

Adherence of Bacteria to Pediatric Intravenous Catheters and Needles and Its Relation to Phlebitis in Animals

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ABSTRACT. The adherence of bacteria to pediatric IV catheters and needles was studied. Scanning electron micrographs showed that bacteria adhered well to the catheters and needles, mainly to non-smooth surface areas. *In vitro* quantitative determination, with the use of radiolabeled bacteria, revealed differences in the affinity of bacteria for the various IV cannula materials. The adherence per square area was greatest for plastic catheters, less for steel needles, and least for siliconized needles. Mean values for the adherence of *Staphylococcus aureus* to these cannulae were $37.9\text{--}40.3 \times 10^5$ bacteria/cm² for the plastic catheters; 10.2×10^5 bacteria/cm² for the steel needles, and $7.2\text{--}7.6 \times 10^5$ /cm² for the siliconized needles. Removal of the glutaraldehyde-fixed bacteria adhered to the cannulae, after their placement in veins of rabbits, was lower for the plastic catheters than the IV needles. The appearance and severity of venous phlebitis produced by the various cannulae was determined in an animal model. The degree of the inflammatory response elicited correlated with the *in vitro* bacterial adherence, indicating that bacterial adherence plays a role in the appearance of cannula-associated phlebitis.

In view of our results and other previous observations of lower rate of infections with the use of IV needles, it is suggested that needles should be preferred to plastic catheters whenever possible. The described *in vitro* assay for bacterial adherence can be used to determine the adherent properties of IV cannulae, which should be considered in any future cannula design. (*Pediatr Res* 18:1361-1366, 1984)

Insertion of IV catheters and needles has become an integral part of pediatric care in hospitals, used for fluid replacement, administration of drugs, and monitoring of critically ill patients. Over one-fourth of hospitalized patients in the United States were found to receive IV infusions (8). This technique, however, has been associated with numerous complications, the most common of which is thrombophlebitis (5) followed sometimes by life-threatening septicemia. The infections can be caused by contaminated infusion solutions (14, 20), but they are more frequently related to contamination of the IV cannula (catheter or needle) where the microorganisms are partially shielded from the host defense mechanisms, can rapidly proliferate and initiate

infections. The bacteria can reach the cannula both at the moment of insertion and thereafter by migration along the interface between the cannula and the tissue.

Several studies have shown that steel needles ("scalp-vein needles") appear to be safer than plastic catheters, in relation to the frequency of cannula contamination as well as the appearance of thrombophlebitis and septicemia (1, 4, 5, 17). However, controlled comparative studies have not been performed, and the apparent safety of the IV needles may indeed be related to their use for shorter durations or in less severely ill patients, rather than to inherent properties of the needles as compared to the catheters.

In recent years evidence has been obtained that the adherence of bacteria to mammalian surfaces and medical devices is an important initial step in the infectious process (10, 16, 23), and it was shown that bacteria are capable of adhering to IV catheters (19, 22). We therefore thought that it would be useful to quantitatively study bacteria adherence to various types of pediatric IV catheters and needles under controlled *in vitro* conditions, and to relate it to the appearance of phlebitis in an animal model.

MATERIALS AND METHODS

Bacteria. Clinical isolates of bacteria, which are frequently found in cannula-related infections and isolated from foreign body infections, were identified by standard microbiological techniques (7). The bacteria tested were: *Staphylococcus aureus* coagulase-positive encapsulated strain and *Serratia marcescens* (obtained from S. Katz, Hadassah Medical Center, Israel), *Klebsiella pneumoniae* (obtained from M. Weinstein, Beilinson Medical Center, Israel), and *Escherichia coli* 0115:K4 (clinical isolate 7343, obtained from Dr. G. Altmann, Tel-Hashomer Hospital, Israel). All Gram-negative organisms were fimbriated strains. *S. aureus* were grown in 0.5% peptone (Difco Laboratories, Detroit, MI), and *E. coli*, *S. marcescens*, and *K. pneumoniae* were grown in 1% peptone, 0.5% yeast extract and 0.5% NaCl.

Preparation of radiolabeled bacteria. Sixteen hours prior to each experiment, bacteria were transferred from stock agar slants to freshly prepared respective media (15 ml) with the addition of 200 μ l D-[¹⁴C]glucose (0.1 mCi/ml, 239 mCi/mmol; The Radiochemical Centre, Amersham, England), and incubated at 37° C. The bacteria were harvested by centrifugation (9000 \times g, 10 min), and washed three times by suspension in saline and sedimentation as above. The sedimented bacteria were resuspended in saline; their concentration was adjusted photometrically to give a stock solution of 10⁹ bacteria/ml, from which a series of 1:5 dilutions were prepared for the various experiments. The radioactivity of the labeled bacteria was counted in a Tricarb liquid scintillation spectrometer (model 3255, Packard Instru-

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ments, Downers Grove, IL) with 10 ml Triton X-100-toluene scintillation fluid. The usual specific radioactivity obtained was 1 cpm/ $1-2 \times 10^3$ bacteria. Precipitation in 5% trichloroacetic acid showed that more than 90% of the radiolabeled precursor was incorporated into macromolecular components of the bacteria. Glutaraldehyde-fixed radiolabeled bacteria were prepared by resuspension of the washed bacterial sediment in 0.2% glutaraldehyde in isotonic saline and incubation for 20 min. The bacteria were sedimented ($9000 \times g$, 10 min), washed three times with 0.1 M glycine in isotonic saline and finally resuspended in saline to the desired concentration. No growth was observed upon culturing these glutaraldehyde-fixed bacteria on agar plates.

IV catheters and needles. Catheters and needles routinely used in pediatric practice were examined. They included: (a) siliconized steel needles, size 21 and 25 gauge (Deseret Pharmaceutical Co., Sandy, UT); (b) steel needles, size 21 and 25 gauge (Migada, Rehovoth, Israel); (c) Teflon catheters, size 22 and 24 gauge (Quick-Cath, Travenol Laboratories, Deerfield, IL); and (d) Teflon catheters size 19 gauge (Venflon, Viggo AB, Helsingborg, Sweden). The outer and inner diameters of the cannulae tested were obtained from the manufacturers and verified by measuring them with a technical microscope (model 5-A, Hauser, Bienne, Switzerland).

Assay for *in vitro* bacterial adherence to IV catheters and needles. Pediatric catheters (segments of 2.0-cm length, after sterile cut) and needles (the whole needle, without the connecting plastic tube) were preincubated in 4 ml sterile isotonic saline (37°C , 60 min) in 60×15 mm Petri dishes (Falcon Plastics B-D) and agitated in an incubation shaker (New Brunswick Scientific, Edison, NJ) at 100 rpm. Radiolabeled bacteria (1 ml , 10^6-10^9 bacteria/ml, depending on the experiment) were added to the Petri dishes and incubated with the IV cannulae for 20–100 min (37°C , 100 rpm). The cannulae, always tested in duplicates, were picked up with sterile fine forceps and washed five times in sterile saline solution to remove the nonadhered bacteria. This washing procedure was found to be satisfactory, since no further release of radiolabeled bacteria was observed by additional washing. The number of the adhered bacteria was determined by counting the radioactivity of the incubated cannulae in 20-ml vials, as described above. The number of the bacteria adhered to the incubated cannula was divided by its calculated surface area [$3.14 \times (\text{cannula diameter}) (\text{cannula length})$] to obtain the bacteria adhered per cm^2 of the cannula examined, which was compared among the various catheters and needles tested, expressing the affinity of bacteria for the different cannula materials.

In order to follow the *in vivo* situation, in some experiments we preincubated the cannulae in sterile whole blood (4 ml) and compared the results to preincubation with saline. In other experiments, unlabeled bacteria (*S. marcescens*, $1-2 \times 10^9/\text{ml}$) were added to the incubating solution with the radiolabeled *S. aureus*, to find out whether competition plays a role in the adherence process.

Since the IV lengths and surface areas of the various cannulae were different, it seemed important to determine not only the adherence per unit area, but also the adherent capability of the whole IV portion of the cannulae. This was determined by multiplying the adherence per cm^2 by the calculated IV surface area. The adherence index of each cannula was defined as the adherence to its IV surface area divided by that of a 25 gauge siliconized needle (Deseret), which was the lowest and defined as one unit.

***In vivo* studies with an animal model.** Male albino rabbits (weight, 2–3 kg) were used in these experiments. IV catheters and needles were aseptically inserted into the marginal vein of the animal's ear, after shaving the hair over that area and cleaning it with 70% ethyl alcohol. The cannula was securely anchored to prevent any motion, and sterile saline solution was infused through it in a constant rate (2 ml/h) using a Pediatric Voluset (model VS-40, Migada, Rehovoth, Israel; 60 drops/ml), while the animal continued to drink and eat regularly. Preliminary

examinations showed that no local or systemic changes occurred during cannulation for a follow-up period of 72 h.

In order to induce cannula-related phlebitis in the animal, a bacterial suspension of *S. aureus* (0.1 ml , 5×10^9 bacteria/ml) was applied to the skin at the site of cannula insertion. This procedure produced venous phlebitis, extending beyond the cannula tip, and we were able to culture the *S. aureus* from the cannula, using the semiquantitative method (15). The infectivity of the various pediatric IV cannulae was determined by inserting them into the animal vein and recording the local phlebitic response after 72 h, which was classified according to three grades: 0, no changes; 1, mild phlebitis with redness, swelling, and induration; 2, severe inflammation with suppuration. In all the experiments, cannulae were inserted into the veins of both ears, but bacteria were applied only to one ear, the other served as a control.

In a second type of experiment, we studied the *in vivo* removal rate of the adhered bacteria from the various cannulae. Cannulae were incubated with inert glutaraldehyde-fixed radiolabeled bacteria, and were then inserted into the animal's vein. The radioactivity of the adhered bacteria was determined after the *in vitro* assay, before insertion into the animal, as well as following removal of the cannulae from the animal, after 1, 2, or 3 days. The removal rate of bacteria from the different cannulae was compared.

Scanning electron microscopy. Pediatric IV catheters and needles were incubated with suspension of *S. aureus* (37°C , 100 min, 100 rpm), washed and fixed with 2% glutaraldehyde solution (room temperature, 60 min, 100 rpm). After washing in 0.1 sodium cacodylate buffer (pH 7.2) and incubation in 1% osmium tetroxide (30 min), the cannulae were washed again, dehydrated with increasing concentrations of alcohol and dried to a critical point. The cannulae were then fixed to metal planchets, coated with gold, and examined with a scanning electron microscope (model JSM-35C, JEOL, Japan).

RESULTS

***In vitro* bacterial adherence.** Adherence of bacteria to the IV catheters and needles was clearly demonstrated by the scanning electron micrographs. Different types of irregularities were observed (Fig. 1) on the internal and external surfaces of all the cannulae examined. Bacteria adhered easily to these irregularities, sometimes forming small colonies (Fig. 2), but adhered bacteria were also seen on rather smooth areas of the surface of the cannulae examined.

Quantitative assay with radiolabeled bacteria showed that the adherence was temperature (optimal 37°C) and time dependent (Fig. 3). The adherence was minimal after 10-min incubation, and increased gradually with time.

The number of bacteria adhered to the catheters and needles increased when the concentration of *S. aureus* in the incubating suspension was higher (Fig. 4). Similar results were obtained in suspensions of *E. coli* and *K. pneumoniae*.

The quantitative adherence of bacteria to the various pediatric cannulae is summarized in Table 1. It was found that bacteria adhered differently to the various cannula materials under the same experimental conditions. The adherence of *S. aureus* (per cm^2) was lowest to siliconized needles, slightly higher to steel needles and about seven times higher to IV catheters, although experimental variations were found. The adherence to different size catheters of the same manufacturer was very similar. Differences in the adherent capabilities of the various bacteria examined were also noticed (Table 1), but the trend of increased adherence (per unit area) to the catheters, as compared to the needles, remained.

The adherence index, indicating the adherent capability of the whole IV surface area of the cannula, showed even greater differences: the index of the IV catheters was up to 17.3 times higher than the small siliconized needle (Fig. 5). The adherence

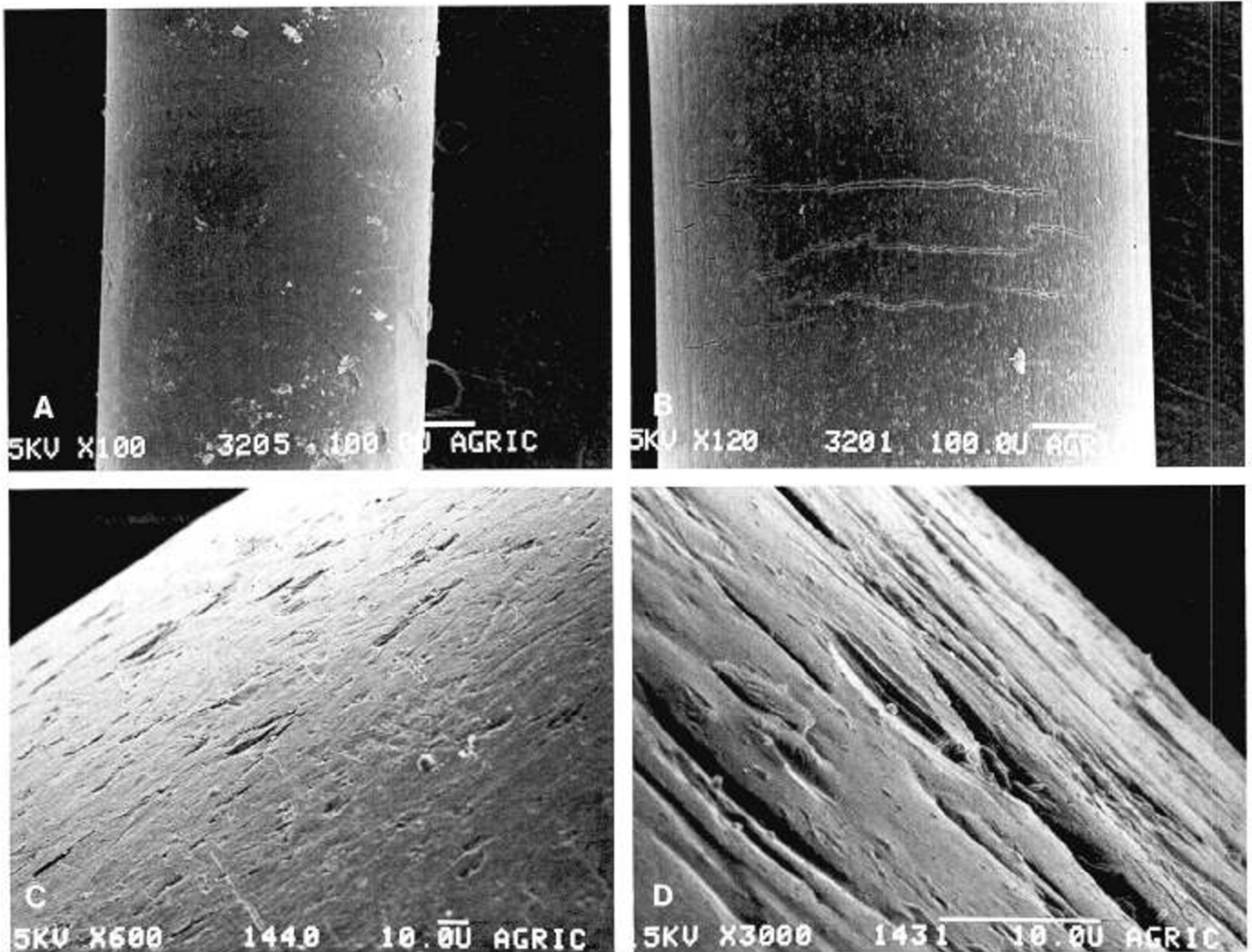


Fig. 1. Scanning electron micrographs of various pediatric IV cannulae, showing different types of irregularities on the surface area of all the cannulae examined. A, Deseret siliconized needle 25 gauge ($\times 100$); B, Quick-Cath catheter 22 gauge ($\times 120$); C, Migada needle 21 gauge ($\times 600$); D, Venflon catheter, 19 gauge ($\times 3000$).

index of the catheters was greater both as a result of increased adherence per unit area and a greater IV surface area.

Preincubation of the cannulae in sterile whole blood, instead of saline caused about 40% increase in the number of bacteria adhered to the IV catheters and needles. Addition of unlabeled bacteria (*S. marcescens*) to the incubating solution inhibited the adherence of radiolabeled *S. aureus* (Fig. 6) to the catheters and needles. No differences in the adherence to the various cannulae were found between the glutaraldehyde-fixed and the viable bacteria.

In vivo studies with an animal model. It was possible to produce cannula-related venous phlebitis in the animal by the procedure described above. The various pediatric IV catheters and needles were inserted into the animal vein and the degree of inflammation was recorded. Different IV cannulae elicited different degrees of inflammatory reaction (Table 2). The "infectivity score," an indicator of the infective properties of the various cannulae, was higher for the IV catheters than the needles, in correlation with the adherence index determined *in vitro*.

Cannulae to which glutaraldehyde-fixed radiolabeled bacteria (*S. aureus*) were adhered did not cause any infection. *In vitro* incubation of these cannulae for 3 days did not affect the number of bacteria adhered. Removal of these cannulae from the animal after 1, 2, or 3 days and counting the bacteria still adhered indicated the *in vivo* removal rate of bacteria from the IV cannulae. Higher percentage of the adhered bacteria were re-

moved from the IV needles compared to the catheters (60% versus 38%, respectively; Fig. 7), but still a significant percentage of the adhered bacteria remained attached to the IV cannulae, apparently due to shielding from the host defense mechanisms.

DISCUSSION

Previous studies using culture methods have shown that bacteria adhered to IV catheters (19, 22). In the present study bacterial adherence to IV catheters and needles, which are routinely used in pediatric practice, was quantitatively determined by the use of radiolabeled bacteria. Our results show that bacteria, which are usually associated with cannula-related infections, adhere with different affinity to the various types of cannula materials, under the same *in vitro* experimental conditions. The adherence (per cm^2) was greatest to plastic catheters and least to siliconized needles. Moreover, since the IV surface area of the various cannulae is different, the adherent capability of the whole IV surface area was determined. This shows even higher differences: the adherence to IV catheters is 11.1 to 17.3 times higher than the adherence to the small siliconized needle (Fig. 5).

The removal of adhered bacteria from the cannulae by the host defense mechanisms, after their insertion into the animal vein, was limited. However, the removal from the IV catheters, which previously adhered more bacteria, was lower than from the needles (38% versus 60%).

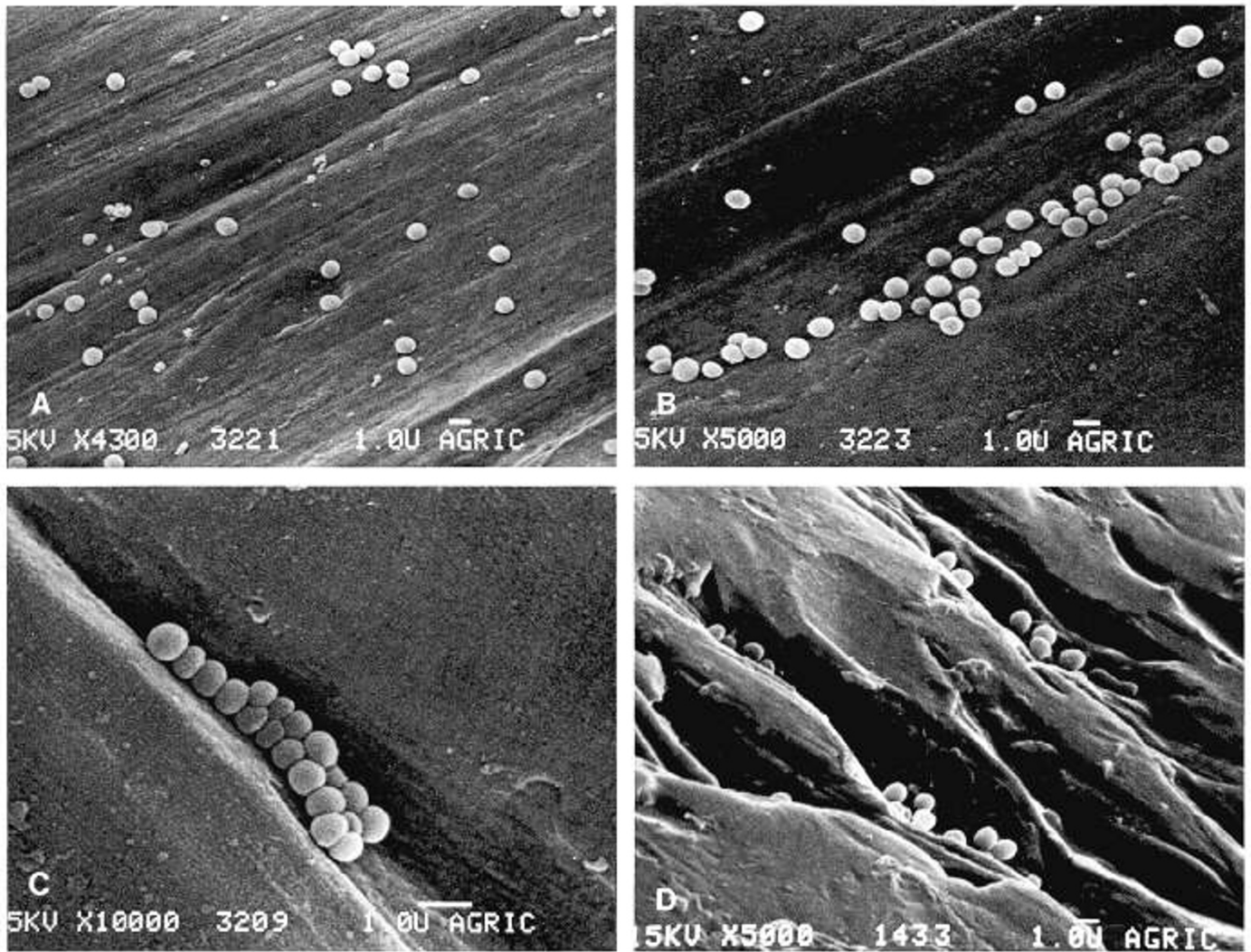


Fig. 2. Adherence of *S. aureus* to IV cannulae as seen by scanning electron microscopy. The experimental conditions and the preparations of the samples are detailed in the text. A, Deseret siliconized needle ($\times 4,300$); B, quick-Cath catheter ($\times 5,000$); C, Migada needle ($\times 10,000$); D, Venflon catheter ($\times 5,000$).

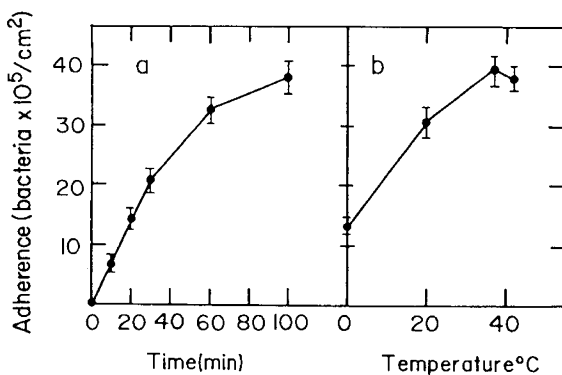


Fig. 3. Time (a) and temperature (b) dependence of the adherence of *S. aureus* (10^9 bacterial/ml) to IV catheter (Quick-Cath 22 gauge), incubated at $37^{\circ}C$ (a), for 100 min (b). Results represent means \pm SEM of three determinations.

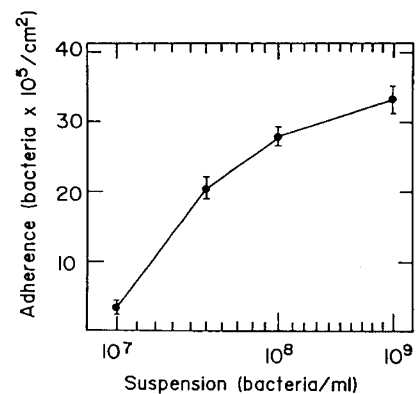


Fig. 4. Adherence of *S. aureus* to IV catheter (Quick-Cath 22 gauge) incubated $37^{\circ}C$, 100 min, 100 rpm) with bacterial suspension of different concentrations. Results represent means \pm SEM of three determinations.

The mechanisms of bacterial adherence to IV cannulae has not as yet been clarified. Scanning electron micrographs showed that bacteria attached to irregularities, found on all the cannulae that were examined by us (Fig. 2). However, bacteria also adhered to the rather smooth parts of the cannulae surfaces, in accordance with the observations of Locci *et al.* (12). Differences were also found between the adherent capabilities of the various types of bacteria examined.

The mechanism of the adherence is currently under investigation in our laboratory. We have observed that bacterial hydrophobicity, which was shown to play a role in adherence to biomaterials (9) and measured by adherence to hydrocarbons (21), is apparently important. Preliminary results indicate that cell surface hydrophobicity of the *S. aureus* and *S. marcescens* strains used in this study was much higher than that of the *E. coli* strain, which indeed adhered less well (Ashkenazi *et al.*,

Table 1. Adherence of bacteria to IV catheters and needles*

Type of cannula	Trade name and size	Adherence (bacteria $\times 10^5/\text{cm}^2$)†			
		<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>S. marcescens</i>
Teflon	Quick-Cath 24g	38.2 \pm 4.3	31.4 \pm 4.8	3.7 \pm 0.4	36.2 \pm 5.1
Teflon	Quick-Cath 22g	40.3 \pm 3.1	29.9 \pm 3.4	3.6 \pm 0.5	35.8 \pm 4.3
Teflon	Venflon 19g	37.9 \pm 3.6	31.3 \pm 4.1	3.4 \pm 0.6	39.1 \pm 3.9
Steel needle	Parperit 21g	10.2 \pm 1.1	10.2 \pm 1.6	1.5 \pm 0.3	11.2 \pm 1.8
Siliconized needle	Deseret 21g	7.6 \pm 0.6	7.5 \pm 0.9	0.8 \pm 0.1	8.1 \pm 1.0
Siliconized needle	Deseret 25g	7.2 \pm 0.8	8.1 \pm 0.9	1.0 \pm 0.2	9.2 \pm 0.8

* The adherence was determined under the standard experimental conditions (10^9 bacteria/ml, 37° C, 100 min, 100 rpm). g, gauge.

† Results represent means \pm SEM of 20 determinations for *S. aureus*, and of six determinations for the other bacteria.

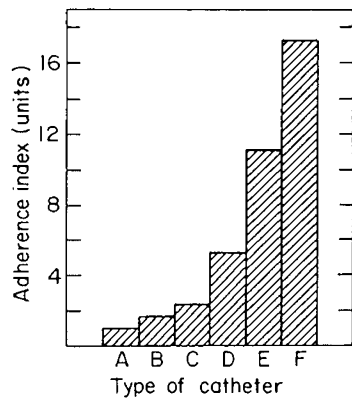


Fig. 5. Comparative adherence of *S. aureus* to IV surface area of different cannulae in units (adherence to 25 gauge siliconized needle, A = 1 unit; B = 21 gauge siliconized needle (Deseret); C = 21 gauge needle (Migada); D = 24 gauge catheter (Quick-Cath); E = 22 gauge catheter (Quick-Cath); F = 19 gauge catheter (Venflon)).

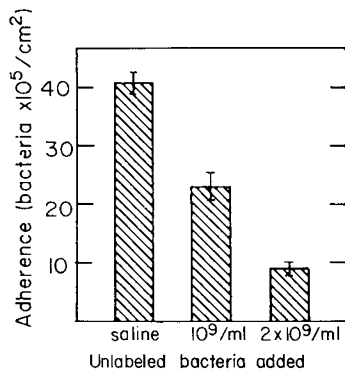


Fig. 6. Inhibition of the adherence of radiolabeled *S. aureus* (10^9 bacteria) to IV catheter (Quick-Cath 22 gauge) by the addition of unlabeled *S. marcescens* ($1-2 \times 10^9$) to the incubating solution. Results represent means \pm SEM of three determinations, under the standard assay conditions (37° C, 100 min, 100 rpm).

manuscript in preparation). The adherence to the cannulae material is probably a nonselective process, as indicated by competition between the different bacteria. Another possible factor was suggested by Christensen *et al.* (2, 3) who have shown that the production of slime may be an important factor for adherence to solid surfaces and production of infections. Preincubation of the IV cannulae with sterile whole blood, instead of saline, augmented the adherence of bacteria. This can be explained by previous observations that a fibrin sleeve usually covered the catheters after their insertion (18) and that bacteria can attach to fibrin cloths (6). Bacteria that adhered to IV catheters have been shown to multiply (19).

In order to evaluate the applicability of the *in vitro* findings to the *in vivo* situation, we have examined the ability of the various cannulae to produce venous phlebitis in an animal model. Our

Table 2. The production of venous phlebitis by the IV cannulae in an experimental model*

Cannula type†	Degree of inflammatory response			Infectivity score‡
	0	1	2	
Catheter		9:10	1:10	11
Needle	4:10	6:10		6

* Venous phlebitis was elicited in rabbits after insertion of the cannulae to their marginal vein of the ear, and the degree of the inflammatory response was recorded after 72 h. Twenty rabbits were used, 10 for each cannula. The detailed experimental conditions are explained in the text.

† Catheter, Quick-Cath 22 gauge needle. Needle, siliconized needle, 21 gauge.

‡ Infectivity score is the summation of the degree of inflammatory response found in the rabbits.

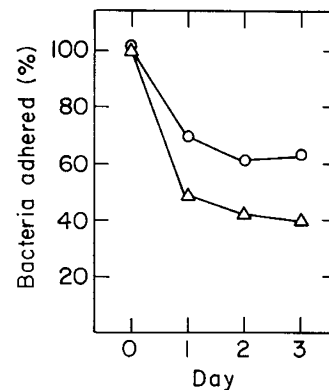


Fig. 7. Removal of glutaraldehyde-fixed *S. aureus* adhered to IV cannulae following their placement in veins of rabbit. For further details see the text. Adherence before insertion into the animal = 100%. *In vitro* incubation of the cannulae with the glutaraldehyde-fixed adhered bacteria for 3 days had no effect on the number of the adhered bacteria. O, catheter (22 gauge Quick-Cath); Δ , siliconized needle (21 gauge Deseret).

results indicate that the degree of the inflammatory response in the animal correlates well with the adherent capability of the various cannulae. These findings are in accordance with previous observations that both local thrombophlebitis and septicemia in humans are more frequent with the use of catheters than IV steel needles (4, 11, 13, 17). Since the development of cannula-associated infections is affected by multiple factors, such as duration of cannulation, its location, type of infusion fluid, and host defense mechanisms, it was not clear whether the different rates of infections are related to inherent properties of the cannulae. Our findings indicate that the adherent properties of the IV cannulae, which are dependent on physical, structural, and chemical properties, may play a role in the appearance and severity of inflammation associated with their use.

In view of the present findings, together with previous observations in children that IV needles were less frequently complicated by infections (17), it is suggested that IV needles should be used whenever possible, especially in infants, where they can

easily be introduced into scalp veins. Indiscriminate use of plastic catheters, solely for convenience, should be discouraged. However, catheters are generally more secure, and may be indicated in critically ill children for administration of drugs and fluids and for monitoring.

Further investigations are needed in order to clearly understand the mechanisms of bacterial adherence to IV cannulae and to devise ways to prevent it. Our assay for *in vitro* bacterial adherence to cannulae can be used in order to help to produce better designed cannulae, with minimal adherent properties and inflammatory complications.

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The Effect of Postnatal Age on the Adherence of *Shigella flexneri*, *Escherichia coli* 0124, and *E. coli* 0128 to Guinea Pig Intestinal Cells

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ABSTRACT. Since postnatal development of the gastrointestinal tract has an important effect on its microbial flora and may influence the types of intestinal infections, we examined the effect of age on bacterial adherence to intestinal epithelial cells. Radiolabeled bacteria were incubated with guinea pig enterocytes released by treating loops of the intestine with solutions containing EDTA, dithiothreitol, and citrate. Nonbound bacteria were separated from intestinal cells by sedimentation on a Percoll gradient. The colonic cells avidly bound *Shigella flexneri* (64 bacteria per cell), *Escherichia coli* 0124 (59), and *E. coli* 0128 (53).

The adherence process was Ca²⁺ and temperature dependent, was inhibited by fucose, glucose, and mannose, and was shown to be mediated by a carbohydrate-binding protein (lectin) on the colonic cells. Adherence of these bacteria to intestinal cells of newborn animals was only 15–25% of the adherence to adult animal cells and increased gradually, reaching adult values at about 2 weeks of age. The lectin activity, which was determined by agglutination of bacteria, was secreted with the colonic mucus. It was undetectable in the newborn animal, appeared gradually with age, and its titer correlated with the adherent capability of the colonic cells.

E. coli 0128 was the only one of the bacteria tested which significantly adhered to the ileum (19 bacteria per cell) in a process inhibited by mannose. This adherence

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