Rapid Detection of Contaminated Intravenous Fluids Using the Limulus In Vitro Endotoxin Assay

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Intravenous fluids and administration sets may become contaminated with gram-negative bacteria during use and result in a life-threatening situation to the patient. The Limulus in vitro assay for endotoxin was used in two patients whose parenteral fluids had become contaminated with Pseudomonas aeruginosa. This test allowed rapid detection of the contaminated intravenous fluids and demonstrated a concomitant endotoxia in both patients. The same strains of pseudomonas were subsequently cultured from each patient's blood, intravenous catheter tip, and parenteral fluid and administration set. A different serotype of pseudomonas was unique to each patient, indicating two separate and unrelated cases of accidental contamination of the administration sets. Endotoxin-like activity was also demonstrated from several brands of commercial human serum albumin, which may contribute low-level activity detectable by the Limulus assay.

Contamination of parenteral fluids by various organisms, including gram-negative bacteria, has been shown to occur in modern, well-equipped hospitals (3, 4, 11). Documentation of contaminated intravenous fluids in the hospital has been limited to either gross visualization of turbidity or culture methods for viable microorganisms. The latter method is too time consuming to be practical for examining "in-use" parenteral fluids. Standard culture methods may also fail to detect nonviable organisms or those with special growth requirements, such as anaerobes.

The Limulus assay has been shown to be a promising method for the rapid detection of endotoxin in patients suspected of gram-negative sepsis (8, 9, 13). At present, this method is the most sensitive test known for the detection of endotoxin (14, 16) and can be applied easily to the sampling of parenteral solutions (1, 2). Both endotoxin associated with the intact cell walls of viable gram-negative bacteria and solubilized or "free" endotoxin may be detected by the Limulus assay (J. H. Jorgensen and R. F. Smith, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 92, 1972).

This communication describes two separate cases of septicemia due to intravenous fluids in which the Limulus assay was used as a rapid method for documenting the presence of contaminated fluids.

MATERIALS AND METHODS

Patients studied. The two patients involved in this study were both females, aged 4 and 7 years. Each patient was recuperating from serious burn injuries and was afebrile and asymptomatic of serious infectious processes, until both patients developed sudden high fever (106.0 °F) and tachycardia, and became very restless and irritable. Both were receiving a combination of 5% dextrose in 0.2% sodium chloride (McGraw Laboratories, Glendale, Calif.) with normal human serum albumin (Abbott Laboratories, North Chicago, Ill.) added, and 5% dextrose in 0.45% saline (McGraw Laboratories, Glendale, Calif.). These solutions had been prepared and their administration begun long before significant symptomatology was observed (Table 1). The solutions showed no grossly visible evidence of contamination at the time that they were discontinued and sampled.

In an attempt to define the cause of the sudden deterioration of these patients, blood was obtained for culture and endotoxin assay. Intravenous catheters were removed and cultured, and the entire fluid administration sets were removed and immediately cultured and tested for endotoxin.

Limulus assay. Horseshoe crabs were purchased...
from the Marine Biological Laboratory, Woods Hole, Mass. Amebocyte lysate was prepared by methods previously described (7). Lysate used in this study could detect as little as 1 ng of Escherichia coli 055:B5 endotoxin per ml (Boivin extract, Difco). Samples of intravenous fluids were obtained directly from the bottles and from two sites of the accompanying tubing (Fig. 1). These samples were assayed by adding 0.1 ml of the untreated infusion fluid to 0.1 ml of Limulus lysate in disposable glass test tubes (10 by 75 mm) previously rendered sterile and pyrogen free (7).

Heparinized plasma samples were diluted in serial twofold increments in sterile, pyrogen-free 0.85% sodium chloride (Travenol Laboratories, Morton Grove, Ill.) after treatment by the pH-shift method of Reinhold and Fine (13) for removal of plasma inhibitors. One-tenth milliliter of each dilution (1:2 thru 1:128) was added to a 0.1-ml sample of Limulus lysate. A negative control tube containing only 0.1 ml of Limulus lysate and 0.1 ml of the saline diluent was also included. All Limulus assays were incubated for 1 h at 37°C. After incubation, the presence of a gel or a marked increase in viscosity and turbidity was considered a positive test for endotoxin (7).

**Culture methods.** Intravenous fluid specimens from each of the four sampling points on the infusion apparatus were cultured for the presence of viable bacteria. Samples (0.1 ml) of each specimen were applied to 5% human blood in Columbia agar base (BBL), phenylethyl alcohol 5% human blood in Columbia agar base (BBL), and MacConkey agar (BBL) by using the spread plate technique of inoculation. A tube of fluid thioglycolate medium (BBL) was also inoculated with each sample.

Blood cultures were performed by using B-D Vacutainer blood culture tubes, followed by subculture to the above media at 24 h for characterization and identification. Polyethylene cannulae were removed from the patients, and the tips were cultured in fluid thioglycolate medium and subsequently identified in a similar manner.

**RESULTS**

**Administration apparatus.** Figure 1 shows the results of the Limulus assay and cultures taken from the four sampling sites on the apparatus. In both cases, a strong gelation of the Limulus lysate was observed after only 30 min of the 1-h incubation period, indicating a relatively high endotoxin content of the fluid. In both cases, Pseudomonas aeruginosa was isolated from the same sample points that evidenced a positive endotoxin assay. Cultivation of the parenteral fluid yielded confluent growth on the plates, indicating a probable viable count exceeding 10,000 bacteria/ml of fluid. In both cases, the infusion solution containing the albumin additive was contaminated, whereas the bottle of 5% dextrose in 0.45% NaCl was sterile and free of endotoxin. Therefore, the albumin itself was cultured and tested for endotoxin in an effort to localize the source of contamination. This was accomplished by applying the same procedures to the examination
of samples from other bottles of undiluted albumin from the same manufacturer and lot (Abbott, lot no. 17510). Other bottles of albumin from this lot were found to be sterile. However, this material produced endotoxin-like activity by the Limulus assay. This activity was determined to be equivalent to 16 ng of the E. coli 055:B5 endotoxin per ml, by serial twofold dilutions in pyrogen-free saline.

In an effort to determine if this activity was indeed bacterial endotoxin or a natural artifact of the human serum albumin, samples of albumin from other manufacturers and lots were similarly cultured and Limulus tested. All of these fluids proved to be sterile, but varying amounts of endotoxin-like activity were observed among these products (Table 2). However, samples from one manufacturer (Dow) were repeatedly shown to give a negative Limulus assay.

**Patient samples.** In both patients, endotoxemia was demonstrated by positive Limulus assays. Positive endotoxin tests were noted up to a dilution of 1:32 in both cases. However, the significance of the actual endotoxin titer in a patient's plasma has not been firmly established. *P. aeruginosa* was isolated from blood cultures and intravenous cannulae from both patients. The pseudomonas isolates of each patient appeared identical from all sources, i.e., parenteral fluid, blood, and catheter tip, as determined by homologous antibiograms of these isolates. However, the pseudomonads isolated from one patient did not appear identical to those of the other patient, based on antibiotic susceptibility profiles. This observation was confirmed by serotyping the isolates using the Verder-Evans serotyping schema (15). The isolates from patient T.W. were group III, serotype 2108, whereas the strains from patient Y.F. were group I, serotype 359/2243.

**DISCUSSION**

The two occurrences of contamination of intravenous fluids represented by these two patients were isolated instances not occurring simultaneously. The source of the pseudomonads in both instances was thought to be accidental contamination of the fluids with viable cells during addition of the albumin to the saline solution, rather than the albumin itself. Both of these pseudomonas serotypes are commonly encountered among patients in our hospital. The observation that certain human serum albumin preparations produce positive Limulus assay reactions is presumptive evidence that this activity is due to trace amounts of bacterial endotoxin. However, it has been shown by other methods that bovine serum albumin and human gamma globulin may frequently have trace endotoxin contamination (5, 10).

The fact that these two patients had been receiving parenteral solutions contaminated with gram-negative bacteria and their associated endotoxin for several hours is an obvious explanation for their sudden severe sepsis. The isolation of the same bacterium from each patient's blood, catheter tip, and parenteral fluid and administration set established the source of the two infections. However, the detection of endotoxin in the patients' blood and the intravenous fluids by the Limulus assay, within 90 min of sampling, permitted this same deduction to be made at a time when it could influence the diagnosis and subsequent treatment of these patients. The rapidity of the Limulus assay in similar situations would seem invaluable, especially in burn patients where fatal complications of intravenous therapy have become more common (12). Parenteral fluids, other than whole blood or plasma, may be very simply assayed for endotoxin by using the Limulus method, since treatment of these fluids to remove plasma inhibitors is not required. The Limulus test, therefore, allowed rapid detection of "in-use" parenteral fluids contaminated with endotoxin associated with gram-negative bacilli in two separate patients.

| Brand and lot no. | No. of bottles tested | Results |
|------------------|-----------------------|---------|
| Abbott 17519     | 4                     | 16 ng/ml|
| 17528            | 3                     | 16 ng/ml|
| 17533            | 2                     | 16 ng/ml|
| 17540            | 1                     | 8-10 ng/ml|
| 17565            | 1                     | 6 ng/ml  |
| Hyland-Travenol  | 3                     | 16 ng/ml|
| 0489R007A1       |                       |         |
| Armour H68509    | 2                     | 4-6 ng/ml|
| H69010           | 11                    | 0-2 ng/ml|
| Cutter K8400     | 4                     | Negative|
| K8482            | 1                     | 4 ng/ml  |
| Dow 174053       | 5                     | Negative|
| 174057           | 25                    |         |
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