# Bacterial Migration along Solid Surfaces

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An in vitro system was developed to study the migration of uropathogenic *Escherichia coli* strains. In this system an aqueous agar gel is placed against a solid surface, allowing the bacteria to migrate along the gel/solid surface interface. Bacterial strains as well as solid surfaces were characterized by means of water contact angle and zeta potential measurements. When glass was used as the solid surface, significantly different migration times for the strains investigated were observed. Relationships among the observed migration times of six strains, their contact angles, and their zeta potentials were found. Relatively hydrophobic strains exhibited migration times shorter than those of hydrophilic strains. For highly negatively charged strains shorter migrating strain with respect to glass was allowed to migrate along solid surfaces differing in hydrophobicity and charge, no differences in migration times were found. Our findings indicate that strategies to prevent catheter-associated bacteriuria should be based on inhibition of bacterial growth rather than on modifying the physicochemical character of the catheter surface.

Catheter-associated bacteriuria is the most common hospital-acquired infection (9). The pathogenesis of this infection is not fully understood (15, 21), but it is generally accepted that the migration of bacteria extraluminally in the periurethral space is the major pathway of infection in properly managed closed urinary drainage systems (8, 15). The adhesion of bacteria originating from the urethral meatus onto the outer catheter surface is thought to be an important event in the pathogenesis of this infection (8, 9, 15, 21). The mechanism of bacterial migration along the catheterized urethra into the bladder has not been resolved. Migration of bacteria to the bladder may take place by a pumplike action caused by movements of the catheter with respect to the urethra. Bacteria may also grow along the catheter surface (6, 18, 19). Another possibility is that bacteria migrate into the space between the catheter and the urethral wall by means of chemotaxis (16). It is also possible that all these mechanisms are involved. Bacterial migration along catheter surfaces in an experimental setting has not often been studied. From results in experiments with mice Cooper et al. (5) suggested that bacterial migration from the skin to the subcutaneous part of the catheter was due to the capillary action between the catheter surface and dermal tunnel. Wilkins et al. (23) studied bacterial migration along monofilaments embedded in a gel. They found different migration distances using various bacterial species.

The aim of this study was to get more insight into the migration of *Escherichia coli*, the predominant pathogen of catheter-associated urinary tract infections (9, 21), along the catheterized urinary tract. To this end an in vitro system was developed to study the migration of *E. coli* along various surfaces. In the system an aqueous agar gel (1% [wt/vol]) is in contact with a solid surface, allowing bacteria to migrate along the gel/solid surface interface. Six *E. coli* strains varying in hydrophobicity and surface charge and four solid surfaces, also varying in hydrophobicity was measured by means of the

water contact angle, whereas the surface charge was determined by measuring the zeta potential, which is a measure of the surface charge.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** Six strains of *E. coli* isolated from patients with either catheter-associated or non-catheter-associated urinary tract infections were biotyped (7) and serotyped according to the method of Ørskov et al. (20). For each experiment bacteria were grown without aeration at 37°C for 18 h in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and harvested by centrifugation at 2,000 × g for 10 min. Then bacteria were washed three times with 0.01 M KCl buffered with  $8 \times 10^{-4}$  M Na<sub>2</sub>HPO<sub>4</sub> and  $2 \times 10^{-4}$  M KH<sub>2</sub>PO<sub>4</sub>, pH 7.4. Finally, bacteria were resuspended in the same buffer to an  $A_{540}$  of 1.0 (PM6; Carl Zeiss, Oberkochen, Germany), representing approximately 10° CFU/ml, as determined by culturing and plate counting.

**Generation times.** Generation times of the strains grown in MacConkey broth (Difco) were determined as follows. At 1-h intervals aliquots (1 ml) were 10-fold serially diluted in buffer (0.01 M KCl buffered with  $8 \times 10^{-4}$  M Na<sub>2</sub>HPO<sub>4</sub> and  $2 \times 10^{-4}$  M KH<sub>2</sub>PO<sub>4</sub>, pH 7.4). Of each dilution 0.1 ml was plated on MacConkey agar (Difco). Plates were incubated at 37°C for 18 h. Then CFU were counted, and generation times were calculated.

**Motility.** Motility was measured by using a clear and transparent medium made of 3 g of beef extract (Oxoid L29; Oxoid, Ltd., Basingstoke, United Kingdom), 10 g of peptone (Oxoid L37), 5 g of sodium chloride (pro analysi [p.a.]; Merck, Darmstadt, Germany), and 4 g of agar (Merck 1615) in 1,000 ml of deionized water. Tubes with 10 ml of sterilized medium were inoculated with a straight wire. A single stab down the center of the tube to about half the depth of the medium was made. After incubation at  $37^{\circ}$ C for up to 18 h, the motility was assessed. The bacterial strain giving diffuse hazy growth throughout the entire medium was classified as very motile (++). When only a straight line of growth along

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the stab and diffuse outgrowth were observed, the motility was designated +. Bacterial motility was classified as +/- when sharply defined fingerlike outgrowths from the stab into the medium were seen and no clefts were present. When growth was restricted to the stab line, the bacteria were considered to be nonmotile (-).

Zeta potentials. Zeta potentials were determined as a measure for the net charge of the bacteria. Washed bacteria were suspended in 0.01 M KCl buffered with  $8 \times 10^{-4}$  M Na<sub>2</sub>HPO<sub>4</sub> and  $2 \times 10^{-4}$  M KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, at a concentration of 10<sup>7</sup> CFU/ml. The zeta potentials were calculated from the velocity of the particles, measured with a Lazer Zee meter 501 (PenKem, Bedford Hills, N.Y.), by using the Smoluchowski equation (13).

Water contact angles. Bacteria were harvested by centrifugation, washed three times with deionized water, and finally resuspended in deionized water. Bacteria were deposited on membrane filters (Millipore HA, 0.45-µm pore size; Millipore Corp., Bedford, Mass.) by using negative pressure in order to produce a lawn of 50 to 100 stacked cells. After a standard drying time of 20 min, plateau water contact angles were determined at 25°C by the sessile drop technique (1, 4).

Solid surfaces. Methyl methacrylate and trimethylaminoethyl methacrylate chloride (TMAEMA-Cl) were purchased from Polyscience Inc., Warrington, Pa. Methacrylic acid (MAA) was purchased from Fluka Chemie AG, Buchs, Switzerland. Three methacrylate polymers-poly(methyl methacrylate) (PMMA) and copolymers of methyl methacrylate with either 15% TMAEMA-Cl (PMMA-TMAEMA-Cl, 85:15) or 15% MAA (PMMA-MAA, 85:15)-with various hydrophobicities and charges were synthesized and characterized as described previously (10, 12). Briefly, streaming potentials of polymer surfaces in contact with 0.01 M KCl buffered with 8  $\times$  10<sup>-4</sup> M Na<sub>2</sub>HPO<sub>4</sub> and 2  $\times$  10<sup>-4</sup> M KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, were obtained by using an apparatus in which two glass plates either coated or noncoated with polymer were placed in parallel positions. Zeta potentials were calculated from the streaming potentials with the assumption that the surface conductance is negligible. Receding contact angles of polymer surfaces were determined at 21°C by the Wilhelmy plate technique (1).

For the experiments with the in vitro system either polymer-coated or non-polymer-coated glass rods and tubes were used. Glass rods (length, 18 cm; diameter, 6 mm) and tubes (length, 16.5 cm; outer diameter, 16 mm; inner diameter, 11 mm) were cleaned ultrasonically in a 1% (vol/vol) RBS 25 (Perstorp Analytical, Oud-Beijerland, The Netherlands) soap solution. After rinsing with water the surfaces were further cleaned by immersion in a mixture of hydrochloric acid (37%, p.a.; Merck) and nitric acid (65%, p.a.; Merck), ratio 3:1 (vol/vol), for 18 h. After rinsing with double-deionized water they were ready for use. Glass rods and tubes used for the preparation of the polymer coatings were rinsed in double-deionized water and ethanol (p.a.; Merck). After drying in vacuo at 60°C for 3 h, glass rods and tubes were silanized with n-propyltrimethoxysilane (Polyscience) in the case of PMMA and with  $\gamma$ -aminopropyltriethoxysilane (Janssen Chimica, Beerse, Belgium) in the case of PMMA-MAA. Polymer coatings were prepared by a dip coating procedure as described previously (10, 12). The coated glass rods and tubes were dried in vacuo at 60°C for 18 h.

In vitro system. A device was designed to mimic the extraluminal space between the inserted catheter and the epithelial cell surface to study bacterial migration along various surfaces. The in vitro system is schematically given



FIG. 1. In vitro migration system.

in Fig. 1. A glass tube, with a ground joint fitting into the middle neck of a three-necked round-bottomed flask (50 ml) and a screw thread at the other end, was attached to the middle neck of the flask. A longer glass tube containing a silicone rubber ring (outer diameter, 11 mm; inner diameter, 7 mm) at the lower site was fitted into a screw cap containing a silicone rubber gasket. The silicone rubber ring was necessary to keep the solidified agar column in the system. The screw cap with the long glass tube was positioned in the flask via the screw thread of the shorter glass tube already attached to the flask. The long glass tube could be closed at the upper site by Parafilm (American National Can, Greenwich, Conn.). One side neck of the flask was closed with a silicone rubber cap. All glassware was made of borosilicate glass and was of standard quality.

The components of the system were cleaned ultrasonically in 1% (vol/vol) RBS 25 soap solution, extensively rinsed in hot tap water, soaked in a mixture of ethanol and water (70:30), and finally rinsed with deionized water. After assembly, the system was sterilized in an autoclave at 121°C for 15 min. The three-necked round-bottomed flask was filled with 25 ml of ice-cold sterile buffer (0.01 M KCl buffered with 8 ×  $10^{-4}$  M Na<sub>2</sub>HPO<sub>4</sub> and 2 ×  $10^{-4}$  M KH<sub>2</sub>PO<sub>4</sub>, pH 7.4), which was necessary to solidify the agar solution when this was poured into the glass tube. Thereafter, the glass tube containing the silicone rubber ring at the lower site was placed into the buffer.

The long glass tube was filled with an autoclaved solution of agar (Bacto Agar B 140; Difco) either in buffer (1.0%, wt/vol) or in MacConkey broth (1.0%, wt/vol) and allowed to cool to 40 to  $45^{\circ}$ C in order to obtain an agar column of 4 cm. To prevent dehydration of the agar, 0.3 ml of sterile buffer or MacConkey broth, pH 7.4, was placed on the agar column immediately after solidification of the agar. The glass tube was closed at the upper site with Parafilm. After 45 min the buffer in the three-necked round-bottomed flask was re-

No. and strain	Catheter associated	Generation time (min) <sup>a</sup>	Motility <sup>b</sup>	Water contact angle (°) <sup>c</sup>	Zeta potential (mV) <sup>d</sup>
1. O2K2	+	18	++	$56.9 \pm 1.8$	$-53.0 \pm 1.7$
2. O8K(A)28	+	24	++	$45.8 \pm 7.6$	$-36.1 \pm 1.6$
3. O83K?	+	20	++	$54.3 \pm 1.0$	$-56.8 \pm 3.7$
4. O111K58	_	25	_	$18.3 \pm 2.9$	$-9.4 \pm 2.7$
5. O157K-	_	22	+	$18.9 \pm 1.4$	$-13.4 \pm 4.6$
6. O161K-	-	21	++	$26.3 \pm 1.6$	$-53.6 \pm 3.7$

 TABLE 1. Generation times, motilities, water contact angles, and zeta potentials of six E. coli strains isolated from patients with bacteriuria

<sup>a</sup> Strains had grown in MacConkey broth.

<sup>b</sup> ++, very motile; +, motile; -, nonmotile.

<sup>c</sup> Measured on lawns of bacteria; mean values  $\pm$  standard deviation (n = 3).

<sup>d</sup> Determined at pH 7.4; mean values  $\pm$  standard deviation (n = 3).

placed by 22.5 ml of sterile buffer at 37°C. The long glass tube was then positioned so that the silicon rubber ring just touched the surface of the buffer. The system was placed into an incubator at 37°C. After 1.5 h of equilibration 4 ml of MacConkey broth was put on top of the agar column, representing residual urine in the bladder (upper compartment). After 30 min of further equilibration, 2.5 ml of bacterial suspension in buffer, with a concentration of approximately  $10^9$  CFU/ml, was added to the buffer in the three-necked round-bottomed flask in order to obtain a final concentration in the flask of  $10^8$  CFU/ml. To avoid pressure differences in the system when the bacterial suspension was added, the second side neck was only loosely stoppered.

The bacterial migration was determined as follows. From the MacConkey broth on top of the agar column, aliquots (0.2 ml) were taken at different time periods up to 72 h. Of these aliquots 0.1 ml was spread on MacConkey (Difco) and brain heart infusion (Difco) agar plates. Two plates per sample were used, providing a detection limit of 20 CFU/ml. The plates were incubated at 37°C for 18 h. When colonies were seen on the plates, it was concluded that bacteria had reached the upper compartment. The migration time is defined as the period necessary for bacteria to migrate from the suspension into the upper compartment. Experiments were performed with strain O2K2, and measurements were carried out at different time periods up to 72 h.

Growth ratios of bacteria adherent at solid surfaces. In order to investigate bacterial growth at surfaces, perfusion systems as described by Hogt et al. (11) containing the test surfaces were first perfused with a bacterial suspension in buffer (0.01 M KCl buffered with  $8 \times 10^{-4}$  M Na<sub>2</sub>HPO<sub>4</sub> and  $2 \times 10^{-4}$  M KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) to achieve an adhesion of approximately 500 bacteria per mm<sup>2</sup>. To obtain surfaces with the required number of initially adherent bacteria, the concentration of the bacterial suspension and the time period for adhesion used were adjusted for each bacterial strain and test surface. For glass, PMMA, and PMMA-MAA a bacterial suspension of 10° CFU/ml was used, whereas PMMA-TMAEMA-Cl was exposed to a bacterial suspension of 10<sup>8</sup> CFU/ml. For each combination of bacterial strain and test surface, two perfusion systems were applied. After the initial adhesion, the systems were rinsed with buffer for 30 min. To determine the number of adherent bacteria after the rinsing step, one system was perfused with a 2% (vol/vol) glutaraldehyde solution in buffer for 15 min and then rinsed with double-deionized water for 50 min. After the test surfaces were dried in air, adherent bacteria were counted microscopically. In order to investigate the growth of the adherent bacteria, the other system was perfused with MacConkey

broth, pH 7.4, for 180 min. After this period of time the system was rinsed with buffer for 30 min and then perfused with 2% (vol/vol) glutaraldehyde in buffer for 15 min to fix the adherent bacteria present at the surface. Finally, the systems were rinsed with double-deionized water for 50 min. Adherent bacteria were counted microscopically. Bacterial growth was expressed as the growth ratio, defined as the number of adherent bacteria on a certain surface at the time of evaluation divided by the number of adherent bacteria at t = 0. Each series of experiments using a combination of one strain and one test surface was carried out three times.

#### RESULTS

**Generation time and motility.** Generation time and motility of each strain are shown in Table 1. Generation times ranged from 18 to 25 min. Strain O111K58 was the only strain which lacked motility, whereas the other strains were motile. Strain O157K- was less motile than the other motile strains.

Contact angles and zeta potentials. The three catheterassociated uropathogenic strains O2K2, O8K(A)28, and O83K? had relatively high water contact angles (Table 1). The non-catheter-associated uropathogenic strains O111 K58, O157K-, and O161K- had much lower contact angles. Strains O2K2, O83K?, and O161K- had high negative zeta potentials, whereas strains O111K58 and O157K- had low negative zeta potentials. Strain O8K(A)28 had an intermediate negative zeta potential. The zeta potential of the PMMA-MAA surface was more negative than the zeta potential of the PMMA surface, whereas the zeta potential of the PMMA-TMAEMA-Cl surface had a positive value (Table 2). This is in agreement with what is expected because

 
 TABLE 2. Zeta potentials and water contact angles of the solid surfaces

Surface	Zeta potential (mV) <sup>a</sup>	Water contact angle (°) <sup>b</sup>
PMMA	$-13.6 \pm 0.6$	$58.0 \pm 1.2$
РММА-МАА	$-36.7 \pm 2.7$	$27.6 \pm 2.7$
PMMA-TMAEMA-Cl	$+16^{c}$	$18.6 \pm 4.5$
Glass	$-40.3 \pm 2.5$	0

<sup>*a*</sup> At pH 7.4, calculated from streaming potentials; mean values  $\pm$  standard deviation (n = 3).

<sup>b</sup> Receding contact angle as determined by the Wilhelmy plate technique; mean values  $\pm$  standard deviation (n = 3).

 $^c$  Zeta potential becomes rapidly less positive in contact with buffer, and after 18 h the zeta potential has become  $-4.6 \ mV.$ 

28

26

24





FIG. 2. Relation between the migration time of strain O2K2 with respect to the agar/glass interface and the length of the agar column.

the carboxyl groups of MAA will be partly deprotonated in contact with a buffer with a pH of 7.4, whereas TMAEMA-Cl will be fully dissociated in an aqueous environment. The glass surface had the highest negative zeta potential. Receding water contact angles of the charged methacrylate polymers were lower than that of the uncharged PMMA. The glass surface had a water contact angle of 0°.

Migration. The migration time for strain O2K2 along the interface between the agar and the glass tube applied to the system with an agar column prepared from agar and buffer was  $35.4 \pm 3.7$  h (n = 6). When the agar column was prepared from agar and MacConkey broth, the migration time was 22.1  $\pm$  1.8 h (n = 8). The long migration time obtained with the first experiment is most probably due to the fact that it takes a certain time period before the MacConkey broth on top of the agar column reaches the bacteria present in the gel/solid surface interface. In order to circumvent this effect, migration experiments were performed with an agar column prepared from agar and Mac-Conkey broth. MacConkey broth was placed on top of the agar column.

In order to study the influence of the bacterial concentration on the migration time, various bacterial concentrations  $(5 \cdot 10^7, 1 \cdot 10^8, \text{ and } 5 \cdot 10^8 \text{ CFU/ml})$  of strain O2K2 were applied to the device. Measurements were performed in triplicate. No significant differences between migration times were observed (22.0  $\pm$  2.6, 22.1  $\pm$  1.8, and 21.0  $\pm$  2.3 h, respectively).

The length of the column was varied from 2 to 8 cm in order to study the influence of the length of the agar column on the bacterial migration time. Measurements were performed in triplicate. Figure 2 shows that the migration time of strain O2K2 increased with an increasing length of the agar column.

The migration times of the six E. coli strains were studied with an agar column (length, 4 cm) prepared from agar and MacConkey broth. The results are shown in Table 3. Each strain was tested on two different days in quadruplicate. Significant differences in migration time were observed between the various strains. Within 72 h no migration was observed for strains O111K58 and O157K-. After this time period the experiment was discontinued because the agar column started to come out of the glass tube.

TABLE 3. Migration times and growth ratios of E. coli strains with respect to glass

No. and strain	Migration time $(h)^a$	Growth ratio <sup>b</sup>	
1. O2K2	$22.1 \pm 1.8$	12	
2. O8K(A)28	$36.0 \pm 4.0$	ND	
3. O83 <b>K</b> ?	$25.9 \pm 3.7$	4.5	
4. O111K58		<1	
5. O157K–	_	<1	
6. O161K-	$31.1 \pm 1.2$	ND	

<sup>a</sup> Mean values  $\pm$  standard deviation (n = 8). —, migration was not detected within 72 h.

<sup>b</sup> Of strains adherent on glass after a growth period of 180 min. The growth ratio is defined as the number of bacteria adherent on a surface after a period of time in which growth is allowed divided by the number of adherent bacteria at t = 0. ND, not determined.

In order to study the effect of solid surfaces with various properties on the migration time, glass tubes were coated with different polymers and tested as described above. Table 4 shows the migration times for strain O2K2 with respect to the four solid surfaces used. Experiments were performed in triplicate on two different days. The four materials were tested on the same day. No significant differences between the migration times with respect to the four solid surfaces were observed.

All bacteria that were detected in the upper compartment were very motile, as was observed microscopically.

Growth ratios of E. coli adherent on various solid surfaces. Among the strains tested growth ratios of bacteria adherent on glass surfaces ranged widely (Table 3). Two strains (O111K58 and O157K -) showed no increase in the number of adherent bacteria with time, whereas strains O2K2 and O83K? had rather high growth ratios. The growth ratios for strain O2K2 adherent on the various surfaces ranged from 3 to 12 (Table 4).

Physicochemical characteristics of the bacterial strains in relation to their migration times. When the contact angles of the bacterial strains are plotted against the migration times of the corresponding bacterial strains, relatively hydrophobic strains show shorter migration times than hydrophilic strains (Fig. 3A). When the zeta potentials are plotted against the migration times, highly negatively charged strains show shorter migration times than less negatively charged strains (Fig. 3B).

## DISCUSSION

The migration times of six E. coli strains along a MacConkey agar/glass interface differed significantly. The four motile strains showed migration in the in vitro system used, whereas the one nonmotile strain and the one less motile

TABLE 4. Migration times and growth ratios of E. coli O2K2 with respect to various surfaces

Solid surface	Migration time (h) <sup>a</sup>	Growth ratio <sup>b</sup>
Glass	$22.1 \pm 1.8$	12
PMMA-MAA	$21.6 \pm 2.3$	8
РММА	$22.0 \pm 3.3$	5
PMMA-TMAEMA-Cl	$23.6 \pm 3.3$	3

<sup>a</sup> Mean values  $\pm$  standard deviation (n = 6).

<sup>b</sup> Growth ratio is defined in Table 3, footnote b.



FIG. 3. Contact angles (A) and zeta potentials (B) of six *E. coli* strains in relation to their migration times with respect to the agar/glass interface. Numbers in the plots correspond with numbers in Tables 1 and 3. Arrows indicate that no migration was detected within 72 h.

strain showed no migration. Relatively hydrophobic strains showed shorter migration times than hydrophilic strains, and highly negatively charged strains showed shorter migration times than less negatively charged strains. From these results it may be expected that motile strains which are rather hydrophobic and highly negatively charged migrate relatively fast in this in vitro system.

The question of by which mechanism bacteria migrate in our system arises. It was postulated by Cooper et al. (5) that the rapid migration of staphylococci along a catheter placed through the skin of mice was caused by capillary action. This mechanism can be excluded in our system because the space between the glass surface and MacConkey agar was entirely filled with fluid before bacteria were applied to the system. Another possibility is migration induced by chemotaxis (16). In general, this requires a gradient of nutrients. However, in our system such a gradient is not present. It is possible that metabolically active bacteria create their own gradient (16). Because of the relatively high salt concentration of Mac-Conkey broth, chemotaxis of bacteria in MacConkey broth is unlikely (2, 3). Bacterial migration can also be caused by growth through the fluid present in the narrow space between the agar and the solid surface. It is assumed that this fluid has a composition approximately similar to that of MacConkey broth. The generation times of the strains grown in MacConkey broth did not differ significantly. Therefore, the different migration times of the various strains cannot be due merely to bacterial growth in the space between the glass surface and the MacConkey agar column. In principal, differences in migration times may also be governed by differences in motility among the strains, since the motility test used does not reveal differences in motility among the very motile strains. It is also possible that the growth of bacteria adherent on the solid surface plays a role in the migration process. In Table 3 it is shown that strains with high growth ratios when adherent on glass have short migration times. The migration times for strain O2K2, having the shortest migration time along the glass surface, did not differ with respect to the four solid surfaces used, whereas the growth ratios for this strain adherent on the surfaces differed significantly (Table 4). Apparently, the migration rate of this strain is not significantly influenced by the character of the solid surface used as well as by the surface-associated growth of this strain. Therefore, the role of the growth of bacteria adherent on solid surfaces as a factor in the migration process is doubtful. On the basis of these results it is most likely that the migration process is governed mainly by the motility of planktonic bacteria. Results of clinical studies revealed that the rate of bacteriuria was not affected by the type of catheter material used (latex, hydrophilic polymer-coated latex, and polyvinyl chloride) (17, 22).

From preliminary experiments performed with this system it appeared that no migration occurred when bacteria were not allowed to grow. The use of silver oxide-coated catheters significantly delayed the onset of catheter-associated bacteriuria (14), also indicating that migration did not take place when the bacterial growth was inhibited. Migration was observed in the system after the bacteria had the opportunity to grow. However, it appeared that the presence of a glass rod was not necessary for migration, because bacteria were able to migrate along the interface between the glass tube and the agar column. Therefore, a system without a glass rod was chosen for further experiments. From chemotaxis experiments (unpublished results) it appeared that our strains were not able to move through an agar gel with 0.4% (wt/vol) agar, which is in agreement with the literature (2, 3). Therefore, migration through the agar column used (1%, wt/vol) could be excluded. Varying the bacterial concentra-tion in our system from  $5 \cdot 10^7$  to  $5 \cdot 10^8$  CFU/ml had no influence on the migration time. Apparently, the number of bacteria that initially penetrated the agar/solid surface interface was in all cases sufficient to allow migration. Varying the length of the agar column from 2 to 8 cm influenced the migration time considerably (Fig. 2). Extrapolation of the migration time to a column length of zero showed that bacterial migration started after an adaptation time of 18.2 h. After this adaptation period, strain O2K2 migrated with an average rate of 1.1 cm/h. The differences in migration times for the other strains may be due to differences in adaptation times or in migration rates.

Wilkins et al. (23), using an in vitro system to investigate bacterial migration along monofilament tails of intrauterine contraceptive devices, reported significant differences in migration among different bacterial species. Motile species such as *E. coli* and *Serratia marcescens* migrated over a longer distance than nonmotile species such as *Staphylococ*- cus aureus and Moraxella (Branhamella) catarrhalis. Only E. coli showed different migration rates with respect to various materials, whereas the other strains did not. Migration rates of the different bacteria were similar among various materials tested, except for E. coli. However, it cannot be completely excluded that the observed differences in migration are caused by spreading along the monofilaments during handling of the system, as also was observed in earlier versions of our system (unpublished results).

This study demonstrates that migration of six *E. coli* strains in an in vitro system along an agar/glass interface differs significantly. It is concluded that bacterial migration through the agar/glass interface depends both on the growth in suspension and the motility of the bacterial strains. With respect to the prevention of catheter-associated bacteriuria, our findings indicate that strategies to prevent this infection should be based on inhibition of bacterial growth rather than on modifying the physicochemical character of the catheter surface.

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