Laboratory Diagnosis of Peritonitis in Patients on Continuous Ambulatory Peritoneal Dialysis

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The clinical course and laboratory diagnosis of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis was studied in 32 consecutive episodes. Peritonitis was associated with a failure in aseptic technique in eight episodes and with an exit-site infection in four episodes. Intraperitoneal vancomycin and ceftazidime were safe, effective, and convenient. Most patients administered their antibiotics at home, and symptoms usually resolved by day 4. Culture of the deposit obtained by centrifugation of 50 ml of effluent after leukocyte lysis provided the best rate of recovery (84% culture positive) but was technically demanding. Filtration of the same volume without leukocyte lysis was simple to perform and almost as effective. Enrichment was less satisfactory (65% culture positive) owing to the presence of antibiotic or infection with fastidious microorganisms. Culture of 50 ml of effluent after concentration by a commonly used laboratory technique, centrifugation without leukocyte lysis, performed poorly (59% culture positive at 48 h), as this method caused sequestration and death of microorganisms within the leukocytes. Culture of nearly 1 liter of effluent from 33 asymptomatic patients by the same techniques yielded no microoganisms.

Peritonitis is the most serious complication of continuous ambulatory peritoneal dialysis (CAPD) (1). However, culture of dialysis effluent by standard techniques yielded organisms in only 50% of the 121 episodes of peritonitis in our patients in 1985. Our routine method consisted of direct culture of a standard loopful of liquid (0.007 ml) and culture of the centrifuged deposit of 20 ml of effluent (7). Direct culture was positive in 33% of episodes, and culture of the centrifuged deposit increased the recovery to only 50%, and often without the anticipated increase in microbial numbers. Both methods frequently provided fewer than five colonies per plate, a level readily confused with contamination. Other workers have reported similar difficulties (2, 4, 10).

Centrifugation or filtration of large volume (100 ml) of effluent has been reported to increase the recovery rate to 81 to 98% (5, 14, 20), suggesting that low concentrations of bacteria are involved. However, similar improvements have been reported by enrichment of small (5-ml) volumes of effluent (2, 10). Without an assessment of the rate of laboratory contamination, the significance of these results is questionable because the commonest infecting organisms, coagulase-negative staphylococci, are also common laboratory contaminants of enrichment media (17). Furthermore, 25 to 37% of specimens from patients without peritonitis have yielded positive cultures (14, 20), raising the possibility that microorganisms in the effluent may not always cause peritonitis. Other workers have improved the recovery of microorganisms by treating the effluent with a leukocyte-lysing agent, suggesting that intracellular sequestration of microbes was an important cause of false-negative cultures (6).

We therefore decided to investigate these questions by culturing fluid from symptomatic and asymptomatic patients by both conventional (direct culture and culture after concentration by centrifugation) and alternative techniques. In addition, we evaluated the quantitation of the leukocytes in the effluent at presentation, the leukocyte differential count, and the Gram stain of the centrifuged deposit. We also collected clinical information at presentation and followed the response to therapy and correlated these with the laboratory findings.

MATERIALS AND METHODS

Patients. The patients studied were those in the CAPD program at St. Thomas' Hospital, London, all of whom are adults.

(i) **Symptomatic group.** Patients were asked to come to the Renal Unit if their dialysate effluent became cloudy or if they had abdominal pain.

The following information was collected at presentation: presenting symptoms and duration, occurrence during the previous 2 weeks of any illness, antibiotics taken, use of additives to the effluent, breaches in aseptic technique during bag exchanges, and number of exchanges performed away from home.

A presumptive diagnosis of bacterial peritonitis was made when microscopy confirmed that the cloudiness of the effluent was due to an elevated leukocyte count or when abdominal pain was accompanied by tenderness or guarding. Antibiotics were administered intraperitoneally, vancomycin (50 mg/liter to each bag) when gram-positive bacteria were seen in a Gram stain of the centrifuged deposit of the effluent, or ceftazidime (50 mg/liter to each bag) when gram-negative bacteria were seen. When no organisms were seen, both antibiotics were given until culture results were available, when the inappropriate agent was stopped. Other antibiotics were given when indicated by antimicrobial susceptibility testing. Patients were admitted to the hospital if they were in severe pain or unable to administer their antibiotics at home. Therapy was continued for 10 days. Patients who failed to respond by this time were admitted for review, and their further clinical progress was recorded.

(ii) **Asymptomatic group.** All patients routinely attend the Renal Unit clinic every 6 weeks. Patients attending the clinic were questioned as before to provide a control group. The statistical significance of any difference between the symptomatic and asymptomatic group was established by standard error of percentage difference (18). Effluent from those

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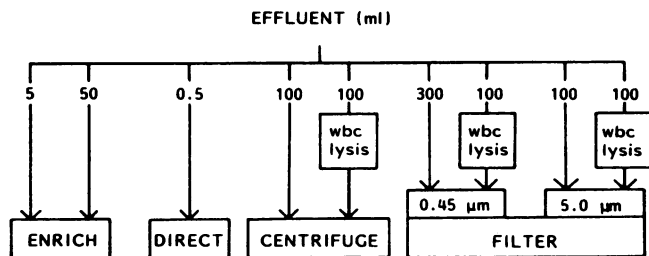* Corresponding author.

EFFLUENT (ml)

FIG. 1. Culture techniques. wbc, Leukocyte.

not receiving antibiotic was examined by the same methods used for the symptomatic group.

Bacteriological methods. The entire bag of effluent was sent to the laboratory and examined within 2 h of drainage. The injection port was cleaned with methylated 70% ethanol, and fluid was withdrawn with a sterile cannula and syringe.

(i) Microscopy. Leukocytes and erythrocytes were counted in a modified Fuchs-Rosenthal chamber. A Gram-stained smear (for the detection of microorganisms) and a Giemsa-stained smear (for leukocyte differential count) were made from the centrifuged deposit.

The leukocyte count was repeated after 1 h of incubation with the lysing solution, and the percentage of leukocytes remaining intact was recorded. Leukocytes were lysed by the addition of an equal volume of lysing medium to the effluent and incubation for 1 h at 37°C. The lysing medium consisted of a detergent, Tween 20 (Sigma Chemical Co., St. Louis, Mo.), at a final concentration of 0.7% with a proteolytic enzyme, Rhozyme P41 (Genencor Inc., Le Vesinet, France), at a final concentration of 0.5%, as described by Zierdt (21).

(ii) Culture. Figure 1 summarizes the culture techniques. For enrichment, 5 ml of effluent was added to 10 ml of Robertson cooked meat medium (Southern Group Laboratories, London, England), and 50 ml of effluent was added to 17 ml of quadruple-strength nutrient broth (number 2; Oxoid, Ltd., Basingstoke, England). Broths were incubated aerobically with 5% CO_2 at 37°C and subcultured after 1, 2, 3, and 7 days to blood agar (Oxoid Columbia agar base with 6% horse blood) incubated aerobically with 5% CO_2 at 37°C.

For direct culture, 0.5 ml of effluent was dispensed on the surface of a blood agar plate and incubated aerobically with 5% CO_2 at 37°C.

Unlysed effluent (100 ml) was transferred to four 30-ml sterile plastic universal conical-bottom containers (Sterilin, Ltd., Middlesex, England) and centrifuged at 4,000 rpm (3,500 × g) for 30 min in a centrifuge (Damon/IEC, Inc., Needham Heights, Mass.). The deposit was divided for aerobic culture with 5% CO_2 and anaerobic culture on blood agar at 37°C and for culture in room air at 30°C and room temperature. Anaerobic culture was performed in an anaerobic cabinet (model 1028; Forma Scientific, Inc., Marietta, Ohio). Effluent (100 ml) was similarly treated after the addition of the leukocyte-lysing agent.

Mixed cellulose ester membranes (Millipore Intertech, Inc., Bedford, Mass.) were used for filtration of 50-ml volumes of unlysed effluent. The 0.45-μm-pore-size filter was supplied sterile; the 50-μm-pore-size filter was not and was sterilized by treatment with ethylene oxide. Filters were transferred to blood agar and incubated aerobically with 5% CO_2 at 37°C. Further 50-ml volumes were filtered for anaerobic culture at 37°C. These procedures were duplicated after the addition of the leukocyte-lysing agent to further 50-ml volumes of effluent. An additional 50-ml volume of unlysed

effluent was filtered through a 0.45-μm-pore-size filter and cultured in room air at 30°C. The survival of the infecting microorganisms in unlysed effluent held at 4°C was studied by filtration through a 0.45-μm-pore-size filter of 50-ml volumes after 24, 48, and 72 h of storage, so that the final volume of unlysed effluent examined by 0.45-μm filtration was 300 ml.

All cultures were examined for growth after 1, 2, 3, and 7 days of incubation. Colony counts were made and related to the volume examined to give the microbial concentration provided by each culture technique. Great care was taken to avoid plate contamination, defined as growth outside the area of inoculation or the recovery of only one colony of an organism from within this area on all culture plates. Organisms were identified by standard methods (3) (API Laboratory Products, Ltd., Montalieu-Vercieu, France).

Antibacterial activity in the effluent was detected by inhibition of growth of three test organisms: the Oxford staphylococcus (NCTC 6571), *Bacillus subtilis* (ATCC 6633), and *Escherichia coli* (NCTC 10418). Effluent was delivered to a well cut into a blood agar plate previously seeded with the test organism. Plates were examined after overnight incubation at 37°C, and any inhibition of growth of the test organism was noted.

RESULTS

During a period of 3 months, 32 consecutive episodes of peritonitis involving 24 patients were studied. A second specimen from one patient with persistent peritonitis was also received. All patients presented with cloudy effluent, and six had no other symptom. Only six patients required hospitalization (owing to severe abdominal pain), and the patients in the other 26 episodes administered their own antibiotics at home. Symptoms resolved after 4 days of therapy in 25 episodes, but among 7 episodes in which symptoms had not resolved by this time only 1 resolved without complication. The patients in three of the seven episodes showed no response after 10 days of treatment with antibiotics appropriate for their infections (*Staphylococcus aureus*, treated with intraperitoneal vancomycin, *Pseudomonas aeruginosa*, treated with intraperitoneal ceftazidime, and *Candida parapsilosis*, treated with oral flucytosine), and symptoms resolved only after removal of the Tenckhoff catheter. Two patients recovered, but peritonitis recurred with the same organism (*S. aureus* and *Klebsiella* sp). One patient was found to have a persisting infection with a ceftazidime-resistant *Moraxella urethralis* strain when the effluent was recultured after 4 days of ineffectual treatment with this antibiotic. Symptoms resolved after 3 days of therapy with intraperitoneal gentamicin. The final patient had tuberculous peritonitis and was successfully treated with oral antituberculous therapy and catheter removal.

The five episodes of culture-negative peritonitis were not distinguishable from the culture-positive episodes in clinical, epidemiological, or other laboratory findings. Symptoms resolved in all by day 3 of combined antibiotic therapy.

Effluent from 33 asymptomatic patients was examined. A total of 20 of the 24 patients in the symptomatic group were included during an asymptomatic interval before or after the bout of peritonitis included in the study. Fluid was also examined from 13 other (unmatched) patients, all of whom had suffered previous bouts of peritonitis.

Leukocyte count and differential. All symptomatic patients had an effluent leukocyte count above 50×10^6/liter, whereas the count was below this figure in all asymptomatic

TABLE 1. Organisms isolated

Organism	Episodes
At presentation	
Gram positive	
Staphylococcus epidermidis	7
Staphylococcus aureus	4
Streptococcus mitior	1
Gram negative	
Pseudomonas fluorescens	3
Pseudomonas putida	1
Pseudomonas aeruginosa	1
Klebsiella sp.	2
Enterobacter sp.	1
Moraxella osloensis	1
Haemophilus parainfluenzae	1
Mixed bacteria	
Moraxella urethralis, Pseudomonas testosteroni, Pseudomonas stutzeri, Acinetobacter calcoaceticus subsp. anitratus, and Escherichia coli	1
Pseudomonas fluorescens and Pseudomonas stutzeri	1
Pseudomonas aeruginosa and Staphylococcus epidermidis	1
Mycobacteria (Mycobacterium tuberculosis)[a]	1
Fungi (Candida parapsilosis)	1
Repeat culture (Moraxella urethralis)	1
Culture negative	5

[a] Isolated from enrichment culture only, after 5 weeks of incubation.

TABLE 2. Comparison of culture techniques (after 48 h of incubation)[a]

Method of culture	No. of specimens examined	No. culture positive (%)	No. yielding <5 CFU/50ml (%)
Enrichment	32	21 (66)	NA[b]
Quantitative culture without leukocyte lysis			
Direct culture	17	14 (82)	7 (41)
Centrifugation	32	19 (59)	6 (19)
Filtration	32	27 (84)	1 (3)
After leukocyte lysis			
Centrifugation	32	27 (84)	1 (3)
Filtration	32	27 (84)	1 (3)

[a] The specimen yielding M. tuberculosis is not included.
[b] NA, Not applicable.

patients. Only five asymptomatic patients had a count above 10 × 10⁶/liter: two had recently had a Tenckhoff catheter inserted and two others had recently recovered from an episode of peritonitis.

Mononuclear cells predominated in asymptomatic patients (and the percentage was less than 90% in only four subjects), whereas polymorphs predominated (>60%) in all but three of the symptomatic patients, of whom one had tuberculous peritonitis, another was receiving immunosuppressive treatment and was infected with Staphylococcus epidermidis, and the third had noninfective peritonitis secondary to recent trauma associated with the insertion of a Tenckhoff catheter.

Gram stain. Organisms were seen in the Gram-stained smear of the centrifuged deposit in 9 of the 28 culture-positive specimens (32%), including 8 of the 14 yielding gram-positive bacteria, but in only 1 of the 13 yielding gram-negative bacteria (although the concentration of both on culture was similar). The number of organisms observed was proportional to the numbers grown on culture, and no organism was seen when the culture yielded fewer than 40 CFU/ml by lysis techniques.

Leukocyte count after lysis. Intact leukocytes were seen in only two specimens after 1 h of treatment with the lysing agent; 1.2 and 6% of the leukocytes remained unaffected in these specimens.

Culture. Of the 33 specimens from the symptomatic patients, 28 were culture positive, a recovery rate of 84%. The organisms isolated in each episode are shown in Table 1. The

33 specimens from the asymptomatic patients were sterile on culture.

No technique yielded a false-positive culture result; in every culture-positive specimen, the same organisms were recovered by all techniques yielding growth. However, false-negative results (when a technique failed to yield the infecting organism) were seen (Table 2).

Enrichment techniques. The results of culture of 5 and 50 ml of effluent were identical and yielded growth at the first subculture (except for one slow-growing Moraxella sp., positive at the second subculture). No false-positive cultures were seen, but there were seven false-negatives. Five false-negatives were due to inappropriate culture conditions: three P. fluorescens strains did not grow at 37°C, one Haemophilus influenzae strain did not grow on blood agar, and one Mycobacterium tuberculosis strain was recovered only after prolonged incubation of the enrichment medium. Colonies of tubercle bacilli were apparent on blood agar after 14 days of aerobic incubation at 37°C when the broths were subcultured after 5 weeks of incubation. Two false-negatives were attributed to the presence of an antibiotic in the effluent to which the organisms were susceptible.

Quantitative techniques. In addition to the number of false-negatives, Table 2 presents the number of episodes when the yield of a technique was less than 5 CFU/50 ml, a level at which confusion with laboratory contamination is possible.

Although direct culture achieved a satisfactory culture-positive rate, colony counts of the infecting organism were frequently very low (Table 2). The number of organisms obtained was always proportional to that obtained by filtration without lysis (but to no other technique).

Centrifugation without lysis was also unsatisfactory, yielding on average 100-fold-fewer colonies than lysis centrifugation, or filtration with or without leukocyte lysis. On eight occasions, the infecting organism was not recovered after 48 h of incubation, and even after 7 days of incubation, five samples remained culture negative.

Filtration without lysis yielded, on average, about half the number of organisms obtained by lysis filtration or centrifugation, but there were wide variations from specimen to specimen. Colony counts were the same for all specimens whether filtered through the 0.45- or 5.0-μm-pore-size filter. Filter blockage occurred on 12 occasions with the 0.45-μm-pore-size filter and on 5 occasions with the 5.0-μm-pore-size filter. However, filter blocking never occurred with the first 10 ml of effluent with the 0.45-μm-pore-size filter or with the first 20 ml for the larger pore size, and these volumes passed
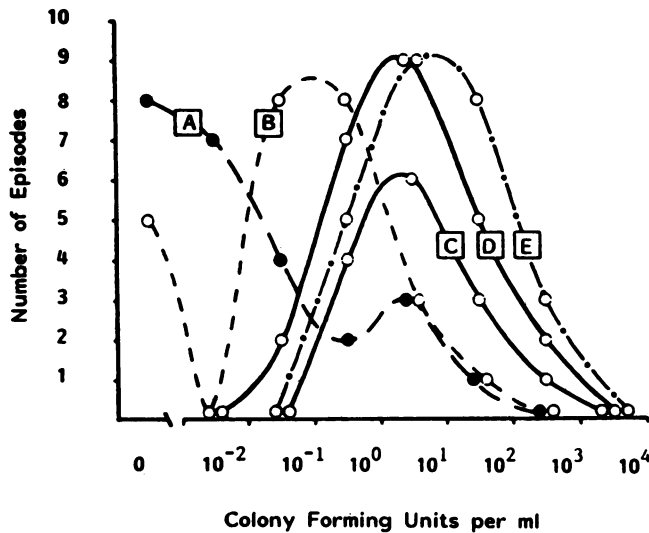
FIG. 2. Comparison of culture techniques in 25 culture-positive episodes. A, Centrifugation without lysis (24 h); B, centrifugation without lysis (7 days); C, direct culture (24 h); D, filtration without lysis (24 h); E, lysis centrifugation and filtration (24 h).

rapidly, in an average of 11 s. When blocking occurred, we found that pouring off the unfiltered part did not result in the loss of organisms already filtered.

The results of lysis centrifugation and lysis filtration (0.45- and 5.0-μm pore sizes) were equivalent and produced the highest numbers of organisms. Unlike filtration without leukocyte lysis, loss of organisms owing to the larger pore size of the 5.0-μm-pore-size filter was observed, but for *Moraxella* and *Pseudomonas* spp. only, for which recovery was markedly reduced. Filter blocking did not occur, and the 100-ml mixture of effluent and lysing medium passed rapidly, even through the 0.45-μm-pore-size filter (12 s on average).

We were surprised to find that the appearance of organisms capable of forming visible growth after overnight incubation was often delayed, resulting in increasing total counts over the 7 days of incubation. This phenomenon was seen with filtration and centrifugation without lysis and direct culture, but not with the leukocyte lysis techniques. The phenomenon was observed only when the counts were reduced compared with those obtained by the leukocyte lysis techniques and became more pronounced as the difference increased. This difference was greatest with the technique of centrifugation without lysis, and here the total count often increased 100-fold during the week of incubation (but always falling far short of the numbers obtained by the lysis techniques.) The phenomenon was also seen with filtration without lysis, but not in the 17 occasions when the counts were identical with those of the lysis techniques.

The overall performance of the various culture techniques at 24 h is compared for 25 episodes in Fig. 2. The identical efficiency of direct culture (C) and filtration without leukocyte lysis (D) is evident. The superior efficiency of the lysis techniques (E) and the comparatively poor performance of centrifugation without lysis (A) are shown, and the improvement with this technique after 7 days of incubation (B), owing to the delayed appearance of colonies, is also demonstrated.

Antimicrobial susceptibilities of infecting bacteria to vancomycin and ceftazidime. None of the 13 gram-positive isolates were resistant to vancomycin. Of the 18 gram-negative

isolates, 2 were resistant to ceftazidime: *M. urethralis* was resistant on first isolation, and *P. aeruginosa* had acquired resistance after treatment of three previous episodes with this antibiotic.

Survival of infecting organisms in effluent. The majority of organisms showed a gradual decline in numbers in stored effluent with time. An average of 72% of the original numbers (range, 10 to 100%) were recovered after 24 h at 4°C, 63% (range, 10 to 100%) at 48 h, and 46% (range, 0 to 100%) at 72 h. Only one organism (*Moraxella osloensis*) failed to grow after 72 h of storage.

Antimicrobial activity. An antibacterial effect was detected in the fluid of 8 of the 28 patients with culture-positive peritonitis and 3 of the 5 patients with culture-negative peritonitis. The antibiotic effect was unexpected in four symptomatic patients, two of whom had forgotten recent courses of antibiotics for chest infections. The other two patients admitted surreptitious self-treatment with intraperitoneal antibiotics. No antibacterial activity was detected in the effluent of the 33 asymptomatic patients.

Correlation of clinical and laboratory findings. In the 2 weeks before presentation, 8 of the 32 patients with peritonitis and 14 of the 33 asymptomatic patients had been unwell. Eleven patients with peritonitis and 10 asymptomatic patients had exchanged their CAPD bags away from home, and 8 patients in each group had used an additive to their bags. These differences are not statistically significant ($P > 0.1$ and $P > 0.5$, respectively). In contrast, eight patients with peritonitis reported a breach in aseptic technique, whereas none of the asymptomatic patients did so, a statistically significant difference ($P < 0.01$). Six patients had either experienced an accidental line disconnection or had touched the connector during a bag exchange. The organisms recovered from these patients proved to be recognized skin commensals (*S. epidermidis, Acinetobacter* spp., and *C. parapsilosis*). In contrast, mixed gram-negative bacilli (Table 1) of types found in household water (15) were recovered from the dialysate of two patients who had heated their fresh bags of dialysate in hot water, but had first removed the protective wrapper for speedier warming.

We attempted to correlate all the clinical data collected: the severity of symptoms at presentation, the number of days to resolution of symptoms, effluent leukocyte count, organism count per milliliter after centrifugation with and without leukocyte lysis, and the degree of depression of the microbial count after centrifugation without lysis. We found a correlation between two pairs only: a high effluent leukocyte count predicted a large depression in the colony counts after centrifugation without leukocyte lysis, and a low colony count after centrifugation without leukocyte lysis predicted a more rapid resolution of symptoms. Of the eight patients with a profound reduction in counts (no growth after 24 h of incubation), symptoms had resolved by day 3 of treatment in all but one case. Symptoms resolved in this patient by day 4 although she had been given vancomycin (in error) for an infection with a vancomycin-resistant *Pseudomonas putida* strain (MIC, >128 mg/liter). Conversely, the unresolving infections (*S. aureus, P. aeruginosa*, and *C. parapsilosis*) were associated with comparatively small reductions in counts.

Four episodes of *S. aureus* peritonitis occurred in three patients, all of whom had a chronic exit-site infection with the same strain.

DISCUSSION

We have previously described the successful use of intraperitoneal vancomycin and ceftazidime to treat CAPD peritonitis (7), findings confirmed in the present study. This regimen, although expensive, is convenient. A response is usually obtained by day 4, and if symptoms persist, further cultures should be obtained. We have not seen resistance to vancomycin among gram-positive bacteria, and resistance to ceftazidime among gram-negative organisms remains uncommon in our unit (9% of isolates in 1986).

A semiquantitative effluent leukocyte count is sufficient for the laboratory diagnosis of peritonitis; occasional bags are turbid owing to erythrocytes or fibrin. We found that more precise quantitation added no useful information. Although all patients in the study presenting with peritonitis had effluent leukocyte counts of greater than 50×10^6/liter, occasional patients with microbial peritonitis have presented to our unit with much lower counts, and it seems unwise to exclude a diagnosis of infective peritonitis on the basis of a lower leukocyte count.

The leukocyte differential count is similarly fallible in predicting the nature of the infecting organism: an immunosuppressed patient with a low effluent leukocyte count (56×10^6/liter) proved to be infected with a staphylococcus, yet had only 0.5% neutrophils, and although the effluent from the patient with tuberculous peritonitis had a preponderance of mononuclear cells, polymorphs may predominate in this condition (11). In view of these limitations, we do not consider that a special stain for leukocyte differentiation is justified.

The value of the Gram stain of the centrifuged deposit has been questioned. Advocates of the technique have observed microorganisms in 38 and 47% of episodes (6, 9), but others have been less successful, with rates of 7 to 16% (4, 10, 12, 14). We found a carefully prepared and examined Gram stain valuable, allowing an immediate choice of a single antibiotic in one-third of culture-positive episodes (only 1 of 139 culture-positive episodes in 1986 yielded mixed gram-positive and gram-negative organisms).

Our study and several recent reports (4, 6, 8, 13) support the suspicion of earlier workers (14) that the culture of microorganisms from the effluent of asymptomatic patients is almost always due to laboratory contamination. We obtained no microorganisms from nearly a liter of effluent from each of 33 asymptomatic patients.

The main aim of the study was to establish the reasons for the poor performance of standard laboratory culture techniques when applied to CAPD peritonitis and to select the best alternative. Our previous direct culture technique had employed only one standard loopful of effluent and achieved a culture-positive rate of only 30%. The explanation for its inefficiency was provided by analysis of the results obtained by direct culture of the larger volume in the present study. It was our practice not to report low numbers of organisms on the routine culture plates. We found that in only one-third of the episodes was the count sufficient to yield five or more colonies of the infecting organism when sampling only one loopful of fluid.

The comparatively inefficient performance of the standard laboratory concentration technique, centrifugation without lysis, was surprising. Compared with filtration (with or without lysis) and lysis centrifugation, counts were usually depressed 100-fold, and false-negative culture results were also obtained. Our results indicate that in the majority of specimens most organisms are initially extracellular (as shown by direct culture and filtration without lysis) but that after centrifugation without leukocyte lysis almost all the organisms are inside the phagocytic leukocytes (as observed by microscopy of the stained deposit). We suspect that intracellular organisms are either killed or suppressed as long as the leukocyte remains intact. The gradual emergence of surviving organisms from deteriorating phagocytes would account for the phenomenon of delayed appearance of growth, which was most marked with this technique. We noticed that in these cultures (only) growth often appeared along the lines produced by streaking out and not in the pool, even in specimens with no antibacterial activity. Total counts were also often higher at the lower temperatures and after anaerobic incubation. Others have noted the same for specimens plated on MacConkey bile salt medium incubated at 37°C (9), and we assume that these conditions are hostile to the leukocyte and favor the microbe. Four recent studies have shown a similar increase in yield of microorganisms by centrifugation when the leukocytes are lysed (6, 9, 16, 19) but have not noted that the low counts obtained by centrifugation without lysis are an artifact of the technique itself.

Our previous impression that episodes of peritonitis that were culture negative by routine methods responded promptly to antibiotics was confirmed, and we suggest that the extent of the in vitro suppression noted after centrifugation without leukocyte lysis predicts the vigor of the host response in vivo.

The difficulty in anticipating the culture requirements of the diversity of microbes accounted for a further cause of false-negative cultures by routine methods. Three *P. fluorescens* strains were unable to grow in the laboratory at 37°C, one *Haemophilus parainfluenzae* strain was unable to grow on blood agar, and the tubercle bacillus required prolonged incubation. A further cause of failure of culture which we observed was the presence of antibiotics, often undeclared, in the effluent to which the infecting microorganism was susceptible.

The lysis techniques produced, overall, the highest yield of organisms, owing to the liberation of those organisms initially intracellular. Ten milliliters of fluid proved an adequate volume for culture; on only one occasion was the yield less than 1 organism per ml (owing to surreptitious self-treatment before presentation). Lysis centrifugation was the most satisfactory single technique. Unlike filtration, the technique is familiar, the deposit can be easily divided for culture under different conditions, early growth is easy to see, colonies are of familiar morphology, and streaking out from the inoculum makes detection of mixed growths straightforward. In addition, the specimen for culture may be counterbalanced in the centrifuge by an aliquot for Gram stain. Unfortunately, we cannot recommend our lysis technique for routine use: the method involves a 1-h delay during incubation with the lysing solution, which was also difficult to prepare. Other methods of lysis have been described, but all have disadvantages. Gould and Casewell (6) incubated effluent for 0.5 h at room temperature with Triton X-100 before centrifugation for 0.5 h, but this agent is toxic to microorganisms (21) and their technique yielded fewer positive cultures than enrichment. Rescue of intracellular organisms after centrifugation by leukocyte lysis with distilled water has been reported to be effective (9) but requires centrifugation in two stages, and a commercially available system employing saponin and reported to give good results is expensive (16). Disruption of leukocytes by ultrasonication before centrifugation has been found effective (19), but

the technique is not simple and requires access to a sonicator.

We agree with Knight et al. (10) that culture by enrichment is simple and inexpensive, effective with small volumes of effluent, and when performed with care to avoid contamination, represents an improvement upon conventional culture techniques. However, enrichment performed comparatively poorly in this study, and although we have found a commercially available system (Signal System; Oxoid) more successful (13a), the results were again inferior to filtration. Furthermore, the results of identification and sensitivity testing must always be delayed when an enrichment technique is used.

Although filtration of unlysed effluent produced no false-negative culture, its efficiency compared with that of the lysis techniques varied widely. Occasional specimens yielded very low counts compared with the lysis techniques. Presumably, in these cases the majority of the organisms were initially intracellular, for with these specimens the total count increased daily owing to the delayed appearance of growth. A further difficulty which we experienced was filter blocking in specimens with high leukocyte counts. The 5.0-μm-pore-size filter only doubled the rate of filtration and volume that could be filtered before blocking. However, analysis of the culture results indicates that filtration of 10 ml of effluent would have produced no false-negative result and would have yielded fewer than five colonies of the infecting organism on only two occasions. We did not experience blocking of the 0.45-μm-pore-size filter with this volume. We therefore recommend filtration of this amount to laboratories possessing the necessary equipment and culture of 5 ml of effluent by enrichment to those which do not, while we await the development of a simple, efficient, and inexpensive lysis centrifugation technique.

ACKNOWLEDGMENTS

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