspension vortexed for 30-60 sec. The *E. coli* concentration was adjusted to 10^9 ml^{-1} (total count). Epithelial cells prepared in PBS were adjusted to approximately 10^6 per 0.9 ml. To a glass test tube, 0.9 ml of epithelial cell suspension was added, followed by 0.1 ml of *E. coli* suspension to give an *E. coli*:epithelial cell ratio of 100:1. A control included *E. coli* plus 0.9 ml of PBS. The tubes were sealed with parafilm and shaken at room temperature for 60 min with a shaker (Laboratory Rotator - Model G-2, New Brunswick Scientific Co. Inc., New Brunswick, N.J.). All tubes were then centrifuged at 60 x g for 5 min in a Lourdes centrifuge (Lourdes, Beta Fuge, Model A-2-Centrifuge, U.S.A.) at room temperature to sediment the epithelial cells. An aliquot of the supernatants was carefully removed without disturbing the epithelial cell pellet, after which the *E. coli* colony forming units (CFU) ml^{-1} was determined.

$$\text{\% adherence} = \left| \frac{\text{CFU in epithelial cell free supernatant}}{\text{CFU in control supernatant}} \times 100 - 100 \right|$$

D. Viability Adherence Assay

a) Preparation of Epithelial Cells

Human buccal mucosal epithelial cells swabbed from epithelium of cheeks, and vaginal epithelial cells obtained by swabbing the vaginal introitus with a sterile cotton-tipped swab were washed in 4.5 ml of sterile PBS (without NaN_3). Uroepithelial cells were obtained from freshly voided midstream urine specimens. Cells were sedimented and washed by centrifugation at room temperature for 10 min at 300 x g. Supernatant was pipetted off and the cell pellet was washed three times in 5 ml of PBS (after cells are pooled, and supernatant discarded, the cells were resuspended in .5 ml, pools were collected, and the pool was washed three times). Cells were resuspended to contain 1.1×10^6 cells/ml, using a spectrophotometer (Unicam SP 800B Ultraviolet Spectrophotometer, Unicam Instruments Limited, Cambridge, England) (O.D. at .15 at 650 nm).

b) Standardization of *E. coli*

Using a previously prepared culture of *E. coli*, we added PBS to a large tube and swabbed the *E. coli* plate. Then, we washed the swab in PBS. We adjusted the concentration to 1.2×10^9 cells/ml per tube (comparing to McFarland standard #4).

E. ^{125}I-*E. coli* - Epithelial Cell Adherence Assay

a) Radiolabelling of *E. coli*

The controls' and non-colonized patients' *E. coli* was isolated from a rectal swab, while the colonized patients' *Escherichia coli* was isolated from a periurethral swab. The patient's or control's organism was grown on Mueller-Hinton agar for 24 hours at 37°C. The plate was swabbed and the swab was suspended in PBS (pH 7.0 without sodium azide). The suspension was adjusted to 80 Klett Units (filter number 56, 540-590 nm) with PBS and the suspension was centrifuged at 10,000 rpm (Model head no. IEC-872) (Lourdes, Beta-Fuge, Model A-2) for 20 minutes and resuspended in 1 ml of PBS.

The enzymobead reagent (Bio-rad Laboratories) was rehydrated with 0.5 ml sterile distilled water at least one hour before use. (The reagent can be stored at 4°C for 2 weeks or aliquots can be frozen at -20°C).

One percent β-D glucose (1 gram β-D-glucose per 100 ml of sterile distilled water) was then made up.

Following the preparation of the reagents, they were added into a disposable test tube in the following order

0.2 M PBS	pH 7.2	50 µl
E. coli		1 ml
Enzymobead reagent		50 µl
1.0 mCi Na^{125}I		10 µl
1% β-D-glucose		25 µl

Mixed and iodination was allowed to proceed at room temperature for 45 min. The reaction was quenched by filtering the mixture through a .45 μ Nalgene filter and washing the filter with PBS (pH 7.0 containing NaN_3). The bacteria remaining on the filter were resuspended in PBS and the suspension was centrifuged at 10,000 for 20 min. (Model head no. IEC-872). The labelled bacteria were washed three times with cold PBS.

The labelled bacteria were resuspended in PBS and diluted to yield 1.2×10^9 bacteria/ml (McFarland standard #4). 10 μl aliquots of the suspension were removed and counted for radioactivity. Five ml aliquots were removed, and centrifuged for 20 min at 10,000 rpm, and 10 μl aliquots of the supernatant were removed and counted for radioactivity (Beckman Gamma Counting System, Scientific Instruments Division, Irvine, California, U.S.A.) (David and Resifeld, 1974).

b) ^{125}I-*E. coli*-Epithelial Cell Adherence Procedure

In the test situation, 0.9 ml of epithelial cells (1.1×10^6 cells/ml) were combined with 0.1 ml of ^{125}I-*E. coli* (1.2×10^9 cells/ml). In the control, 0.9 ml PBS and 0.1 ml of ^{125}I-*E. coli* were combined in a test tube. Both control and test samples were incubated for 60 min in shaker at room temperature. Following shaking, all tubes were removed and spun for 5 min at 500 rpm (Lourdes centrifuge) (Model head no. IEC-872). The supernatant was then separated from the pellet in the test samples. The radioactivity of the pellet, supernatant and control were then taken.

$$\frac{\%}{\text{adherence}} = \frac{\text{Counts per min in pellet}}{\text{Counts per min in control}} \times 100$$

Non-specific adherence to the tube itself was determined by an additional control containing .9 ml of PBS and .1 ml ^{125}I-*E. coli* (1.2 and 10^9 cells/ml). Following centrifugation for 5 min at 500 rpm, the PBS was then removed from the tube, placed in another separate test tube, and counts of both the PBS and the test tube of bacteria adhering to the sides were monitored. In this way, the per cent adherence of bacteria adhering to the test tube itself was determined.

$$\frac{\%}{\text{adherence}} = \frac{\text{Counts per min of supernatant removed tube}}{\text{Counts per min in control}}$$

F. Carbohydrate Inhibition Assay.

In an attempt to determine the effect of a carbohydrate on bacterial-epithelial cell adhesion, D-mannose was used as a blocking agent. D-mannose was added to *E. coli* suspensions in Minimum Essential Medium (MEM) to achieve a final concentration of 2.5% wt./vol., which has been shown by Schaeffer *et al* (1980) to result in complete inhibition on adherence. The *E. coli* suspension was then washed once, and combined with epithelial cells. Carbohydrate-treated bacterial suspensions were compared with non-treated controls.

G. Statistical Analysis

The mean level of adherence in each study group was regarded as the arithmetic average of the mean level of adherence in each subject in the group.

The Student t test was used for all statistical comparisons, and P values were determined by reference to a standard table of critical values. Also, the Pearson correlation coefficient was used to determine degree of association between buccal epithelial, vaginal epithelial cells, and uroepitheal cells.

RESULTS

RESULTS

A. Cell Storage Technique

The effect of the storage technique on adherence and viability was investigated by performing the assay with fresh samples and subsequently with samples from the same cell pools after the addition of DMSO to MEM (5% vol./vol.) and freezing. Adherence ($\rho > .05$) and viability were not affected when cells were stored in dimethyl sulfoxide for up to three months (Table I).

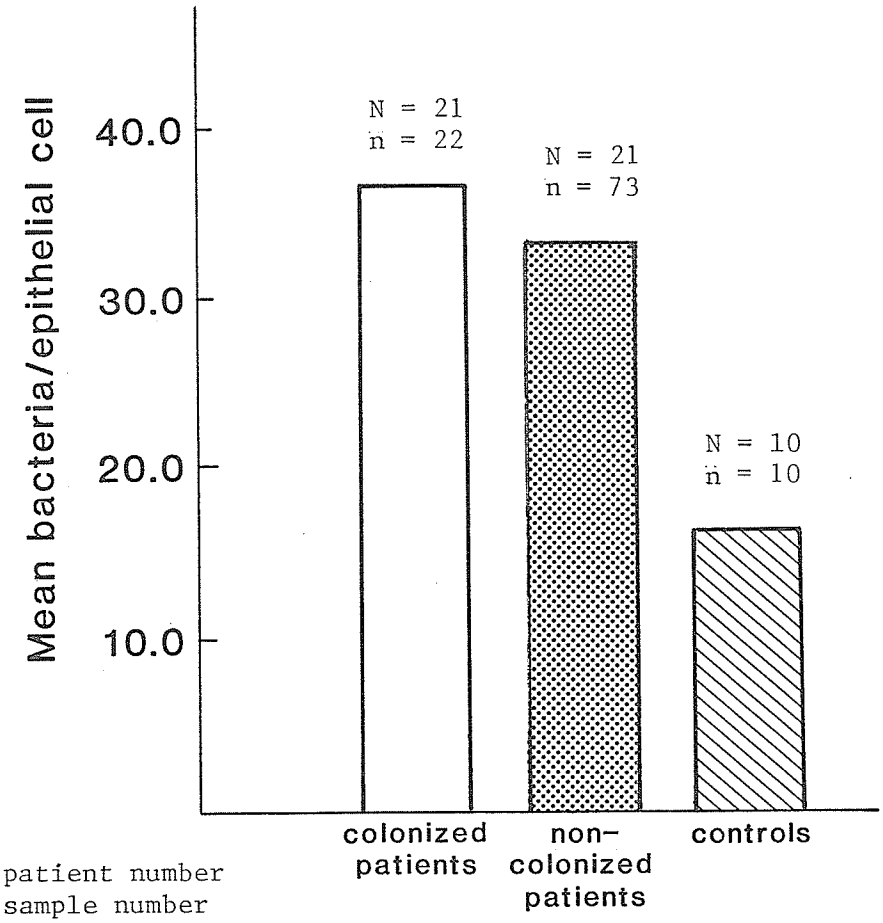
B. Determination of Bacterial-Adherence Assay

Uroepithelial cells

The mean number of *E. coli* bacteria per cell was based on an average of 4 determinations in each of 21 patients (and a single determination in each of 10 controls (Figure I). Adherence values from colonized patients with a history of recurrent UTI's was significantly higher than the level observed in control cells (35.6 ± 1.9 vs. 15.3 ± 4.0) bacteria per cell (mean $\pm$ S.D.), $\rho < .005$ (Figure I) (Table 2). A similar trend was observed in non-colonized patients with a history of recurrent UTI's vs. control cells, 33.4 ± 1.4 vs. 15.3 ± 4.0 bacteria per cell ($\rho < .005$). In comparing colonized patients with non-colonized patients, the level of adherence (bacteria/epithelial cell) was not significantly different (35.6 ± 1.9 vs. 33.4 ± 1.4) ($\rho > .05$).

Table I. Comparison of frozen and non-frozen epithelial cell samples for adherence and viability

	^{125}I (% Adherence)		Viability (cell number)	
Sample	Frozen (3 months)	Fresh sample	Frozen (3 months) (Cell no.)	Fresh sample (Cell no.)
1	17.3	22.6	6.8×10^5	1.1×10^6
2	25.0	19.1	8.7×10^5	1.1×10^6
3	15.0	19.4	7.7×10^5	1.1×10^6
4	23.5	22.8	6.2×10^5	1.1×10^6
5	22.4	19.3	8.1×10^5	1.1×10^6



N = patient number
n = sample number

Figure 1: In vitro adherence of E. coli to uroepithelial cells from patients with recurrent urinary-tract infections and healthy controls

Vaginal Epithelial Cells

The mean number of ^{125}I-*E. coli* adherent vaginal epithelial cell was based on an average of 4 determinations in each of 21 patients and a single determination in each of 10 control subjects. The cells from the colonized patients had a mean level of adherence that was significantly higher than the level observed in control cells (35.8 $\pm$ 1.9 vs. 15.5 $\pm$ 2.7 bacteria per cell) (mean $\pm$ S.D.) $\rho < .005$. Also, cells from non-colonized patients revealed an adherence level that was significantly higher than that of control cells (31.7 $\pm$ 2.6 vs. 15.5 $\pm$ 2.7) bacteria/cell, $\rho < .005$. As was found with uroepithelial cells, no significant difference existed between patients with active disease (colonized) and inactive disease (non-colonized) (35.6 $\pm$ 1.9 vs. 33.4 + 1.4) (Table II) (Figure 2).

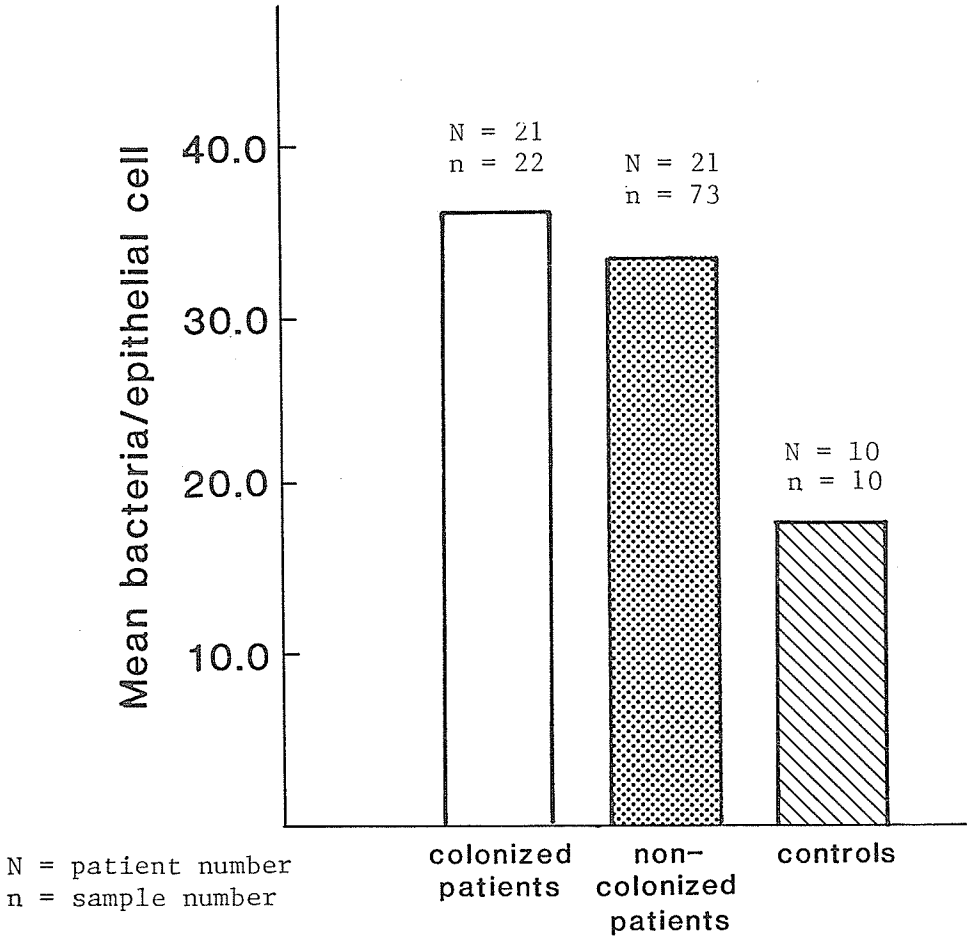


Figure 2: In vitro adherence of E. coli to vaginal cells from patients with recurrent urinary-tract infections and healthy controls

Buccal cells

To establish whether there was a generalized change in the mucosal adhesive characteristics of the cells, buccal cell receptivity was determined. The mean level of adherence, based on an average of 4 determinations in each of 21 patients and a single determination in each of 10 control subjects, comparing control cells with cells from patients (colonized and non-colonized) demonstrated that cells from colonized and non-colonized patients were significantly more receptive than those of control cells (32.8 $\pm$ 1.9 and 32.7 $\pm$ 1.5 vs. 15.9 $\pm$ 3.1, respectively), $\rho < .005$ (Figure 3).

Comparing adherence values for vaginal epithelial, buccal epithelial and uroepithelial cells for each group (patients with active and inactive disease, and controls), no significant difference between cell types was observed ($\rho > .05$)(Figures 1,2,3) (Table II). Colonized patients': vaginal, buccal and uroepithelial cells (35.8 $\pm$ 1.9, 32.8 $\pm$ 1.9, 35.6 $\pm$ 1.9 bacteria/cell, respectively). Non-colonized patients: 31.7 $\pm$ 2.6, 32.7 $\pm$ 1.5, 33.4 $\pm$ 1.4 bacteria/cell) and control group (15.5 $\pm$ 2.7, 15.9 $\pm$ 3.1, 15.3 $\pm$ 4.0) (Figures 1,2,3).

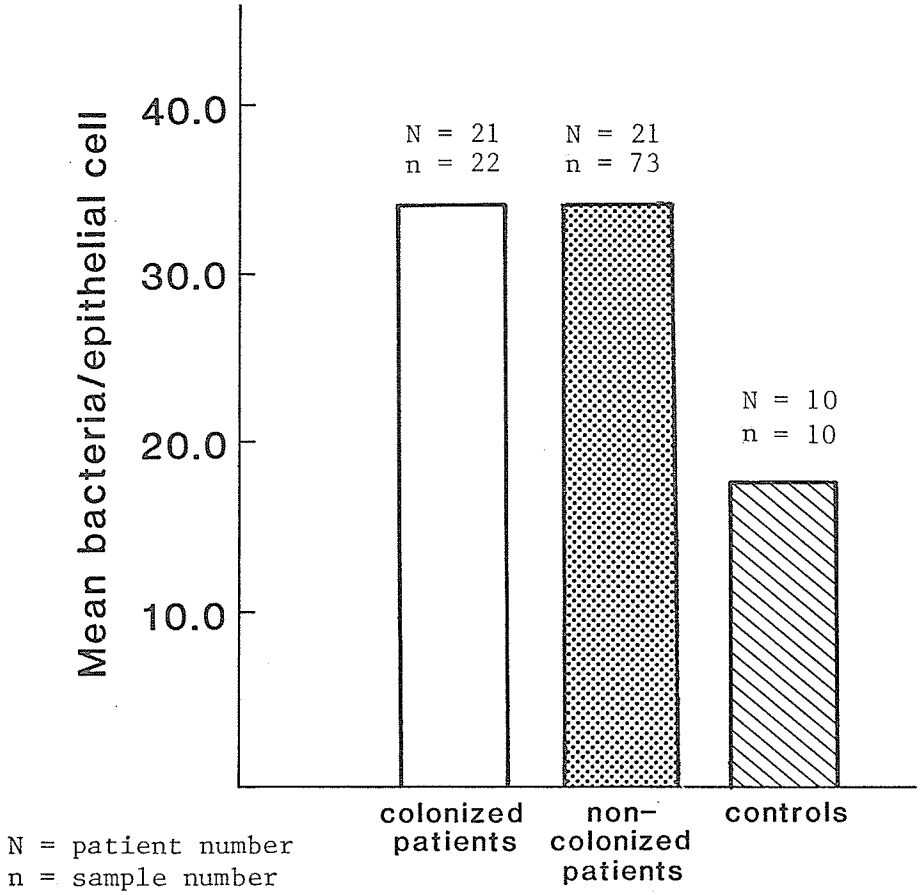


Figure 3: In vitro adherence of E. coli to buccal cells from patients with recurrent urinary-tract infections and healthy controls

Table II: Per cent adherence per epithelial cell (raw data) as determined by the ^{125}I-*E. coli* Adherence Method

Patient no.	Pre- or post-menopausal	Cell type	Colonized	Non-colonized	Range	Mean
1	pre	buccal	30.0	32.8	30.0-32.8	31.4
		vaginal	39.4	34.9	34.9-39.4	37.2
		uroepithelial	35.2	33.8	33.8-35.2	34.5
2	pre	buccal	38.6	30.3/36.4/27.2	27.2-38.6	33.1
		vaginal	35.2	40.5/39.0/45.0	35.2-45.0	36.1
		uroepithelial	51.9	35.5/33.0/41.0	33.0-51.9	40.3
3	pre	buccal	36.3	31.1/33.6/34.8/ 30.3/30.7	30.3-36.3	32.8
		vaginal	36.3	30.3/31.5/34.6/ 35.0/25.1	25.1-36.3	32.1
		uroepithelial	37.2	33.6/37.3/38.2/ 34.9/29.0	29.0-38.2	35.0
4	pre	buccal	46.9	34.6/29.2	29.2-46.9	36.9
		vaginal	35.8	30.5/46.9	35.9-40.9	37.7
		uroepithelial	35.9	30.0/34.8	30.0-35.9	33.6
5	pre	buccal	30.0	38.3/39.6/42.4/ 40.2/36.0	30.4-42.4	37.8
		vaginal	35.0	33.6/39.2/33.6/ 35.6/31.0	31.0-39.2	34.7
		uroepithelial	38.0	36.5/40.0/34.0/ 36.8/40.2	34.0-40.2	37.6
6	pre	buccal	24.3	24.9	24.3-24.9	24.6
		vaginal	22.9	17.7	17.7-22.9	20.3
		uroepithelial	43.7	26.6	26.6-43.7	35.2
7	pre	buccal	21.6	29.0	21.6-29.0	25.3
		vaginal	20.1	22.5	20.1-22.5	21.3
		uroepithelial	23.0	29.6	23.0-29.6	26.3
8	pre	buccal	27.5	33.4/31.8/30.6/ 34.2/32.0	27.5-32.0	31.6
		vaginal	30.0	27.7/27.4/29.4/ 31.6/27.4	27.4-31.6	28.9
		uroepithelial	32.6	28.2/31.0/28.9/ 29.4/23.5	28.2-32.6	28.9
9	pre	buccal	31.5	22.1/26.3	22.1-31.5	26.6
		vaginal	28.8	30.0/30.6	28.8-30.6	29.8
		uroepithelial	34.0	21.6/24.2	21.6-34.0	30.0

Continued ....

Table II: (Continued)

Patient no.	Pre- or post-menopausal	Cell type	Colonized	Non-colonized	Range	Mean
10	pre	buccal	27.5	34.6/35.8/36.4	27.5-36.4	33.6
		vaginal	20.1	32.7/34.9/33.5	20.1-34.6	30.3
		uroepithelial	32.3	32.8/35.2/33.4	32.3-35.2	33.4
11	post	buccal	19.7	36.1/37.2/39.7/ 32.4/40.1	19.7-40.1	34.2
		vaginal	39.8	35.3/32.0/36.9/ 38.4/37.9	32.0-38.4	36.7
		uroepithelial	38.4	31.0/30.6/34.2/	30.6-34.2	33.1
12	post	buccal	36.9	37.7	36.9-37.7	37.3
		vaginal	30.9	34.9	32.9-34.9	33.9
		uroepithelial	37.0	35.6	35.6-37.0	36.3
13	post	buccal	33.3	34.8/38.2/36.9/33.3	33.3-38.2	35.3
		vaginal	37.1	33.1/36.0/32.8/34.5	32.8-37.1	34.7
		uroepithelial	28.6	22.8/27.9/24.2/20.3	20.3-28.6	24.8
14	post	buccal	23.9	33.8/36.2	23.9-36.2	31.3
		vaginal	42.9	41.0/45.2	41.0-45.2	43.0
		uroepithelial	19.6	50.2/62.6	19.6-62.6	44.1
15	post	buccal	29.4	33.4/39.7/32.0/ 35.7/30.0/33.2/ 36.8	29.4-39.7	33.8
		vaginal	35.0	22.6/39.4/31.2/ 34.6/27.4/36.8/ 26.8	22.6-39.4	31.7
		uroepithelial	33.9	31.5/35.6/32.9/ 29.7/38.2/31.2/ 28.4	28.4-38.2	32.7
16	post	buccal	38.2	39.8/45.2/44.6/ 38.1/34.6/42.5	34.6-45.2	40.4
		vaginal	42.6	38.6/33.4/37.8/ 34.6/38.4/32.1	32.1-42.6	36.8
		uroepithelial	43.6	40.8/41.6/43.4/ 47.2/39.6/38.2	38.2-47.2	41.8
17	post	buccal	29.1	19.0	19.0-29.1	24.1
		vaginal	25.6	18.1	18.1-25.6	21.9
		uroepithelial	44.2	34.2	34.2-44.2	39.2
18	post	buccal	47.9	23.0/25.2	23.0-47.9	32.1
		vaginal	55.5	50.9/54.9	54.9-55.5	53.8
		uroepithelial	43.0	42.2/44.4	42.2-44.4	43.2

Continued ....

Table II: (Continued)

Patient no.	Pre- or post-menopausal	Cell type	Colonized	Non-colonized	Range	Mean
19	post	buccal	40.4	34.8/38.2/35.2/34.0/34.6/38.0	34.0-40.4	36.5
		vaginal	33.6	18.5/22.4/20.6/20.2/18.0/17.3	18.5-33.6	21.5
		uroepithelial	17.8	26.3/25.8/31.4/26.8/21.0/32.5	17.8-32.5	25.9
20	post	buccal	23.6	36.5/40.2/36.4/34.8/34.2/37.0/43.4	23.6-43.4	35.8
		vaginal	35.9	30.6/33.4/29.7/34.0/28.6/32.1/32.8	28.6-35.9	32.1
		uroepithelial	30.3	31.6/33.0/34.8/30.9/32.6/37.2/28.1	28.1-37.2	32.3
21	post	buccal	30.5/32.5	40.4/42.6	30.5-42.6	36.5
		vaginal	27.6/30.0	56.3/53.8	27.6-56.3	41.9
		uroepithelial	29.6/31.6	51.2/49.7	29.6-51.2	40.5

c) Comparison between Vaginal cell, Buccal Cell, and Uroepithelial Cell Receptivity

To determine whether high vaginal receptivity was associated with high buccal receptivity, adherence values for buccal and vaginal cells obtained simultaneously from each of 21 patients and 10 control subjects were compared. Colonized patients, vaginal cells (35.8 $\pm$ 1.9 vs. 32.8 $\pm$ 1.9, respectively); non-colonized patients, (31.7 $\pm$ 2.6 vs. 32.7 $\pm$ 1.5) and control group,(15.5 $\pm$ 2.7 vs. 15.9 $\pm$ 3.1, respectively). The Pearson correlation coefficient revealed a direct nonlinear relation between buccal and vaginal adherence in controls (γ = .543) and in patients (γ = .696) (Table III(a)).

Also, in an attempt to determine whether high vaginal receptivity was associated with high uroepithelial receptivity, adherence values for vaginal and uroepithelial cells obtained simultaneously from each subject were compared. Colonized patients, vaginal and uroepithelial cells (35.8 $\pm$ 1.9 vs. 35.6 $\pm$ 1.9, respectively); and control group, (15.5 $\pm$ 2.7 vs. 15.3 $\pm$ 4.0, respectively). The correlation coefficient revealed a direct relation between vaginal and uroepithelial adherence in controls (γ = .743) and in patients (γ = .893). A similar direct nonlinear relation was observed between buccal and uroepithelial cell adherence in controls (γ = .830) and in patients (γ = .646) (Table III(b)).

Table III. (a) Relation between vaginal cell and buccal cell receptivity in patients with recurrent urinary tract infections and healthy controls

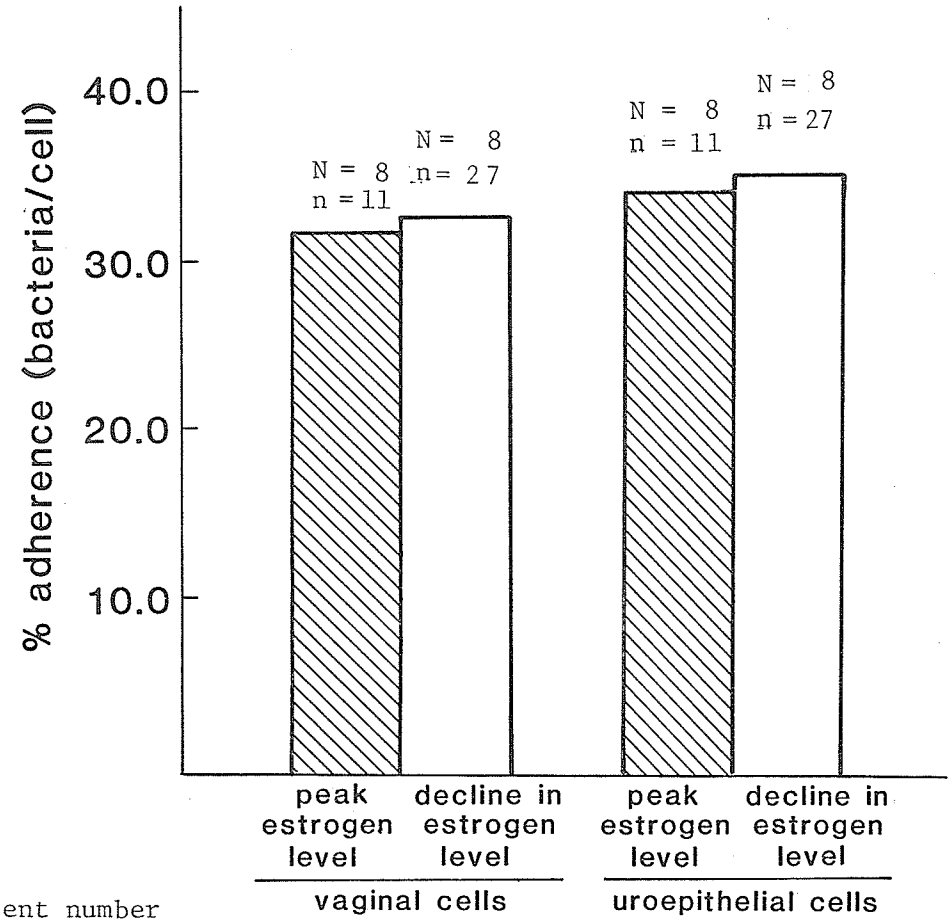
Study Group	Pearson Correlation Coefficient
Controls	.543
Patients	.696

Table III. (b) Relation between vaginal and uroepithelial and buccal and uroepithelial cell receptivity in patients with recurrent urinary tract infections and healthy controls.

Study Group	Correlation Coefficient
Controls: (vaginal vs. uroepithelial)	.743
Controls: (buccal vs. uroepithelial)	.830
Patients: (vaginal vs. uroepithelial)	.893
Patients: (buccal vs. uroepithelial)	.646

D. Effect of Hormonal Status on Adherence

Attempting to determine whether hormonal levels coincident with the menstrual cycle affected adherence, pre-menopausal patients (age range, 22 to 45 years) were examined for mean level of adherence/epithelial cell at peak estrogen level (2 weeks post-menses) and during a decline in estrogen level. The data revealed a lack of correlation between the degree of *E. coli* adherence and the day of the menstrual cycle. No significant difference was found in the mean number of bacteria per vaginal epithelial or uroepithelial cell comparing maximal hormonal level with a decline (vaginal cells, 31.8 ± 1.9 vs. 30.6 ± 31.1, uroepithelial cells, 32.7 ± 2.1 vs. 34.0 ± 1.6) (mean $\pm$ S.D.) (Figure 4).



N = patient number
n = sample number

Figure 4: In vitro adherence of E. coli to vaginal and uroepithelial cells from pre-menopausal patients with recurrent urinary-tract infections during peak estrogen level and decline in hormonal level

E. Effects of Antimicrobial Prophylaxis on Adherence

In evaluating the effects of antibiotic on adherence, 8 patients receiving a nightly dose of either trimethoprim (100 mg), trimethoprim (40 mg) in combination with sulfamethoxazole (200 mg) or nitrofurantoin (50 mg) to prevent recurrent urinary infection were compared with 10 patients who were not receiving prophylaxis. The level of adherence (bacteria/vaginal cell) in patients who received antimicrobial prophylaxis during the period of cell collection was significantly lower than the level in patients who were not given antibiotics (26.7 ± 1.3 vs. 35.1 ± 1.3, $\rho < .005$), but adherence was still greater than in controls (26.7 ± 1.3 vs. 15.5 ± 2.7, $\rho < .005$) (Figure 5(a).

A similar trend was observed in the level of adherence (bacteria/uroepithelial cell) in patients who received antimicrobial prophylaxis and those who did not receive antimicrobial prophylaxis (30.4 ± 1.0 vs. 34.6 ± 1.0, $\rho < .005$) (Figure 5(a).

Examining bacteria per buccal epithelial cell, no significant difference was found between patients given preventive therapy and those not receiving antimicrobial prophylaxis (34.1 ± 1.0 vs. 34.0 ± 1.1, $\rho = .35$) (Figure 5(a).

We also evaluated the effects of antimicrobial prophylaxis on five patients receiving a nightly dose of either trimethoprim (100 mg), trimethoprim (40 mg) in combination with sulfamethoxazole (200 mg), or nitrofurantoin (50 mg) to prevent recurrent urinary infection comparing them to when they were off prophylaxis (at least one month following antimicrobial prophylaxis).

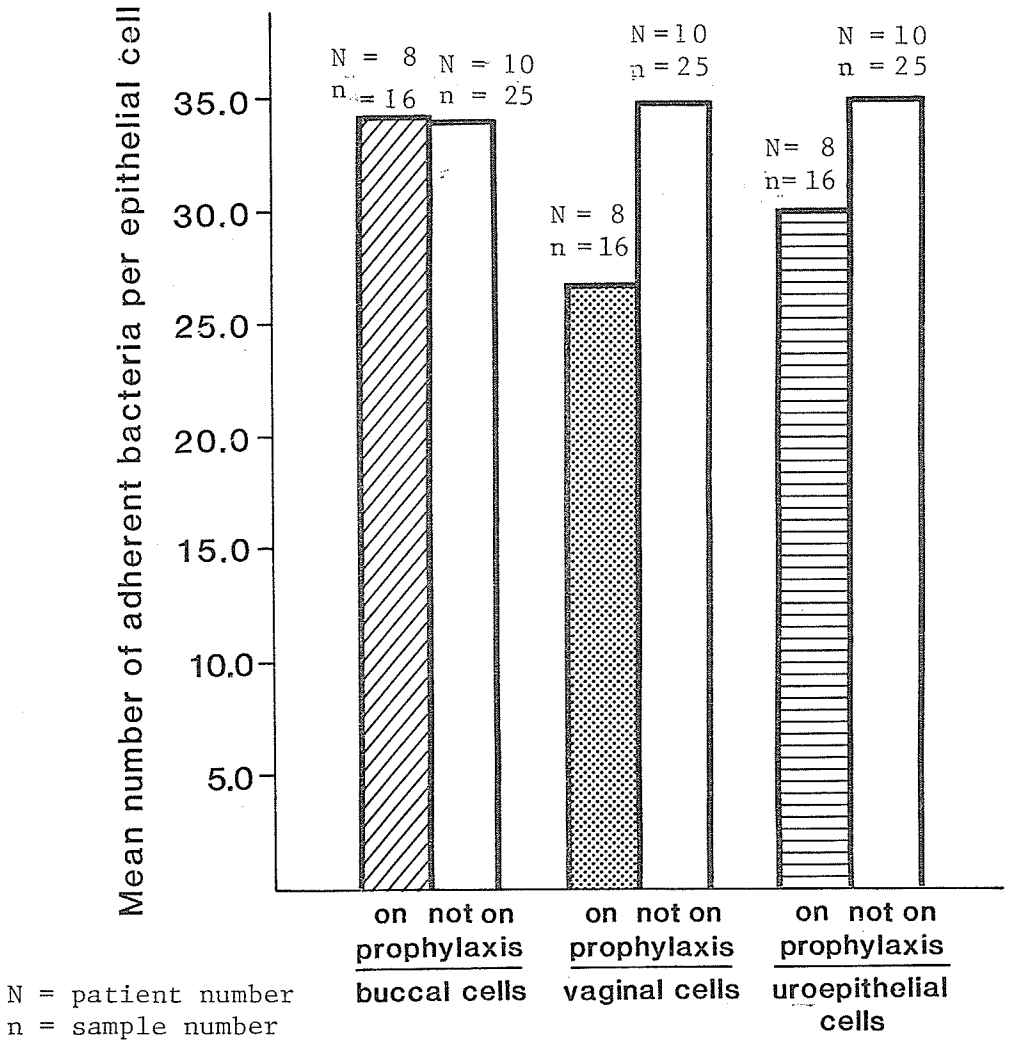
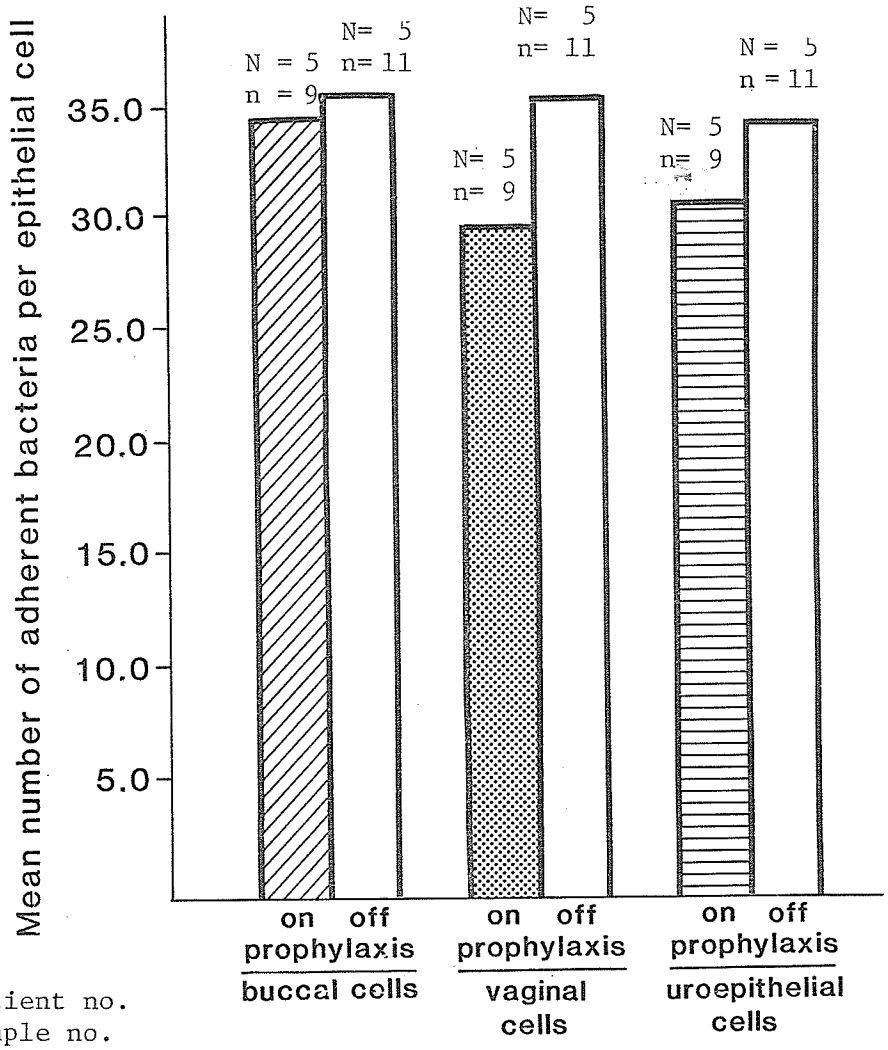


Figure 5(a) In vitro adherence of E. coli to vaginal, buccal and uroepithelial cells from patients with recurrent UTI receiving antimicrobial prophylaxis and those not on prophylaxis.

The mean level of adherence per vaginal epithelial cell was significantly lower ($\rho < .005$) for patients receiving antimicrobial prophylaxis, as compared to when they were no longer receiving antimicrobial prophylaxis (28.4 ± 1.6 vs. 34.6 ± 1.3) (Figure 5(b)). The same trend was observed for adherence levels with uroepithelial cells (29.9 ± 2.2 vs. 33.2 ± 2.5) receiving and not receiving antimicrobial prophylaxis ($\rho > .05$). There was no significant difference ($\rho > .05$) in the mean number of bacteria adhering to buccal epithelial cells in patients when they were receiving antimicrobial prophylaxis, and following prophylactic treatment (Figure 5(b)).



N= patient no.
n= sample no.

Figure 5(b) In vitro adherence of E. coli to vaginal buccal, and uroepithelial cells from the same patients with recurrent UTI receiving antimicrobial prophylaxis and a minimum of four weeks following prophylaxis.

F. Comparison of Viability vs. ^{125}I (Radioisotope Adherence Assay)

In an attempt to compare the sensitivity of two *in vitro* adherence assays, all three cell types, buccal, vaginal, and uroepithelial cells from patients were examined for mean number of adherent bacteria per epithelial cell by both methods. The results revealed a significantly higher sensitivity for adherence by the ^{125}I method as opposed to that of the viability method for all cell types, buccal, vaginal and uroepithelial (34.1 $\pm$ 2.6 vs. 21.4 $\pm$ 21.5, $\rho < .01$; 32.1 $\pm$ 5.1 vs. 10.9 $\pm$ 9.5, $\rho < .005$; 36.7 $\pm$ 2.2 vs. 16.1 $\pm$ 11.4, $\rho < .005$, respectively) (Figure 6).

As well, intra-patient mean comparison reveals a significantly lower variation of adherent bacteria per epithelial cell for all three cell types when measured by the ^{125}I-*E. coli* adherence method compared to the Viability Adherence Method (21.5 vs. 2.69, 9.5 vs. 5.1, 11.4 vs. 2.2, (deviation from the mean) for buccal, vaginal and uroepithelial cells, respectively) ($\rho < .05$) (Figures 1,2,3) (Table IV)

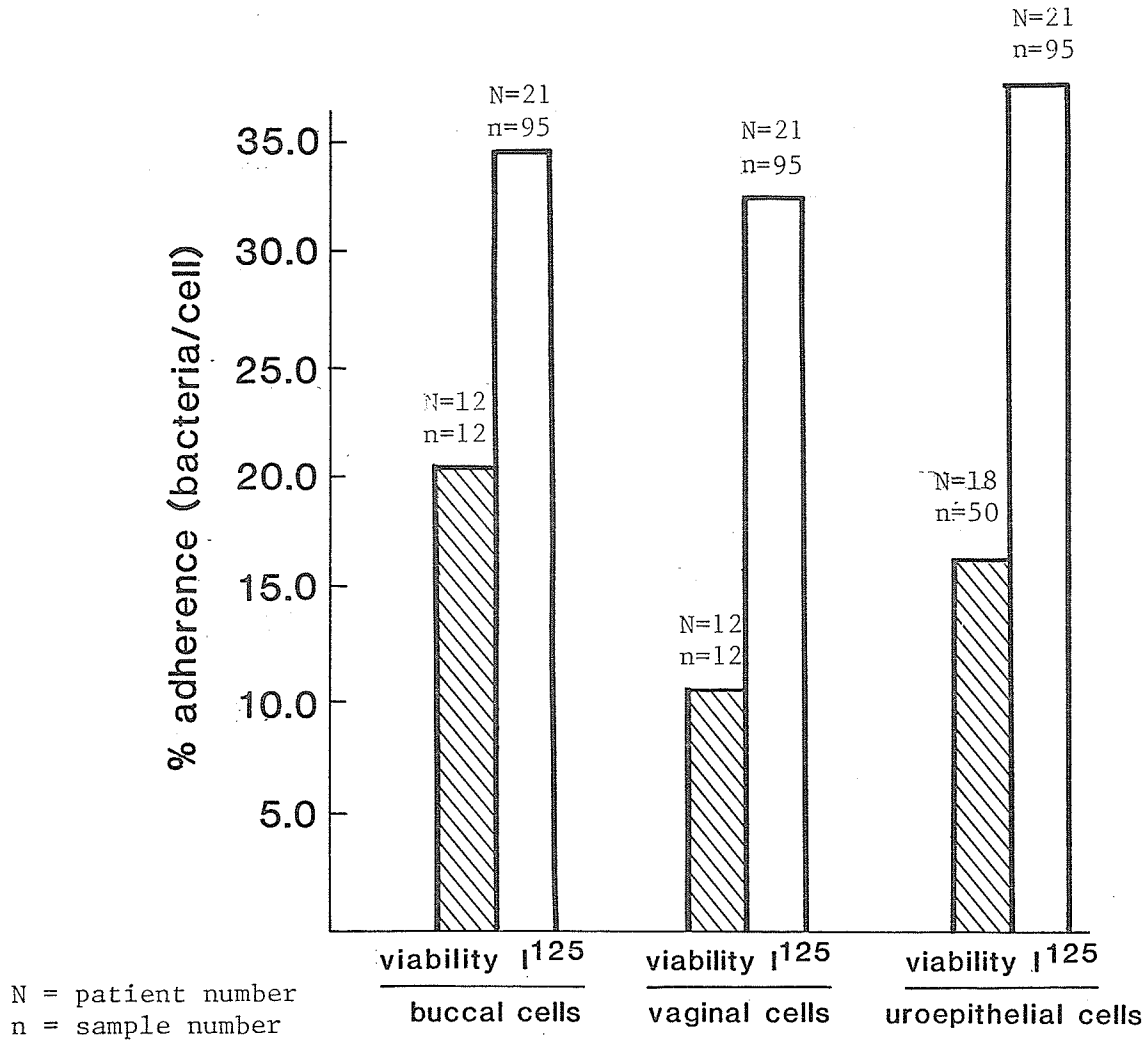


Figure 6: In vitro adherence of E. coli to buccal, vaginal and uroepithelial cells from patients with recurrent UTI. ^{125}I adherence method compared to radioisotope method.

Table IV: Per cent Adherence per epithelial cell (raw data) determined by the Viability Adherence Method

Patient number	Vaginal epithelial (mean)	Buccal epithelial (mean)	Uroepithelial	Mean (Uroepithelial)
1	15.8	50.0	66.6/75.0/0	47.2
2	1.0	16.0	0 / 0 / 50.0 / 0 / 0	10.0
3	7.9	38.3	0 / 0 / 0 / 20.0	5.0
4	0	0	0 / 0 / 16.6	5.5
5	1.0	0	11.1 / 78.4 / 20.0	36.5
6	15.8	0	5.3 / 5.5 / 0	3.6
7	27.7	43.0	10.0/ 0	5.0
8	0	26.8	20.0 / 0	10.0
9	62.5	81.0	22.2 / 7.6	9.9
10	0	0	5.3 / 5.5	5.4
11	0	2.8	40.0 / 37.5 / 28.5	35.3
12	0	0	20.0 / 10.0	15.0
13	-	-	9.1 / 50.0 / 0	19.7
14	-	-	0 / 66.6 / 0	22.2
15	-	-	14.2 / 11.1	12.7
16	-	-	44.4 / 28.5	36.5
17	-	-	10	5.0
18	-	-	9.1 / 8.3 / 0	5.8
Mean ± S.D.	10.9 ± 9.5	21.4 ± 21.5		16.1 ± 11.4

G. Effect of Physiological Age on Adherence

In determining whether physiological age affected adherence of *E. coli* to epithelial cells, pre-menopausal patients (age range, 22 to 45 years) and post-menopausal patients (age range, 45 to 70 years) were examined with respect to mean level of adherence (bacteria/cell) using buccal, vaginal and uroepithelial cells obtained simultaneously from each subject. No significant difference in the number of bacteria adhering per epithelial cell was found in comparing pre- and post-menopausal patients for all of the cell types tested, (buccal epithelial cells, 34.2 $\pm$ 3.3 vs. 31.6 $\pm$ 2.3; vaginal epithelial cells, 36.2 $\pm$ 2.5 vs. 31.0 $\pm$ 3.3; and uroepithelial cells, 38.9 $\pm$ 2.4 vs. 32.5 $\pm$ 2.8) (mean $\pm$ S.D.). The mean number of bacteria/cell in the pre-menopausal group was slightly higher than that of the post-menopausal patient group, but it was not a significant difference (Figure 7).(ρ = .30).

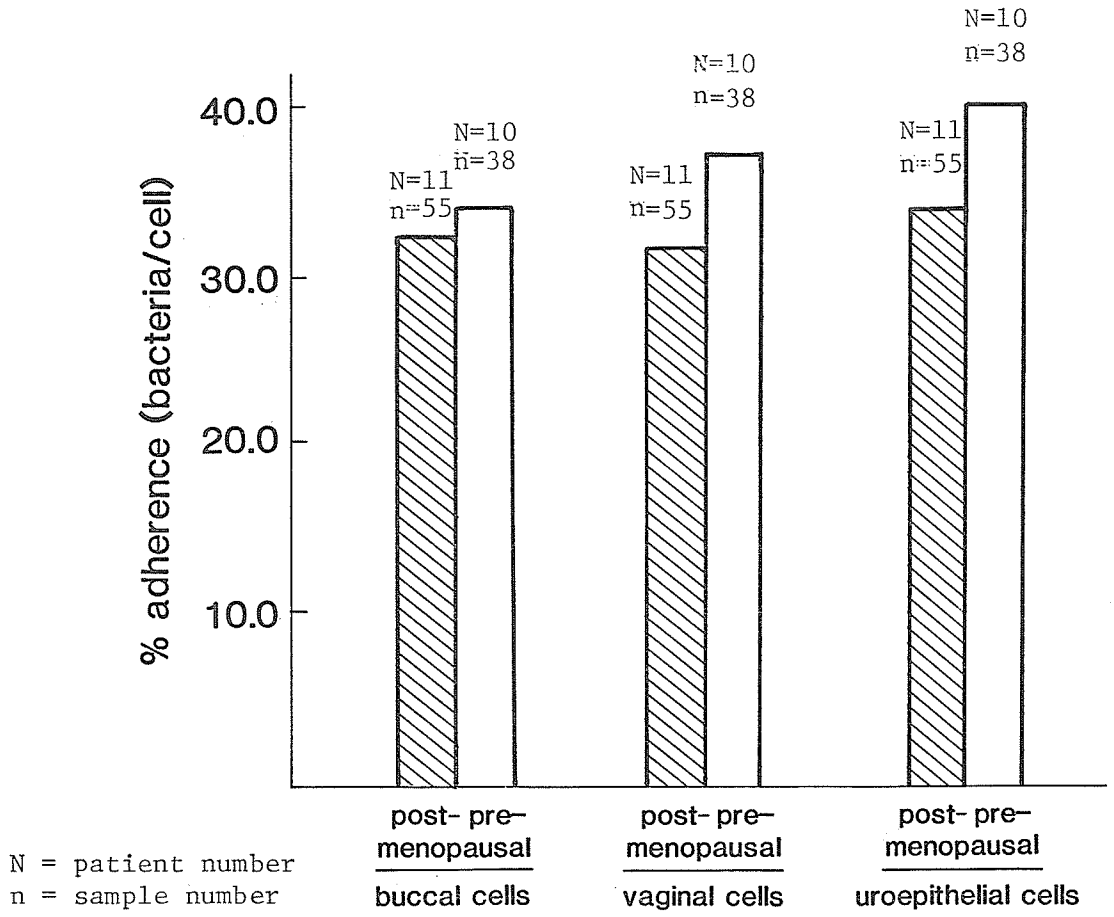


Figure 7: In vitro adherence of E.coli to buccal, vaginal and uroepithelial cells from pre-menopausal and post-menopausal patients with recurrent urinary tract infections

H. Inhibition of Adherence with 2.5% D-mannose

D-mannose was added to an E. coli suspension (serotype 018) in MEM to achieve a final concentration of 2.5% (wt./vol.) when combined with epithelial cells. Carbohydrate-treated bacterial suspensions were compared with nontreated controls.

The mean level of adherence using non-carbohydrate treated bacterial suspension combined with patients' (non-colonized) buccal epithelial, vaginal epithelial, and uroepithelial cells was observed to be 32.2 $\pm$ 1.5, 29.8 $\pm$ 1.4, and 31.9 $\pm$ 1.3, respectively((mean $\pm$ S.D.). In the carbohydrate-treated group, adherence was inhibited completely (0% adherence) by pre-incubation of E. coli (018) with D-mannose 2.5% (wt./vol.) (Table V).

Table V: Mannose Inhibition (2.5% wt./vol.) final concentration pre-incubated with *E. coli* (018)

Cell type	Adherence (mean bacteria/ cell) in the absence of D-mannose	% Adherence (in the presence of D-Mannose)
Buccal epithelial	32.2 ± 1.5	0%
Vaginal epithelial	29.5 ± 1.4	0%
Uroepithelial	31.9 ± 1.3	0%

n = 158

DISCUSSION

A. Introduction

Microbial colonization of mucosal surfaces has been studied in diarrheal, respiratory, oral pharyngeal, and urogenital diseases. The ability of a microorganism to adhere to a mucosal surface is considered to be a prerequisite to the colonization of some mucosal surfaces. This interaction between the bacterial and mucosal surfaces, which promotes adhesion, has been shown to be mediated by surface characteristics of the bacteria and of the mucosal surface, and by the presence of antibody, particularly secretory IgA (Fowler and Stamey, 1977).

It has been established that bacteriuria in young girls and women is preceded by colonization of the vaginal introitus by the specific species of Enterobacteriaceae that produces the infection (Stamey, 1972). The susceptibility of the vaginal introitus to colonization by urinary pathogens from the fecal reservoir appears to be the biologic defect that separates women with recurrent urinary infections from those resistant to recurrent infection (Stamey and Sexton, 1975).

Our study examines the role of bacterial adherence to human uroepithelial, buccal epithelial, and vaginal epithelial cells in the pathogenesis of recurrent urinary tract infections. In evaluating bacterial adherence, a number of parameters such as whether or not the patient was receiving antimicrobial prophylaxis, physiological age, and hormonal status were observed for their effect on bacterial adherence to epithelial cells in patients with recurrent urinary tract infections and in normal healthy controls.

In examining E. coli adherence to buccal cells obtained simultaneously from these same subjects, we investigated a possible generalized change in mucosal adhesive characteristics.

Also, in an attempt to corroborate our study with past findings on adherence, we examined the effect of 2.5% (wt./vol.) D-mannose on adherence of E. coli (018) (Kindly donated by Dr. A.J. Schaeffer, Northwestern University Medical School, Chicago, Illinois).

There are a variety of methods which can be used to detect in vitro adherence of bacteria to epithelial cells. Our study compared the viability adherence assay (Slack and Wheldon, 1977) with the radiolabelled bacteria method (Swanson et al, 1975); The radio-labelling method (Swanson et al, 1975) was found to be signigicantly ($\rho < .005$) more sensitive than the viability method (Slack and Wheldon, 1977) in the detection of in vitro adherence to epithelial cells (Figure 6).

Also, intra-patient comparison of mean number of adherent bacteria per epithelial cell revealed a significantly ($\rho < .05$) smaller deviation from the mean using the ^{125}I-Radioisotope method allowing for a greater accuracy of adherence determination (Figure 6) (Table (IV).

However, validation of the Radioisotope method with repeated tests using the same epithelial cells and E. coli was not possible since we were not able to obtain a large enough cell pool (epithelial cell numbers) from individual subjects.

The use of labelled bacteria eliminates the problem of removing the indigenous organisms from the surface of the epithelial cells, and the subjectivity associated with counting the colony forming units required in the determination of adherence in the viability adherence assay. Unlike Parsons et al (1979) who used ^{14}C-labelled E. coli, and Schaeffer (1981) who used ^{3}H-uridine labelled E. coli, our study utilized radiolabelling of E. coli with ^{125}I. The advantage to ^{125}I-labelling compared to that of ^{14}C and ^{3}H is that ^{125}I allows for higher counts per unit of time, therefore allowing for the use of smaller amounts, and resulting in greater sensitivity. Even though the measurement of adherence is determined on a ratio basis, the greater energy emission of ^{125}I (gamma emittor) as opposed to ^{14}C or ^{3}H (beta emittors) allows for a greater accuracy of measurement. This is exemplified by comparison of Parsons' (1979) and Schaeffer's (1981) results with those determined by the ^{125}I method in our study. Parsons et al (1979) found no statistically significant differences among values for adherence of E. coli 04 to the vaginal cells of control and cystitis-prone women (76.5 ± 59.9 vs. 74.3 ± 64.4) at pH 4.0. Although Schaeffer et al (1981) were able to show a significant difference in the mean number of adherent bacteria per vaginal and buccal cell for patients with a history of recurrent UTI compared to that of normal healthy controls (vaginal cells, 10.1 ± 0.9 vs. 3.8 ± 0.4), (buccal cells, 11.7 ± 1.2 vs. 7.1 ± 0.4, $\rho = .002$), his adherence system (^{3}H-uridine labelled E. coli 018) appeared to be less sensitive than the ^{125}I-radio-labelling method employed in our study (adherent bacteria per

vaginal epithelial cell from colonized patients and controls, 35.8 $\pm$ 1.9 vs. 15.5 $\pm$ 2.7) and adherent bacteria per buccal epithelial cell from colonized patients and controls, 32.8 $\pm$ 1.9 vs. 15.9 $\pm$ 3.1, $\rho < .005$).

Parsons *et al* (1979) examined the *in vitro* adherence of ^{14}C-labelled *Escherichia coli* in normal and cystitis-prone females. His study population consisted of six female patients whereas our study attempted to give a more accurate representation of epithelial cell receptivity by evaluating 21 patients with recurrent UTI for 2 to 46 weeks. Schaeffer *et al* (1981) evaluated 24 patients from 7 to 59 weeks also allowing for a more valid determination of adherence, but his study unlike ours used only *E. coli* (018) for all experimental trials. In contrast to Schaeffer *et al* (1981), our study involved the use of the patients' and controls' own *E. coli* in combination with the subjects' cells. The use of the patients' own organism parallels the *in vivo* situation more closely than the use of a standard serogroup of *E. coli* in the determination of *in vitro* adherence.

The bacteria (in Brain Heart Infusion and 1% glycerol) and epithelial cells (dimethyl sulfoxide and MEM, 5% vol./vol.) were stored at -20°C in order to minimize changes in adherence and viability from assay to assay. Freezing did not significantly alter either viability or adherence (Table I).

B. Detection of *E. coli* Adherence to Uroepithelial, Vaginal and Buccal Epithelial Cells

Schaeffer *et al* (1981) compared *in vitro* adherence of one strain of *E. coli* (018) to vaginal cells obtained frequently from healthy women and patients with recurrent urinary tract infections, using ^{3}H-uridine labelled *E. coli* in the adherence assay. They also examined adherence to buccal cells obtained simultaneously from these same subjects. Vaginal cells from the patients had a mean level of adherence that was significantly higher than the level observed in control cells. No significant difference was observed in the mean number of adherent bacteria in colonized patients and non-colonized patients, but both differed significantly from controls. Also, buccal cells from patients were significantly more receptive when compared with control cells. Schaeffer also observed a pattern of increased buccal cell receptivity with higher vaginal cell receptivity.

In a previous study employing a visual test system, Källenius (1978) investigated the adhesion of *Escherichia coli* to human epithelial cells *in vitro*, observed that bacterial adherence to epithelial cells from infection-prone females had a significantly higher mean number of adhering bacteria than the healthy controls ($\rho < .01$).

In our study it was also found that adherence to vaginal cells was greater in patients (colonized and non-colonized) than

in controls (35.8 $\pm$ 1.9 and 31.7 $\pm$ 2.6 vs. 15.5 $\pm$ 2.7, bacteria per cell, respectively, (mean $\pm$ S.D.), $\rho < .005$, (Figure 2), as was adherence to buccal cells (32.8 $\pm$ 1.9 and 32.7 $\pm$ 1.5 vs. 15.9 $\pm$ 3.1, $\rho < .005$ (Figure 3) and uroepithelial cells (35.6 $\pm$ 1.9 and 33.4 $\pm$ 1.4 vs. 15.3 $\pm$ 4.0, $\rho < .005$ (Figure 1). The results support the concept that increased receptivity of epithelial cells for bacteria may have a role in the increased frequency of vabinal colonization observed in susceptible patients (Schaeffer et al, 1980; Svanborg-Edén et al, 1979; Källenius and Winberg, 1978; Fowler and Stamey, 1977).

The fact that increased levels of adherence persisted whether or not the patients had urinary tract infections (colonized or non-colonized) (Figures 1-3), suggests that host defence mechanisms are instrumental in determining the outcome of vaginal colonization, and this finding correlates with the clinical observation that urinary tract infections usually recur despite spontaneous or pharmacologically induced remissions (Kraft and Stamey, 1972).

Our observation that the receptivity of buccal cells was significantly greater in patients with recurrent urinary tract infections than in controls suggests that susceptibility to recurrent urinary tract infections is associated with a widespread alteration in the surface characteristics of mucosal epithelial cells. Furthermore, a direct non-linear association that was observed between vaginal cell and buccal cell receptivity (Table III (a) suggests that the adhesive characteristics of epithelial

cells may be controlled in part by the same factor or factors (Schaeffer et al, 1981; Rutter et al, 1975).

Although the cause of the variation in adherence is not known, previous in vitro studies have indicated that there may be a difference among uroepithelial cells in the number of receptor sites, the degree of affinity that the receptors have for bacteria, or both (Schaeffer et al, 1980). Glycoproteins (Williams and Gibbons, 1975) or glycolipids (Svanborg-Edén et al 1980) and immunoglobulin A (Svanborg-Edén and Hanson, 1978) in oral and vaginal secretions may be able to coat cell surfaces and alter the receptivity of cells in vivo and in vitro.

Susceptibility to recurrent urinary tract infection in women may be related to a widespread fluctuating alteration in the surface characteristics of mucosal epithelial cells.

C. Effects of Hormonal Status on Adherence

Schaeffer et al (1979) in evaluating the adherence of E. coli to human urinary tract epithelial cells examined the adherence of E. coli to human uroepithelial cells obtained from midstream urine specimens of healthy women. When adherence was correlated with the day of the menstrual cycle, a repetitive, cyclical pattern became apparent. Adherence from cycle to cycle appeared to be maximal during the estrogen-dependent phase and diminished after ovulation. The impact of hormones on bacterial colonization of rat vaginal epithelium has been studied by Larsen et al (1977). Colonization varied cyclically and peaked during the preestrus and estrus phases. Mardh and Weström (1976) demonstrated that a peak in estrogen secretion was reflected by maximum colonization. Marrie and Swantee (1980) studied in vivo and in vitro bacterial adhesion to uroepithelial cells from healthy females in three physiological age groups, premenarche, reproductive, and menopausal. In vivo, the mean number of bacteria adherent per cell was 9.1, 6.3, and 17.2 for the premenarche, reproductive, and menopausal groups, respectively. They also observed that adhesion of E. coli to reproductive cells peaked during week two of the menstrual cycle. Taken together, these observations may suggest that receptor sites on uroepithelial cells are more available during certain stages of cell development.

On the other hand, Schaeffer et al (1981) in evaluating in vitro E. coli adherence to vaginal and buccal epithelial cells

from patients with recurrent urinary tract infections and healthy controls observed that hormonal levels coincident with the menstrual cycle did not affect adherence. Svanborg-Edén *et al* (1980) also observed a lack of correlation between the degree of *E. coli* adherence and the day of the menstrual cycle in studying *E. coli* adherence to human uroepithelial cells.

In the present study, no significant difference was found between physiological age groups (post-menopausal and pre-menopausal) for bacterial adherence to buccal, uroepithelial, or vaginal epithelial cells (31.6 $\pm$ 2.3 vs. 34.2 $\pm$ 3.3, 32.5 $\pm$ 2.8 vs. 38.9 $\pm$ 2.4, and 31.0 $\pm$ 3.3 vs. 36.2 $\pm$ 2.5, respectively) (Figure 7).

Consistent with Schaeffer *et al* (1981) and Svanborg-Edén *et al* (1980) we observed a lack of correlation between the degree of *E. coli* adherence and the hormonal status of the cell donor. Vaginal and uroepithelial cells were investigated for mean level of adherence during peak estrogen level (two weeks post menses), and following a decline, 30.6 $\pm$ 3.1 vs. 31.8 $\pm$ 1.9, and uroepithelial cells, peak vs. decline, 32.7 $\pm$ 2.0 vs. 34.0 $\pm$ 1.6 (mean $\pm$ S.D.) did not reveal a significant difference in adherence at different hormonal levels (Figure 4).

D. Effects of Antimicrobial Therapy on Adherence

Schaeffer et al (1981) investigated in vitro adherence of E. coli to vaginal and buccal cells from healthy controls and patients with recurrent urinary tract infections. Nine patients, including four who received a nightly dose of either trimethoprim (40 mg) in combination with sulfamethoxazole (200 mg) or cinoxacin (500 mg) to prevent recurrent infection, had 24 documented urinary tract infections (active disease during or within one month of the period of cell collection). The level of mean bacteria per vaginal epithelial cell in patients who received antimicrobial prophylaxis during the period of cell collection was significantly lower than the level in patients who were not given preventive therapy, but adherence in these patients was still greater than in controls. Adherence (mean bacteria/buccal epithelial cell) in patients with inactive disease and those given preventive therapy did not differ significantly from adherence in controls ($\rho = .08$ and $\rho = .07$).

Eisenstein et al (1980) examined the influence of sublethal concentrations of antibiotics on expression of the mannose-specific ligand of E. coli. They observed that the aminoglycoside antibiotics, streptomycin, gentamicin, and neomycin had the most marked effects relative to their minimum inhibitory concentrations, followed by tetracycline. They concluded that antibiotics, at concentrations below their minimum inhibitory concentration, may have profound effects on surface properties of bacteria that may be pertinent for their ability to colonize and infect human

mucosal surfaces. The mechanisms may vary from one drug to another but appear to depend on the classic actions of the antibiotics on inhibiting protein synthesis.

Svanborg-Edén et al (1979) has observed decreased adhesion of E. coli treated with subminimal inhibitory concentrations of ampicillin and amoxicillin. Sandberg et al (1979) confirmed the findings with ampicillin for a larger number of E. coli strains but failed to demonstrate lowered adhesive capacity for E. coli treated with sub-MIC's of either chloramphenicol or nitrofurantoin. In a similar study, Vosbeck et al (1979) examined the adhesion of radiolabelled E. coli (strain SS142) to monolayers of Intestine 407_1, a human epitheloid tissue culture cell line. In this study, the adhesion of bacteria grown in the presence of subminimal inhibitory concentrations of tetracycline, clindamycin, or trimethoprim-sulfametrole was reduced in a manner that was dose-dependent.

In our study, eight patients who received a nightly dose of either trimethoprim (100 mg), trimethoprim (40 mg) in combination with sulfamethoxazole (200 mg), or nitrofurantoin (50 mg) to prevent recurrent urinary tract infection were compared with 10 patients who had not received antimicrobial prophylaxis or who were at least 4 weeks post prophylactic therapy at the time of cell harvest. The level of adherence in vaginal cells from patients who received antimicrobial prophylaxis during the period of cell collection was significantly lower than the patients who

were not given antimicrobial prophylaxis, or whose cell samples were collected at least one month following prophylactic therapy (26.7 $\pm$ 1.3 vs. 35.1 $\pm$ 1.3, $\rho < .005$) (Figure 5(a). Also, the mean number of adherent bacteria per uroepithelial cell from patients receiving antimicrobial prophylaxis differed significantly ($\rho < .05$) from adherence values from patients not receiving antibiotics (29.9 $\pm$ 2.2 vs. 33.2 $\pm$ 2.5) (Figure 5(a)).

The mean number of adherent bacteria per buccal epithelial cell from patients receiving antimicrobial prophylaxis did not differ significantly from adherence values from patients not receiving antibiotics (34.1 $\pm$ .7 vs. 34.0 $\pm$ 1.1, $\rho = .35$) (Figure 5(a)).

A similar trend was observed in evaluating the same five patients when they were receiving antimicrobial prophylaxis, compared to when they were at least one month off prophylaxis. The mean number of adherent bacteria per vaginal and uroepithelial cell was significantly higher when the patients were not receiving antimicrobial prophylaxis as opposed to when they were on prophylaxis (34.6 $\pm$ 1.3 vs. 28.4 $\pm$ 1.6, vaginal cells, $\rho < .005$), (33.2 $\pm$ 2.5 vs. 29.9 $\pm$ 2.2, uroepithelial cells, $\rho < .05$). There was no significant difference in the mean number of adherent bacteria per buccal epithelial cell in patients receiving prophylaxis and when they were off prophylaxis (35.7 $\pm$ 2.8 vs. 34.4 $\pm$ 1.0, $\rho = .35$) (Figure 5(b)).

Vaginal cells and uroepithelial cells from patients given antimicrobial prophylaxis were less receptive for *E. coli* than

were cells from patients not given this therapy; buccal cell receptivity was not affected by antimicrobial agents. The drugs used in this study are concentrated in vaginal fluid (trimethoprim-sulfamethoxazole) or present in high concentrations in urine (nitrofurantoin). The possibility that adherence was reduced by a drug-induced alteration in the indigenous flora or modification of the epithelial cell receptor may explain the results.

E. Effect of D-mannose on Adherence of *E. coli* to Epithelial Cells

Schaeffer *et al* (1980) investigated the effect of carbohydrates on adherence of *Escherichia coli* to human urinary tract epithelial cells. Adherence of *E. coli* to voided uroepithelial cells from healthy women was measured by use of [^{3}H] uridine-labelled bacteria filtered through a polycarbonate membrane filter (5-μm pore size). At a concentration of 2.5% (wt./vol.) D-mannose, D-mannitol, α-methyl-D-mannoside, and yeast mannan completely inhibited adherence of the bacteria to the epithelial cells. Reducing the concentration of D-mannose, or its derivatives, to between 1.0 and 0.1% resulted in partial inhibition in the adherence of the bacteria; a further reduction in the concentration to between 0.01 and 0.001 caused an enhancement of adherence up to 160% of the control level. Bacterial preincubation in 2.5% (wt./vol.) D-mannose for one minute before epithelial cells were added completely inhibited adherence; similar treatment of the epithelial cells had no significant effect on subsequent adherence of the bacteria. The inhibitory effect of D-mannose was decreased if bacterial adhesive ability, or cell receptivity, increased.

In an attempt to corroborate our results with Dr. A.J. Schaeffer's (Northwestern University Medical School, Chicago, Illinois), we followed the experimental design for complete

inhibition of bacterial epithelial cell adhesion as detailed in the "Effect of Carbohydrates on Adherence of *Escherichia coli* to Human Urinary Tract Epithelial Cells", Schaeffer *et al*, (1980). The adhesive *E. coli* strain (018) used in these experiments was kindly donated by Dr. Schaeffer. The epithelial cells were obtained from non-colonized patients, as described on page 30. D-mannose (Sigma Chemical Company) was added to an *E. coli* suspension in MEM to achieve a final concentration of 2.5% (wt./vol.) when combined with epithelial cells. Mannose-treated bacterial suspensions were compared with nontreated controls. Using buccal epithelial, vaginal epithelial, and uro-epithelial cell samples with *E. coli* (018) in the absence of D-mannose, our findings revealed no change in adherence (32.2 $\pm$ 1.5, 29.5 $\pm$ 1.4, 31.9 $\pm$ 1.3, buccal, vaginal, uroepithelial respectively) (mean $\pm$ S.D.). Preincubation of the bacteria with D-mannose completely prevented their adherence to epithelial cells (Table V). These findings are in agreement with those of other investigators who have reported inhibition with D-mannose (Ofek *et al*, 1978; Bar-Shavit *et al*, 1977; Salit and Gotschlich, 1977; Old, 1972). The data suggest that D-mannose inhibits bacterial adherence by competing with mannose-like receptors on the epithelial cell surface and that there may be a difference among epithelial cells in the number of receptor sites, or the degree of affinity, or both, that these receptors have for bacteria (Schaeffer *et al*, 1981).

G. Conclusion

This study was designed to investigate the role of adherence in the pathogenesis of recurrent urinary tract infections, and the effect of physiological age, hormonal status, antimicrobial therapy, and carbohydrate, on E. coli adherence to epithelial cells from patients and controls.

A significantly greater level of adherence was found in patients with recurrent urinary tract infections compared to normal healthy controls using vaginal, buccal, and uroepithelial cells with the subjects' own E. coli.

The increased receptivity may be due to an increased number of carbohydrate-containing receptors (as suggested by the inhibition results (100% inhibition) in the presence of D-mannose, a greater affinity of bacterial to epithelial cells, or both.

Adherence in the pre- and post-menopausal groups did not differ significantly. Also, regardless of the hormonal status of the pre-menopausal group (peak vs. decline in estrogen level), no significant difference in adherence was observed. That is, we were able to show that the availability of the receptors on the epithelial cells were hormonally regulated.

However, it was shown that adherence was significantly lower for vaginal and uroepithelial cells, but not for buccal from patients receiving antimicrobial prophylaxis compared to those who did not receive antibiotics. This may be due to a drug-induced alteration in the indigenous flora or modification of the epithelial cell receptor.

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APPENDIX

APPENDIX A

Phosphate Buffered Saline (PBS) pH 7.2

Stock Buffer	1 M	
Na_2HPO_4		109.6 gm
$NaH_2PO_4H_2O$		31.5 gm
Double distilled water to 4 liters		

Preparation

Stock Buffer	1 M	40.0 ml
NaCl		8.5 gm
Double distilled water to 1 liter		

The preparation was autoclaved at 121°C for 20 min. and stored at 4°C.