THE *IN VITRO* ATTACHMENT OF *ESCHERICHIA COLI* TO HUMAN UROEPITHELIAL CELLS: INVESTIGATION OF HOST AND BACTERIAL CELL FACTORS.

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TO

MUM AND DAD

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ABSTRACT

Escherichia coli is the main causative organism in urinary tract infections (UTI) in adult women. Attachment of E.coli to the uroepithelium is believed to be an important step in the onset of infection, as it enables the bacteria to resist hydrokinetic clearance mechanisms. In the present study, a reproducible *in vitro* test system was used to assay E.coli attachment to uroepithelial cells harvested from the urine of premenopausal women.

Type specific antisera and an indirect fluorescent antibody technique were also used to detect E.coli attached to uroepithelial cells. The results from this study were comparable to those obtained using methylene blue as a bacterial stain. The fluorescent antibody technique was applied to the study of uroepithelial cells from women with symptomatic UTI, and E.coli were seen attached in varying numbers to these cells. Uroepithelial target cells with large numbers of E.coli attached, were found to exist within any given cell population. The capacity to attach to uroepithelial cells appeared to be a property held by the majority of E.coli strains isolated from urinary tract infections.

Receptivity of uroepithelial cells to attachment of certain E.coli strains varied over the menstrual cycle in a repetitive, cyclical pattern, similar to expected oestrogen levels. Highest attachment values were obtained between days 9 and 13, 18 and 28, and lowest values between days 14 and 17 of the menstrual cycle. Uroepithelial cells collected from six women in the first few months of pregnancy were highly receptive to E.coli attachment; whereas cells collected in the later months were less receptive. These results did not follow expected oestrogen levels.

The bladders of a pig, a lamb and a group of mice were examined for the presence of mucopolysaccharides. Periodic acid Schiff's

(PAS) reagent and alcian blue were used to identify a wide range of mucus substances. Several techniques were devised incorporating these two stains in combination with viability or bacterial stains to examine the mucopolysaccharides coating human uroepithelial cells from the urine sediment. These double-staining techniques proved to be highly reproducible. Significantly more viable than nonviable uroepithelial cells were coated with mucus. Attachment of one E.coli strain was greater to mucus coated than to non-coated cells; while attachment of a second strain was apparently unaffected by the presence of a mucus coat.

A proportion of E.coli strains produce a mannose sensitive haemagglutinin (MSHA) detected with guinea pig erythroyctes; while a proportion of E.coli strains produce a mannose resistant haemagglutinin (MRHA) detected with human erythrocytes. The role of these bacterial haemagglutinins in the attachment of E.coli to uroepithelial cells was investigated. The expression of a MSHA was in some cases affected by storage of E.coli for 12 months; while expression of a MRHA was not affected by storage. The presence of a MSHA did not enhance attachment to uroepithelial cells. However, after growth on trypticase soy agar, a number of strains expressed a MRHA and attached in significantly greater numbers to uroepithelial cells. Several experiments were designed to investigate the nature of the MRHA.

Electron microscopy was used to examine E.coli cells for the presence of pili. Strains expressing a MSHA were highly piliated; strains possessing a MRHA were highly piliated, poorly piliated or non-piliated. Further studies were carried out using transmission electron microscopy and scanning electron microscopy to examine bacterial attachment to the uroepithelial cell surface. In the former, ruthenium red was used in one instance to stain the polysaccharides which coat the bacterial and uroepithelial cells.

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