Increased Microbial Yield from Continuous Ambulatory Peritoneal Dialysis Peritonitis Effluent after Chemical or Physical Disruption of Phagocytes

PETER C. TAYLOR,[1]* LAURA A. POOLE-WARREN,[2] AND REGINA E. GRUNDY[1]

Department of Microbiology, The Prince of Wales Hospital, Randwick, New South Wales 2031,[1] and Centre for Biomedical Engineering, The University of New South Wales, Kensington, New South Wales 2033,[2] Australia

The laboratory diagnosis of continuous ambulatory peritoneal dialysis-associated peritonitis is often hindered by either the absence of or the recovery of low numbers of viable microorganisms. This may be the result of sequestration of bacteria within phagocytes. Sonication of clinical specimens prior to culturing or culturing on saponin-containing media resulted in the growth of significantly greater numbers of colonies than standard culturing on conventional media. In addition, the demonstration that microorganisms are sequestered in phagocytes helped to establish the pathogenic nature of such isolates and distinguish them from contaminants even when present in low numbers. A variety of physical and chemical techniques can disrupt phagocytes and improve the sensitivity of laboratory methods used to confirm the diagnosis of peritonitis in continuous ambulatory peritoneal dialysis patients.

Peritonitis is the most common complication of treatment by continuous ambulatory peritoneal dialysis (CAPD) (11, 15) and may be caused by a wide variety of microorganisms (1, 3–5, 7, 9, 11). Laboratory specimens of CAPD effluent obtained shortly after the onset of symptoms of peritonitis usually contain sufficient inflammatory exudate to cause visible turbidity (5, 9, 11). However, the microorganisms recovered from these specimens are characteristically low in number and may be slow growing (5, 6, 9), requiring particular care for their recovery.

Studies have recommended that large volumes of fluid be examined to improve the recovery of pathogenic microorganisms (3–5, 9, 11), and particular reliance has been placed on broth culturing as a sensitive technique (3, 5, 12), despite false-positive results in up to 27% of cases (3, 11), presumably due to exogenous contamination. Reports by Buggy et al. (1), Peterson et al. (8), and Kleiman et al. (6) have referred to the intraleukocytic location of some pathogens associated with CAPD peritonitis. Gram stains of CAPD effluent in our experience and that of others rarely show organisms (5, 6, 9, 11), but when present, the organisms appear to be either adherent to or sequestered inside phagocytes, usually granulocytes.

Most episodes of CAPD peritonitis are associated with organisms normally recognized as commensal skin flora and often regarded as contaminants when isolated only from broth cultures of clinical specimens. The pathogenic significance of such isolates is increased if they are recovered from several media in pure cultures and also if phagocytosis can be demonstrated to have occurred before the specimen was handled (6).

This study was conducted to determine if a variety of chemical and physical methods which would disrupt phagocytes but not damage microorganisms might be usefully incorporated in clinical laboratory methods to improve the yield from specimens of CAPD effluent.

A preliminary study of 21 specimens of infected CAPD effluent carried out between October 1984 and August 1985

examined the relative merits of several chemical and physical methods in disrupting phagocytes before culturing. Each specimen was the intact drainage bag collected before antibiotic therapy for CAPD peritonitis was commenced. A further 14 similar specimens of CAPD effluent from patients with peritonitis were examined between August 1985 and August 1986 to compare the separate and combined effects of sonication and culturing on saponin-containing media with direct culturing on conventional blood agar.

In both studies, intact bags of effluent which had been stored at 4°C were examined within 24 h of collection. Aliquots (15 ml) of well-mixed, turbid fluid were aseptically aspirated and labeled fluid samples. Visible clumps of fibrinopurulent material which settled by gravity were seen in 15 specimens in the preliminary study and in 10 specimens in the subsequent study. After suspension of the bag with the injection port downwards for 30 min, 15 ml of this material was aspirated with some fluid and labeled sediment.

Leukocyte counts were determined for fluid samples in an improved Neubauer counting chamber by using phase-contrast microscopy. Leukocyte counts were always >100/mm³, and at least 50% were granulocytes. Gram-stained smears from all fluid samples were examined. The sediment, which consisted of clumps of pus cells and fibrin, could not be dispersed by simply being flushed through a pipette, and after sonication it acquired a uniform, creamy consistency, so cell counts were not determined. Gram stains were performed for some sediment samples.

Physical disruption of phagocytes was achieved by sonicating 15-ml samples of fluid or sediment packed in ice for 15 s with a model B-30 Sonifier (Branson Sonic Power Co., Danbury, Conn.) at 50% pulsed duty cycle and output control 5 (SON treatment) before culturing; this process also had the potential to disperse bacterial aggregates. The standard microtip sonic probe was washed in 95% ethanol for 10 min and allowed to dry before use. Other fluid samples were frozen at −10°C and then thawed at room temperature over a total period of 30 min in the preliminary study only before being cultured on conventional blood agar (F/T treatment).

* Corresponding author.

TABLE 1. Numbers of colonies isolated from 10 drops of fluid and sediment cultured on HBA, LBA, or MAC before and after different treatments in the preliminary study

Organism[a]	No. of colonies/10 25-µl drops of:									
	Fluid cultured:						Sediment cultured:			
	HBA	LBA	MAC	DOC[b]	F/T[b]	SON[b]	HBA	LBA	MAC	SON[b]
Streptococcus faecalis	8	1,000	1,000	—[c]	1,000	—	—	—	—	—
Streptococcus mitis	1	4	0	—	0	—	—	—	—	—
Acinetobacter sp.	1,000	1,000	1,000	1,000	1,000	—	—	—	—	—
Staphylococcus aureus	2,000	2,000	2,000	400	700	—	0	2,000	2,000	—
CNS	3	13	3	7	13	—	8	146	117	—
S. mitis	0	3	0	2	3	—	6	40	0	—
Enterobacter agglomerans	3	5	2	2	2	—	—	—	—	—
Streptococcus pneumoniae	1,000	—	0	200	400	—	—	—	—	—
Acinetobacter sp.	65	—	650	250	416	—	0	0	600	—
Candida parapsilosis	10	48	36	54	60	—	0	1,000	1,000	—
S. mitis	4	29	0	4	5	—	0	33	0	—
CNS	1	15	12	5	7	—	0	44	760	—
CNS	0	5	5	3	5	—	0	2	92	—
S. aureus	49	146	220	538	228	—	57	2,000	2,000	—
CNS	0	2	0	—	—	—	0	8	8	—
CNS	1	36	32	—	—	—	0	47	92	—
CNS	9	19	24	—	—	—	4	316	261	—
C. guilliermondii	115	180	0	—	—	—	—	—	—	—
Klebsiella aerogenes	0	2	0	—	—	—	11	24	57	—
CNS	1	14	10	—	—	47	0	101	190	198
CNS	69	124	107	—	—	387	72	191	166	—

[a] Each entry represents a single specimen (total, 21). CNS, Coagulase-negative staphylococcus.
[b] Cultured on HBA after treatment.
[c] —, The specimen was not examined by the indicated technique.

Phagocytes were chemically disrupted in the preliminary study by the addition of 2 drops of 10% filtered sodium deoxycholate to 15-ml fluid samples kept at room temperature for a further 30 min then cultured on conventional blood agar (DOC treatment). Turbidity rapidly cleared after the addition of deoxycholate.

The solid culture media used in these studies were conventional blood agar (HBA) consisting of Columbia agar base (CM331; Oxoid Ltd., London, England) layered with an equal volume of tryptone soya agar (CM131; Oxoid) containing 7% defibrinated horse blood (CSL, Melbourne, Australia); saponin-lysed blood agar (LBA) containing 0.05% saponin and 9% horse blood (14); and MacConkey agar (MAC) (CM7; Oxoid). All solid media were poured in 22-ml amounts.

Plates were dried and inoculated with 10 discrete 25-µl drops of each sample. Colonies were counted after 24 and 48 h of incubation in 5% CO_2 in air at 37°C or longer if no growth was apparent. Ten milliliters of each fluid specimen was inoculated into 90 ml of thioglycolate broth (Difco Laboratories, Detroit, Mich.), which was subcultured after 7 days of incubation at 37°C or earlier if visible turbidity was observed.

The coagulase activity of staphylococci in 10% human plasma was determined and other bacterial isolates were identified by the methods of Cowan and Steel (2) or by using API test strips (API Systems SA, Montalieu Vercieu, France). Fungi were identified by the Mycology Reference Laboratory, The Royal North Shore Hospital, Sydney, Australia. Statistical comparison of treatment effects was conducted by analysis of variance. Significance between means was determined by the Student-Neuman-Keuls test (13).

Of 21 fluid specimens examined in the preliminary study (Table 1), 11 showed that the colony counts obtained on HBA were lower than those obtained on LBA or MAC or by

treating the specimens with DOC, F/T, or SON before culturing on HBA. Several streptococcal isolates and one Candida guilliermondii isolate failed to grow on MAC; however, for other organisms, there were no significant differences in the numbers of colonies counted on LBA, MAC, or HBA after DOC, F/T, or SON treatment when fluid specimens were examined. No growth was obtained from 4 of 21 fluid and 9 of 15 sediment samples cultured directly on HBA. Only one sediment specimen was obtained from each bag, and the effects of pretreatment with DOC or F/T were not compared with culturing on LBA or MAC. Greater numbers of organisms were recovered from sediment specimens cultured on LBA, MAC, or HBA (after SON treatment) than on HBA alone (Table 1).

Results from the preliminary study favored LBA and SON as the optimal chemical and physical means of examining specimens to increase the yield from CAPD effluent. To confirm these observations, we collected specimens of effluent from 14 separate episodes of CAPD peritonitis from nine patients (three patients had recurrences) between August 1985 and August 1986 to compare SON and LBA with culturing on HBA. Both fluid and sediment samples from 10 specimens were examined. Scanty, intracellular, gram-positive cocci only were seen in nine sediment samples from which staphylococci were subsequently isolated. Even so, the numbers of organisms seen after lengthy microscopic examination of smears were rarely sufficient to be reported with confidence. No growth was obtained from 4 of 14 (29%) fluid and 5 of 10 (50%) sediment samples cultured on HBA prior to SON; however, all samples produced growth after SON. The greatest viable counts from 13 of 14 fluid and 9 of 10 sediment samples were obtained after SON (Table 2). Viable counts from fluid samples were lower than those from comparable sediment samples. Four specimens in particular had <6 colonies of staphylococci per plate from fluid samples (Table 2) but yielded dramatically higher counts from

TABLE 2. Colony counts in 10 drops of fluid and sediment cultured on HBA and LBA before and after SON

Organism[a]	No. of colonies/10 25-μl drops of:							
	Fluid cultured on:				Sediment cultured on:			
	HBA	LBA	HBA after SON	LBA after SON	HBA	LBA	HBA after SON	LBA after SON
CNS	0	10	22	31	0	383	111	5,300
CNS	1	19	85	211	0	21	337	1,800
CNS	2	1	3	3	17	124	950	1,000
CNS	1	1	41	29	1	110	198	201
CNS	3	8	28	23	0	440	830	1,400
CNS	21	21	47	44	21	213	470	440
CNS	0	1	3	3	0	61	128	183
CNS	2	3	4	5	0	9	197	62
CNS	7	14	16	17	9	900	1,050	750
CNS	3	1	2	0	20	113	414	321
Streptococcus faecalis	0	80	51	237	—[b]	—	—	—
Bacillus cereus	1	15	8	15	—	—	—	—
Sporobolomyces salmonicolor	51	82	133	122	—	—	—	—
Proteus sp.	0	12	100	300	—	—	—	—

[a] Each entry represents a single specimen (total, 14). CNS, Coagulase-negative staphylococcus.

[b] —, The specimen was not examined by the indicated technique.

comparable sediment samples, especially following SON (1,000, 183, 197, and 414 colonies per plate). SON sediment samples cultured on LBA yielded significantly higher counts than all other culture systems ($P < 0.05$). No sample was contaminated during SON.

Figure 1 shows the typical growth and the differences in numbers of colonies of coagulase-negative staphylococci obtained from the sediment of a specimen cultured on HBA and LBA without pretreatment and on HBA after SON.

Saponin-containing LBA is one of the standard gonococcal media (12) used in this laboratory and was selected for this study because of the lack in saponin of microbial toxicity (16), a disadvantage of MAC, DOC, Triton X-100, and other surfactants (5, 6, 16). Sonication has been used to disrupt leukocytes and human tissue cells in phagocytosis assays and during viral passage (10, 12). The results given here show that brief sonication of samples containing large amounts of inflammatory exudate increases the yield from
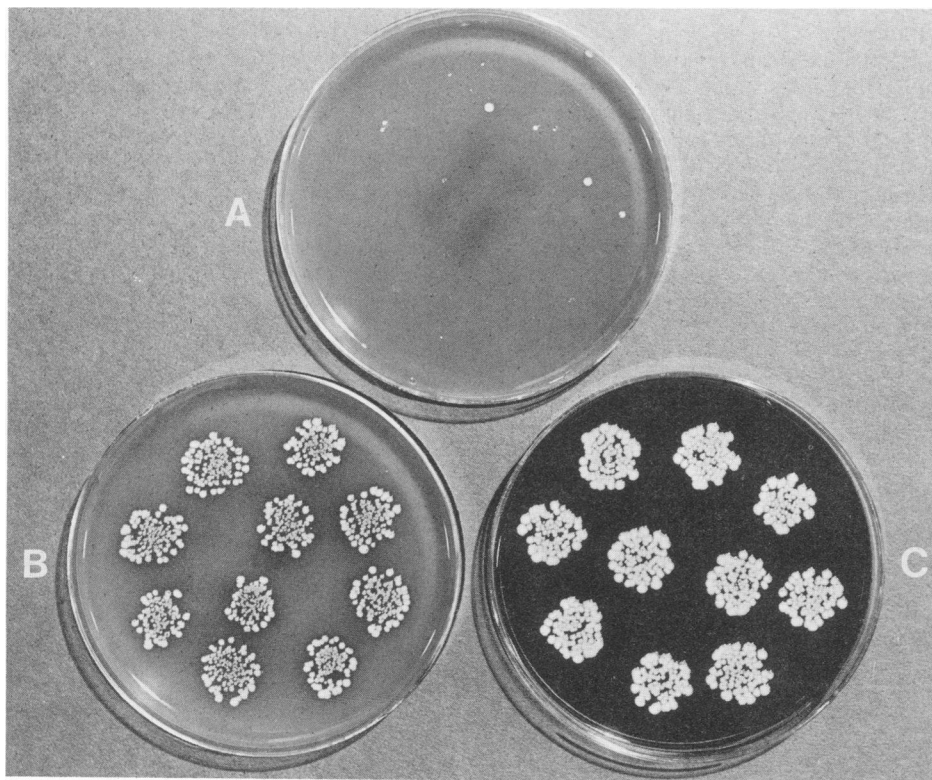

FIG. 1. Colonies of coagulase-negative staphylococci growing from a sample of sediment. Plates A (HBA) and C (LBA) were inoculated with 10 drops of unsonicated sample; plate B (HBA) was inoculated with the same amount of sonicated sample.

CAPD specimens by disrupting phagocytes containing pathogens and possibly by dispersing bacterial aggregates. As most microscopic bacterial aggregates contained less than 10 organisms (usually 2 to 4), their simple dispersion would not account for the much greater differences in viable counts obtained before and after SON when samples were cultured on HBA. Dispersion of aggregates did not play a part in the increased counts from LBA as compared with those from HBA before SON.

It appears that the pathogens of CAPD peritonitis are often adherent to or sequestered in intact leukocytes, aggregated and clumped within fibrin, or both, resulting in falsely low viable counts when conventional media are used. Physical and chemical disruption of phagocytes often greatly increases the microbial yield from samples. Laboratory methods should recognize the low viable counts of pathogens in CAPD specimens, the apparent intracellular location of many organisms, and the diversity of potential isolates. As the sequestered microorganisms appear to be rapidly killed once samples are cultured on conventional media, a mechanism is suggested whereby there is a temporary suspension of the bactericidal powers of phagocytes within the peritoneum of CAPD patients or at least within the effluent drainage bag. Further studies of the nature of this phenomenon are under way in this laboratory; its clinical relevance to the pathogenesis or treatment of CAPD peritonitis has yet to be determined.

In common with others (5), we recommend that intact bags of CAPD effluent from patients with peritonitis be sent to laboratories. When possible, the clumped inflammatory exudate as well as the turbid fluid should be examined by using both physical and chemical methods to disrupt phagocytes before culturing. In our experience, this approach facilitates successful culturing on solid media and more rapid identification of isolates and minimizes the false-positive laboratory diagnoses common with broth culture methods (3, 11).

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