Factors That Influence Microbial Contamination of Fluids Associated with Hemodialysis Machines

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Studies were conducted on the microbiological quality of fluids associated with different types of dialysis systems located in six dialysis centers and 14 homes. Included were (i) single-pass systems employing either parallel flow (Kil or Gambro) or capillary cartridge dialyzers and (ii) recirculating single-pass and batch recirculating systems using coil dialyzers. Microbiological assays were performed on the water used to prepare dialysis fluid, the concentrated dialysate, and either pre- and postdialyzer dialysate (single-pass systems) or the dialysate contained in storage reservoirs and recirculating cannisters (recirculating systems). The levels of microbial contamination consisting of gram-negative bacteria were directly related to the type of dialysis system, method of water treatment, distribution system, and in some instances, the type of dialyzer. Recirculating single-pass and batch recirculating systems consistently contained significantly higher levels of contamination than single-pass systems. These results were directly related to the design of recirculating systems which permits carbon- and nitrogen-containing waste products dialyzed from the patient to accumulate, be used as nutrients by microorganisms, and subsequently allow for 2- to 4-log increases in contamination levels during a dialysis treatment. In contrast, levels of contamination in single-pass machines were related more to the quality of the water used to prepare dialysis fluid and the adequacy of cleaning and disinfection procedures than to the design of the system.

Hemodialysis was utilized solely for acute renal failure until the early 1960's. With the development of the artificial arterial-venous shunt and technological advances in ancillary dialysis equipment, maintenance or chronic hemodialysis has become a common procedure (9). In 1973, approximately 11,000 patients were undergoing dialysis in private or hospital-based centers and in homes throughout the United States. This number may approach 50,000 in the next 2 to 3 years as a result of increased federal aid (3) to patients suffering from chronic renal failure.

Since the 1960's, the technology of hemodialysis has been changing constantly, and this "man-machine interface" has increased significantly within the hospital environment. It was recognized that microorganisms could grow readily in certain fluids associated with dialysis equipment (17, 18), but microbiological parameters were not given serious consideration in the design of these types of systems. We have pointed out in the past (5, 1, 2) that some gram-negative bacteria, notably water bacteria such as Pseudomonas spp., have the capability of rapid multiplication in distilled water. Fluids used in the dialysis procedure consist initially of balanced solutions of salts, sometimes with added glucose; during dialysis these solutions are further enriched by the addition of nitrogen- and carbon-containing waste products dialyzed from the patient's blood. Thus, in a hemodialysis system, the growth potential of water bacteria is at a much higher level than in distilled water. It was of interest, therefore, to conduct a series of studies to determine those factors that influence levels of microbial contamination in dialysis systems.

MATERIALS AND METHODS

These studies were conducted over a period of 1.5 years in hemodialysis centers located in Phoenix, Ariz., San Francisco, Calif., Seattle, Wash., Albuquerque, N.M., and Newark, N.J. The two basic types of dialysis systems used in these centers, including associated dialyzers, are listed in Table 1. The first type is the so-called single-pass system illustrated in Fig. 1 by kinetic diagram and photograph. Water used to prepare dialysis fluid, which is usually treated by softening, deionization, or reverse osmosis, enters the machine and is mixed automatically with concentrated dialysate to produce a dialysate containing one part of concentrate to thirty-four parts of water. This
Table 1. Dialysis centers included in study

| Hospital    | Type(s) of dialysis system          | Dialyzer          | Water treatment system       | Disinfectant          |
|-------------|------------------------------------|-------------------|------------------------------|-----------------------|
| G Home units| Single-pass                        | Kiil (parallel flow) | Reverse osmosis | Chlorine dioxide      |
| S           | Single-pass                        | Kiil (parallel flow) | Softening       | Formaldehyde          |
| F           | Single-pass (central dialysate distribution system) | Capillary cartridge | Deionization     | Sodium hypochlorite   |
| M           | Single-pass                        | Gambro-parallel flow | Reverse osmosis | Chlorine dioxide      |
| A           | Recirculating single-pass          | Coil              | Deionization     | Sodium hypochlorite   |
| B           | Recirculating single-pass plus recirculating batch | Coil              | None            | Sodium hypochlorite   |

Dialysis fluid is then heated, deaerated, and passed through the dialyzer, where waste products subsequently pass from the patient’s blood through the membrane to the dialysis fluid. The waste-rich dialysis fluid then passes through a blood leak detector and is exhausted to drain. The system, consequently, is “single-pass,” with a flow rate of approximately 300 to 500 ml/min.

The second general type is the so-called recirculating or recirculating single-pass system. This latter term is slightly confusing since in this system, as Fig. 2 shows, dialysis fluid does not really make only a single pass through the dialyzer. Incoming water and concentrated dialysate are mixed, manually or automatically. However, the resultant dialysis fluid, which can be fed from a central system or from a 150- to 200-liter reservoir tank, is then pumped to a 8- to 16-liter recirculating cannister. Dialysis fluid flows up through a coil dialyzer, spilling back into the recirculating cannister. Approximately 300 to 500 ml of fresh dialysate per min enter the recirculating cannister and an equal amount is exhausted.

The types of dialyzers employed in the centers studied are illustrated in Fig. 3. Single-pass systems utilized one of three types. The large Kiil dialyzer is a parallel flow type which must be assembled and disassembled with each use. The Gambro dialyzer, also a parallel flow dialyzer, is smaller than the Kiil and was designed as a disposable unit. The third type, the capillary cartridge dialyzer (hollow fiber), also is relatively small and disposable. With recirculating single-pass systems, the coil dialyzer was used exclusively.

In all centers, sodium hypochlorite, chlorine dioxide, or formaldehyde solutions were used to disinfect the dialysis systems.

Sampling methods. In those centers using single-pass systems, samples were collected from the water used to prepare dialysis fluids and from the dialysis fluid at points just before it entered (premembrane dialysate) and left (postmembrane dialysate) the dialyzer. For recirculating single-pass systems, samples were collected from water used to prepare dialysis fluid, the 150- to 200-liter dialysate reservoir tank, and the recirculating cannister. The concentrated salt solutions were not sampled consistently after it was determined that levels of bacterial contamination were extremely low, probably due to the high osmolality.

Samples were usually collected at the beginning and end of a dialysis treatment, which normally ranges from 5 to 7.5 h. Approximately 150 ml were collected in sterile milk dilution bottles to which 0.1 of a 10% (wt/vol) solution of sodium thiosulfate had been added prior to autoclaving to inactive available chlorine. Viable counts were determined using membrane filters (Millipore 0.45-μm); portions ranging from 50 ml of undiluted sample to 10⁻¹ dilutions were assayed. Membrane filters were placed on standard
methods agar (Difco) and incubated at 37 C for 48 h, at which time colonies were counted and representatives were picked for identification. The asparagineacetamide system (4, 5) was used to detect and/or enumerate Pseudomonas aeruginosa. For coliform bacteria, a tube of double-strength lactose broth (Difco) was inoculated with 10 ml of sample and incubated at 37 C for 48 h; growth from positive tubes was streaked into FC medium (Difco) for confirmation.

To determine the effect of patient running time on the levels of microbial contamination, a series of sequential samples were obtained over a 6-h period from a specific dialysis system. In another series of tests designed to measure growth supporting capability of dialysate fluids, pre- and postmembrane dialysate samples were aseptically collected from a single-pass dialysis system located in a hospital center and one located in a private home. Samples were assayed immediately and then were allowed to incubate at 37 C. At various intervals, portions of dialysis fluid were withdrawn and assayed. At center G, a series of assays were conducted on a single-pass system utilizing a Kiil dialyzer which had been disinfected, but which was not in use on that particular day. Samples were collected from various tubings to determine whether "dead end" spots unresponsive to disinfec-

**FIG. 2.** Photograph (a) and diagram (b) of a recirculating single-pass dialysis system; (c) batch type of recirculating dialysis system.

**FIG. 3.** Types of dialyzers used with single-pass and recirculating dialysis systems.
tion existed, which could serve as foci of recontamination.

Although these studies include data collected primarily from dialysis machines located in centers, samples also were collected from single-pass systems using Ki1l parallel flow dialyzers in 14 private homes in the Phoenix area. The patients on these machines had been trained at kidney center G referred to above. Formaldehyde was used to disinfect 12 of the home machines and chlorine dioxide was used for the remaining two.

RESULTS

The levels of bacterial contamination in water used to prepare dialysis fluids varied depending primarily on two factors: type of water treatment system and chlorine levels. Normally, water softeners and deionizers produced water with relatively high (10^4 to 10^5/ml) bacteriological levels. Levels in reverse osmosis systems appeared to show greater variation; high counts were associated with the use of storage tanks and/or extraordinarily long distribution systems. If free chlorine was present, counts were relatively low (<50 organisms per ml); in the absence of free chlorine, the counts ranged from 25 to 2.1 x 10^4/ml. As mentioned previously, the concentrated dialysis fluids contained very few microorganisms (<1/ml).

The levels of bacterial contamination in dialysis fluids of single-pass dialysis systems located in center G and in 14 homes are shown in Table 2. Total viable counts were markedly lower in those machines in the hospital center than in home units; we believe this was due to more scrupulous and consistent cleaning and disinfecting practices at the center, since no direct correlation with levels of contamination in incoming water sources was observed. Invariably, viable counts were higher in postmembrane dialysate samples than in premembrane dialysate suggesting that bacterial growth occurred within the Ki1l dialyzer. Both the levels and frequency of detection of P. aeruginosa also reflected this trend.

In most instances, the duration of dialysis with a single-pass system did not appreciably affect levels of bacterial contamination in the dialysis fluids. However, if initial counts were relatively high (10^4 to 10^5/ml), running time appeared to influence both levels and types of contaminating organisms, especially when the large Ki1l parallel flow dialyzer was used. Sequential samples from a single-pass system located in a private home (Table 3) illustrate differences in initial levels of contamination in pre- and postmembrane dialysis fluids and the effect of patient running time on total viable and P. aeruginosa counts in postmembrane dialysis fluid.

At hospital S, which used single-pass systems

| Location of dialyzer | Sampling point | No. of samples | Mean viable count/ml | Range viable count/ml | Median count/ml | Samples positive for P. aeruginosa (%) |
|----------------------|----------------|----------------|----------------------|-----------------------|-----------------|---------------------------------------|
| Hemodialysis         | Predialyzer    | 28             | 0.14                 | 0 to 28               | 0.20            | 43                                    |
| center G             | Postdialyzer   | 28             | 3.8                  | 0 to 270              | 3.5             | 68                                    |
| Private homes        | Predialyzer    | 14             | 590                  | 0.40 to 13,000        | 2,800           | 71                                    |
|                      | Postdialyzer   | 14             | 890                  | 0.50 to 90,000        | 3,100           | 93                                    |

Table 3. Effect of running time on the level of microbial contamination in fluids associated with a single-pass type kidney system with a Ki1l dialyzer—home patient, Phoenix

| Source               | Total counts per ml for sample interval (h) | P. aeruginosa MPN* per ml for sample interval (h) |
|----------------------|---------------------------------------------|---------------------------------------------|
|                      | 0               | 2               | 4               | 6               | 0               | 2               | 4               | 6               |
| Incoming water       | 2.6 x 10^1      | 2.7 x 10^1      | 6.0 x 10^-1     |                 | 9.0 x 10^-2     | 2.3 x 10^-1     | 1.1 x 10^-1     | 3.0 x 10^-2     |
| Hemotrate (conc. salt solution) | 10^-1 | 2.7 x 10^1 | 3.1 x 10^1 | 3.2 x 10^1 | 9.0 x 10^-2 | 2.3 x 10^-1 | 1.1 x 10^-1 | 3.0 x 10^-2 |
| Predialyzer dialysate| 10^-1 | 1.1 x 10^4 | 1.2 x 10^4 | 3.2 x 10^4 | 10^-3 | 2.4 x 10^2 | 1.1 x 10^3 | 1.1 x 10^3 |
| Postdialyzer dialysate| 10^-1 | 1.1 x 10^4 | 1.2 x 10^4 | 3.2 x 10^4 | 10^-3 | 2.4 x 10^2 | 1.1 x 10^3 | 1.1 x 10^3 |

*MPN, Most probable number.
with capillary cartridge dialyzers, levels of contamination in dialysis fluids were relatively low (<10⁹ organisms per ml). No significant increases in viable counts were observed in pre- and postmembrane dialysis fluids at the end of dialysis.

Data from centers A and B, which utilized recirculating single-pass systems and coil dialyzers, are shown in Table 4. Two major differences were immediately apparent: (i) the dialysis fluids from these systems showed consistently higher levels of contamination than those in single-pass systems, and (ii) the duration of dialysis treatment had a consistent and significant effect on final levels. Initial counts increased by 2 to 4 logs by the end of dialysis. This was in marked contrast to results obtained with single-pass systems located in hospital centers.

Figure 4 shows the data collected from a batch recirculating system (as opposed to a recirculating single-pass system, this type employs a large tank containing a coil and about 150 liters of dialysis fluid which is emptied after approximately 2 h and refilled with fresh dialysis fluid). In this instance, the machine was filled with dialysis fluid and operated for 5 h prior to the start of patient dialysis. At 2-h intervals during dialysis, the dialysis fluid was emptied and fresh dialysis fluid was added. Assays performed at various intervals showed that the levels of microbial contamination increased progressively from approximately 2 × }

![Image](https://via.placeholder.com/150)

**FIG. 4. The effect of running time on the levels of microbial contamination in the dialysate of a batch type recirculating dialysis system.**

**TABLE 4. Levels of microbial contamination in samples of dialysis fluids from recirculating single-pass dialysis systems**

| Dialysis center | Machine | Source | Sampling interval (5 to 7 h) |
|-----------------|---------|--------|-----------------------------|
|                 |         |        | Start (no./ml)       | End (no./ml)       |
| A (Study 1)     | I       | Incoming water⁹ | 10⁻¹ |                   |
|                 |         | Dialysate (reservoir) | 2.4 x 10⁴ | 1.3 x 10⁵ |
|                 |         | Dialysate (recirculating cannister) | 1.2 x 10⁴ | 3.3 x 10⁷ |
|                 |         | Dialysate (reservoir) | 3.2 x 10⁴ | 6.0 x 10⁴ |
|                 |         | Dialysate (recirculating cannister) | 2.0 x 10⁴ | 3.2 x 10⁷ |
|                 |         | Dialysate (reservoir) | 3.5 x 10⁴ | 7.3 x 10⁷ |
|                 |         | Dialysate (recirculating cannister) | 3.3 x 10⁴ | 1.9 x 10⁷ |
| A (Study 2)     | I       | Incoming water | 10⁻¹ |                   |
|                 |         | Dialysate (reservoir) | 1.1 x 10⁴ | 7.0 x 10⁴ |
|                 |         | Dialysate (recirculating cannister) | 9.0 x 10⁴ | 1.3 x 10⁷ |
|                 |         | Dialysate (reservoir) | 2.7 x 10⁴ | 3.9 x 10⁷ |
|                 |         | Dialysate (recirculating cannister) | 1.9 x 10⁴ | 1.2 x 10⁷ |
| B               | I       | Incoming water | 10⁸ |                   |
|                 |         | Dialysate (reservoir) | 9.0 x 10⁴ | 2.0 x 10⁵ |
|                 |         | Dialysate (recirculating cannister) | 8.0 x 10⁴ | 1.5 x 10⁵ |
|                 |         | Dialysate (reservoir) | 1.8 x 10⁴ | 3.0 x 10⁵ |
|                 |         | Dialysate (recirculating cannister) | 1.0 x 10⁴ | 1.6 x 10⁵ |
|                 |         | Dialysate (reservoir) | 1.5 x 10⁴ | 1.6 x 10⁵ |
|                 |         | Dialysate (recirculating cannister) | 5.0 x 10⁴ | 6.0 x 10⁷ |
|                 |         | Dialysate (reservoir) | 1.5 x 10⁴ | 1.5 x 10⁵ |
|                 |         | Dialysate (recirculating cannister) | 7.0 x 10⁴ | 4.5 x 10⁵ |
|                 |         | Dialysate (reservoir) | 1.5 x 10⁴ | 1.5 x 10⁵ |
|                 |         | Dialysate (recirculating cannister) | 1.5 x 10⁴ | 3.1 x 10⁵ |
|                 |         | Dialysate (reservoir) | 8.5 x 10⁴ | 2.0 x 10⁵ |
|                 |         | Dialysate (recirculating cannister) | 5.0 x 10⁴ | 1.8 x 10⁵ |

⁹ Water used to prepare dialysis fluid.
10^7/ml to 3 \times 10^9/ml during the dialysis treatment run.

One of the problems that was immediately evident in certain of the single-pass kidney systems was the use of considerable lengths of tubing with numerous connections and intersections. Many connecting tubes were used only occasionally and, since they were not part of the main flow system, constituted "dead ends" for water or dialysis fluids. Table 5 shows the results of assays when samples were taken from a "T" connection in the tubing of a particular system. The system had been routinely exposed to disinfectant several hours before assay, and the viable counts and levels of *P. aeruginosa* were extremely high (1.5 \times 10^7/ml and 1.1 \times 10^9/ml, respectively). Samples of water back flushed through the "T" connection 2 h after the first sample was taken indicated that microorganisms were reproducing at a relatively fast rate. It was obvious that the disinfectant used (in this case 200 ppm of chlorine dioxide) never reached this portion of the kidney machine; this allowed for the persistence of a massive reservoir of bacterial contamination to seed the dialysis fluid at the start of each treatment.

The types of microorganisms associated with source water and dialysis fluid samples from all centers invariably were gram-negative water bacteria. Fecal coliform bacteria were detected frequently, but their rate of multiplication did not appear to be extensive; *Moraxella* spp. and *Acinetobacter* spp. were detected sporadically. *P. aeruginosa* and other *Pseudomonas* spp., *Achromobacter* spp., and *Xanthomonas* spp. comprised the predominant flora, and as shown in Fig. 5, were capable of attaining high levels in

![Graph](image)

**Fig. 5.** Comparative ratio of growth of the naturally occurring microbial flora in pre- and postdialysis fluid from a hospital and a home based single-pass dialysis system.

either pre- (1.1 \times 10^7/ml) or postmembrane dialysis fluids (2.2 \times 10^9/ml). As anticipated, postmembrane dialysis fluid, enriched with patient waste materials, supported more rapid growth of contaminating organisms than did premembrane dialysis fluids (generation times 0.6 and 0.8 h, respectively).

### DISCUSSION

The results obtained in these studies show that bacterial contamination in dialysis systems is not associated with a single cause but rather results from several factors involving: (i) the water treatment system, (ii) distribution system, (iii) type of dialysis system, and in some cases, (iv) the type of dialyzer. Although this particular study concentrated on the type of dialysis system and the dialyzers employed, mention will be made where applicable to the water supply and distribution system. One clear result shown in Table 4 is that the recirculating type of dialysis systems (recirculating single-pass or completely [batch] recirculating), by the nature of their designs, contribute to relatively high levels of bacterial contamination in dialysis fluids. The coil is contained in a recirculating bath of dialysis fluid that is constantly

#### Table 5. Microbial contamination in tubing of a single-pass kidney machine

| Sample | No. microorganisms per ml | *P. aeruginosa* MPN/ml |
|--------|--------------------------|------------------------|
| A.     |                          |                        |
| No. 1  | 1,500,000                | 1,100                  |
| No. 2  | 5                        | 0.2                    |
| No. 3  | 0.2                      | Detected               |
| No. 4  | 0                        | 0                      |
| B.     |                          |                        |
| No. 1  | 12,000                   | 460                    |
| No. 2  | 0.01                     | 0                      |
| No. 3  | 0                        | 0                      |

* A, Four consecutive 100-ml samples of drained "T" connection; B, three consecutive 100-ml samples of water back flushed through "T" connection 2 h after A.
pooling carbon- and nitrogen-containing waste compounds dialyzed from the patient’s blood; the further addition of small amounts of fresh dialysis fluid (300 to 500 ml/min) essentially creates a chemostat for continuous and rapid growth of microorganisms. High levels of bacterial contamination occurred despite recommended disinfection and cleaning procedures and seemed to be due almost entirely to the design of the systems. On the other hand, the levels of contamination in single-pass systems which had significantly lower levels of bacteria in their dialysis fluids than the recirculating type systems, appeared to depend primarily on the microbiological quality of the incoming water and the method of machine disinfection. When disinfectant was put into the machine at points which did not allow for adequate contact with all tubings, particularly ones that contained “dead end” connections, single-pass machines invariably had high levels of contamination. Although the disinfectants used with these systems were limited to those that do not have an adverse effect on membranes and the components of the machines, the type of disinfectant was not a significant factor contributing to the levels of bacterial contamination. It appeared that disinfection could be accomplished provided that the disinfectant was distributed through all parts of the machine for an adequate period of contact time. Procedures suggested by manufacturers for disinfection of some machines were insufficient because they recommend introducing the disinfectant into the machine in the same manner as the concentrated dialysis fluid. Unfortunately, the incoming water lines from the tap to these suggested points of entrance (constituting about 2/5 of the liquid volume of a machine) are not exposed to disinfectant; thus, environments are provided in which certain gram-negative bacteria can proliferate and act as constant reservoirs of contamination.

The type of dialyzer was not an important factor contributing to microbial contamination. A notable exception was the large Kiil dialyzer in which fluid could be occluded between plates allowing microorganisms to proliferate.

The treatment of the water used to prepare dialysis fluids at these various centers varied from no treatment to softening, deionization, and/or reverse osmosis (Table 1). It has been shown that softeners and deionizers allow for the rapid growth of gram-negative bacteria especially in the absence of residual chlorine (14, 19). Although the process of reverse osmosis tends to produce water that is low in bacteriological content, in the absence of residual chlorine microorganisms can readily multiply in the distribution system downstream from the membrane. Anecdotally, we found that the placement of a variety of string-wound cartridge-type filters in the system usually did not help to lower the level of microbial contamination, but on the contrary, the filter medium per se acted as a surface upon which microbial growth occurred producing a constant reservoir of microorganisms in the system.

The design of a distribution system for the treated water was a significant factor contributing to the levels of bacterial contamination. In several centers, parts of the distribution systems contained either storage tanks or “dead ends,” both of which allow for the multiplication of gram-negative bacteria. These sorts of situations compound the overall contamination problem by allowing either moderately high (100 to 500/ml), or in the case of a storage tank, massive levels (10^4 to 10^7/ml) of contamination to become further amplified in other parts of the distribution system as well as within the dialysis system itself. We also noted that large diameter piping systems used to distribute water at slow flow rates can contribute to levels of bacterial contamination. The use of piping with a small inside diameter increases the velocity of the fluid contents sufficiently to provide a flushing action in the pipe and, at the same time, reduce the volume of fluid in which bacteria can multiply. Another important design criterion is that the entire system must be able to be disinfected easily and efficiently.

One reason that microbiological parameters have not been taken into consideration in the design of hemodialysis equipment might be the view that the levels of bacterial contamination in dialysate do not really pose health hazards to the dialysis patient. However, we believe that there are several reasons why excessively high levels of microbial contamination, especially gram-negative bacteria, in dialysis fluids do constitute potential hazards to patients.

(i) Although intact bacterial cells theoretically are too large to pass through an intact dialysis membrane (9), it has been reported that bacteria can pass through when they are present in large numbers (16). In addition, cells could be introduced directly into the patient’s blood during major or minor blood leaks associated with the integrity of the membrane or its housing (20). The dialysate often is under negative pressure in relation to blood but this pressure differential is a poor protective barrier. Thus, in the case of an overt break in the membrane system, bacterial cells could potentially gain entrance directly into the blood and
cause transient bacteremia or clinically overt septicemia. The exact level of bacterial contamination constituting a potential hazard is not known, but it is obvious that the probability of bacterial cells being introduced into the blood due to a leak in the membrane system is directly proportional to the level of bacterial contamination in the dialysate. In the investigation of an outbreak of septicemia and pyrogenic reactions among patients in center G, which is the subject of another report (6), it was shown that septicemia in two patients was directly due to P. aeruginosa gaining entrance to the blood from the dialysate.

(ii) Gram-negative bacteria associated with water and dialysate have the capability of multiplying rapidly and achieving massive cell levels in dialysate, especially when it contains waste products from the patient’s blood. Consequently, there is a multitude of metabolic products including endotoxins (21) and exotoxins (13) from the bacterial cells themselves which may pass intact membranes and effect pyrogenic reactions. Indeed, the outbreak of septicemia and pyrogenic reactions referred to above (6) clearly documented a direct relationship between the levels of bacterial contamination in the dialysis fluid and the attack rates for pyrogenic reactions among patients. Bacterial endotoxins have a molecular weight of approximately 1 million and theoretically should not pass through a dialysis membrane which has a molecular weight limit of 10,000 to 50,000 (9, 21). Although the term pyrogen is often used to designate bacterial endotoxin, other unidentified products of gram-negative organisms can produce all or some characteristic clinical symptoms of pyrogenic reactions, i.e., fever, rigor, leukopenia followed by leukocytosis, thrombocytopenia, coagulation activation, lactacidosis, and vasomotor collapse. Recently, Quarles and co-workers (15) showed that a growing culture of Serratia marcescens produced materials that passed through the membrane of a commercial capillary cartridge dialyzer and caused acute signs of toxemia, pyrexia, and leukopenia in goats. The maximal molecular size of the toxic material was defined relative to a ridged globular protein of 15,000 in molecular weight and 1.9 nm in hydrodynamic radius, or to a flexible fibrous polyglycol of 5,500 in molecular weight and 2.6 nm in hydrodynamic radius. Kidd (11), in in vitro experiments utilizing a number of bacterial products (acetoin, indole, pyocyanin, chlorhexidine, hemolysin, and urease), was able to detect passage of measurable quantities of each through commercial dialysis membranes. The most convincing evidence that bacterial endotoxins are capable of passing into the bloodstream of patients through dialyzer membranes was provided by Gazenfeldt-Gazit and Eliahou (8), who showed in a serologic study of a group of hemodialysis patients in Israel that there were high serum titers of specific hemagglutinating antibody to bacterial endotoxin prepared from dialysate microflora. Titers were found to be insignificant in a group of nondialyzing patients with chronic pyelonephritis; three individuals studied were seronegative at the start of dialysis but became seropositive within 3 months. These types of investigations need to be continued and could be aided by the use of the limulus lysate test (12).

(iii) Massive levels of actively growing gram-negative bacteria in dialysis fluids could subsequently become amplified in micro-environments in the hospital and transported to other critical areas in a variety of ways. Obviously, a bandage or dressing containing 10⁶ cells of P. aeruginosa would be handled with much respect from the standpoint of containment and isolation. Characteristically, the dialysate of renal dialysis systems is neither contained nor isolated from the environment. Although the role of the environment in nosocomial infections is a very difficult one to explore, it stands to reason that as more sites become contaminated with increasing levels of microorganisms, the chances of opportunistic pathogens reaching a compromised host are likewise increased.

(iv) Another potential problem associated with high levels of gram-negative bacteria in source water and especially dialysate fluids is the definite possibility of transmission of drug resistance factors (R-factors) among various gram-negative bacteria. We believe that it is feasible for multiply resistant strains to become amplified in this type of an environment and constitute a relatively important part of a pyramiding system eventually leading to a more multiply resistant population of gram-negative bacteria in an intramural environment.

Finally, it should be stressed that neither the incoming water nor the dialysate fluids need to be sterile. In this respect we would disagree with Fregerslev (7) who suggested all fluids including hemotrate be sterilized. Rather, we agree with Jones et al. (10) and suggest that the levels of bacterial contamination be maintained at a low level, preferably less than 1,000/ml. Requirements for sterile source water or dialysate fluid would be most unrealistic since no manufacturer can currently meet that specification. Since there is no one bactericidal or bacteriostatic agent that can be added directly to the
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