VIRULENCE OF *ESCHERICHIA COLI* IN ASCENDING URINARY-TRACT INFECTION IN MICE

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SUMMARY. The virulence of *Escherichia coli* strains in ascending urinary-tract infection was studied in mice drinking a 5% glucose solution; factors determining the virulence were examined. Of 33 strains, 8 (group I) infected the bladder and kidney, 10 (group II) infected only the bladder, while the remaining 15 strains (group III) did not cause infection. The adherence of group-I and group-II strains to bladder epithelial cells *in vitro* was inhibited by D-mannose. In group III, 13 strains barely adhered to the epithelial cells, while two strains showed an adherence unaffected by D-mannose. Most strains in groups I and II agglutinated erythrocytes of guinea-pig, chicken, and horse, and cells of *Candida albicans* in a mannose-sensitive manner. All strains in groups I and II had fimbriae. Virulence for the urinary tract was not directly related with O-serotype, intraperitoneal virulence, ability to grow in mouse urine, ability to ferment dulcitol, production of haemolysin, susceptibility to serum bactericidal activity, or susceptibility to antibiotics. These results suggest that the adherence of the *E. coli* to mouse-bladder epithelial cells in a mannose-sensitive manner plays an important role in the development of urinary-tract infection in mice and that the adherence is probably mediated by type-1 or closely related fimbriae.

INTRODUCTION

The majority of urinary-tract infections (UTI), especially of acute and uncomplicated episodes, are caused by *Escherichia coli*. The prevalence of *E. coli* in such infections is assumed to result from its proximity to the gastrointestinal tract (Montgomerie, 1978). Early epidemiological studies suggested that *E. coli* isolated from UTI were those that prevailed in the faeces at the time of infection (Turck, Petersdorf and Fournier, 1962; Vosti *et al.*, 1964), but subsequent reports indicated that pathogenicity of *E. coli* in UTI might correlate with special properties such as the production of haemolysin (Cooke and Ewins, 1975; Minshew *et al.*, 1978), resistance to serum bactericidal activity (Olling, 1977; Björkstén and Kaijser, 1978), and the type or amount of K antigen (Glynn, Brumfitt and Howard, 1971; Kaijser, 1973; McCabe *et*

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al., 1975; Kaijser *et al.*, 1977). More recently, several authors have shown that *E. coli* isolated from the urine of patients with UTI adhere better to epithelial cells from the urinary tract or from the periurethral area than do those isolated from patients with asymptomatic bacteriuria or from faeces of healthy persons, and have suggested that the adherence of *E. coli* to uro-epithelial cells plays an important role in the initiation of UTI (Svanborg Edén *et al.* 1976; Schaeffer, Amundsen and Schmidt, 1979; Källénus, Möllby and Winberg, 1980; van den Bosch *et al.*, 1980). However, the nature of the virulence factors of *E. coli* in UTI is still a matter of conjecture.

The present study was designed to determine possible differences in urinary virulence among *E. coli* strains in a quantitative way and to examine possible factors affecting the virulence. The ascending UTI model in mice drinking the 5% glucose solution of Keane and Freedman (1967) was used as the test of urinary virulence. This model avoids artificial manipulations that have usually been necessary to produce experimental UTI via the ascending route in animals (Cotran, 1969).

MATERIALS AND METHODS

Bacterial strains. A total of 33 strains of *E. coli* were used; 30 were isolated from extraintestinal clinical sources, and three were laboratory strains, NIHJ JC-2 and K12 from our collection, and K12 ML1410 kindly supplied by Dr S. Mitsuhashi (Gumma University, Japan). All strains were maintained on Trypticase Soy Agar (TSA; BBL Microbiology Systems, Cockeysville, MD) slopes at 4°C. As a rule, bacterial strains were cultured in Brain Heart Infusion Broth (BHI; Difco Laboratories, Detroit, MI) overnight at 37°C for testing.

Mice. Five-week-old female CF#1/b mice (Takeda Chemical Industries Ltd, Osaka) weighing 19–23 g were used. They were given 5% glucose solution as the sole source of drinking water from 4 days before an experiment to the end of observation by the method of Keane and Freedman (1967). The mice drank c. 15–20 ml per day. In part of the experiment, mice were given tap water and drank c. 8–10 ml per day. All mice were fed laboratory chow pellets (CLEA Japan Inc., Tokyo) *ad libitum*.

Determination of urinary virulence. Mice were anaesthetised with 50 mg of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL) per kg body weight, given intraperitoneally, were forced to void urine by gentle compression of the bladder through the external abdominal wall and 0.05 ml of bacterial suspension was inoculated into the bladder via the urethra. Five mice were used for each inoculum-dose experiment. The bacterial suspensions were prepared from an overnight BHI culture of test strains by tenfold serial dilution with physiological saline solution. The existence of infection was determined by bacteriological examination of the bladder and kidney at 7–9 days after the inoculation. The bladder and kidney were removed aseptically and the bladder was rinsed with c. 30 ml of saline. The inner surface of the bladder and the cut surface of the kidney were imprinted onto MacConkey Agar (MCA; Eiken, Tokyo) and the plates were incubated overnight at 37°C. The dose required to produce infection in 50% of mice (ID₅₀) was calculated by the method of Behrens and Kärber (1935).

Viable counts of bacteria in the bladder. Five mice were each inoculated with about 10⁷ cfu of bacteria. The bladder was removed 3 h after the inoculation and rinsed three times with c. 30 ml of saline. The washed bladder was homogenised in 2 ml of saline and serial tenfold dilutions of the homogenate were cultured quantitatively by the pour-plate method in MCA. The number of bacteria was expressed as the number of cfu per entire organ.

In-vitro adherence test. Overnight BHI cultures of bacteria were washed twice in phosphate-buffered saline, pH 7.0 (PBS) for 20 min at 1000 *g* and resuspended in PBS to give approximately 10⁹ cfu/ml. The bladder was removed aseptically from a mouse and the inner surface was scraped with a microspatula. The bladder epithelial cells thus removed were washed three times in PBS for 10 min at 50 *g* and resuspended in PBS to a concentration of 10⁵ cells/ml. A mixture of 0.2 ml of the bacterial suspension, 0.2 ml of the epithelial-cell suspension and 0.1 ml

of PBS or 12.5% (w/v) D-mannose was incubated at 37°C for 1 h. Unattached bacteria were removed by centrifugation in PBS for 10 min at 50 *g*. The final pellet was resuspended in a drop of PBS, dropped onto a glass microscope slide, air dried, fixed with methanol and stained with methylene blue. The number of adherent bacteria on 20 epithelial cells was counted by light microscopy.

Agglutination test. Guinea-pig, chicken, horse and bovine blood samples were purchased from Nippon Bio-Supply Center, Tokyo. Human and mouse blood were freshly collected. The erythrocytes were washed three times with PBS and suspended to a concentration of 2% (v/v). A *Candida albicans* cell suspension was prepared by the method of Ofek and Beachey (1978). The *E. coli* strains were cultured overnight either in BHI or on TSA at 37°C and were suspended in PBS to a concentration of about 10⁹ cfu/ml. Serial twofold dilutions of the bacterial suspensions in 25 µl of PBS were made in a microtitration plate (Dynatech Laboratories, Alexandria, VA). To each well, 25 µl of the erythrocyte or *C. albicans* cell suspension were added. To test for mannose sensitivity, serial twofold dilutions of the bacterial suspension were prepared in PBS containing 1.5% (w/v) D-mannose. Agglutination was read after 1 h at 4°C in the case of erythrocytes and at room temperature in the case of *C. albicans* cells. The titre was expressed as the highest dilution showing the agglutination.

Electron microscopy. A drop of overnight BHI culture of bacteria was placed on a Formvar-coated copper grid and allowed to stand for 2 min. The excess suspension was drained off and adherent bacteria were negatively stained for 30 s with freshly prepared 2% (w/v) ammonium molybdate. Grids were examined in an transmission electron microscope (TEM, Jem-100B; SEOL Ltd, Tokyo) at 80 KV.

O serotype was determined by the conventional tube-dilution technique.

Intraperitoneal virulence. Groups of mice were given intraperitoneal injection of about 10⁷ cfu of *E. coli* and mortality was determined 5 days later.

Growth in urine. Urine was collected from mice by gentle compression of the bladder through the external abdominal wall and pooled urine samples were filtered through a membrane filter of pore size 0.45 µm (Millipore Corporation, Bedford, MA). The sterile urine was inoculated with about 10³ cfu/ml. After incubation overnight at 37°C, growth was determined in TSA by the pour-plate method.

Fermentation of dulcitol was detected by Minitek system (BBL Microbiology Systems, Cockeysville, MD).

Production of haemolysin was tested in liquid and solid media containing sheep- or horse-erythrocytes, by the method of Cooke (1968).

Susceptibility to bactericidal activity of serum. Overnight BHI cultures were diluted 1000 times in Hanks's balanced salts solution (Microbiological Associates, Walkersville, MD) and 10 µl of bacterial suspension were added to 100 µl of serum freshly obtained from mouse, rabbit or human blood. After incubation for 1 h the remaining cfu were counted by the pour-plate method in TSA. The results were rated according to the method of Olling (1977); R indicates that less than 50% of the bacteria were killed, I indicates that 50–99% were killed and S indicates that more than 99% were killed.

Susceptibility to antibiotics. Minimal inhibitory concentrations (MICs) of ampicillin (Solucillin, Takeda Chemical Industries, Osaka), streptomycin (Streptomycin Sulfate, Takeda Chemical Industries, Osaka), chloramphenicol (Chloromycetin Powder, Sankyo Seiyaku Co., Tokyo) and tetracycline (Achromycin, Japan Lederle Co., Tokyo) were determined by the agar-dilution method (Tsuchiya *et al.*, 1981). The strains of *E. coli* not inhibited by these antibiotics at a concentration of 25 µg/ml were regarded as resistant.

Statistical methods. Comparisons of the mean number of bacteria recovered from the bladder wall was by Student's *t* test. Comparisons of the adhesive capacity to bladder epithelial cells was by the Kruskal–Wall test. Inhibitory effect of D-mannose on the adherence was statistically analysed by the χ^2 test.

RESULTS

Urinary virulence

The virulence of the *E. coli* in ascending UTI in mice drinking 5% glucose solution

FIG. 1.—Virulence of *E. coli* strains in ascending urinary-tract infections in mice drinking 5% glucose solution.

was tested (fig. 1). All mice survived for 7–9 days after the bacterial inoculation. The 33 strains were divided into three groups on the basis of the infectivity for the bladder and kidney. Eight strains showed an ID50 of less than 10^5 cfu in the bladder and kidney (group I); 10 strains showed an ID50 of less than 10^5 cfu in the bladder and more than 10^5 cfu in the kidney (group II). The remaining 15 strains failed to produce infection in either bladder or kidney (group III).

In mice drinking tap water, none of the strains produced infection even when 10^7 cfu of bacteria were inoculated into the bladder.

Number of bacteria recovered from the bladder wall

About 10^7 cfu of *E. coli* were inoculated into the bladder and the number of bacteria on the bladder wall was counted 3 h after the inoculation (fig. 2). In the eight strains from group I, the number of bacteria recovered ranged from 3×10^3 to 4×10^5 cfu per bladder. In the 10 strains from group II the number of bacteria recovered ranged from 1×10^3 to 5×10^5 cfu per bladder. No difference was observed between the geometric mean number of bacteria from group I and that from group II ($p > 0.8$). In the 15 strains from group III, bacterial counts ranged from 2×10^2 to 6×10^4 cfu and the geometric mean was significantly lower than those from groups I and II ($p < 0.005$).

FIG. 2.—The number of bacteria recovered from bladder wall of mice 3 h after intra-bladder inoculation of *E. coli*. Group I comprises strains highly pathogenic for bladder and kidney, group II only for bladder, and group III for neither (see fig. 1). Each point represents the geometric mean count from five mice. The horizontal bar indicates the geometric mean count for virulence group.

Adherence to bladder epithelial cells

The adhesive capacity of the *E. coli* strains to bladder epithelial cells was compared by the frequency of distribution of bacteria on epithelial cells and by the mean number of bacteria adhering to 20 epithelial cells. Adhering bacteria were clearly seen on the cell surface and were easy to count. Adherence could also be demonstrated by scanning electronmicroscopy (fig. 3). All 18 strains from groups I and II adhered to more than 80% of epithelial cells and showed a mean number of adhering bacteria ranging from 2.4 to 57.2 bacteria per cell (table I). In group III, only two strains possessed high adhesive capacity with a mean number of adhering bacteria of 11.3 and 20.9 bacteria per cell; the remaining 13 strains hardly adhered to the epithelial cells at all. No difference in adhesive capacity was observed between groups I and II ($p > 0.08$) but the adhesive capacities from groups I and II were significantly higher than those from group III ($p < 0.01$). The adherence of 17 of 18 strains from groups I and II was strongly inhibited by D-mannose ($p < 0.005$) while that of one strain was only weakly inhibited ($p < 0.05$). The adherence of the two strains from groups III with high adhesive capacity was unaffected by D-mannose ($p > 0.2$).

FIG. 3.—Scanning electronmicrograph of *E. coli* adhering to mouse bladder epithelial cells ($\times 16\,000$).

Haemagglutination and agglutination of C. albicans

The agglutination patterns of the *E. coli* strains grown in BHI and the effect of D-mannose on agglutination were tested with guinea-pig, chicken, horse, human, and bovine erythrocytes and with *C. albicans* cells (table II). Eight strains from group I and six of the 10 strains from group II strongly agglutinated guinea-pig erythrocytes and *C. albicans* cells and the agglutination was almost completely inhibited by D-mannose. The majority of these strains also agglutinated chicken and horse erythrocytes in a mannose-sensitive manner. The remaining four strains from group II did not agglutinate any species of erythrocyte tested or *C. albicans* cells except two strains which weakly agglutinated the latter. Of 15 strains from group III, four showed mannose-resistant haemagglutination with various erythrocytes, four showed weak mannose-sensitive agglutination, while the haemagglutination patterns of two strains were of a mixed type. The remaining six strains did not agglutinate *C. albicans* or any species of erythrocytes tested.

The agglutination titres of strains with mannose-sensitive agglutination in the

TABLE I
Adherence of *E. coli* to mouse-bladder epithelial cells and the effect of D-mannose on adherence

E. coli of group	strain	In phosphate-buffered saline,						In 2.5% D-mannose,						χ^2 -test for significance of difference between PBS and D-mannose results
		number of epithelial cells to which the number of adherent <i>E. coli</i> was					number of epithelial cells to which the number of adherent <i>E. coli</i> was					mean* number of adherent <i>E. coli</i> /cell $\pm$ SD		
		0	1-5	6-20	21-50	>50	0	1-5	6-20	21-50	>50			
I	TN 629	0	1	9	9	1	40.0 $\pm$ 44.1	10	10	0	0	0	1.0 $\pm$ 1.2	p<0.005
	TN 630	4	15	1	0	0	2.4 $\pm$ 2.0	11	9	0	0	0	1.2 $\pm$ 1.5	p<0.005
	TN 647	2	5	10	3	0	11.0 $\pm$ 8.1	11	9	0	0	0	0.9 $\pm$ 1.5	p<0.005
	TN 654	3	5	12	0	0	6.5 $\pm$ 4.8	4	14	2	0	0	2.1 $\pm$ 2.1	p<0.005
	TN 655	0	3	16	1	0	10.3 $\pm$ 5.4	9	11	0	0	0	1.4 $\pm$ 1.6	p<0.005
	TN 675	0	1	9	9	1	27.8 $\pm$ 25.3	11	9	0	0	0	1.1 $\pm$ 1.4	p<0.005
	TN 684	0	1	7	3	9	54.1 $\pm$ 45.9	2	6	6	4	2	16.6 $\pm$ 19.1	p<0.005
T 7	0	6	13	1	0	8.8 $\pm$ 5.3	6	10	4	0	0	2.9 $\pm$ 3.1	0.025 < p < 0.05	
II	TN 631	2	7	11	0	0	7.2 $\pm$ 5.0	7	12	1	0	0	2.0 $\pm$ 2.7	p<0.005
	TN 632	0	4	10	3	3	19.8 $\pm$ 18.8	9	10	1	0	0	1.7 $\pm$ 2.2	p<0.005
	TN 633	0	2	4	11	3	32.7 $\pm$ 18.4	16	4	0	0	0	0.2 $\pm$ 0.4	p<0.005
	TN 640	1	14	5	0	0	3.8 $\pm$ 3.1	13	7	0	0	0	0.6 $\pm$ 0.9	p<0.005
	TN 645	3	16	1	0	0	2.6 $\pm$ 2.2	15	5	0	0	0	0.4 $\pm$ 0.8	p<0.005
	TN 648	0	0	4	9	7	57.2 $\pm$ 44.5	6	14	0	0	0	1.4 $\pm$ 1.5	p<0.005
	TN 651	1	7	6	2	4	28.3 $\pm$ 42.4	11	9	0	0	0	0.6 $\pm$ 0.8	p<0.005
	TN 656	2	13	5	0	0	4.5 $\pm$ 4.0	12	8	0	0	0	0.5 $\pm$ 0.6	p<0.005
	TN 657	1	5	13	1	0	9.8 $\pm$ 6.4	7	10	3	0	0	2.3 $\pm$ 2.5	p<0.005
	TN 661	0	13	7	0	0	5.4 $\pm$ 3.5	10	10	0	0	0	1.1 $\pm$ 1.6	p<0.005
	III	TN 634	12	8	0	0	0	0.7 $\pm$ 1.0	...	...	...	...	...	...
TN 635		12	8	0	0	0	0.6 $\pm$ 0.9	...	...	...	...	...	...	...
TN 637		12	8	0	0	0	0.9 $\pm$ 1.3	...	...	...	...	...	...	...
TN 638		14	6	0	0	0	0.6 $\pm$ 1.2	...	...	...	...	...	...	...
TN 639		8	10	2	0	0	2.2 $\pm$ 2.5	...	...	...	...	...	...	...
TN 642		11	8	1	0	0	1.2 $\pm$ 1.8	...	...	...	...	...	...	...
TN 644		1	5	7	3	4	20.9 $\pm$ 23.7	2	3	12	2	1	14.4 $\pm$ 17.4	p>0.2
TN 650		11	9	0	0	0	1.0 $\pm$ 1.6	...	...	...	...	...	...	...
TN 652		16	4	0	0	0	0.2 $\pm$ 0.4	...	...	...	...	...	...	...
TN 653		12	8	0	0	0	0.7 $\pm$ 1.0	...	...	...	...	...	...	...
TN 666		1	6	10	3	0	11.3 $\pm$ 9.2	...	...	...	...	...	...	...
TN 671		10	8	2	0	0	1.1 $\pm$ 1.9	...	...	...	...	...	...	...
NIH JC-2		16	4	0	0	0	0.3 $\pm$ 0.6	...	...	...	...	...	...	...
K 12		17	3	0	0	0	0.2 $\pm$ 0.2	...	...	...	...	...	...	...
K 12 ML		7	13	0	0	0	1.7 $\pm$ 1.7	...	...	...	...	...	...	...
1410		...	...	...	...	...	...	...	...	...	...	...	...	...

... = Not determined.

*Mean of counts on 20 cells.

TABLE II

Agglutination patterns of E. coli strains with erythrocytes and yeast cells, bacterial fimbriation and O serotype

<i>E. coli</i> of group strain		Agglutination pattern of indicated erythrocytes in										Fimbriation	O Serotype
		broth culture						agar culture					
		Gp	Ch	Ho	Hu	Bo	Mo	Ca	Gp	Ca			
I	TN 629	S	s	s	—	—	—	s	s	—	+	0-8	
	TN 630	S	s	S	S	—	—	S	s	s	+	0-156	
	TN 647	S	s	—	—	—	—	s	s	—	+	0-18	
	TN 654	s	s	—	R	r	—	s	—	—	+	0-25	
	TN 655	S	s	—	—	—	—	S	—	—	+	0-6	
	TN 675	S	s	—	—	—	—	S	s	s	+	NG	
	TN 684	S	s	s	—	—	—	S	s	s	+	R	
	T 7	S	s	—	—	—	—	S	—	—	+	0-30, -48	
II	TN 631	S	s	—	—	—	—	s	—	—	+	0-30, -48	
	TN 632	S	—	—	—	—	—	s	s	—	+	R	
	TN 633	S	S	s	—	—	s	S	s	—	+	0-75	
	TN 640	—	—	—	—	—	—	s	—	—	+	0-18	
	TN 645	—	—	—	—	—	—	s	—	s	+	0-114	
	TN 648	S	s	s	—	—	S	S	s	—	+	0-48	
	TN 651	—	—	—	—	—	—	—	—	—	+	0-75	
	TN 656	—	—	—	—	—	—	—	—	—	+	0-23	
	TN 657	S	s	S	r	—	—	S	s	s	+	R	
	TN 661	S	S	—	—	—	—	S	—	s	+	NG	
III	TN 634	—	—	—	—	—	—	—	—	—	+	0-6	
	TN 635	—	—	—	—	—	—	—	—	—	+ or —	NG	
	TN 637	s	—	—	—	—	—	—	—	—	—	NG	
	TN 638	—	—	—	—	—	—	—	—	—	—	0-11	
	TN 639	s	—	—	—	—	—	s	—	—	+	NG	
	TN 642	r	r	r	r	r	—	—	r	—	+	NG	
	TN 644	—	—	—	—	s	—	—	—	—	+	0-91	
	TN 650	s	—	—	—	—	—	s	—	—	+	0-40	
	TN 652	—	—	—	—	—	—	—	—	—	—	0-75	
	TN 653	r	r	r	—	—	r	—	r	—	- or +	0-91	
	TN 666	s	—	r	R	r	r	s	R	—	+	0-18	
	TN 671	R	r	r	R	r	r	—	R	—	- or +	R	
	NIHJ JC-2	—	—	—	—	—	—	—	—	—	—	R	
	K 12	r	r	r	r	r	r	—	—	—	—	R	
	K 12 ML 1410	r	s	r	r	s	s	s	r	—	+	R	

Gp=guinea-pig; Ch=chicken; Ho=horse; Hu=human; Bo=bovine; Mo=mouse; Ca=*Candida albicans*.

S=mannose-sensitive agglutination with high (≥ 16) titre; s=mannose-sensitive agglutination with low (< 16) titre; R=mannose-resistant agglutination with high (≥ 16) titre; r=mannose-resistant agglutination with low (< 16) titre; --no agglutination.

+ = fimbriate - = non-fimbriate.

R=autoagglutinated in saline; NG: not groupable.

absence of D-mannose were always higher when the strain was grown in BHI than when it was grown on TSA but the titres of the strains with the mannose-resistant agglutination were almost invariable.

Electronmicroscopy

Electronmicrographs of *E. coli* strain TN 629 from group I and *E. coli* strain TN 652 from group III grown in BHI overnight at 37°C are shown in figs 4 and 5,

FIG. 4.—Electronmicrograph of *E. coli* strain TN629 with fimbriae ($\times 40\,000$).

respectively. All 18 strains of groups I and II, and eight of 15 strains from group III possessed similar fimbriae as shown in fig. 4. In the remaining seven strains, including strain TN 652, these appendages were not observed (table III).

Other biological properties

No significant correlation was found between urinary virulence and other biological properties, such as O serotype (table II), lethality on intraperitoneal injection in mice, ability to grow in mouse urine, ability to ferment dulcitol, production of haemolysin, susceptibility to serum bactericidal activity, and susceptibility to antibiotics (table III).

FIG. 5.—Electronmicrograph of *E. coli* strain TN652 without fimbriae ($\times 60\,000$).

TABLE III

Some biological properties of E. coli strains of groups I, II and III (as defined in the text)

Test	Number of strains giving positive reaction among		
	8 strains of group I	10 strains of group II	15 strains of group III
Mouse lethality by intraperitoneal injection of tap water	7	4	6
5% glucose	7	3	4
Growth in mouse urine in tap water	0	0	0
5% glucose	8	10	15
Dulcitol fermentation	7	8	10
Haemolysin production in broth medium	3	1	4
agar medium	3	1	4
Resistance to bactericidal activity of serum from human serum	3	3	3
rabbit serum	7	7	6
mouse serum	8	10	15
Resistance to antibiotics*	3	8	10

*Ampicillin, streptomycin, chloramphenicol and tetracycline.

DISCUSSION

Strain-specific differences in the ability of *E. coli* to induce UTI in experimental animals have been reported by several authors. In most of these studies, however, inoculation by the haematogenous route was used (Brumfitt and Heptinstall, 1960;

Kimball, Turck and Petersdorf, 1964; Prat *et al.*, 1965; Fried *et al.*, 1971; van den Bosch, de Graaff and MacLaren, 1979). The ascending route of infection was used in our study because it most closely parallels the usual clinical situation. The *E. coli* strains tested were classified into three groups according to their ability to induce UTI in mice drinking 5% glucose solution. Of 33 strains, eight strains (group I) induced UTI involving the bladder and the kidney and 10 strains (group II) induced infections localised to the bladder, while the remaining 15 strains (group III) did not cause infection. Nicholson and Glynn (1975) investigated the renal pathogenicity of seven strains of *E. coli* using an ascending model in mice with foreign bodies in the bladder, and found differences among the strains in the dose infecting 50% of kidneys. Guze *et al.* (1973) tested the virulence of 22 strains of *E. coli* using the same model as in the present study and showed that there were marked differences among the strains in the ability to produce renal infection and death. In our study, none of the strains, at the inoculum sizes tested, caused death. This discrepancy may be due to strain differences of the mice or of the *E. coli* strains used.

Various biological properties of *E. coli* are reported as factors influencing virulence for the urinary tract (Montgomerie, 1978). Of several properties examined in the present study, the adhesive capacity of the strains to bladder epithelial cells related positively with their ability to induce UTI. The strains of groups I and II showed significantly higher counts than the group-III strains in bacterial recovery from the bladder wall 3 h after inoculation. Furthermore, strains of groups I and II adhered well *in vitro* to bladder epithelial cells in a mannose-sensitive manner, but most of the group-III strains barely adhered to the epithelial cells. These observations suggest that adherence of *E. coli* to bladder epithelial cells plays an important role in the development of experimental UTI in mice. In recent years, evidence has accumulated, for various bacterial infections, that adherence of bacteria to mucosal surfaces of a susceptible host is an important step in colonisation and infection (Ofek and Beachey, 1980). Although the role of bacterial adherence in the pathogenesis of human UTI is not thoroughly clear, several authors have suggested a more or less positive correlation between the clinical pathogenicity of *E. coli* in UTI and its adhesive capacity to uroepithelial cells (Svanborg Edn *et al.*, 1976; Schaeffer *et al.*, 1979; Kllenius *et al.*, 1980; van den Bosch *et al.*, 1980). The results presented here support these epidemiological findings.

The ability of *E. coli* to adhere to epithelial cells is often associated with surface structures called fimbriae which can be classified into various types by dimension, composition, stability, antigenicity, adhesive specificity, and agglutination patterns with erythrocytes and yeast cells (Duguid and Old, 1980). All strains of groups I and II possessed fimbriae and their adherence to bladder epithelial cells was inhibited by D-mannose. Furthermore, most of these strains agglutinated erythrocytes of guinea-pig, chicken, and horse, and *C. albicans* cells in a mannose-sensitive manner; the agglutination titres were always higher when the *E. coli* had been grown in BHI than on TSA. These results indicate that most strains of groups I and II possess fimbriae closely related to the so-called type-1 fimbriae. On the other hand, the adherence and haemagglutination of two group-III strains with high adhesive capacity were not affected by D-mannose. From these findings, it appears that the adherence of most strains of groups I and II to bladder epithelial cells is mediated by type-1 or closely related fimbriae, which play an important role in the development of UTI in

mice. Aronson *et al.* (1979) showed that the incidence of UTI with *E. coli* in diuresed mice was markedly reduced by inoculation into the bladder of bacteria suspended in D-mannose solution, probably because of inhibition of bacterial adherence to the bladder mucosal surfaces by D-mannose. Salit and Gotschlich (1977*a* and *b*) demonstrated that purified type-1 fimbriae from *E. coli* attached to monkey-kidney cells in tissue culture and agglutinated guinea-pig erythrocytes in a mannose-sensitive manner. Fader, Avots-Avotins and Davis (1979) and Fader and Davis (1980) found that the fimbriae of *Klebsiella pneumoniae*, similar to type-1 fimbriae of *E. coli*, play an integral role in the ability to cause cystitis in rat by mediating the attachment of the bacteria to the uroepithelial surface. In contrast, the adherence of *E. coli* isolated from human UTI to human uroepithelial cells has not always been found to be inhibited by D-mannose (Svanborg Edn, 1978, van den Bosch *et al.*, 1980). rskov, rskov and Birch-Andersen (1980) suggested that a new type of fimbria, referred to as F7, mediated the adherence of *E. coli* to human uroepithelial cells and they state that *E. coli* with type-1 fimbriae specifically adhered to urinary mucus rather than to the actual cell surface; the adhesive property of type-1 fimbriae does not seem to be essential for the development of human UTI. It is known that the adherence of some pathogens varies from host to host and from tissue to tissue (Ofek and Beachey, 1980). Korhonen, Leffler and Svanborg Edn (1981) have found that four pilated strains of *E. coli* and *Salmonella typhimurium* attached to human buccal cells, but two strains with a mannose-resistant agglutination adhered only to human urinary tract cells and another two strains with mannose-sensitive agglutination adhered only to rat urinary-tract cells. The discrepancy between animal models and human infection may be related to differences between the epithelial cells used in the studies.

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