THE ROLE OF CERVICOVAGINAL ANTIBODY IN THE PATHOGENESIS OF RECURRENT URINARY TRACT INFECTION

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THE ROLE OF CERVICOVAGINAL ANTIBODY IN THE PATHOGENESIS OF RECURRENT URINARY TRACT INFECTION

BY

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ABSTRACT

Absence of local cervicovaginal antibody in patients with recurrent infections may allow the adherence of Escherichia coli (E.coli) to periurethral cells, and thus promote colonization and predispose to recurrent urinary tract infection (UTI). Cervicovaginal washings from 22 patients with a history of recurrent UTI and 29 normal controls with no history of UTI were examined for specific local antibody using indirect immunofluorescence. No significant difference in antibody was found in these populations.

Consequently, a sensitive radioimmunoassay technique was developed to detect cervicovaginal antibody. A solid phase was prepared by coupling a pool of 8 serogroups of E.coli which are frequently implicated in UTI to Sepharose 4B. Serial dilutions of cervicovaginal washings were reacted with the solid phase, and the absorbed anti-E.coli antibodies were detected by the uptake of ^{125}I-labeled anti-human IgG or anti-human IgA. The antibody levels were quantitated by interpolation on a standard curve prepared using immunospecifically purified human anti-E.coli antibodies. 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.

In these studies, colonization was not related to cervicovaginal antibody.

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TABLE OF CONTENTS

	Page
ABSTRACT	i.
LIST OF TABLES	vii.
LIST OF FIGURES	viii.
INTRODUCTION	1
LITERATURE REVIEW	6
A. Virulence of E.coli Causing Urinary Tract Infection (UTI)	6
B. Serum Antibody Response in UTI	11
C. Enterobacterial Colonization of the Introital Mucosa	14
D. Factors Mediating Introital Colonization	16
a) Bacterial Growth Rate	16
b) Microbial Interference	17
c) Cervicovaginal Antibody	18
d) Bacterial Adherence to Mucosal Surfaces	24
MATERIALS AND METHODS	29
A. Study Population	29
B. Collection of Specimens	30
a) Collection of Swabs	30
b) Collection of Cervicovaginal Washings	31
C. Laboratory Procedures	32
a) Processing of Swabs	32
b) Processing of Cervicovaginal Washings	32

	Page
D. Indirect Immunofluorescence Procedure	34
E. Radioimmunoassay Procedure	36
a) Purification and Labeling of Rabbit Anti-Human IgG and IgA	36
b) Preparation of Bacterial Antigens and Coupling to Solid Phase	38
c) Radioimmunoassay for Quantitating Antibody Levels in Cervicovaginal Washings	40
i) Preliminary Studies	40
ii) Radioimmunoassay Procedure	44
F. Statistical Methods	46
RESULTS	47
A. Detection of Specific Antibody in Cervicovaginal Washings by Indirect Immunofluorescence	47
B. Quantitation of Antibody to E.coli in Cervicovaginal Washings by Radioimmunoassay	52
C. Comparison of Antibody Levels in Cervicovaginal Washings from Three Groups as Detected by Radioimmunoassay	59
D. Comparison of Radioimmunoassay and Immunofluorescence Results	61
E. Specificity of Antibody	63
DISCUSSION	69
A. Introduction	69
B. Detection of Specific Antibody in Cervicovaginal Washings by Indirect Immunofluorescence	73

Page

C. Comparison of Antibody Levels in Cervicovaginal Washings from Three Groups as Detected by Radioimmunoassay 77

D. Comparison of Radioimmunoassay and Immunofluorescence Results 79

E. Specificity of Antibody 79

F. Conclusion 82

BIBLIOGRAPHY 84

APPENDICES 93

LIST OF TABLES

Tables		Page
I.	Comparison of Cervicovaginal Washings of Patients and Controls for the Presence of Antibody as Detected by Indirect Fluorescence Assay (IFA).	48
II.	Comparison of Amicon Concentration and Lyophilization Treatments of Cervicovaginal Washings on the Detection of Antibody in IFA.	50
III.	Distribution of Antibody to E.coli in Lyophilized Cervicovaginal Washings as Detected by IFA.	51
IV.	Comparison of Radioimmunoassay (RIA) and Immunofluorescence (FA) Results.	62
V.	(a) Relative Levels of IgG Antibody Against E.coli, E.coli Outer Membrane Fraction and an E.coli Fraction Containing Pili, Flagella and Capsular Material.	64
	(b) Relative Levels of IgA Antibody Against E.coli, E.coli Outer Membrane Fraction and an E.coli Fraction Containing Pili, Flagella and Capsular Material.	64
VI.	(a) Relative Levels of IgG Antibody Against Pseudomonas aeruginosa and Streptococcus fecalis in Cervicovaginal Washings.	66
	(b) Relative Levels of IgA Antibody Against Pseudomonas aeruginosa and Streptococcus fecalis in Cervicovaginal Washings.	66
VII.	Relative IgG Antibody Levels to E.coli, S.fecalis and P.aeruginosa in Untreated and Absorbed Cervicovaginal Washings.	68

LIST OF FIGURES

Figure		Page
1.	Estimation of *E.coli* Antigen Density in Organisms/ml Sepharose 4B for Quantitative Absorption of Antibody in Human Serum.	41
2.	Estimation of the Amount of ^{125}I-Anti-Human IgG required to Saturate Anti-*E.coli* IgG Antibody Bound to Sepharose 4B-*E.coli* at 10^8 CFU/ml gel.	43
3.	Standard Curve for Quantitating IgG Antibodies Against *E.coli* Urinary Serogroups in Cervicovaginal Washings.	53
4.	Standard Curve for Quantitating IgA Antibodies Against *E.coli* Urinary Serogroups in Cervicovaginal Washings.	54
5.	IgG Antibody Titration of a Cervicovaginal Washing and the Cervicovaginal Washing Containing a Known Amount of Purified Antibody.	56
6.	(a) IgG Antibody Titration of a Cervicovaginal Washing and the Cervicovaginal Washing Containing a Known Amount of Purified Antibody.	57
	(b) IgA Antibody Titration of the Cervicovaginal Washing and the Cervicovaginal Washing Containing a Known Amount of Purified Antibody.	58
7.	Antibody Levels Against *E.coli* Urinary Serogroups in Cervicovaginal Washings of Controls, Non-Colonized Patients and Colonized Patients.	60

INTRODUCTION

Infections of the urinary tract encompass a wide variety of clinical entities whose common denominator is microbial invasion of any of the tissues of the tract extending from the urethral meatus to the renal cortex (Kunin, 1972). Infection may predominate at a single site such as the kidney (pyelonephritis), the bladder (cystitis), or be restricted to the urine (bacteriuria), but the entire system is always at risk of invasion by bacteria once one of its parts is infected.

Recurrent urinary infection is a significant problem in approximately 10% of the adult female population (Waters, 1969). Recurrent infections may be classified as (a) relapses--infections resulting from the survival of the infecting organism in the urinary tract; or (b) reinfections--infections resulting from re-entry of bacteria into the urinary tract. Reinfections occur at irregular intervals with no temporal relationship to previous therapy. This study addresses itself to the subject of pathogenesis of recurrent reinfection in adult females.

It is now generally accepted that urinary infection most commonly results from ascending periurethral invasion of the bladder by potentially pathogenic organisms present in the fecal flora (Stamey et al., 1971; Harding and Ronald, 1974; Cattell et al., 1974). Recurrent

reinfections are usually infections with serologically different strains from those causing previous infections (Bergstrom et al., 1967). In longitudinal studies on young girls and women, Stamey and his colleagues (1971, 1975) observed a higher incidence of colonization of the introitus (vaginal vestibule and the periurethral mucosa) with Enterobacteriaceae in women with recurrent urinary infection compared to normal control women with no history of recurrent urinary infection. To account for recurrent infection, Stamey has suggested that susceptibility of the vaginal introitus to colonization by enterobacteria from the fecal reservoir is the biologic defect that separates women who experience recurrent urinary infection from those resistant to recurrent infection (Fowler and Stamey, 1977).

The factors which mediate the ability of a bacterial species to colonize the vagina or any mucosal surface include (1) bacterial growth rate, (2) microbial interference, (3) antibody, and (4) bacterial adherence to the mucosal surface (Fowler and Stamey, 1977). Stamey and Timothy (1975) investigated factors that may influence bacterial growth rates. They demonstrated that the pH of the vaginal introitus and the glycogen and potassium concentrations in the vaginal fluid were similar in control and patient groups. Studies were also carried out to determine the influence of indigenous microbes on

colonization of the introitus by enterobacteria (Fowler et al., 1977). No significant difference in the frequency of the common indigenous bacteria was reported between the patient and control groups. In addition, there was no significant correlation between the incidence of colonization with Enterobacteriaceae and the incidence of colonization with common non-pathogenic organisms.

Stamey and Howell (1976) failed to show any differences in agglutination titers of antibody to Escherichia coli (E.coli) in cervicovaginal washings from patients with recurrent infections and from normal controls. In the former, specific O-antigens of E.coli colonizing the vaginal introitus were used for agglutination; in the latter, O-antigens from fecal E.coli were used. Using a more specific technique--indirect immunofluorescence--Stamey and his co-workers (1978) were able to detect a significant difference in cervicovaginal antibody in the two groups. Cervicovaginal antibody was found in only 26% of subjects susceptible to urinary infections. In contrast, 77% of control subjects demonstrated antibody-coating of their fecal Enterobacteriaceae when exposed to cervicovaginal antibody.

Concurrent studies in our laboratory showed no significant difference in specific local antibody in cervicovaginal washings from patients with recurrent infection and from controls. Techniques similar to

Stamey's were employed for processing the washings and indirect immunofluorescence was used to detect antibody. In addition, various methods of processing specimens were compared and a radioimmunoassay procedure was developed to quantitate antibody to E.coli in cervicovaginal washings.

Tuttle et al. (1978) used a double antibody solid phase radioimmunoassay to quantitate total IgA levels in vaginal washings from girls. Vaginal IgA levels were reported to be significantly lower in girls with recurrent urinary infections compared to an age-matched group of controls who had no history of urinary infection. However, total IgA in vaginal washings was quantitated without reference to antibody specificity.

The adherence of bacteria to epithelial cells is an important prerequisite for the colonization of mucosal surfaces. Fowler and Stamey (1977) have shown that E.coli adhere more avidly to washed mucosal cells from the introitus of susceptible women than to introital cells from control resistant women. There is evidence that bacterial adherence is influenced, in part, by surface mucosal antibody. Williams and Gibbons (1972) have shown that preparations of secretory immunoglobulin A isolated from human parotid fluid specifically inhibited the adherence of Streptococcus spp. to epithelial cells. Tramont (1976) reported that local genital antibodies to infecting strains of Neisseria gonorrhoeae inhibited

the attachment of gonococci to epithelial cells for at least three weeks to four months.

Our hypothesis is that the lack of local antibody production in patients with recurrent urinary tract infections can allow the adherence of Enterobacteriaceae to periurethral cells, and thus promote colonization, which predisposes to recurrent urinary tract infections. The aim of the present study was to determine if there was a relationship between enterobacterial colonization and vaginal antibody. Antibody in cervicovaginal washings from patients with recurrent infections and from controls with no history of recurrent infections was quantitated using a radioimmunoassay procedure--a more sensitive and specific technique than those employed by other investigators.

LITERATURE REVIEW

A. VIRULENCE OF *E.coli* CAUSING URINARY TRACT INFECTION (UTI)

The question as to whether or not the bacteria that cause urinary tract infection have special characteristics making them more virulent for the urinary tract has long been discussed. Most of the unobstructive cases of UTI are caused by *E.coli*, and patients are usually infected by the O-serogroup which predominates in their bowel (Kunin *et al.*, 1964; Hanson *et al.*, 1977). Hanson *et al.* (1975) have found with few exceptions, that the strains found in the urine of children with acute pyelonephritis are smooth, and 80% of these strains belong to the eight most prevalent O-groups (01, 02, 04, 06, 07, 016, 018 and 075). In contrast, *E.coli* of such O-groups are seen in the flora of stools of only 28% of healthy children. Furthermore, Hanson and his colleagues (1975) found that rough strains (spontaneously agglutinating) were much more common in asymptomatic bacteriuria patients. Loss of carbohydrate components by mutation can change the O-antigen characteristics of the bacterium resulting in spontaneous agglutination and sometimes in a rough colony on agar plates. The changed cell wall makes the bacterium less virulent, although virulence is polygenic in character (Roantree, 1971).

Resistance to the bactericidal activity of serum has been reported to be a virulence factor of *E.coli* strains tested in intraperitoneal infections in mice (Rowley, 1954)

and experimental pyelonephritis in rats (Henkel and Commichau, 1971). The sensitivity to the bactericidal activity of normal serum differs among E.coli strains causing different clinical forms of UTI. Strains causing pyelonephritis were much more resistant to the bactericidal activity of normal human serum than the strains in patients with asymptomatic bacteriuria (Lindberg et al., 1975). The presence of more sensitive strains in patients with asymptomatic UTI indicates that these patients differ in their host-parasite relationship from those with symptomatic infections. In some patients with untreated asymptomatic bacteriuria, the strains tended to become more sensitive to serum. This probably reflects changes in the bacterial cell wall.

The factors involved in the complement-dependent bactericidal effect of serum are not well defined. However, it is known that lipopolysaccharide is able to trigger the alternate pathway of complement activation, probably via properdin (Pillemer et al., 1955; Lachmann and Nichol, 1973). Variations in the bacterial envelope may make the strains more or less susceptible to the action of complement, thus explaining the findings of Machie and Finkelstein (1931) that resistance or sensitivity is a characteristic of the individual strain and not of the species.

Further evidence suggesting differences in surface characteristics (antigenic determinants) between E.coli

strains causing acute pyelonephritis and those causing asymptomatic bacteriuria were noticed when O-antigens were prepared from them. Human red blood cells coated with O-antigens from strains that caused pyelonephritis were used to detect high levels of O-antibodies in the patients (Lindberg *et al.*, 1975). However, the O-antigen from strains that produced asymptomatic bacteriuria gave only low titers with the same antisera. Hanson *et al.* (1977), in addition, found that preparations of O-antigen from *E.coli* strains isolated from patients with acute pyelonephritis absorbed the O-antibodies from rabbit antisera to such strains better than did strains that produced asymptomatic bacteriuria that were of the same O-group.

Hanson and his colleagues (1977) have noticed differences in the antigenic patterns of *E.coli* strains of the same O-group isolated from patients with asymptomatic bacteriuria compared with strains isolated from patients with pyelonephritis. The differences were demonstrated with both immunoelectrophoresis and crossed immunoelectrophoresis.

Capsular bacterial antigens are virulence factors, and hence attention has been given to *E.coli* K-antigens in relation to urinary tract infections. In 1971, Glynn *et al.* reported that strains causing pyelonephritis in adults contained higher amounts of K-antigen than strains

causing cystitis and strains present in the normal fecal flora. Kaijser (1973) using specific and quantitative crossed immunoelectrophoresis, found K-antigens K1, K2a, K2c and K13 more often among the strains of E.coli causing pyelonephritis than among strains from urine of patients with cystitis or from the stools of healthy school children. Hanson et al. (1977) reported 50-70% of urinary isolates of E.coli had only one of ten K-antigens. The K1 antigen was the most common, dominating in strains causing acute pyelonephritis.

The mechanism by which K-antigen promotes virulence is uncertain, but it may be related to an organism's ability to resist phagocytosis and the bactericidal activity of serum (Glynn et al., 1971; Kaijser, 1973). The studies suggesting that the K-antigen is a significant virulence factor seem to be in conflict with other studies indicating the dominance of certain serogroups of E.coli in the stool and in urinary tract infections. This dilemma may be resolved in light of recent observations of Ørskov (1978) that the K and O serogroups appear to be inter-related.

Enterobacteria synthesize a number of organic iron-chelating substances which are necessary for their uptake of environmental iron. These compounds are of interest since the growth of pathogenic strains in vivo depends on their capacity to obtain the iron held by substances

like lactoferrin. Rogers (1973) investigated two strains of E.coli, one virulent and the other relatively avirulent for the mouse. In chemically defined media containing some iron, the first strain produced substantial amounts of iron chelators, the second, little. The virulence of the second strain was enhanced when it was injected together with a preparation of E.coli iron chelator. In preliminary studies examining the virulence of E.coli in the urinary tract of the mouse, Montomerie (1978) was unable to correlate virulence with the presence of iron-binding catechols in E.coli.

B. SERUM ANTIBODY RESPONSE IN UTI

There have been a number of studies concerned with the serum antibody response in urinary tract infections. Kunin (1962) investigated the distribution of antibodies in man to 11 different E.coli O-antigen groups, including six groups commonly observed in urinary tract infections. Antibodies to all 11 groups were demonstrated in the serum of almost all of the patients over the age of two years. Girls with previously documented urinary tract infections were found to have antibody titers similar to those of other females of the same age groups. These observations indicate that urinary tract infections occur independently of pre-existing antibody in serum.

Using passive hemagglutination and bacterial hemagglutination, investigators have attempted to correlate the patient's serum antibody titer with the site of urinary tract infection (Winberg et al., 1963; Percival et al., 1964). These authors found elevated serum antibody titers to E.coli O-antigens more often in patients with pyelonephritis than in those with cystitis. In contrast, others (Fairley et al., 1971; Clark et al., 1971) were unable to demonstrate elevated antibody titers in patients with pyelonephritis. Antibody against the common enterobacterial antigen, an antigen shared by most strains of Enterobacteriaceae (Kunin, 1963), has also been detected and used as an indication of renal bacterial infection

(Whang and Neter, 1963; Saito, 1967; Kudo, 1970). However, Andersen (1966) and Carter et al. (1968) have been unable to confirm this.

There is evidence that serum antibodies to the O- and particularly the K-antigens of E.coli protect against infection in experimental pyelonephritis in rabbits (hematogenous infections) and rats (ascending infections) (Hanson et al., 1977; Kaijser and Olling, 1973). The fact that 80% of the recurrences in patients with UTI are due to serologically different strains (Bergstrom et al., 1967) may be the result of the protective effect of such antibodies produced in earlier infections. Thus, strains which have different antigenic determinants than the strains causing previous urinary infections may be selected for, because of their resistance to antibody directed against the previous infecting strains. Changes in antigens of Vibrio cholerae strains isolated during infection of gnotobiotic mice have been attributed to the selective effect of antibody within the intestinal lumen (Sack and Miller, 1969). Antigenic changes in E.coli strains isolated during asymptomatic bacteriuria have been documented (Lindberg et al., 1975). In several instances, a smooth strain of E.coli of a common O-group became a spontaneously agglutinating strain and also became more sensitive to the bactericidal activity of serum. Possible mechanisms responsible for antigenic variation in bacteria include highly mutable genes, phase

changes, episomal control, or other intracellular control systems (Beale and Wilkinson, 1961). Corbeil *et al.* (1975) have suggested that organisms change by one or more of these mechanisms and that population changes occur *in vivo* because of selective pressures due to the immune response.

C. ENTEROBACTERIAL COLONIZATION OF THE INTROITAL MUCOSA

It is now generally accepted that urinary infection most commonly results from ascending periurethral invasion of the bladder by potentially pathogenic organisms normally present in the fecal flora (Stamey *et al.*, 1971; Harding and Ronald, 1974; Cattell *et al.*, 1974). The postulated sequence of events is: initial colonization of the introitus and periurethral area, extension of colonization to the urethra, transfer of bacteria to the bladder during micturition or coitus, and, when bladder defense mechanisms are impaired, the establishment and multiplication of bacteria in bladder urine (Cattell *et al.*, 1974).

In longitudinal studies on young girls and women, Stamey and his colleagues have shown that the introital flora differs between normal women and patients with recurrent infection (Stamey *et al.*, 1971; Stamey and Sexton, 1975). Women susceptible to urinary infection were more likely to carry Enterobacteriaceae and *Streptococcus fecalis* (*S.fecalis*) on the introital mucosa of the vagina than control women of similar age and endocrine status who had never experienced urinary infection. Introital carriage in patients was characterized by greater numbers of bacteria and the colonization persisted for longer periods. Other investigators have also demonstrated a difference in introital flora (Bailey

et al., 1973; Bruce et al., 1973; Elkins and Cox, 1974). To account for recurrent infection, Fowler and Stamey (1977) have suggested that susceptibility of the vaginal introitus to colonization by enterobacteria from the fecal reservoir is the biologic defect that separates women who experience recurrent urinary infection from those resistant to recurrent infection.

Some workers have failed to show striking differences in enterobacterial carriage between normal subjects and women with recurrent infection (Cox, 1966; Cox et al., 1968; O'Grady et al., 1970; Marsh et al., 1972; Cattell et al., 1974). However, O'Grady et al. (1970) observed that recurrent infection was commoner in patients who were persistent carriers of enterobacteria. This apparent conflict could be due to differences in the selection of patients and the timing of studies in relation to episodes of urinary infection.

D. FACTORS MEDIATING INTROITAL COLONIZATION

a) Bacterial Growth Rate

Stamey and his co-workers have investigated factors that may influence bacterial growth rates. They reported no difference in introital pH between controls and women susceptible to urinary infections (Stamey et al., 1971). An analysis of mucosal pH of the vaginal introitus in relation to enterobacterial carriage showed that colonization with more than 100 enterobacteria per ml was more likely to occur at an introital pH more than 4.4 than less than or equal to 4.4 (Stamey and Timothy, 1975). This data suggested the possibility that the common E.coli O-groups might be more likely to survive on the vaginal mucosa than the uncommon groups in the presence of an altered vaginal pH (more than 4.4). Stamey and Kaufman (1975) showed that when vaginal fluid was adjusted at pH increments between 4.0 and 5.0 and innoculated with E.coli, the uncommon O-groups that never cause urinary infections were significantly more inhibited by the lower vaginal pH than were the common O-groups. Furthermore, they reported that vaginal fluid collected from control healthy females was bactericidal to E.coli at pH 4.0. When the pH was increased to 6.5, the same vaginal fluids supported bacterial growth.

In an effort to find a biologic explanation for the differences in pH associated with greater introital

colonization, Stamey and Timothy (1975) measured glycogen concentrations in vaginal fluid from women susceptible to recurrent urinary infections and from controls known to be resistant to urinary infections. They found no statistical difference in vaginal glycogen and potassium content between the two groups. Thus, susceptibility to introital colonization and recurrent urinary infections in women is not caused by failure of glycogen synthesis nor is it likely due to a defect in glycogenolysis.

b) Microbial Interference

Enterobacteria colonizing the vaginal introitus must compete with indigenous microbial flora for survival. The indigenous microbes may inhibit the growth of potentially pathogenic organisms by producing antimicrobial substances (bacteriocins), using essential nutrients or interfering with the ability of the pathogenic bacteria to adhere to the mucosal surface (Savage, 1972). Fowler et al., (1977) have compared quantitative aerobic cultures of the vaginal introitus of patient and control groups in regard to the influence of indigenous organisms on colonization of the introitus by enterobacteria. There was no significant difference in the frequency of the common indigenous bacteria between the patient and control groups. Moreover, the frequency of the common indigenous bacteria was unaffected by the presence of enterobacteria in the same culture. Available data indicates that none of the common

indigenous vaginal bacteria promote or suppress colonization of the vagina (Fowler and Stamey, 1977).

c) Cervicovaginal Antibody

The existence of a local secretory immune system that is independent of that responsible for the production of circulating antibodies is now well established (Heremans, 1968; Tomasi and Grey, 1972; Waldman and Ganguly, 1974). This system is confined to epithelial surfaces and is characterized by the predominance of IgA antibodies which are secreted into external secretions by plasma cells indigenous to the secretory organ; during its transepithelial passage the IgA becomes linked to a glycoprotein, known as secretory component (Vaerman and Férin, 1975).

Does the concept of the "secretory immunologic system" apply to the female genital tract? Straus (1961) demonstrated the local production of anti-salmonella antibodies following immunization by the vaginal route. Waldman et al. (1972) studied natural and locally induced antibodies to Candida albicans and found in both cases, that the antibody in cervical mucus was predominantly of the IgA class. These data strongly support the existence of a local immune response.

Can the female genital tract function as a typical immune secretory system? It seems unlikely that the vagina itself could constitute a secretory immune system. First, its epithelium is of the stratified squamous type,

instead of the classical columnar type, and there are no glands associated with the vaginal mucosa (Vaerman and Férin, 1975). Immunofluorescence studies (Tourville et al., 1970; Lippes et al., 1970) disclosed that IgA-and IgM-plasma cells were absent beneath the vaginal epithelium. Pure vaginal "secretions" are normally difficult to obtain (Ogra and Ogra, 1973), being always contaminated by cervical and other secretions. However, in three completely hysterectomized women Waldman et al. (1972) reported that the IgA/IgG ratio was as high in pure vaginal secretions as in cervicovaginal secretions. Secretory component was detected in such fluids, but it could not be ascertained if this secretory component was free, or bound to IgA or both. More recently, Jalanti and Isliker (1977) examined cervicovaginal secretions from 12 women who had a total hysterectomy and they detected only minute quantities of IgA and no secretory component. Thus, the available evidence militates against the vagina being well suited as a secretory antibody site.

The cervical mucosa appears to be able to function as a local secretory immune system. Columnar epithelial cells are found in the cervix and immunofluorescence frequently disclosed subepithelial IgA- and IgG-plasma cells (Tourville et al., 1970; Lippes et al., 1970). Some workers have found that IgA-producing cells predominate in the cervix (Masson and Férin, 1969), whilst others have found IgG to be the predominant immunoglobulin (Tourville

et al., 1970). Pozzuoli et al. (1968) were able to detect only a few IgA-producing plasma cells in the uterine cervix but this group (Pozzuoli et al., 1971) and others (Lippes et al., 1970) were able to identify secretory component in epithelial cells of the oviduct, endometrium and endocervix.

If the cervix is able to function as a local immune system one would expect a significantly higher IgA/IgG ratio in cervical secretions than that found in serum. In earlier data these ratios were rather low and not significantly different from the serum ratio (Chodirker and Tomasi, 1963; Masson et al., 1969; Hulka and Omran, 1969), whereas more recent data seem to agree more with the expected ratio (Govers and Girard, 1971; Waldman et al., 1972; Schumacher, 1973; Behrman and Lieberman, 1973).

There is some disagreement on the influence of the menstrual cycle on the IgA/IgG ratio. A number of investigators have reported highest ratios at midcycle, around ovulation and low ratios throughout anovulatory cycles (Hulka and Omran, 1969; Schumacher, 1973; Jalanti and Isliker, 1977). Jalanti and Isliker (1977) found that IgG does not vary significantly during the cycle and the high IgA/IgG ratio at midcycle was attributed to increased IgA secretion. These findings were, however, not apparent in a large series reported by Waldman et al. (1972).

Quantitation of immunoglobulins in cervicovaginal secretions, in all studies (Hulka and Omran, 1969; Govers and Girard, 1972; Chipperfield and Evans, 1975; Stamey and Howell, 1976) but one (Waldman et al., 1972) shows that IgG is present in greater concentrations than IgA. IgM has not been consistently reported as a significant immunoglobulin in cervicovaginal secretions.

The surface epithelium of the endometrium and uterine tubes consists of a single layer of cylindrical cells (Vaerman and Férin, 1975). Immunofluorescence (Tourville et al., 1970; Lippes et al., 1970) revealed strong IgG staining in the stroma and along the basement membrane particularly during the secretory phase. IgA and IgG cells were very sparsely distributed and were almost only found during the early proliferative phase. Hence, the endometrium does not seem suitable to function as a local immune system, being normally short of local plasma cells.

It seems clear, therefore, that the major source of immunoglobulins in the female genital tract is a secretory system localized to the endocervix. A mechanism furnishing mucus with locally produced IgA provides a defense against superficially proliferating infectious agents. The linkage to IgA of secretory component confers a protection against proteolysis upon the immunoglobulin molecule; secretory IgA has therefore a considerable biological advantage over serum IgA or IgG in body secretions and when present on epithelial surfaces appears to possess considerable

antibacterial activity. This action appears to be mediated partly by the ability of IgA to activate the complement alternate pathway, and in the presence of activated complement and lysozyme (both of which are present in cervical mucus), cause bacterial lysis; further the coating of organisms by secretory IgA causes agglutination thus inhibiting growth, reducing their invasive capacity and enhancing granulocytic phagocytosis (Hanson and Brandtzaeg, 1979). However, the major role of secretory IgA is its prevention of bacterial adherence to mucosal surfaces.

Is introital carriage with Enterobacteriaceae in women susceptible to recurrent urinary tract infections related to the absence of specific cervicovaginal antibody? Stamey and Howell (1976) using bacterial agglutination, failed to show any differences in antibody titers in vaginal fluid to E.coli colonizing the vaginal introitus of patients and to E.coli cultured from the rectum of controls. Furthermore, longitudinal observations on individual strains in patients during the course of introital colonization, did not disclose any relationship between colonization and specific antibody in vaginal fluid. The antibody titers in these studies were low and equal titers were obtained to E.coli serogroups infrequently implicated in urinary infection (0X9 and 0103), suggesting that the agglutination was non-specific.

Using a more specific technique, indirect immunofluorescence with fluorescein-conjugated antisera to human

immunoglobulins, Stamey and his co-workers (1978) were able to detect a difference in cervicovaginal antibody to E.coli in the two groups. Cervicovaginal antibody, as measured by antibody-coating of Enterobacteriaceae that colonize the vaginal vestibule, was found in only 26% of subjects susceptible to recurrent reinfections. In contrast, 77% of control subjects demonstrated antibody-coating of their fecal Enterobacteriaceae when exposed to cervicovaginal antibody. However, the amount of specific antibody coating the bacteria in these studies was not large. Antibody titers in cervicovaginal fluid were never higher than 1:32; IgA titers were 1:4 or less. Thus a significant quantitative difference in specific antibody contained in cervicovaginal fluid was not demonstrated.

Tuttle et al. (1978) quantitated the amount of IgA in the vaginal fluid of girls using a double antibody solid phase radioimmunoassay technique (Den Hollander and Schuurs, 1970; Koistinen, 1975). Vaginal IgA levels were significantly lower in girls with recurrent urinary infections compared to an age-matched group of controls who had never experienced bacteriuria. However, total IgA was measured without reference to antibody specificity to the infecting organism. An overlap in IgA values between the two groups was also observed supporting the idea of multifactorial etiology in recurrent urinary tract infections.

d) Bacterial Adherence to Mucosal Surfaces

The ability of a microorganism to adhere to a mucosal surface is considered a prerequisite to colonization of some mucosal surfaces. Microbial colonization of mucosal surfaces has been studied for enteropathogenic E.coli in the gut, for gonococci in the urogenital tract and for streptococci in the oral cavity, among others.

Certain strains of E.coli are enteropathogenic and cause infectious diarrhea (Taylor, 1966). Clinical disease is associated with a proliferation of enteropathogenic E.coli in the upper small intestine (Smith and Halls, 1967), and it is likely that bacteria proliferate more readily if they can adhere to the mucosal surface (Savage, 1972).

Interactions between the surface of Neisseria gonorrhoeae and cells of the human host play a critical role in the pathogenesis of gonorrhea. Kellogg et al. (1963, 1968) correlated virulence with specific gonococcal colony forms. Gonococci which grew in colonies with types 1 and 2 morphology caused gonorrhea in human volunteers, whereas gonococci from types 3 and 4 colonies did not. This correlation was extended by the studies of Swanson et al. (1971) and Jephcott et al. (1971) who demonstrated pili on the gonococci of types 1 and 2 and the absence of pili on organisms from types 3 and 4 colonies. These observations suggest that pili may correlate with virulence by influencing interactions between the gonococcal surface and host epithelial cells.

Bacteria indigenous to the human mouth display distinct tropisms for colonizing various oral surfaces. Streptococcus salivarius preferentially colonizes the dorsal surface of the tongue; Streptococcus sanguis and Streptococcus mutans have the teeth as their preferred habitat, and Streptococcus mitis is the predominant species colonizing the buccal mucosa (Gibbons and van Houte, 1975). Several studies have shown that the predilection of a number of oral species for colonizing a given oral surface is related to their specific ability to attach to the surface (Gibbons and van Houte, 1971; van Houte et al., 1971; Gibbons and van Houte, 1975).

Fowler and Stamey (1977) have examined the role of bacterial adherence to human vaginal epithelial cells in the pathogenesis of introital colonization by Enterobacteriaceae. They demonstrated that Staphylococcus epidermidis, a normal indigenous organism for the vaginal introitus, readily adhered to vaginal epithelial cells in vitro. Samples of vaginal cells from patients and control women were at times covered with bacteria so adherent that they could not be washed from the cells. These results suggest that bacteria that commonly colonize the vagina do so in part, because of a greater tendency to adhere to the vaginal mucosa. Different bacteria were shown to vary in their adhesive properties. E.coli O-groups that commonly cause urinary infection showed greater adherence as a group than the O-groups that rarely

cause infection; however, the difference was not statistically significant. The most striking observation made by the investigators was 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. However, the frequency with which a particular E.coli O-serogroup produces urinary infection cannot be explained primarily by its ability to adhere to vaginal epithelial cells. Rather, it is likely related to the frequency with which an E.coli O-serogroup appears in the rectal flora (Gruneberg et al., 1968). It seems fair to conclude that microbial adherence to the vaginal mucosa is important in the organism's ability to colonize the surface.

Kallenius and Winberg (1978) investigated bacterial adherence to periurethral cells of urinary tract infection-prone girls and healthy controls. Both in vivo and in vitro, statistically higher numbers of bacteria adhered to periurethral cells of infection-prone girls than to the cells of healthy girls. These findings suggest that the previously demonstrated disposition of infection-prone girls to bacterial colonization in the periurethral area is associated with an enhanced binding capacity of their periurethral cells for bacteria in vivo and potential uropathogens such as various strains of E.coli in vitro.

Mårdh and Westrom (1976) have studied the adhesive properties of bacterial species that cause infections of

the female genital tract using an *in vitro* method. Bacterial species associated with infection of the lower genital tract, *Neisseria gonorrhoeae*, group B Streptococci and *Corynebacterium vaginale*, were shown to be more adherent to vaginal cells than non-pathogenic vaginal flora. In a recent investigation, Fowler and Stamey (1978) were unable to show a correlation between the adhesive properties of an *E.coli* strain and the affinity of that strain to colonize the vaginal introitus.

There is evidence that bacterial adherence is influenced, at least in part, by surface mucosal antibody. Williams and Gibbons (1972) demonstrated that preparations of secretory IgA isolated from human parotid fluid specifically inhibited the adherence of *Streptococcus spp.* to epithelial cells. Tramont (1976) reported that local genital antibodies to infecting strains of *Neisseria gonorrhoeae* inhibited the attachment of gonococci to epithelial cells for at least 3 weeks to 4 months.

Local secretory IgA is thought to be a determining factor in bacterial adherence to introital mucosal cells of the vagina. In support of this theory is the observation by Stamey *et al.* (1977) that lactobacilli and *S.epidermidis* normal indigenous vaginal flora are not coated with antibody when treated with cervicovaginal fluid. Furthermore, absence of vaginal colonization in normal women appears to be related to cervicovaginal antibody against their fecal bacteria (Stamey *et al.*, 1978).

The surface characteristics of the vaginal mucosa and components of the bacterial surface have also been implicated as important factors that influence bacterial adhesiveness (Fowler and Stamey, 1977).

Parallelism has been observed between the abilities to produce symptoms of urinary tract infection and to attach to human urinary epithelial cells *in vitro*. Svanborg Edén *et al*. (1976) demonstrated that *E.coli* causing symptomatic urinary infection adhere in larger numbers to epithelial cells from the urinary tract than do *E.coli* isolated from urine of patients with asymptomatic bacteriuria. Recently, this group reported that serum IgG, milk IgA and isolated IgG and secretory IgA fractions of urine from patients with acute pyelonephritis inhibited the adhesion of *E.coli* to human urinary tract epithelial cells (Svanborg Edén, 1978). Antibodies to the O-antigen more efficiently inhibited adhesion than anti-K antibodies.

MATERIALS AND METHODS

A. STUDY POPULATION

The patient group consisted of twenty-nine females cared for in the Urinary Infection Clinic at the Health Sciences Centre, who had at least two symptomatic urinary tract infections within the preceding twelve months. Twenty-two patients were colonized with Enterobacteriaceae at the time of collection of cervicovaginal washings. The mean age of the patients was 35.7, with a range of 21 to 66 years. Intravenous pyelograms were obtained in twenty-six patients, all were normal with one exception where calyceal distortion was observed. Twenty-six patients were premenopausal, one had had a hysterectomy, and two had juvenile-onset diabetes.

Thirty-one healthy young non-pregnant female volunteers, with no history of urinary tract infections, were selected as controls. None were colonized with Enterobacteriaceae during the study. The mean age of the control subjects was 37.8 years, with a range of 19 to 57 years. Thirty control women were premenopausal and two had had hysterectomies.

B. COLLECTION OF SPECIMENS

At each visit the following specimens were obtained in the following order: (1) periurethral swab, (2) vaginal swab, (3) rectal swab, (4) cervicovaginal washing, and (5) 5 cc's of blood. Specimens were not collected during the menstrual cycle or during antimicrobial therapy.

a) Collection of Swabs

Periurethral Swab

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 phosphate buffered saline, pH 7.2 (PBS, Appendix A).

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 1 ml of PBS.

Rectal Swab

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

b) Collection of Cervicovaginal Washings

Cervicovaginal washings were collected as a 50 ml distilled water wash of the cervix and vagina. This was accomplished by inserting a speculum into the vagina, then placing a Rob Nel Number 16 red rubber catheter[1] in the mid-vagina, and gently injecting and withdrawing 50 ml sterile distilled water in a syringe. The syringe was emptied into a sterile plastic urine specimen container.

1 Argyle, Division of Sherwood Medical, Saint Louis, Missouri.

C. LABORATORY PROCEDURES

a) Processing of 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 innoculated 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 Enterobacteriaceae were isolated and stocked.

b) Processing of Cervicovaginal Washings

Two techniques for processing cervicovaginal washings have been reported: Tramont (1976) concentrated washings by filtration[2] and Stamey et al. (1978) lyophilized cervicovaginal fluid. In order to compare these methods, the specimens were aliquoted and processed using both methods. The samples were stood at 4°C for 2-3 hours to allow cells to settle, and the supernatant was then filtered through a 0.45μ Nalgene filter.[3]

2 Amicon PM-10 Filter, Amicon Corporation, Scientific Systems Division, Lexington, Massachusetts.

3 Nalge, Sybron Corporation, Rochester, New York.

Twenty-five milliliter aliquots of the filtrate were poured into sterile plastic urine specimen containers. One aliquot was lyophilized, stored at room temperature, and reconstituted with 0.5 ml sterile water (concentration 50X). The second aliquot was concentrated to fifty times using an Amicon minicon B15 filter[4] and was stored at $4^{o}C$ until use.

In view of the fact that radioimmunoassay is a more sensitive technique, concentrating the specimens was not necessary. Cervicovaginal washings that were to be quantitated for antibody using the radioimmunoassay procedure were centrifuged at 6000 x *g* for 10 minutes and the supernatant was stored at $-20^{o}C$.

4 Amicon Corporation, Scientific Systems Division, Lexington, Massachusetts.

D. INDIRECT IMMUNOFLUORESCENCE PROCEDURE

The presence of antibody in cervicovaginal washings in initial studies was determined by the indirect immunofluorescene technique as described by Thomas et al. (1974) for measuring serum antibody.

The controls' rectal Enterobacteriaceae and the patients' predominant introital Enterobacteriaceae were suspended to a concentration of 10^8 Colony-Forming Units (CFU)/ml PBS. Drops of these suspensions were placed on clean glass slides, air dried and heat fixed. The fixed organisms were flooded with the individual's cervicovaginal washing and the slides were incubated in a moist dark chamber at 37°C for thirty minutes, washed twice with PBS, air dried and treated with a 1:5 dilution of fluorescein-conjugated horse anti-human gammaglobulin.[5] After incubation at 37°C for thirty minutes, the slides were washed twice with PBS, air dried, and one drop of Bacto FA mounting fluid[6] was applied and a coverslip affixed. The slides were immediately examined for

5 Roboz Surgical Instrument Company, Inc., Washington D.C.

6 Difco Laboratories, Detroit, Michigan.

fluorescence with a Leitz microscope[7] at 1000X magnification.

The intensity of fluorescence was recorded as positive or negative and positives were subjectivity graded as 1+ to 3+. A sample was considered positive if $\geq$ 1+ fluorescence was seen on at least five cells. If more than ten fluorescing cells were seen, the percentage of fluorescing organisms was estimated. Negative and positive controls were included with each run of specimens. Appropriate controls of bacteria unexposed to cervicovaginal washings were used to measure nonspecific fluorescence. As a positive control, a patient's serum which was previously demonstrated by indirect immunofluorescence to have antibody to the patient's introital Enterobacteriaceae was run against the colonizing organism.

7 Ernst Leitz Wetzlar, Germany.

E. RADIOIMMUNOASSAY PROCEDURE

A radioimmunoassay procedure--a more sensitive and objective technique--was developed to quantitate antibody to E.coli in cervicovaginal washings from patient and control groups. The procedure involves: (a) reacting antibodies contained in cervicovaginal washings with a solid phase consisting of Sepharose 4B[8] to which a pool of E.coli urinary serogroups were coupled (Stage and Mannik, 1974) and extensively washing the solid phase after equilibrium; and (b) quantitating the antibody bound to the solid phase by addition of an excess of the appropriate ^{125}I-labeled anti-human immunoglobulin.

a) Purification and Labeling of Rabbit Anti-Human IgG and IgA

Rabbit anti-human IgG and rabbit anti-human secretory IgA were obtained from Cappel Laboratories Inc., Cochranville, Pennsylvania. Purification of rabbit anti-human IgG antibody was achieved by absorption onto a Sepharose 4B-IgG immunosorbent. IgG was purified from normal human

8 Pharmacia Fine Chemicals, Piscataway, New Jersey.

serum by absorption onto a Sepharose 4B-Protein A immunosorbent and elution with acetate buffer, pH 4.0 (Appendix B). The IgG preparation was concentrated by filtration (Amicon XM100A filter) and $NaHCO_3$-Na_2CO_3 buffer, pH 9.7 (Appendix C) was added to displace the acetate buffer. The IgG fraction was coupled to Sepharose 4B (Stage and Mannik, 1974) and the Sepharose 4B-IgG immunosorbent was suspended in the rabbit anti-human IgG. After gentle shaking at 4°C for 16 hours, the immunosorbent was washed with cold PBS (Appendix D) and rabbit anti-human IgG was eluted with glycine-HCl buffer, pH 2.8 (Appendix E). The pH of the eluate was immediately adjusted to neutrality and the preparation was dialyzed against PBS. Similarly, purified rabbit anti-human IgA was obtained by absorption with Sepharose 4B to which was coupled a fraction rich in human IgA.[9] The monospecificity of both purified anti-immunoglobulin preparations was confirmed by Ouchterlony and immunoelectrophoretic analyses. The purified anti-human IgG and IgA preparations were shown to react with human IgG and IgA, respectively.

9 Cappel Laboratories Inc., Cochranville, Pennsylvania.

The purified rabbit anti-human IgG and IgA were labeled with iodine-125[10] by the method of Greenwood et al. (1963). Approximately 90 mg of purified anti-human IgG or IgA was labeled, using 16 mg of chloramine T and 2.0 mCi of ^{125}I. The reaction was stopped by the addition of 24 mg of sodium metabisulphite. Separation of ^{125}I-labeled rabbit anti-human immunoglobulins from free ^{125}I was carried out by gel filtration through a column of BioGel P60.[11] Specific radioactivities of 1.1-1.5 x 10^6 cpm/µg were recorded for the ^{125}I-labeled preparations.

b) Preparation of Bacterial Antigens and Coupling to Solid Phase

A pool of 8 common E.coli urinary serogroups (01, 02, 04, 06, 07, 016, 018 and 075) were used as antigens. The E.coli 02, 016 and 018 strains were obtained from the Center for Disease Control, Atlanta, Georgia. Types 01, 04, 06, 07 and 075 were isolated from patients' periurethral and rectal swabs. Cultures were grown at 37°C for 18 hours in brain-heart infusion broth (BHI).[12]

10 The Radiochemical Center, Amersham, England.

11 Bio-Rad, Richmond, California.

12 Difco Laboratories, Detroit, Michigan.

A late log-phase culture was obtained by innoculating 50 ml of BHI with 0.01 ml of overnight cultures of each serogroup and incubating for 18 hours. The culture was harvested by centrifugation at 12,000 x *g* for 10 minutes, washed twice with $NaHCO_3$-Na_2CO_3 buffer, pH 9.7 and resuspended to a concentration of 10^8 CFU/ml $NaHCO_3$-Na_2CO_3 buffer using a McFarland No. 1 opacity standard.

The pooled serogroups were covalently bound to Sepharose 4B by a modification of the method of Stage and Mannik (1974). The Sepharose 4B was activated by the addition of cyanogen bromide (80 mg CNBr/ml packed gel) dissolved in acetonitrile. The reaction proceeded at 20°C and the pH was maintained at 10-11 by addition of concentrated NaOH. The reaction was terminated after 10 minutes by rapid washing of the Sepharose 4B with 10 volumes of cold bicarbonate buffer. The activated Sepharose 4B was equilibrated in bicarbonate buffer, centrifuged at 800 x *g* for 2 minutes, suspended in an equal volume of the bacterial preparation containing 10^8 CFU/ml, and then gently agitated at 4°C for 16 hours. The slurry was centrifuged and the supernatant was saved and counted for bacteria to ensure that the coupling had been successful. An equal volume of 0.5M ethanolamine in $NaHCO_3$-Na_2CO_3 buffer was added to the Sepharose 4B-E.coli conjugate to neutralize any remaining CNBr-activated groups on the Sepharose and the suspension was stirred at 4°C for 3 hours.

Following neutralization, the gel was sequentially washed on a Buchner funnel with 0.05M $NaHCO_3$-Na_2CO_3, 0.40M glycine-HCl (pH 2.8), PBS, then equilibrated in assay buffer (Appendix F) for the radioimmunoassay. The Sepharose 4B-*E.coli* suspensions were stored at 4°C and periodically checked for leaching of organisms.

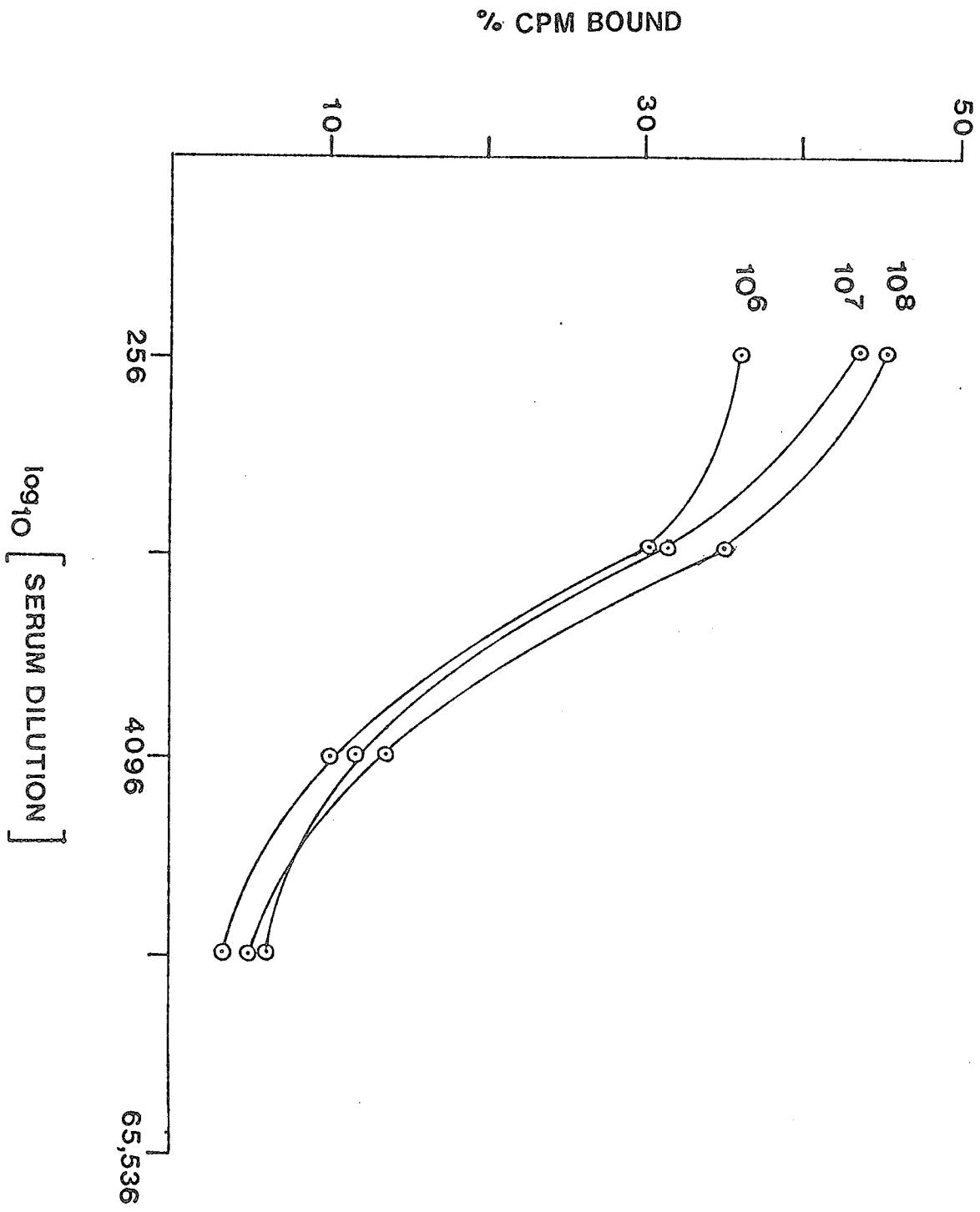
c) Radioimmunoassay for Quantitating Antibody Levels in Cervicovaginal Washings

i) Preliminary Studies

Preliminary assays were performed to determine the optimal amounts of *E.coli* antigen/ml of solid phase and the optimal amounts of labeled anti-immunoglobulin to use in the radioimmunoassay procedure.

In order to determine the optimum amount of *E.coli*/Sepharose 4B, increasing amounts of *E.coli* were coupled to Sepharose 4B in the range 10^6-10^8 CFU/ml solid phase. Volumes of 0.2 ml of a 1:1 suspension (volume/volume) of the solid phase in assay buffer were reacted with 0.2 ml of serial dilutions of a positive serum sample for 16 hours at 25°C, washed three times with cold assay buffer and then reacted with an excess of ^{125}I-rabbit anti-human IgG (2×10^5 cpm) for 16 hours at 25°C. The solid phases were washed three times with cold assay buffer before measuring the bound radioactivity. There was no significant increase in bound radioactivity in antibody excess (low serum dilution), when the antigen concentration was increased from 10^7 to 10^8 CFU/ml Sepharose 4B (Figure 1),

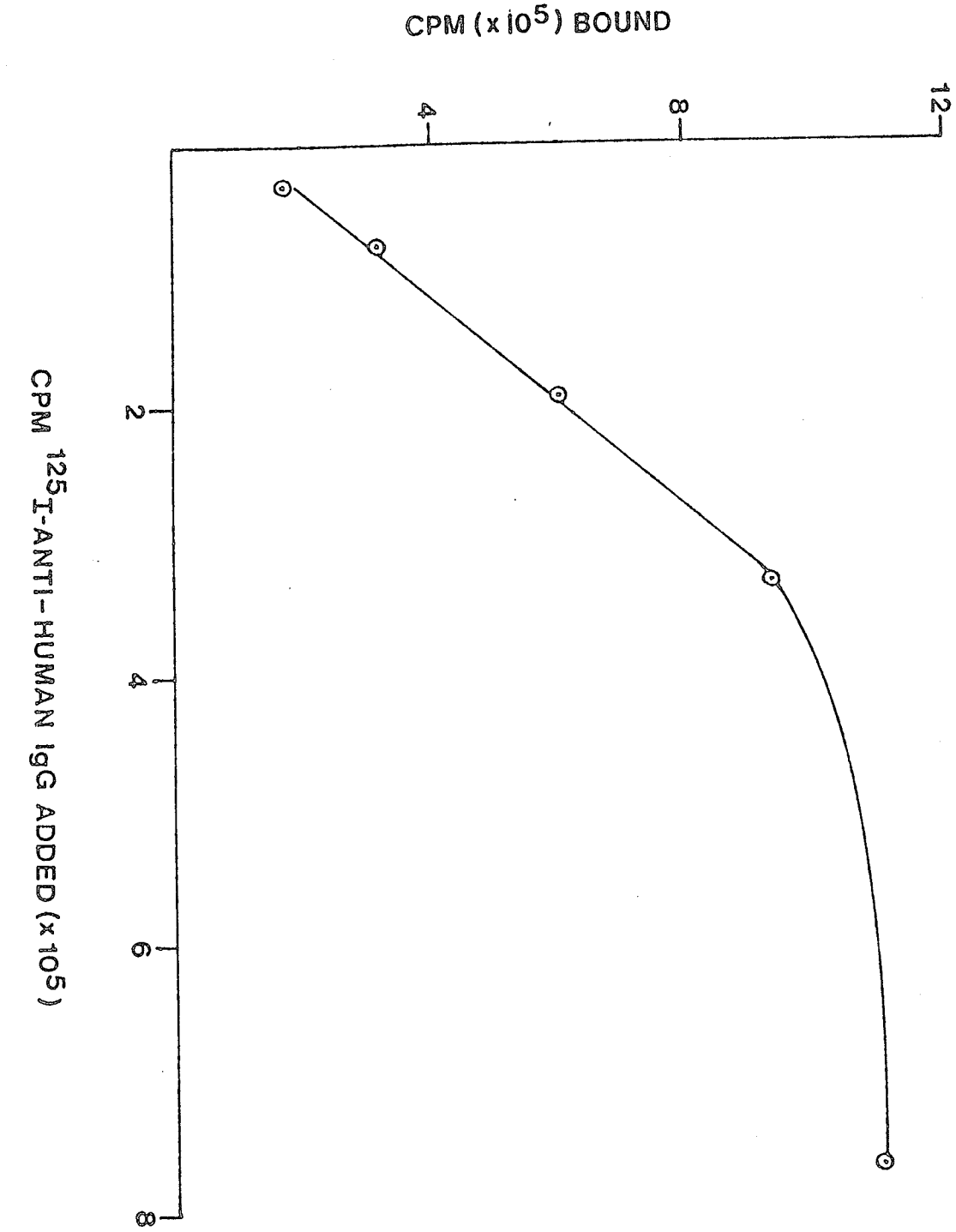
Figure 1 Estimation of *E.coli* Antigen Density in Organisms/ml Sepharose 4B for Quantitative Absorption of Antibody in Human Serum. Volumes of 0.2 ml of Sepharose 4B-*E.coli* suspension were reacted with 0.2 ml of serum dilution, washed, then reacted with 2.0×10^5 cpm ^{125}I-anti-human IgG. Concentrations of 10^6, 10^7, and 10^8 CFU/ml packed gel were tested.


% CPM BOUND
10
30
50
10^6
10^7
10^8
256
4096
65,536
$\log_{10}$ [SERUM DILUTION]

indicating that a concentration of 10^7 CFU/ml Sepharose 4B was sufficient for maximal absorption of anti-E.coli antibody. Maximal binding of ~ 50% cpm ^{125}I-anti-human IgG indicates that this labeled preparation was ~50% pure with respect to antibody activity. The remaining 50% may have been denatured in the immunospecific isolation procedure and subsequent ^{125}I-labeling.

The amount of labeled anti-immunoglobulin to be used in excess of bound antibody was determined by reacting 0.2 ml volumes of Sepharose 4B-E.coli (10^8 CFU/ml packed gel) with 0.2 ml of a 1:2000 dilution of a positive serum sample for 16 hours at 25°C and washing three times with cold assay buffer. The washed solid phases were then reacted with 0.1 ml volumes of increasing amounts of ^{125}I-rabbit anti-human IgG (4.0×10^4 - 7.7×10^5 cpm) for 16 hours at 25°C, washed three times with cold assay buffer and measured for bound radioactivity. The counts bound to absorbed antibody increased with the addition of increasing amounts of labeled anti-immunoglobulin, reaching a maximum after addition of 3.0×10^5 cpm (Figure 2). Therefore, adding amounts in excess of 3×10^5 cpm should ensure accurate quantitation of absorbed anti-E.coli antibody.

Figure 2 Estimation of the Amount of ^{125}I-Anti-Human IgG Required to Saturate Anti-*E.coli* IgG Antibody Bound to Sepharose 4B-*E.coli* at 10^8 CFU/ml gel.


CPM (x 10^5) BOUND
4
8
12
2
4
6
8
CPM ^{125}I-ANTI-HUMAN IgG ADDED (x 10^5)

ii) Radioimmunoassay Procedure

The cervicovaginal washings were serially diluted in assay buffer containing 0.1% bovine serum albumin. Samples (0.2 ml) of each dilution were added to duplicate glass culture tubes containing 0.2 ml of Sepharose 4B-E.coli. The tubes were incubated at room temperature (25°C) for 16 hours with gentle shaking. After incubation, the solid phase was sedimented by centrifugation at 800 x *g* for two minutes, the supernatant removed by aspiration, and the solid phase was washed three times with 1 ml of cold assay buffer. ^{125}I-rabbit anti-human IgG or IgA was diluted in assay buffer to a concentration of 2.0 x 10^6 cpm/ml. Samples of 0.2 ml (4 x 10^5 cpm) of ^{125}I-anti-immunoglobulin were added to each tube. The tubes were again incubated at 25°C for 16 hours with gentle shaking. The tubes were washed three times with cold assay buffer and the bound radioactivity was counted.[13]

Nonspecific binding of ^{125}I-rabbit anti-human immunoglobulin to the solid phase was measured by including controls which had assay buffer instead of sample dilutions added to the Sepharose 4B-E.coli.

13 Beckman gamma 8000, Beckman Instruments Inc., Fullerton, California.

The bound radioactivity was calculated using the following equation:

$$\% \text{ radioactivity bound} = \frac{B - C}{C_T} \times 100$$

B = labeled anti-human IgG or IgA bound to antigen

C_T = amount of labeled anti-human IgG or IgA added to each tube

C = nonspecific binding

iii) Preparation of Standard Curves

Antibody levels in cervicovaginal washings were quantitated by interpolation on a standard curve. Purified anti-E.coli antibodies were isolated from pooled human sera by absorption with Sepharose 4B to which an excess ($>10^{12}$ CFU/ml packed gel) of urinary E.coli serogroups had been coupled. Antibodies were subsequently eluted with 0.4M glycine-HCl buffer, pH 2.8. The pH of the eluate was immediately adjusted to 7.4 and after Amicon concentration, the preparation was dialyzed against PBS. Serial dilutions of the purified antibody solution were reacted with Sepharose 4B-E.coli and the appropriate ^{125}I-anti-immunoglobulin in the radioimmunoassay, and standard curves were prepared by plotting % radioactivity bound versus the $\log_{10}$ purified antibody dilution.

F. STATISTICAL METHODS

Fisher's exact test was used for statistical comparison of the indirect immunofluorescence results and one way analysis of variance was employed to analyze the radioimmunoassay data.

RESULTS

A. DETECTION OF SPECIFIC ANTIBODY IN CERVICOVAGINAL WASHINGS BY INDIRECT IMMUNOFLUORESCENCE

Cervicovaginal washings from fifteen patients with recurrent urinary infections and twenty-one normal controls were tested by indirect immunofluorescence for the presence of specific antibody. One of the fifteen patients (7%) demonstrated antibody-coating of their introital Enterobacteriaceae after exposure to cervicovaginal washings processed by Amicon concentration. Of the twenty-one control subjects, four (19%) demonstrated antibody-coating of their fecal Enterobacteriaceae when exposed to cervicovaginal washings (Table I). No significant difference in specific antibody, as measured by antibody-coating of Enterobacteriaceae after exposure to Amicon concentrated cervicovaginal washings, was detected between the two groups ($p = 0.3$).

Specific antibody was detected in three of the fifteen patients' (20%) lyophilized cervicovaginal washings. Seven of the twenty-one controls (33%) demonstrated antibody-coating of their fecal Enterobacteriaceae when exposed to lyophilized washings (Table I). Statistical analysis showed no significant difference between patient and control groups ($p = 0.3$).

More specimens were positive after lyophilization than after Amicon concentration; however, this difference is not significant ($p = 0.1$). A comparison of the degree

TABLE I

Comparison of Cervicovaginal Washings of Patients and Controls for the Presence of Antibody as Detected by Indirect Fluorescence Assay (IFA)

Group	Amicon FA+/Total	Lyophilization FA+/Total
Patients*	1/15 (7%)	3/15 (20%)
Controls**	4/21 (19%)	7/21 (33%)
Total	5/36	10/36

*Introital Enterobacteriaceae used for bacteria.

**Predominant fecal E.coli used for bacteria.

and number of fluorescing organisms in the positive specimens using the two techniques is shown in Table II. There appears to be a greater degree of fluorescence and/or a larger percentage of cells fluorescing in the lyophilized samples. This trend indicated that lyophilization was a superior concentrating technique and therefore, subsequent cervicovaginal washings were lyophilized. The results from analysis of an additional 15 washings combined with previous data is summarized in Table III. These data show no significant difference in specific vaginal antibody between patient and control groups ($p = 0.16$).

TABLE II

Comparison of Amicon Concentration and Lyophilization Treatments of Cervicovaginal Washings on the Detection of Antibody by IFA

Specimen		Amicon Concentration	Lyophilization
Patients:	A	1+; 6 cells	1+; 1%
	B	--	2+; 5%
	C	--	1+; 1%
Controls:	A	1+; 7 cells	1+; 6 cells
	B	1+; <1%	1+; 10%
	C	1+; 8 cells	1+; 20%
	D	1+; 1%	--
	E	--	1+; 7 cells
	F	--	1+; 5%
	G	--	1+; <1%
	H	--	1+; <1%

TABLE III

Distribution of Antibody to E.coli in Lyophilized Cervicovaginal Washings as Detected by IFA

Group	Number of Subjects	Number FA+	% FA+
Patients	22	3	14
Controls	29	10	34

B. QUANTITATION OF ANTIBODY TO E.coli IN CERVICOVAGINAL WASHINGS BY RADIOIMMUNOASSAY

Standard curves were prepared by reacting serial dilutions of the purified antibody solution isolated from human sera with Sepharose 4B-E.coli and the appropriate labeled anti-immunoglobulin in the radioimmunoassay and plotting % radioactivity bound versus the $\log_{10}$ purified antibody dilution. Figures 3 and 4 are the standard curves used for quantitating IgG and IgA antibody, respectively. Each point represents the mean for the 10 replicates and the lines denote the standard deviations. In the assay of antibody levels in samples, bound radioactivity was plotted as a function of sample dilution, the points were best fitted to the standard curve, and antibody levels were determined by interpolation using the ascending portion of the curve where ^{125}I-anti-immunoglobulin is in excess. Six percent bound was taken as the lower limit for assigning a positive result; the value (6%) represents radioactivity nonspecifically bound to the solid phase. Since no attempt was made to quantitate in absolute amounts (µg/ml) the purified antibody used for preparation of the standard curves, antibody in specimens was quantitated relative to the standard. Relative antibody levels were reported as % bound radioactivity X reciprocal cervicovaginal washing dilution.

Figure 3 Standard Curve for Quantitating IgG Antibodies Against E.coli Urinary Serogroups in Cervicovaginal Washings.

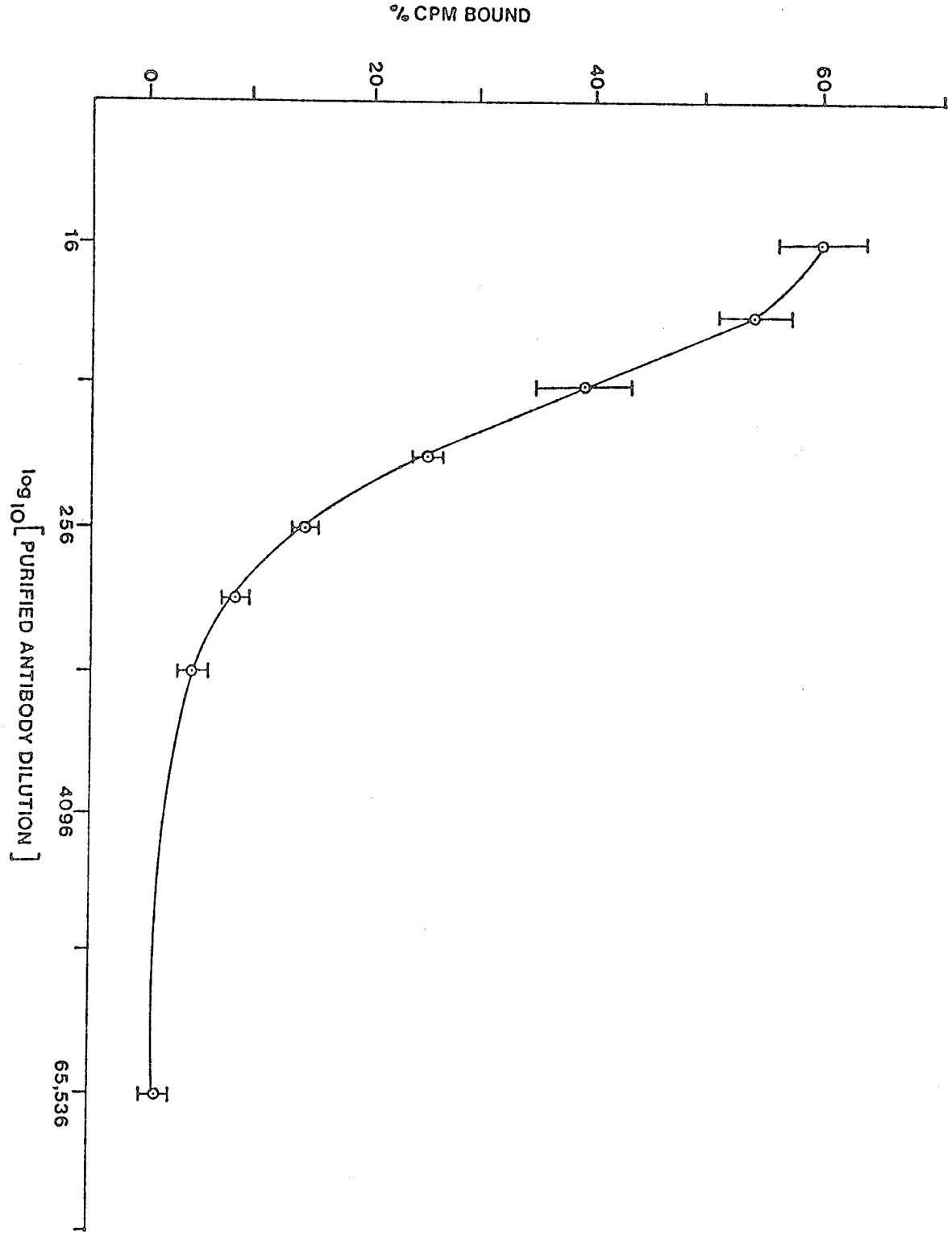
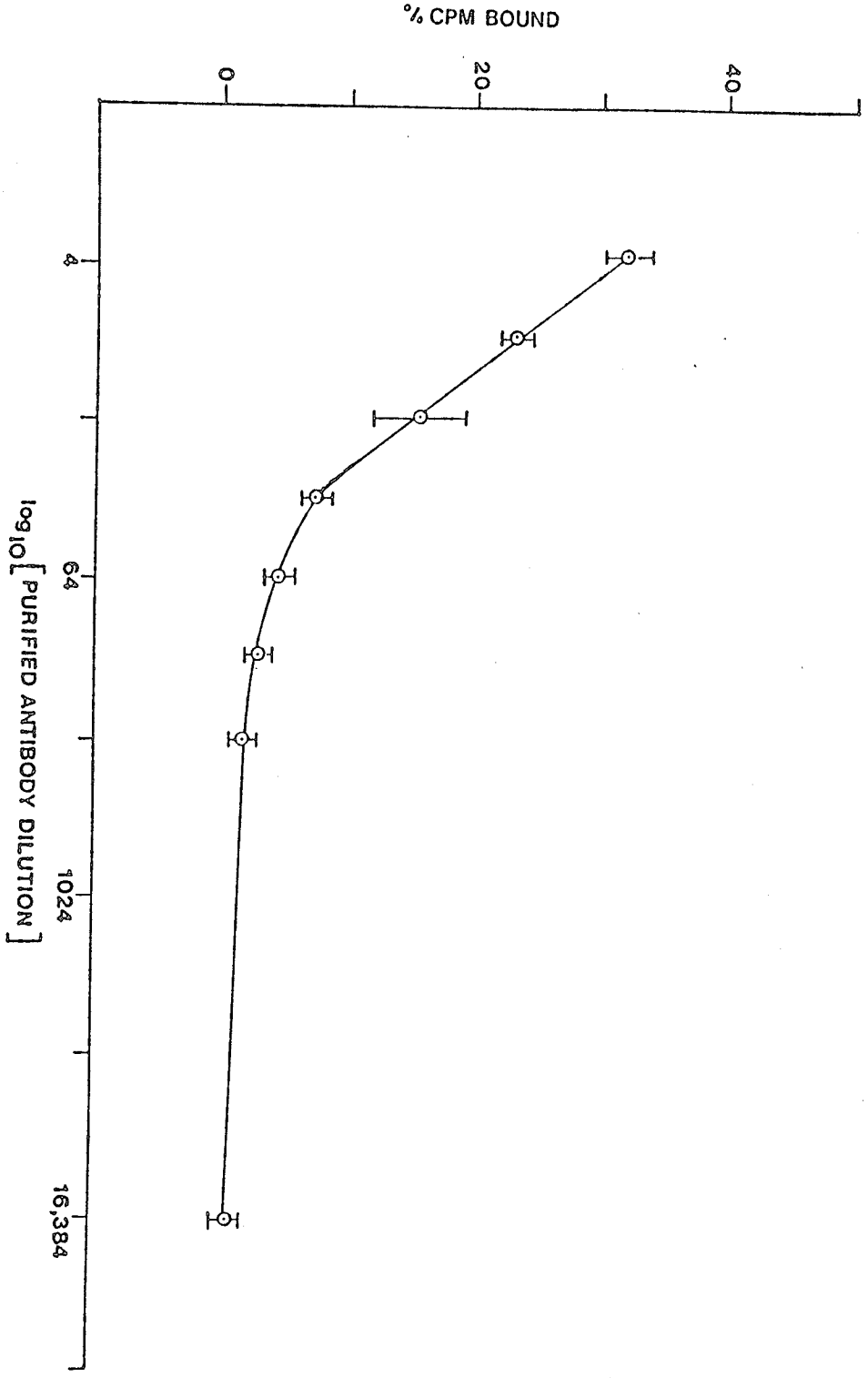

% CPM BOUND
0
20
40
60
16
256
4096
65,536
log10[PURIFIED ANTIBODY DILUTION]

Figure 4 Standard Curve for Quantitating IgA Antibodies Against E.coli Urinary Serogroups in Cervicovaginal Washings.


% CPM BOUND
0
20
40
4
64
1024
16,384
log10 [PURIFIED ANTIBODY DILUTION]

A known amount of the purified antibody preparation was added to cervicovaginal washings, and then subjected to radioimmunoassay. The washings were previously determined to be positive or negative by radioimmunoassay. The titration curve for the purified antibody contained in a specimen which was found to be negative for anti-E.coli antibodies (Figure 5), was the same as the curve obtained for the purified antibody preparation alone (Figure 3). The amount of antibody measured in a positive cervicovaginal washing to which was added a known amount of purified anti-E.coli antibody was equal to the sum of the two antibody concentrations (Figures 6a and 6b). Therefore, there are no factors in these cervicovaginal washings interfering with the absorption and detection of anti-E.coli antibodies.

Figure 5 IgG Antibody Titration of a Cervicovaginal Washing (△) and the Cervicovaginal Washing Containing a Known Amount of Purified Antibody (○).

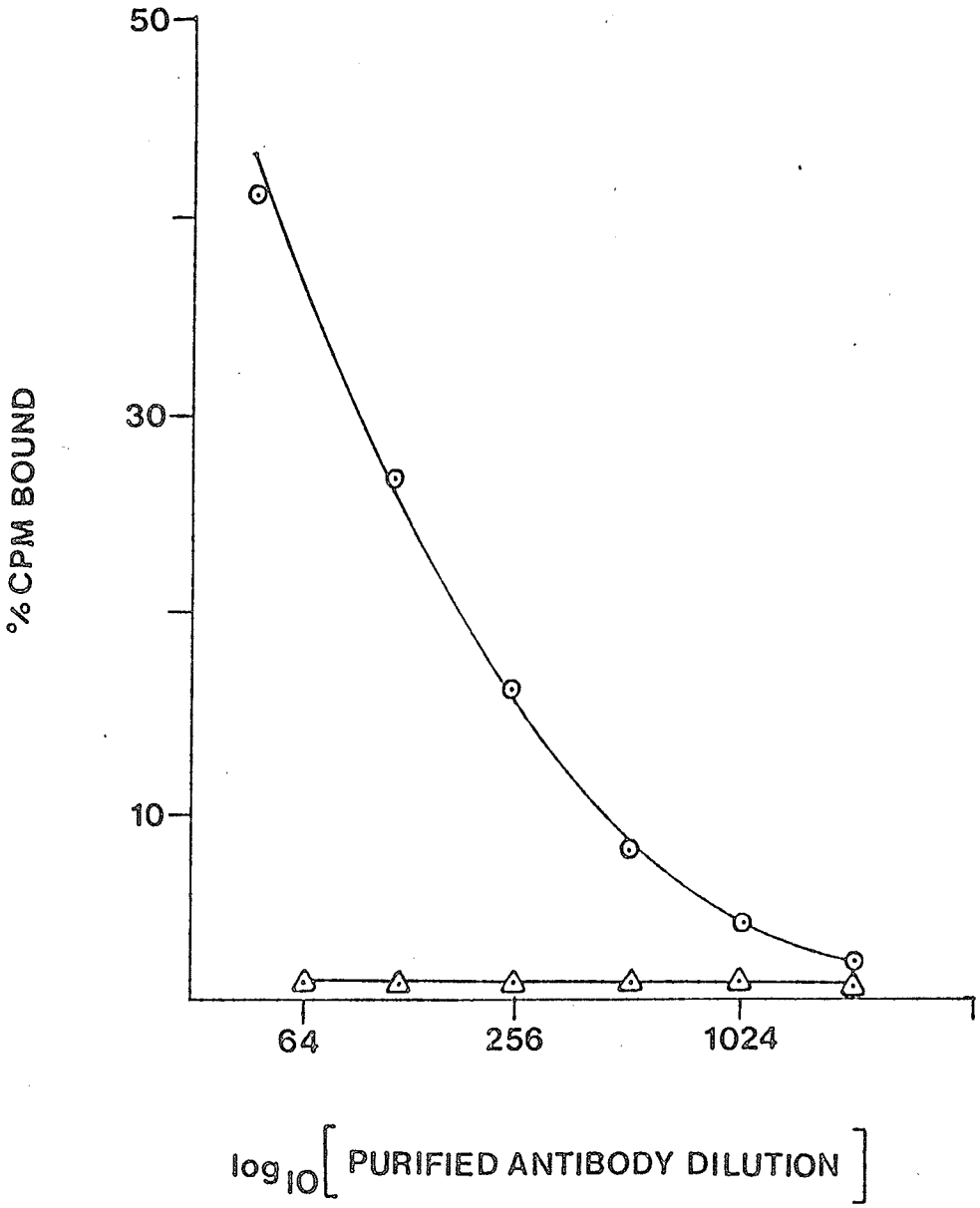

50
30
10
% CPM BOUND
64
256
1024
log10[PURIFIED ANTIBODY DILUTION]

Figure 6a IgG Antibody Titration of a Cervicovaginal Washing (△) and the Cervicovaginal Washing Containing a Known Amount of Purified Antibody (○).

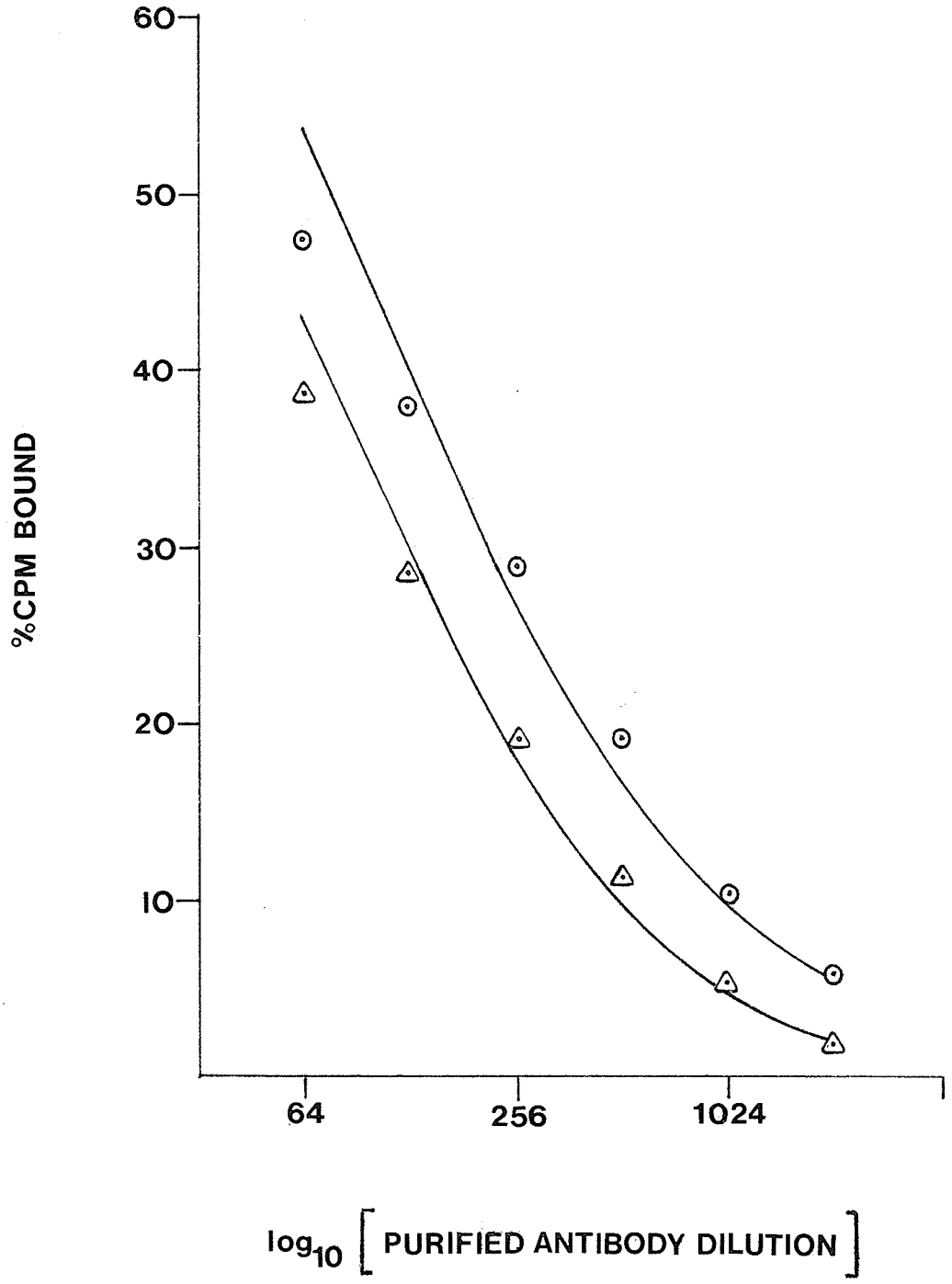
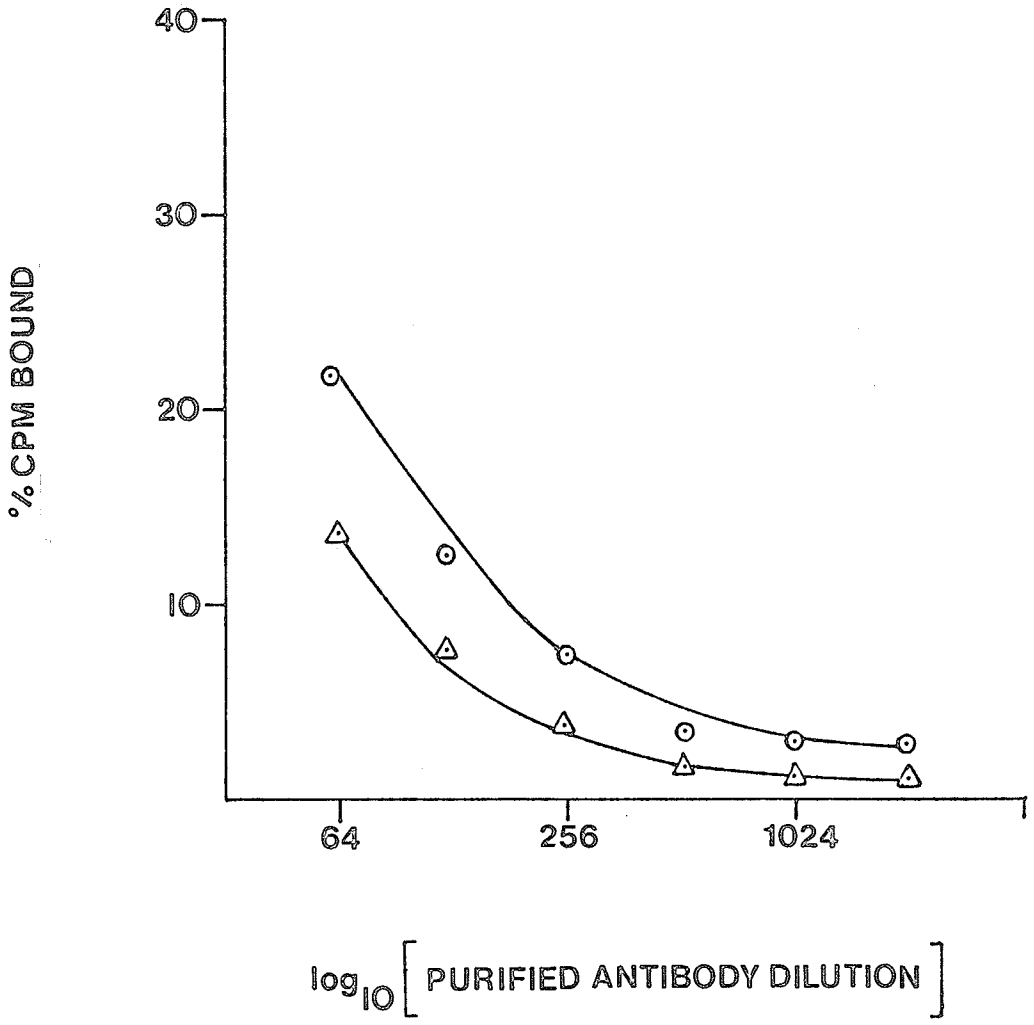

60
50
40
30
20
10
%CPM BOUND
64
256
1024
log10 [PURIFIED ANTIBODY DILUTION]

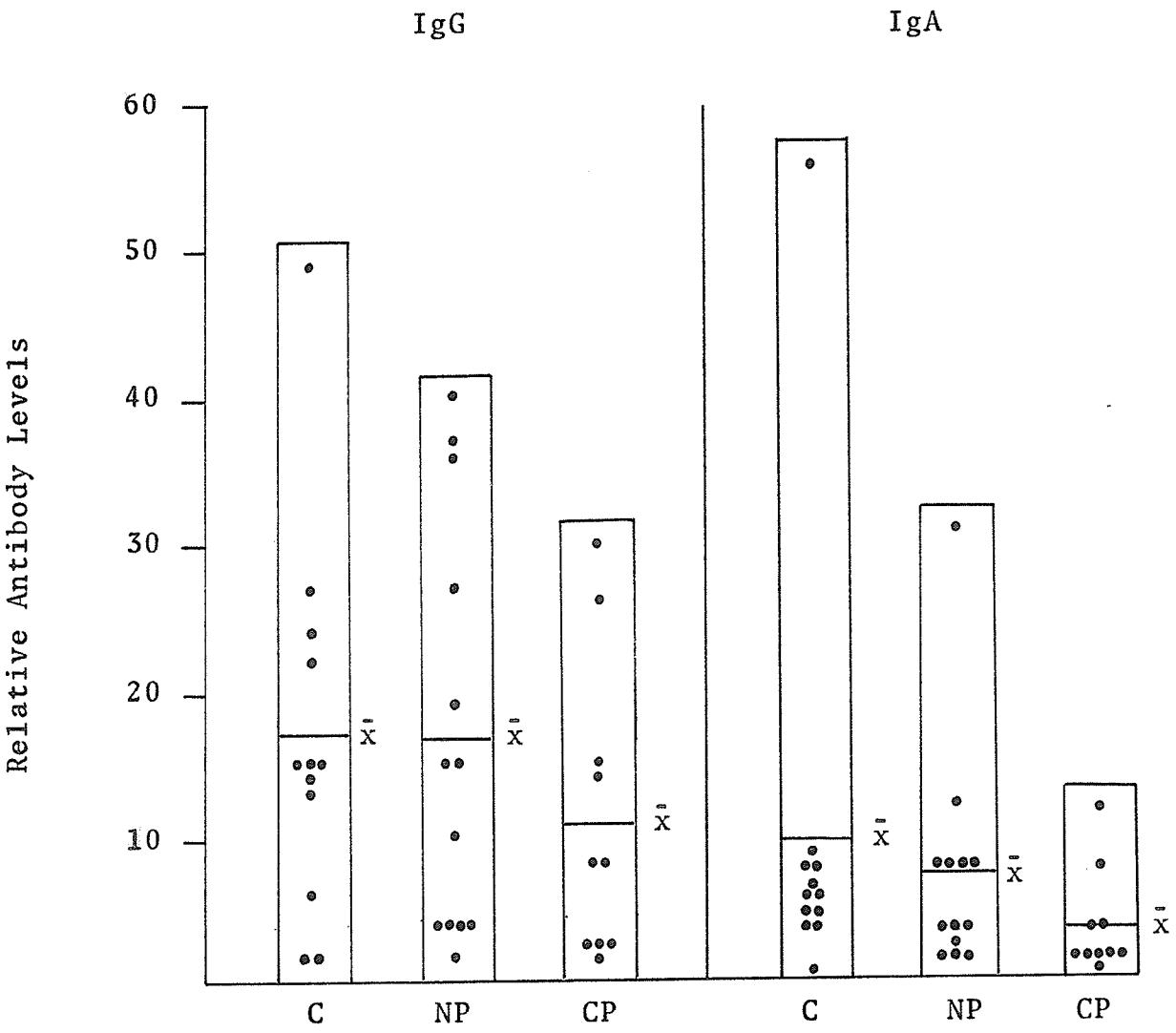
Figure 6b IgA Antibody Titration of the Cervicovaginal Washing (△) and the Cervicovaginal Washing Containing a Known Amount of Purified Antibody (○).


40
30
20
10
% CPM BOUND
64
256
1024
log10 [PURIFIED ANTIBODY DILUTION]

C. COMPARISON OF ANTIBODY LEVELS IN CERVICOVAGINAL WASHINGS FROM THREE GROUPS AS DETECTED BY RADIOIMMUNOASSAY

IgG and IgA antibodies were quantitated in cervicovaginal washings from: 10 females with a history of recurrent reinfections of the urinary tract and whose introitus was colonized with E.coli; 13 non-colonized females with a history of recurrent reinfections; and, 12 females with no history of recurrent reinfections who were not colonized. Immunoglobulin levels in specimens from the three groups are shown in Figure 7. The mean level of IgG in cervicovaginal washings from colonized patients was 10.8, non-colonized patients 16.7 and 17.0 in controls ($p > 0.05$). The mean IgA levels were 3.9 for colonized patients, 7.6 for non-colonized patients and 9.9 for controls ($p > 0.05$). Therefore, there was no significant difference in IgG and IgA levels among the three groups. In addition, the mean IgG levels were greater than the mean IgA levels in each group.

Figure 7 Antibody Levels Against E.coli Urinary Serogroups in Cervicovaginal Washings of Controls (C), Non-Colonized Patients (NP) and Colonized Patients (CP).


IgG
IgA
Relative Antibody Levels
60
50
40
30
20
10
C
NP
CP
C
NP
CP
x̄

D. COMPARISON OF RADIOIMMUNOASSAY AND IMMUNOFLUORESCENCE RESULTS

Cervicovaginal washings from 10 colonized patients, 10 non-colonized patients and 8 controls were examined for the presence of anti-E.coli antibody by both indirect immunofluorescence and radioimmunoassay. The immunofluorescence procedure detects predominantly IgG antibody, whereas the radioimmunoassay procedure detects either IgG or IgA antibody. Thus, specimens were considered positive by radioimmunoassay for antibody activity if IgG antibody levels were >6 (radioactivity nonspecifically bound). Results of the two methods are shown in Table IV. More specimens were positive for anti-E.coli antibody by radioimmunoassay (64.3%) than by indirect immunofluorescence (17.9%); this difference is significant ($p < 0.02$).

TABLE IV

Comparison of Radioimmunoassay (RIA) and Immunofluorescence (FA) Results

Group	RIA+/Total	FA+/Total
Colonized Patients	6/10	0/10
Non-Colonized Patients	6/10	4/10
Controls	6/8	1/8
Total	18/28 (64.3%)	5/28 (17.9%)

E. SPECIFICITY OF ANTIBODY

To determine if the antibody detected by the radioimmunoassay is specific for a particular component of the cell envelope, cervicovaginal washings were reacted with cell envelope fractions covalently coupled to Sepharose 4B in the radioimmunoassay. A fraction containing flagella, pili and capsular material was isolated from a 1000 ml culture of the eight E.coli urinary serogroups (10^8 CFU/ml) by treatment in a Sorvall omnimixer.[14] The outer membrane was then removed by lysozyme-ethylenediamine-tetraacetic acid treatment, aggregated by lowering the pH to 5.0, and recovered by centrifugation (Wolf Watz et al., 1973). Representative results from quantitating samples for antibody to the two envelope fractions are shown in Tables Va and Vb. There is no evident difference in levels of IgG or IgA antibody to the outer membrane fraction and to the pili, flagella and capsular fraction.

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TABLE Va

Relative Levels of IgG Antibody Against *E.coli*, *E.coli* Outer Membrane (OM) Fraction and an *E.coli* Fraction Containing Pili, Flagella and Capsular Material (PFC)

		IgG Antibody Levels Detected with:	
Specimen	*E.coli*	OM Fraction	PFC Fraction
1	50	32	49
2	18	40	32
3	10	23	26

TABLE Vb

Relative Levels of IgA Antibody Against *E.coli*, *E.coli* Outer Membrane (OM) Fraction and an *E.coli* Fraction Containing Pili, Flagella and Capsular Material (PFC)

		IgA Antibody Levels Detected with:	
Specimen	*E.coli*	OM Fraction	PFC Fraction
A	20	10	4
B	14	8	2

Are antibodies to Streptococcus spp., organisms which are frequently found in the vagina and cervix, and to Pseudomonas spp. which rarely populate these regions, present in the cervicovaginal washings? Specimens were collected from women who had previously been exposed to S.fecalis, but not P.aeruginosa, as determined by periurethral and vaginal cultures. The solid phases used in the radioimmunoassay were prepared by covalently coupling suspensions of S.fecalis and P.aeruginosa (10^8 CFU/ml packed gel) to CNBr-activated Sepharose 4B. IgG or IgA antibodies to S.fecalis and P.aeruginosa in specimens were quantitated relative to the standard. Typical results are shown in Tables VIa and VIb. Both IgG and IgA antibodies to S.fecalis and P.aeruginosa were demonstrated in cervicovaginal washings.

The surface of E.coli possesses many different antigenic determinants, each of which can potentially elicit the formation of antibody. If two organisms happen to have one or more determinants in common, they could cross-react with antibody. To test for antigenic cross-reactivity with the antibody detected by the radioimmunoassay, two cervicovaginal washings, previously shown to be positive for anti-E.coli antibody, were absorbed in separate experiments with E.coli (pooled urinary serogroups), S.fecalis or P.aeruginosa. The absorption involved centrifuging 2.0 ml of the bacterial

TABLE VIa

Relative Levels of IgG Antibody Against *Pseudomonas aeruginosa* and *Streptococcus fecalis* in Cervicovaginal Washings

	IgG Antibody Levels Detected with:	
Specimen	*P.aeruginosa*	*S.fecalis*
1	26	22
2	15	27
3	8	19

TABLE VIb

Relative Levels of IgA Antibody Against *Pseudomonas aeruginosa* and *Streptococcus fecalis* in Cervicovaginal Washings

	IgA Antibody Levels Detected with:	
Specimen	*P.aeruginosa*	*S.fecalis*
A	8	8
B	6	4

suspensions containing 1.8 x 10^9 CFU/ml, resuspending the packed cells in 2.0 ml of the specimen and incubating at 4^oC for 16 hours. After centrifugation, the absorbed sample was removed and tested for antibody activity against E.coli, S.fecalis and P.aeruginosa. The absorption process resulted in very little, if any dilution of the specimen. The absorbed cervicovaginal washings showed a marked decrease in the levels of antibody to the three organisms as measured in the radioimmunoassay (Table VII). The antibody levels dropped 58-96% after absorption with each of the organisms.

TABLE VII

Relative IgG Antibody Levels to *E.coli*, *S.fecalis* and *P.aeruginosa* in Untreated and Absorbed Cervicovaginal Washings

Specimen	Specimens absorbed with:	IgG Antibody Levels Detected with: *E.coli*	*P.aeruginosa*	*S.fecalis*
1	Untreated	24	14	25
	E.coli	3	2	2
	P.aeruginosa	10	5	6
	S.fecalis	2	2	2
2	Untreated	48	32	50
	E.coli	6	2	2
	P.aeruginosa	12	2	6
	S.fecalis	2	2	2

DISCUSSION

A. INTRODUCTION

Susceptibility to introital colonization is considered to be one of the principal factors predisposing individuals to recurrent urinary tract infections. Clearly, it is important to determine why females with recurrent infections are more frequently colonized with Enterobacteriaceae than normal females, so that preventive and therapeutic measures can be taken. Studies with Neisseria gonorrhoeae (Tramont, 1976) and oral Streptococci (Williams and Gibbons, 1972) demonstrated that local antibody can prevent the adherence of bacteria to epithelial cell surfaces. Similarly, this phenomenon may be involved in determining degrees of resistance to colonization of the vaginal introitus with Enterobacteriaceae.

Our hypothesis is that patients with recurrent urinary tract infections have diminished or absent local antibody, thus facilitating the adherence of Enterobacteriaceae to vaginal epithelial cells and promoting colonization which predisposes to recurrent urinary infections. To investigate whether differences between patients with recurrent infections and normal women who are not susceptible to urinary infections are mediated by antibody, it was proposed to quantitate (accurately and specifically) antibody present in cervicovaginal washings from both groups.

There are a variety of techniques which can be used to measure antibody in body fluids. Bacterial agglutination

was used by Stamey and Howell (1976) to measure antibody in cervicovaginal washings from patients with recurrent urinary infections and from normal controls. Agglutination was performed by reacting the patients' introital E.coli or the controls' predominant rectal E.coli with serial dilutions of the appropriate washing. No significant differences in agglutination titers between patients and controls was reported. In addition, equal antibody titers to those using specific E.coli were obtained in washings from patients and controls when rare O-groups which infrequently cause urinary infection were used in agglutination. Thus, the agglutination technique employed by these investigators did not demonstrate antibody titers specific to the colonizing organism and correlating with the state of infection. Furthermore, the antibody titers were low, indicating the insensitivity of the technique.

Immunofluorescence is a widely used and more sensitive method for detecting antibody. In our initial studies, an indirect fluorescence assay was used to detect specific antibody in cervicovaginal washings from patients and controls. The patients' introital Enterobacteriaceae and the controls' predominant fecal Enterobacteriaceae were fixed to glass slides, reacted with the individuals cervicovaginal washing and specific antibody coating the organisms was detected using fluorescein-labeled anti-human γ-globulin. No significant difference in specific antibody, as measured by indirect immunofluorescence, was observed in cervicovaginal

washings from patients and from controls. However, the immunofluorescence technique has a number of limitations. First, interpretation of fluorescence is subjective and can lead to bias in reading results. In addition, immunofluorescence on fixed samples is not easily quantitated and particularly not in this investigation since for positive specimens, both the degree of fluorescence and the percentage of fluorescing organisms were low. Therefore, the possibility that there was a quantitative difference in antibody levels in cervicovaginal washings from patients and controls could not be excluded.

Radioimmunoassay offers a unique combination of sensitivity, specificity and precision for the quantitation of antibody to infectious organisms in body fluids. The radioimmunoassay procedure used to quantitate antibody in cervicovaginal washings involved reacting serial dilutions of the specimens with Sepharose 4B-E.coli suspensions and detecting the absorbed antibody by addition of the appropriate ^{125}I-anti-human immunoglobulin.

Radioimmunoassay is the most sensitive of the various methods used for measurement of antibody, generally measuring in the nanogram (10^{-9} gm) range. The radioimmunoassay procedure employed in this study detected approximately 4.0×10^{-5} gm antibody/0.2 ml in cervicovaginal washings. This value was obtained by multiplying the concentration of the purified antibody (.11 mg/0.2 ml) by the dilution

factor which corresponded to a bound radioactivity just greater than 6% (radioactivity nonspecifically bound) (Figure 3) and it represents the minimum sensitivity assuming the purified preparation is 100% antibody. Thus, the technique is sensitive allowing detection of minute amounts of antibody in specimens. The radioimmunoassay was capable of detecting antibody directly from cervicovaginal washings so no prior concentration of specimens, e.g. lyophilization, was required.

Tuttle et al. (1978) using a radioimmunoassay technique, demonstrated a quantitative difference in IgA levels in cervicovaginal washings from children with recurrent urinary infections and from normal children. However, in contrast to our procedure, total IgA levels were measured without reference to antibody specificity to the infecting organism. In our radioimmunoassay antibody was absorbed onto Sepharose 4B to which was coupled a pool of E.coli serogroups which are frequently implicated in urinary tract infection; thus, antibodies which could influence introital colonization and hence the course of urinary infection were specifically quantitated.

Unlike immunofluorescence, radioimmunoassay is a quantitative method. IgG and IgA antibody to specific E.coli serogroups were quantitated by interpolation on standard curves and antibody levels were recorded relative to the standard. Therefore, using the radioimmunoassay procedure, quantitative differences in anti-E.coli antibody

in cervicovaginal washings from patients and controls could be determined.

In summary, the radioimmunoassay procedure has the advantage over immunofluorescence in allowing an objective, specific and accurate analysis of antibody in cervicovaginal washings.

B. DETECTION OF SPECIFIC ANTIBODY IN CERVICOVAGINAL WASHINGS BY INDIRECT IMMUNOFLUORESCENCE

Stamey et al. (1978) reported that cervicovaginal antibody was inversely related to introital colonization. Using indirect immunofluorescence, cervicovaginal antibody to the predominant fecal Enterobacteriaceae was found in 77% of normal controls with no history of urinary infection. In contrast, 26% of patients with recurrent infections demonstrated antibody in cervicovaginal washings to Enterobacteriaceae colonizing the vaginal vestibule. The differences in the presence of antibody between specimens from controls and patients were statistically significant. Thus, introital colonization with Enterobacteriaceae was associated with an absence of cervicovaginal antibody. However, there were exceptions to this inverse relationship; 26% of the colonized patients studied had antibody in their cervicovaginal washings and antibody was not detected in specimens from 33% of the controls. These may be too many exceptions for the relationship between antibody and colonization to be considered valid.

In our initial study, no significant differences in the presence of specific local antibody, as measured by immunofluorescence, were demonstrated in cervicovaginal washings from patients with recurrent urinary infections and normal controls. Fourteen percent (3/22) of the patients with recurrent infections demonstrated antibody-coating of their introital Enterobacteriaceae after exposure to lyophilized cervicovaginal washings. In the control group, 34% (10/29) demonstrated antibody-coating of their fecal Enterobacteriaceae when exposed to lyophilized washings (Table III). The amount of antibody detected in all specimens was not large as evidenced by the low intensity of fluorescence and the low percentage of fluorescing organisms obtained with positive specimens (Table II). These results suggest that introital colonization with Enterobacteriaceae in susceptible women, a prerequisite for urinary tract infection, is not associated with diminished or absent local antibody.

The apparent conflict in the results of these two studies cannot be attributed to differences in methods. The number of females selected for each group and the number of premenopausal and hysterectomized females in each group were similar in both studies.

Cervicovaginal washings were collected from females in both studies as 50 ml distilled water washes of the cervix and vagina. Stamey et al. lyophilized the samples

and later reconstituted them to 1 ml; the 1 ml reconstitution approximated the Na and K concentrations of natural vaginal fluid. Other investigators have concentrated the cervicovaginal washings by Amicon filtration (Tramont, 1976). In order to compare the two techniques, aliquots of the samples were either Amicon concentrated or lyophilized in our study. The lyophilized specimens were reconstituted to the same concentration (50X) as the specimens in Stamey's study. Comparison of Amicon concentration and lyophilization showed no significant differences in the numbers of cervicovaginal washings positive for specific antibody (Table I). However, in specimens which had detectable antibody, the lyophilized washings showed a greater degree of fluorescence and/or a larger percentage of bacteria fluorescing (Table II). This trend suggests that lyophilization is a superior concentrating technique which possibly results in less denaturation of antibody.

In both studies, indirect immunofluorescence was used to detect antibody in cervicovaginal washings. The procedures were adapted from the technique of Thomas et al. (1974) which measured serum antibody. Fluorescence was subjectively graded by visual examination in both cases. Since the immunofluorescence technique was subjective, there may have been some variation in reading results.

There were no substantial differences in the amounts of antibody which were detected in cervicovaginal washings

in both studies. The amount of antibody detected in cervicovaginal washings in Stamey's study was not large. If positive specimens were diluted >1:32, no fluorescing bacteria were demonstrated. Similarly, the degree of fluorescence and the percentage of fluorescing organisms obtained with positive specimens in this study were low and when the specimens were diluted 1:2, antibody was not detected.

In the investigation by Stamey et al., specific antibody was detected in cervicovaginal washings in 26% of the colonized patients with recurrent infections. In our study, 14% of the patients whose introitus was colonized, demonstrated specific antibody in their cervicovaginal washings. These results negate the argument that antibody prevents colonization. However, the levels of specific antibody may be much lower in the specimens from colonized patients compared to the levels in specimens from normal women. The indirect immunofluorescence technique employed in the studies could not detect quantitative differences in specific local antibody in cervicovaginal washings from the two groups and thus the studies are inconclusive. A quantitative analysis of the cervicovaginal washings from patients and controls using a more sensitive and quantitative technique, namely the radioimmunoassay procedure which was previously discussed, would enable us to better determine the relationship between antibody and introital colonization.

C. COMPARISON OF ANTIBODY LEVELS IN CERVICOVAGINAL WASHINGS FROM THREE GROUPS AS DETECTED BY RADIOIMMUNOASSAY

Tuttle et al. (1978), using a double antibody radioimmunoassay, demonstrated a quantitative difference in the amount of IgA in the vaginal fluid from girls with recurrent urinary infections and from girls who had never experienced urinary infections. Vaginal IgA levels were significantly lower in specimens from patients when compared to controls. However, an overlap in IgA levels was noted in the two groups.

The double antibody radioimmunoassay quantitated total IgA without regard to antibody specificity to the infecting organism. IgA antibody specific for organisms other than the colonizing pathogen may be present in the samples, as will be discussed later. In the study, no attempt was made to determine the specificity of the antibody detected by the radioimmunoassay. Thus, antibodies which are able to react with the infecting organism (E. coli) and inhibit the adherence of the organism to the vaginal introitus were not specifically quantitated. Therefore, their results do not convincingly demonstrate any relationship between specific antibody and the degree of colonization.

In our study, IgG and IgA antibody directed against E. coli were quantitated by radioimmunoassay in cervicovaginal washings from patients with recurrent urinary infections who were colonized, from non-colonized patients and from normal females with no history of recurrent infections. No significant quantitative differences in IgG and IgA antibody were demonstrated among the three groups

(Figure 7). The mean IgG and IgA antibody levels were greater in specimens from the control group compared to those from non-colonized patients, and particularly those from colonized patients. One might infer that by increasing the population numbers, a significant difference in antibody levels would be demonstrated. However, it must be emphasized that there was a large degree of overlap in antibody levels among the three groups; and, further high levels of antibody were found in specimens from colonized patients and low levels of antibody were found in specimens from non-colonized patients and from controls. These results do not support the hypothesis that cervicovaginal antibody is inversely related to introital colonization.

The mean IgG levels were greater than the mean IgA levels in specimens from all three groups (Figure 7). This is in agreement with other studies (Hulka and Omran, 1969; Govers and Girard, 1972; Chipperfield and Evans, 1975; Stamey and Howell, 1976) where quantitation of immunoglobulins in cervicovaginal secretions showed that IgG was present in greater concentrations than IgA. IgM was not quantitated in this study because it has not been consistently reported as a significant immunoglobulin in cervicovaginal secretions.

The IgA/IgG ratio found in the cervicovaginal washings was approximately 0.48. This ratio is significantly higher than that found in serum, which lies around 0.2-0.16 (Vaerman and Férin, 1975), suggesting that the antibody in cervicovaginal washings quantitated by the radioimmunoassay was derived from the vagina and cervix.

D. COMPARISON OF RADIOIMMUNOASSAY AND IMMUNOFLUORESCENCE RESULTS

The radioimmunoassay was compared with the immunofluorescence method for the detection of anti-E.coli antibody in cervicovaginal washings from the three groups. More specimens were positive for anti-E.coli antibody by radioimmunoassay than by indirect immunofluorescence (Table IV); this difference is quite significant and can be attributed to the greater sensitivity of the radioimmunoassay technique, especially where, in the majority of cases, the levels of antibody present are low, at or below the level of detectability by immunofluorescence. In addition, the immunofluorescence technique uses a single E.coli serogroup (compared to eight in the radioimmunoassay) and so may be only detecting that fraction of anti-E.coli antibody specific to the serogroup. However, as will be discussed later, antibodies were found to be specific for antigenic determinants common to the different serogroups.

E. SPECIFICITY OF ANTIBODY

Capsular antigens (K-antigens) and somatic antigens (O-antigens) have both been implicated in the pathogenesis of recurrent urinary tract infections (Hanson et al., 1977). Thus, it was of considerable interest to determine if the antibody detected in the cervicovaginal washings by the

radioimmunoassay was specific for a particular component of the E.coli cell envelope.

Cervicovaginal washings were reacted with an E.coli outer membrane fraction and an E.coli fraction containing pili, flagella and capsular material which were coupled to Sepharose 4B and used in the radioimmunoassay. Specimens did not contain IgG or IgA antibody to exclusively one fraction. Using either the outer membrane or pili, flagella and capsular fractions (or whole E.coli) in the solid phase, similar antibody titers were obtained by radioimmunoassay (Table Va and Vb), suggesting that the antibodies present were reacting with antigens common to both fractions, and that no detectable antibodies were present that were directed against unique antigenic determinants of either fraction.

The vaginal introitus is usually colonized by several aerobic organisms of limited urinary pathogenicity. Streptococcus spp. are frequently cultured from the vaginal introitus, whereas Pseudomonas spp. are infrequently cultured from this area (Fowler et al., 1977). Cervicovaginal washings from women who were previously exposed to Streptococcus spp. but not Pseudomonas spp. should contain some antibody activity to the former. Indeed, antibody against Streptococcus fecalis was demonstrated by radioimmunoassay in such washings (Table VIa and VIb). However, antibody to Pseudomonas aeruginosa

was also detected. This may indicate that the women were previously exposed to the organism or that the antibodies are directed against antigens common to the two species.

Absorption experiments indicated that there is extensive antigenic cross-reactivity with the antibody detected by the radioimmunoassay. Antibody against E.coli was partially or completely removed when cervicovaginal washings were absorbed with other organisms (Pseudomonas aeruginosa or Streptococcus fecalis (Table VII), indicating that the organisms have at least one common antigenic determinant. These results suggest that perhaps antibody could play a role in introital colonization. Antibody, specific to surface antigens common to infecting organisms and other non-pathogenic organisms, which is present in secretions may prevent colonization of E.coli on the vaginal introitus. This may be a "universal" defense mechanism which is present in all women. However, differences in susceptibility to infection were not attributed to differences in antibody levels by this investigation, suggesting that the role played by antibody is a minor one.

It should be emphasized that even though the antibodies detected by the radioimmunoassay were not uniquely specific to the infecting organism (E.coli), they were antibodies which reacted with E.coli. This is supported by the

observation that when positive cervicovaginal washings were absorbed with E.coli, the majority of detectable antibody was removed (Table VII). Thus, antibodies which could play a role in the pathogenesis of urinary infection were quantitated by the radioimmunoassay.

It has been suggested that there may be differences in antibody to a specific component which mediates the adherence of pathogens to the vaginal introitus. Such antibody has not been detected because the whole organism was used in the radioimmunoassay. However, because the majority of detectable antibodies were removed by absorption with other organisms, the fraction of antibody directed against specific antigens of the infecting organism would be negligibly small to play a significant role in the inhibition of adherence.

F. CONCLUSION

This investigation was designed to study the role of cervicovaginal antibody in the pathogenesis of recurrent urinary tract infections. In an initial study employing indirect immunofluorescence, no significant differences in specific local cervicovaginal antibody were demonstrated between patients with recurrent urinary infections and normal controls with no history of urinary infections.

Subsequently, a sensitive, specific and quantitative radioimmunoassay technique was developed to quantitate

cervicovaginal antibody. No significant quantitative differences in IgG and IgA antibody were found in cervicovaginal washings from patients with recurrent infections and normal controls. Both high and low levels of antibody were found in colonized and non-colonized patients.

In these studies, introital colonization which predisposes to recurrent urinary tract infections was not related to cervicovaginal antibody.

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APPENDICES

APPENDIX A

Phosphate Buffered Saline (PBS), pH 7.2

Stock Buffer:

Na_2HPO_4	109.6 gm
$NaH_2PO_4 \cdot H_2O$	31.5 gm
Double distilled water to 4 liters	

Preparation:

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

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

APPENDIX B

Acetate Buffer, pH 4.0

$NaOAc \cdot 3H_2O$	4.8 gm
NaCl	11.7 gm
CH_3COOH	10.2 gm

Double distilled water to 4 liters

APPENDIX C

$NaHCO_3$ - Na_2CO_3 Buffer, pH 9.7, 0.1M

$NaHCO_3$	8.4 gm
Na_2CO_3	10.6 gm

Double distilled water to 1 liter

The buffer was stored at $4^{o}C$.

APPENDIX D

Phosphate Buffered Saline (PBS) for Radioimmunoassay, pH 7.4

NaCl	70.14 gm
K_2HPO_4	11.71 gm
KH_2PO_4	1.74 gm

Double distilled water to 8 liters

APPENDIX E

Glycine-HCl Buffer, pH 2.8, 0.40M

Glycine	30.03 gm

Add double distilled water to almost 1 liter.

Adjust pH to 2.8 with concentrated HCl.

Accurately adjust volume to 1 liter with double distilled water.

The buffer was stored at $4^{o}C$.

APPENDIX F

Assay Buffer, pH 7.4

Bovine serum albumin	2.0 gm
NaN_3	2.0 gm
Tween 20	10.0 gm
$Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$	37.2 gm

Double distilled water to almost 2 liters with PBS (Appendix D)

Adjust pH to 7.4 with concentrated NaOH.

Double distilled water to 2 liters