JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1980, p. 725-731
0095-1137/80/12-0725/07$02.00/0
Vol. 12, No. 6

Potential Hazards Associated with Microbial Contamination of In-Line Filters During Intravenous Therapy

C. J. HOLMES,[1,2] R. B. KUNDSIN,[3,4]* R. K. AUSMAN,[5] AND C. W. WALTER[1]

Department of Surgery[1] and Department of Microbiology and Molecular Genetics,[3] Harvard Medical School, and Department of Surgery[2] and Department of Medicine,[4] Affiliated Hospitals Center, Inc., Boston, Massachusetts 02115, and Departments of Surgery, Medical College of Wisconsin and The Deaconess Hospital, Milwaukee, Wisconsin 53233[5]

The survival and multiplication of *Enterobacter agglomerans*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Pseudomonas aeruginosa* in 0.45- and 0.22-μm in-line filter sets during simulated infusion were studied to evaluate the ability of each filter type to prevent infusion of these bacteria into patients. Bacteria were found to proliferate in the upstream compartment of sets housing both filter porosities. None of the 0.22-μm in-line filters was penetrated by the test bacteria. In contrast, *P. aeruginosa* was observed to penetrate each 0.45-μm in-line filter examined within 12 h of continuous infusion. Tribe *Klebsielleae* organisms penetrated a proportion of the 0.45-μm filters usually between 48 and 72 h of infusion. In addition, the elution of endotoxin from gram-negative bacteria trapped in the filter set during infusion is reported. Collected infusion filtrate exhibited a trend of increasing endotoxin-like activity with an increasing duration of infusion. In the case of *E. agglomerans*, mean peak levels of approximately 65 pg of *Escherichia coli* endotoxin per ml were attained after 72 h. Other bacteria produced similar results, except mean peak levels ranged from 5 to 30 pg/ml. It was noted that endotoxin-like activity was not detected in filtrate eluted from contaminated filter sets during the initial 24 h of infusion. We conclude that to avoid potential hazards of bacterial penetration and endotoxin production during continuous use of in-line filter sets, the 0.22-μm filter type must be employed and replaced every 24 h.

Connection of an in-line filter between the administration set and intravenous catheter during intravenous infusion therapy has been proposed as a means of trapping microorganisms and particulate matter that otherwise may be infused into patients. Rates of positive culture of in-line filters have been reported in the range of 2.8 to 29.1% (12, 14, 16, 26). Additionally, a higher incidence of contamination and septicemia has been reported (3, 5) due to manipulation of in-line filters which is often unavoidable when flow rates are reduced or air blockage occurs.

Terminal filters are available with several pore sizes, including 0.45 and 0.22 μm. The 0.45-μm filter is reported to retain most fungi and bacteria, whereas the 0.22-μm filter ostensibly traps all microorganisms.

In 1975, Rusmin et al. reported that a 0.45-μm in-line filter had been penetrated by *Escherichia coli* and *Pseudomonas aeruginosa* (21). The authors postulated that bacteria retained on the filters grew through the filter structure to multiply downstream. A 0.22-μm filter was reported to prevent such penetration.

The existence of a population of gram-negative bacteria on an in-line filter is suggested by this hypothesis. It is reasonable to suspect that endotoxin might be eluted from such a population. The majority of microorganisms that survive and proliferate in simple intravenous solutions are gram-negative bacteria.

This study examines the survival and multiplication of selected gram-negative bacteria in 0.45- and 0.22-μm in-line filters during simulated infusion and the ability of each porosity of filter to prevent the penetration of bacteria. In addition, the elution of endotoxin from bacteria trapped on the filters is reported.

MATERIALS AND METHODS

Preparation of bacterial suspensions. Clinical isolates of *Enterobacter agglomerans*, *Serratia marcescens*, *Klebsiella pneumoniae*, and *P. aeruginosa* were obtained from the Specialty Microbiology Laboratory, Affiliated Hospitals Center, Inc., Boston, Mass. After growth in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at 35°C for 18 h, cells were harvested by centrifugation at 180 × *g* for min, washed three times, and suspended in pyrogen-free, sterile water for injection (Travenol Lab-

oratories, Inc., Deerfield, Ill.). The resulting suspension was diluted serially with pyrogen-free, sterile water to the desired concentration of viable cells per milliliter.

Assembly of intravenous infusion systems. An intravenous set was connected to a 1-liter Viaflex bag (Travenol Laboratories, Inc.) of either 5% dextrose in water or lactated Ringer solution (Travenol Laboratories, Inc.). The luer slip of this set was attached to an extension set, with the final filter having a mean pore diameter of either 0.45 or 0.22 μm (Travenol Laboratories, Inc., code 2C-02-40, lot B318F3, and code 2C-02-4C, lot B304P2). The extension set was then connected to a field monitor (Millipore Corp., Bedford, Mass.) containing a 0.22-μm membrane filter (Fig. 1). A sampling tube was attached to the downstream port of the field monitor to collect filtrate in a sterile, pyrogen-free 2-liter flask.

Glassware was depyrogenated by dry heat at 250°C overnight.

Simulation of infusion. Immediately before the infusion was started, the in-line filter sets were seeded with a 1-ml suspension of bacteria containing approximately 100 viable cells. Infusion was commenced and maintained at a rate of 1 liter per 12 h for 72 h. At each 12-h interval, bags, field monitors, and collection flasks were replaced. A 3-ml amount of tryptose phosphate broth (Difco Laboratories, Detroit, Mich.), prepared at a concentration of 1.5 times the normal strength, was injected into the field monitors upon removal, and these were incubated at 35°C and inspected for growth at 24 and 48 h. Colonies isolated were identified by using standard laboratory techniques. A 1-ml amount of collected filtrate was transferred to a depyrogenated test tube and stored at −20°C for endotoxin testing. Three 1-ml samples of collected filtrate were used to make pour plates in Trypticase soy agar (BBL) for sterility tests.

Upon completion of 72 h of continuous infusion, 1 ml of fluid was removed from the upstream side of the filter set and quantitatively assessed for bacteria by using the serial dilution and pour plate methods.

Aseptic technique was used for all manipulations.

Assessment of ELA of collected filtrates. Frozen samples of filtrates were thawed to room temperature and assayed for endotoxin-like activity (ELA) by using the *Limulus* amoebocyte lysate assay. Three 0.1-ml samples of filtrate were reacted in depyrogenated test tubes with 0.1 ml of *Limulus* amoebocyte lysate (Hyland Therapeutics, Div. of Travenol Laboratories, Inc., Glendale, Calif.). A colorimetric determination of the precipitated protein was then made by the Oyama-Eagle modification (18) of the Lowry assay (10) as follows. After incubation in a 37°C water bath for 30 min, the test tubes were centrifuged at 12,500 × *g* for 10 min. The supernatant in each tube was discarded, and the pellet was dissolved in 0.2 ml of 0.2 N NaOH. A 1-ml amount of Lowry copper solution was added and incubated for 10 min; 0.1 ml of 1 N phenol reagent solution (Folin-Ciocalteu) was then added. After a 15-min incubation, absorbance at 660 nm was measured in a microsampling spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Absorbance readings were interpreted by interpolation with a standard curve of *E. coli* O55:B5 endotoxin (Difco) which was determined simultaneously with the collected filtrate samples. This colorimetric method has been described as an accurate, sensitive, and reproducible technique for the detection of endotoxin-precipitated protein (15, 24).

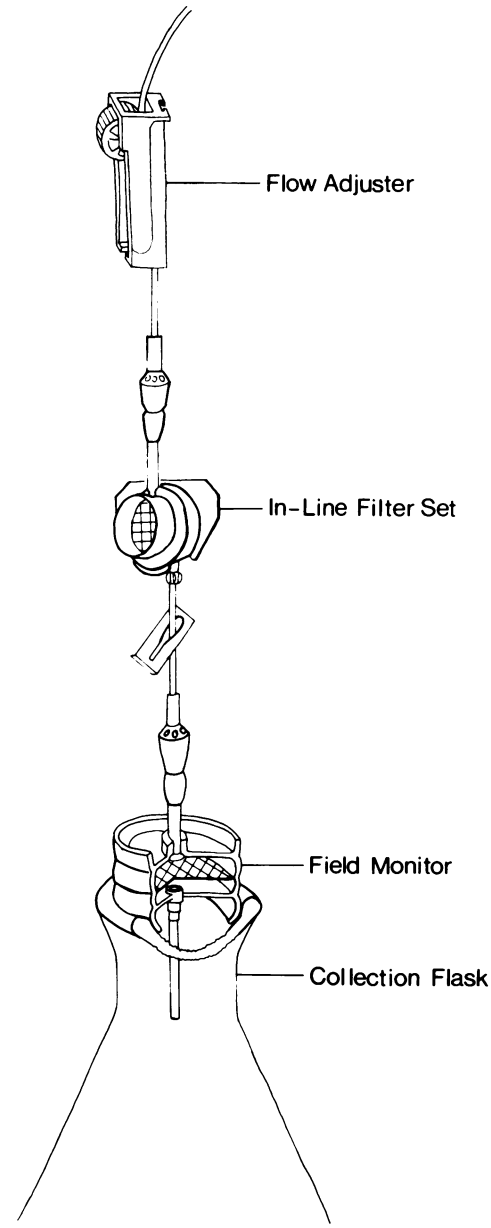

FIG. 1. *Arrangement of in-line filter set, field monitor, and collection flask.*

For each test organism, 12 0.45-μm in-line filter sets were studied in four groups of three. In addition, one nonseeded filter set was included in each group as a control. Twelve 0.22-μm in-line filter sets were likewise

studied with identical controls.

Before the proposed experiment could be undertaken, it was considered necessary to ensure that: (i) membrane filters contained in the field monitors were not inhibitory to the test microorganisms; (ii) the test systems employed were pyrogen-free before use; and (iii) the lots of in-line filter sets employed were not defective as obtained from the manufacturer. Thus, the following additional control experiments were performed.

Growth inhibition by membrane filters. Five 0.22-μm membrane filters employed in the field monitors were wetted with sterile distilled water and placed on Trypticase soy agar plates previously streaked with the selected bacteria in the manner of an agar disk susceptibility test. The plates were incubated at 35°C and inspected for growth at 24 and 48 h. None of the membrane filters produced zones of inhibition with any of the test bacteria.

Endotoxin contribution of intravenous infusion system. Administration sets, in-line filters, and field monitors were rinsed individually with 10 ml of pyrogen-free, sterile water for injection (Travenol Laboratories, Inc.), and each rinse was assayed for ELA.

No endotoxin was detected in rinses of administration sets and in-line filters. In contrast, field monitors contributed, in general, between the equivalent of 50 and 100 pg of *E. coli* endotoxin standard per ml in the first 10-ml rinse. Usually, activity was reduced to approximately 5 pg/ml after five successive 10-ml rinses and eliminated after an additional five rinses. Consequently, each field monitor was rinsed with 200 to 300 ml of pyrogen-free, sterile water before use.

Bubble point testing of in-line filters. In-line filter sets from each lot employed were saturated with distilled water, and the outlet tubes of the sets were immersed in water. Nitrogen under controlled pressure was applied into the filter housing until bubbles appeared at the outlet. This was designated as the bubble point. For filters with a mean pore diameter of 0.45 μm the bubble point specification is 33 lb/in^2, and for filters with a diameter of 0.22 μm it is 55 lb/in^2 (13).

Fifty in-line filter sets of each type, selected from the same lots used for the study, were bubble point tested as obtained from the manufacturer. All of the 0.45-μm filter sets passed the test. Except for two 0.22-μm filter sets, which ruptured at the junction with the filter housing seal, all of this type of filter set passed specifications.

After this initial testing, each in-line filter set was connected to an administration set and infusion container as previously described. Fluid was infused through the filter set for 72 h at a rate of 1 liter every 12 h when bubble point testing was repeated. None of the filters failed this second examination.

RESULTS

Growth of selected bacteria in in-line filter sets during continuous infusion. Preliminary experiments demonstrated that clinical isolates of *E. agglomerans*, *K. pneumoniae*, and *S. marcescens* proliferated in 5% dextrose in water when stored at room temperature (Fig. 2).

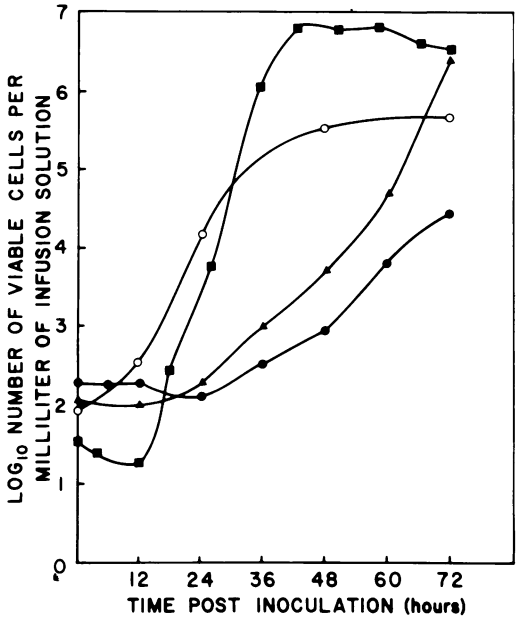

FIG. 2. *Growth of gram-negative bacteria in simple intravenous infusion solutions. Symbols: ■, E. agglomerans in 5% dextrose in water; ▲, S. marcescens in 5% dextrose in water; ●, K. pneumoniae in 5% dextrose in water; and ○, P. aeruginosa in lactated Ringer injection.*

In contrast, clinical isolates of *P. aeruginosa* lost viability in 5% dextrose in water but proliferated in lactated Ringer solution. Thus, 5% dextrose in water was employed for experiments involving the tribe *Klebsielleae* bacteria, and lactated Ringer solution was employed for *P. aeruginosa*. American Type Culture Collection strains of *S. marcescens* (ATCC 8100) and *K. pneumoniae* (ATCC 13883) either lost viability within 72 h of storage in 5% dextrose at room temperature or exhibited a decrease in their viable concentrations. Consequently, American Type Culture Collection strains were not chosen for the study.

Each of the selected bacteria was found to proliferate in the upstream compartments of 0.45- and 0.22-μm filter sets. *E. agglomerans* and *S. marcescens* attained concentrations of approximately 10^7 cells per ml during 72 h of continuous infusion (Table 1). Concentrations of *P. aeruginosa* and *K. pneumoniae* were in the order of 10^5 and 10^4 cells per ml, respectively, after 72 h. These results were consistent with the preliminary growth studies (Fig. 2).

Microbial integrity of in-line filters. None of the 0.22-μm in-line filters was penetrated by any of the test bacteria. Of 12 0.45-μm in-line filters challenged with *E. agglomerans*, 1 permitted the passage of 18 colony-forming units

TABLE 1. *Growth of selected gram-negative bacteria in in-line filter sets over 72 h of simulated infusion*

Bacterium	Filter type (μm)	No. of viable cells inoculated per in-line filter set	No. of viable cells recovered per ml of fluid present in in-line filter set at 72 h[a]
E. agglomerans	0.45	89[b]	1.65×10^7
	0.22	97	4.70×10^7
S. marcescens	0.45	89	8.16×10^6
	0.22	105	9.33×10^6
K. pneumoniae	0.45	110	6.40×10^3
	0.22	109	1.71×10^4
P. aeruginosa	0.45	111	1.74×10^5
	0.22	79	3.37×10^5

[a] Mean of 12 experiments.
[b] Between 1 and 4 ml of fluid was present in each in-line filter set.

(CFU) between 60 and 72 h, and another permitted the passage of 24 CFU between 60 and 72 h.

During the first 48 h of infusion, none of the 0.45-μm filters was penetrated by *K. pneumoniae*. Between 48 and 72 h of infusion, three filters permitted bacterial escape, resulting, in one instance, in the collection of 94 CFU by the field monitors (Table 2).

Of 12 0.45-μm in-line filters, 8 failed to retain *S. marcescens* during 72 h of infusion. In half of these tests, bacterial penetration occurred between 24 and 36 h of infusion, and half occurred between 48 and 60 h. Between 4 and 159 CFU were collected on individual field monitors over the study period (Table 3).

Table 4 shows that each 0.45-μm in-line filter examined was penetrated by *P. aeruginosa* within 12 h of infusion, and in 5 of 12 cases, the number of CFU collected on field monitors were too numerous to count by this time. It was determined that the area of a field monitor could accommodate approximately 100 discrete colonies of this organism. "Too numerous to count" indicates, therefore, a count of 100 or more CFU.

Control experiments. No microorganisms were recovered from nonseeded in-line filter sets, field monitors, or collected filtrate of any control experiments.

ELA of infusion filtrate. ELA of collected filtrate from nonseeded in-line filter sets ranged from the equivalent of 0 to 4 pg of *E. coli* endotoxin per ml. Despite meticulous care to avoid contamination during assembly and simulated infusion, this base-line activity could not be eliminated and probably reflects the inherent variability of the *Limulus* amoebocyte lysate assay employed. When either pore size filter was seeded with *E. agglomerans*, endotoxin levels of filtrate began to rise above the base-line concentration between 24 and 48 h from the onset of infusion (Fig. 3a and b). A trend of increasing ELA with an increasing duration of infusion was noted, attaining mean peak levels equivalent to approximately 65 pg of *E. coli* endotoxin per ml after 72 h. Similarly, with filters intentionally contaminated with *P. aeruginosa*, *S. marcescens*, or *K. pneumoniae*, noticeable increases in ELA of collected filtrate occurred between 24 and 48 h from the onset of infusion. Mean peak levels of ELA for these bacteria were lower than those recorded for *E. agglomerans*, ranging from 5 to 30 pg/ml (Fig. 3c and d and 4).

No significant difference in ELA of collected filtrates existed between experiments employing either 0.45- or 0.22-μm in-line filters ($P > 0.05$).

DISCUSSION

Gram-negative bacteria belonging to the tribe *Klebsielleae* are capable of rapid proliferation in dextrose-containing solutions, presumably by adaptation to low pH and an ability to fix atmospheric nitrogen (11). Likewise, several species of *Pseudomonas* are known to multiply rapidly in lactated Ringer solution (6). Such bacteria commonly are encountered in the hospital environment and often have been identified as contaminants of intravenous infusion fluids (6, 11, 25). Consequently, these microorganisms were selected for the study to assess the in-line filters with a pertinent, severe challenge.

The results demonstrate that selected microorganisms retained on in-line filters proliferate on the membrane surface and in the fluid present in the filter set. Multiplying populations of

TABLE 2. *Passage of K. pneumoniae through 0.45-μm in-line filters during 72 h of simulated infusion*

Period of infusion (h)	No. of CFU of *K. pneumoniae* recovered from in-line filter set filtrate in expt:											
	1	2	3	4	5	6	7	8	9	10	11	12
0–48	0	0	0	0	0	0	0	0	0	0	0	0
48–60	0	18	0	0	0	0	12	0	0	0	0	0
60–72	0	94	18	0	0	0	18	0	0	0	0	0

TABLE 3. *Passage of S. marcescens through 0.45-μm in-line filters during 72 h of simulated infusion*

Period of infusion (h)	No. of CFU of *S. marcescens* recovered from in-line filter set filtrate in expt:											
	1	2	3	4	5	6	7	8	9	10	11	12
0–24	0	0	0	0	0	0	0	0	0	0	0	0
24–36	0	6	0	0	4	0	0	6	0	4	0	0
36–48	0	18	0	0	5	0	6	0	0	19	0	0
48–60	84	29	0	0	0	6	12	9	0	24	16	0
60–72	116	114	0	0	29	9	14	84	0	19	159	0

TABLE 4. *Passage of P. aeruginosa through 0.45-μm in-line filters during 72 h of simulated infusion*

Period of infusion (h)	No. of CFU of *P. aeruginosa* recovered from in-line filter set filtrate in expt:											
	1	2	3	4	5	6	7	8	9	10	11	12
0–12	5	TNTC[a]	TNTC	12	20	8	18	TNTC	TNTC	8	84	TNTC
12–24	21	TNTC	TNTC	45	TNTC	12	TNTC	TNTC	TNTC	17	TNTC	TNTC
24–72	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC

[a] TNTC, Too numerous to count (more than 100 CFU).

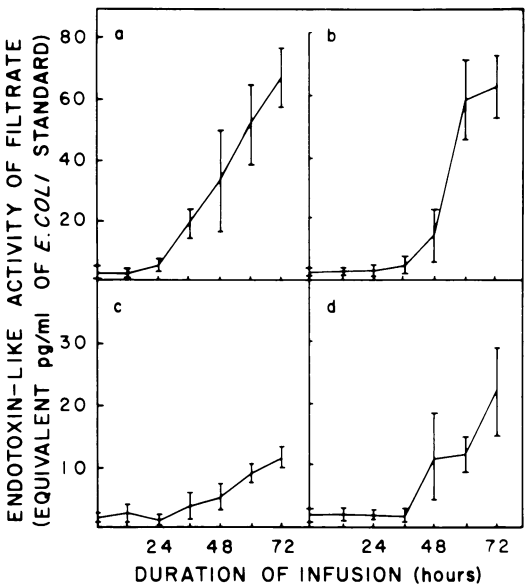

FIG. 3. *Endotoxin activity of infusion fluid collected after passage through contaminated in-line filters. (a) 0.22-μm in-line filters contaminated with E. agglomerans; (b) 0.45-μm in-line filters contaminated with E. agglomerans; (c) 0.22-μm in-line filters contaminated with P. aeruginosa; (d) 0.45-μm in-line filters contaminated with P. aeruginosa. Values are expressed as the mean ± standard error of the mean of 12 results.*

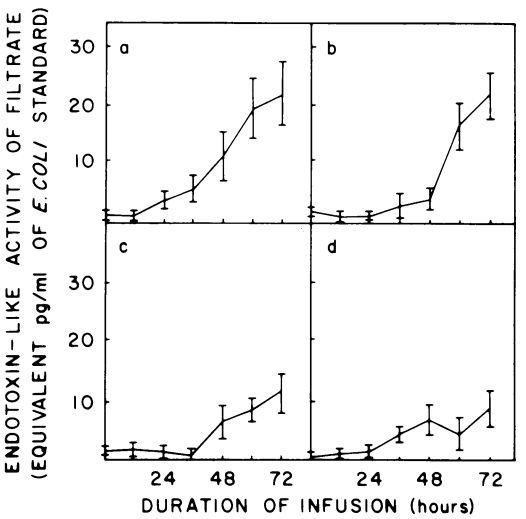

FIG. 4. *Endotoxin activity of infusion fluid collected after passage through contaminated in-line filters. (a) 0.22-μm in-line filters contaminated with S. marcescens; (b) 0.45-μm in-line filters contaminated with S. marcescens; (c) 0.22-μm in-line filters contaminated with K. pneumoniae; (d) 0.45-μm in-line filters contaminated with K. pneumoniae. Values are expressed as the mean ± standard error of the mean of 12 results.*

each bacterium studied were found capable of penetrating 0.45-μm in-line filters and confirmed the understanding that such filters are not absolute. This fact, considered in conjunction with the previous report of Rusmin et al. (21) of 0.45-μm filter penetration by *E. coli* and *P. aeruginosa*, suggests that similar results may be observed with other unstudied microbes that are capable of growth in intravenous infusion fluids.

The data demonstrate that a relationship exists between the degree of filter penetration and cell size. *P. aeruginosa* was observed to penetrate a significantly larger proportion of 0.45-μm filters than did *S. marcescens* ($P < 0.05$).

Likewise, *S. marcescens* demonstrated a significantly higher rate of filter penetration than did either *K. pneumoniae* or *E. agglomerans* (*P*

< 0.05).

A comparison of cell dimensions, as described in *Bergey's Manual of Determinative Bacteriology*, reveals that *E. agglomerans* and *K. pneumoniae* are the largest of the test microorganisms, possessing average cell sizes of 0.5 to 0.7 by 0.8 to 2.5 μm and 0.3 to 0.5 by 5.0 μm, respectively (1, 9). The relatively larger dimensions of these two types of bacteria appear to have hindered their passage through the filter structure. *S. marcescens* and *P. aeruginosa* are defined as the smallest of the bacteria tested, having average cell sizes of 0.5 to 1.0 by 0.5 μm and 0.5 to 0.6 by 1.5 μm, respectively (1, 2). Although these values do not account for the higher incidence of 0.45-μm filter penetration by *P. aeruginosa*, the bacterial populations employed in the study may not have conformed to textbook standards as many factors are known to influence the bacterial cell size of an individual species. Errington et al. have demonstrated that several species of gram-negative bacteria, including *P. aeruginosa* and *S. marcescens*, approximately double in size between times of cell fission (4).

The average cell size of a population of bacteria has been reported to increase during the log phase of growth and subsequently decrease during periods of declining growth (7). In addition, the composition of the culture medium can influence the cell size (20).

Differences in the motility of each test bacterium may have influenced filter penetration. Little information is available concerning the relative motilities of bacteria although *P. aeruginosa* is often described in the literature as being an actively motile organism. Oguiti has defined the average velocity of *P. aeruginosa* to be 47.5 μm/s at 20°C in liquid medium as compared with 28 μm/s for *E. coli* (17). The active motility of *P. aeruginosa* may have assisted the passage through the filter structure although the observation that a number of 0.45-μm filters failed to retain a population of *K. pneumoniae*, a nonmotile organism, suggests that motility is not a requisite for filter penetration.

Rusmin et al. speculated that filter penetration may be associated with bacterial growth and multiplication into the filter. Indeed, penetration may be assisted if bacterial multiplication in the depth of the filter structure occurs; presumably, the growth characteristics of the microbial contaminant in the infusion fluid, such as the lag phase and generation times, would determine the extent that this mechanism would assist the passage of bacteria through the filter barrier. The comparatively faster rate of penetration of 0.45-μm filters by *P. aeruginosa* may be due, in part, to the characteristic early onset of cell multiplication of the particular strain employed in lactated Ringer solution.

The membrane filters used in this study are composed of mixed esters of cellulose (13). None of the test bacteria is known to possess cellulase activity, and enzymatic digestion of the filter material cannot be implicated as a mechanism of filter penetration (2).

The possibility that some of the 0.45-μm in-line filters were manufactured improperly, leading to a failure of retention during the study, seems improbable as all filters of this type that were challenged with *P. aeruginosa* permitted the passage of this bacterium. Assessment of the integrity of control groups of uncontaminated filters by using bubble point determinations, before and after 72 h of continuous infusion, confirmed that the incidence of retention failure could not be attributed to improper manufacture.

No instance of 0.22-μm filter penetration was observed in this study. Until recently, filters of this pore size have been assumed to retain all forms of fungi and bacteria. However, *Pseudomonas diminuta*, *S. marcescens*, and other waterborne bacteria have been reported to penetrate filters with mean pore diameters ranging from 0.22 to 0.15 μm (19, 23; R. Duberstein, Annu. Conv. Parenter. Drug Assoc., Inc., New York, 1979).

This study indicates that if the technique of terminal filtration is adopted during intravenous infusion therapy, the use of 0.22-μm in-line filters is mandatory, particularly as the recent introduction of filter sets of this pore size employing increased surface areas and venting systems ostensibly minimizes the problems of declining flow rates and air blockage.

Rusmin and DeLuca (22) have reported that endotoxin is eluted from in-line filter sets contaminated with gram-negative bacteria upon flushing with carbenicillin or gentamicin. The present study demonstrates that a continuous infusion of endotoxin can originate solely from a population of gram-negative bacteria present in an in-line filter set.

The clinical relevance of infusing endotoxin into patients at the doses and rates described in this study is not known. It is unclear what minimal amount of endotoxin constitutes a pyrogenic dose for humans or whether patient reactions are dependent upon the rate of infusion of endotoxin. However, it has been reported that levels of 100 pg and higher per ml, when injected intravenously in large volumes, can be detected by the USP XIX rabbit test (24), and several investigators have chosen 50 pg of *E. coli* stand-

ard per ml as the pass-fail criterion for the *Limulus* assay (8, 24).

During the first 24 h of infusion, ELA above base-line values was not detected in the filtrate eluted from contaminated filter sets. Levels above base-line values were detectable, however, in some experiments by 48 h. To avoid this potential hazard of terminal filtration, in-line filter sets should be changed every 24 h.

LITERATURE CITED

1. **Breed, R. S., and E. G. D. Murray.** 1957. Family IV. *Enterobacteriaceae* Rahn 1937, p. 332-393. *In* R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), Bergey's manual of determinative bacteriology, 7th ed. The Williams & Wilkins Co., Baltimore.
2. **Burkholder, W. H.** 1957. Genus *I. Pseudomonas* Migula, 1894, p. 89-152. *In* R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), Bergey's manual of determinative bacteriology, 7th ed. The Williams & Wilkins Co., Baltimore.
3. **Collins, J.** 1973. Effect of a Millipore filter on complications of intravenous therapy using in-line filters. Br. Med. J. **24**:456-458.
4. **Errington, F. P., E. O. Powell, and N. Thompson.** 1965. Growth characteristics of some gram-negative bacteria. J. Gen. Microbiol. **39**:109-123.
5. **Freeman, J. B., and A. A. Litton.** 1974. Preponderance of gram-positive infections during parenteral alimentation. Surg. Gynecol. Obstet. **139**:905-908.
6. **Guynn, J. B., D. M. Poretz, and R. J. Duma.** 1973. Growth of various bacteria in a variety of intravenous fluids. Am. J. Hosp. Pharm. **30**:321-325.
7. **Henrici, A. T.** 1928. Morphological variation and the rate of growth of bacteria (Microbiology monographs: general, agricultural, industrial, vol. I). Baillière, Tindall and Cox, London.
8. **Kundsin, R. B., and C. W. Walter.** 1980. Detection of endotoxin on sterile catheters used for cardiac catheterization. J. Clin. Microbiol. **11**:209-212.
9. **Lelliott, R. A.** 1974. Genus XII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, p. 332-340. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
10. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
11. **Maki, D. G., D. A. Goldman, and F. S. Rhame.** 1973. Infection control in intravenous therapy. Ann. Intern. Med. **79**:867-887.
12. **Miller, R. C., and J. B. Grogan.** 1975. Efficiency of in-line bacterial filters in reducing contamination of intravenous nutritional solutions. Am. J. Surg. **130**:585-589.
13. **Millipore Corp.** 1972. Millipore application manual. High-volume pharmaceutical and biological filtration. Catalog no. LAM 2020/0:11-15. Millipore Corp., Bedford, Mass.
14. **Myers, J. A.** 1972. Millipore infusion filter unit: interim report of clinical trial. Pharm. J. **208**:547-549.
15. **Nandan, R., and D. R. Brown.** 1977. An improved *in vitro* pyrogen test: to detect picograms of endotoxin contamination in intravenous fluids using *Limulus* amebocyte lysate. J. Lab. Clin. Med. **89**:910-918.
16. **Newman, M. S., G. Dempsey, and J. Walker.** 1975. Microbial and particulate contamination during prolonged use of I.V. infusion sets. J. Hosp. Pharm. **33**:1-5.
17. **Oguiti, K.** 1936. Untersuchungen über die Geschwindigkeit der Eigenbewegung von Bakterien. Jpn. J. Exp. Med. **14**:19-28.
18. **Oyama, V. I., and H. Eagle.** 1956. Measurement of a cell growth in tissue culture with a phenol reagent (Folin-Ciocalteau). Proc. Soc. Exp. Biol. Med. **91**:305-307.
19. **Pall, D. B.** 1975. Quality control of absolute bacteria removal filters. Bull. Parenter. Drug Assoc. **29**:192-204.
20. **Reti, A. R., and T. J. Leahy.** 1979. Validation of bacterially retentive filters by bacterial passage testing. J. Parenter. Drug Assoc. **33**:257-272.
21. **Rusmin, S., M. B. Althauser, and P. P. DeLuca.** 1975. Consequences of microbial contamination during extended intravenous therapy using in-line filters. Am. J. Hosp. Pharm. **32**:373-377.
22. **Rusmin, S., and P. P. DeLuca.** 1975. Effect of antibiotics and osmotic change on the release of endotoxin by bacteria retained on intravenous inline filters. Am. J. Hosp. Pharm. **32**:378-380.
23. **Wallhauser, K. H.** 1979. Is the removal of microorganisms by filtration really a sterilization method? J. Parenter. Drug Assoc. **33**:156-171.
24. **Weary, M., and B. Baker.** 1977. Utilization of the Limulus lysate test for pyrogen testing large volume parenterals, administration sets, and medical devices. Bull. Parenter. Drug Assoc. **31**:127-133.
25. **Wilkinson, W. R., L. L. Flores, and J. N. Pagones.** 1973. Growth of microorganisms in parenteral nutritional fluids. Drug Intell. Clin. Pharm. **7**:226-231.
26. **Wilmore, D. W., and S. J. Dudrick.** 1969. An in-line filter for intravenous solutions. Arch. Surg. **99**:462-463.