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Lysis-Filtration Blood Culture Versus Conventional Blood Culture in a Bacteremic Rabbit Model

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Thirteen representative pathogenic bacterial species were used to create septicemia in rabbits, by injecting 10^6 colony-forming units into the marginal ear vein. At a selected time, usually 30 to 60 min after injection, heart blood was drawn into heparin and dispensed in 5.0-, 0.5-, and 0.1-ml volumes into duplicate bottles of commercial brain heart infusion broth with sodium polyanetholesulfonate, and into duplicate bottles of a newly developed blood-lysing solution. Lysed blood was filtered, and the filter membranes were cultured in brain heart infusion broth. At the 5.0-ml blood inoculum level, of 126 total culture bottles (63 rabbits) for each system, 83 conventional cultures versus 109 lysis-filtration cultures were positive. At the 0.5-ml blood inoculum, 20 of 126 conventional culture bottles were positive, versus 66 of 126 lysis-filtration cultures. At the 0.1-ml blood inoculum, 2 of 126 conventional culture bottles were positive, versus 30 of 126 lysis-filtration cultures. Overall, 105 of 378 conventional cultures and 205 of 378 lysis-filtration cultures were positive. The advantage of the lysis-filtration system was striking for both gram-positive and gram-negative organisms at all inoculum concentrations, but was greater for gram-positive organisms. Most significant was the rate of recovery by this new system, when the number of bacteria in the blood was reduced to the point where recovery by conventional culture was unlikely. It is postulated that the superiority of lysis-filtration culture may be due to release of bacteria by lysis of phagocytes, preventing continued loss of pathogens by intracellular destruction during the first hours of blood culture.

A previous report (5) favored a blood culture technique in which the blood sample was first treated with a lysing solution and then membrane filtered. The membrane was retrieved and cultured in a medium fortified to replace nutrients usually contributed by the blood. The new system had the advantage of separating bacterial pathogens from all antibacterial properties of the blood, including antibodies, complement, phagocytes, and antibiotics. It was demonstrated that phagocytes were killed during lysis, and intracellular bacteria were released into the lysate. After filtration, erythrocyte stroma, ruptured leukocytes, and bacteria remained on the filter.

Clinical trial of a lysis-filtration blood culture technique supported other experimental evidence of its value (1). To study this phenomenon under more controlled experimental conditions, we used a rabbit model to test the relative efficacy of conventional and of lysis-filtration blood culture techniques in recovering microorganisms from low-level bacteremia. This report is concerned with the results of this study.

MATERIALS AND METHODS

Lysing solution. The lysing solution included Tween 20 (0.7%, vol/vol; Sigma Chemical Co., St. Louis, Mo.) and Rhozyme P-11 (0.25%, wt/vol; Rohm and Haas Co., Philadelphia, Pa.) in 0.01 M sodium phosphate buffer, pH 8.0 (4).

Culture suspensions. Newly isolated bacterial and yeast strains were obtained from the diagnostic laboratory and were subcultured to horse blood agar plates with incubation overnight at 36°C. Microbial suspensions were made in saline to the turbidity of a suspension of Pyrex glass particles, lot 5D, developed for international standardization of *Haemophilus influenzae* vaccine, having 10 opacity units at a light wavelength of 530 nm and 9 opacity units at 420 nm. The turbidity standard was supplied by Margaret Pittman, Bureau of Biologics, Bethesda, Md. The stock suspension had approximately 10^8 colony-forming units per ml. This was diluted 1:100 with saline, and 1.0 ml was injected into the marginal ear vein of a rabbit. The organisms studied were *Staphylococcus epidermidis, S. aureus, Streptococcus pyogenes, S. faecium, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Aeromonas hydrophila, Acinetobacter calcoaceticus, Pasteurella multocida,* and *Candida albicans*. The study was confined to

aerobic microorganisms. Anaerobes are the subject of current studies.

Blood samples. Sampling 30 to 60 min after injection of the organism indicated a very low level of bacteremia. Earlier blood sampling resulted in all culture-positive blood samples, and later sampling resulted in all culture-negative blood samples. The selected sampling time and inoculum size allowed all injected organisms to be phagocytized, and phagocytic killing was well advanced, leaving only the lowest level of detectable bacteremia. Fifteen minutes before cardiac puncture, the rabbit was anesthetized with 100 mg of Ketamine (Vetalar, Parke, Davis & Co.) per kg, given intramuscularly. The skin over the rib cage was clipped and soaked with 70% ethanol, followed by iodine solution (Povidone), followed by 70% ethanol. The skin was allowed to approach dryness between applications. Into a syringe containing 1 ml of heparin (1,000 USP U/ml) was drawn 35 ml of blood. The needle was replaced with another sterile 19-gauge needle.

Culture and lysis. Duplicate 50-ml bottles of brain heart infusion broth with sodium polyanetholesulfonate (SPS; Difco Laboratories) were inoculated with 5.0-, 0.5-, and 0.1-ml volumes of the heparinized blood. Equal blood volumes were inoculated into duplicate 50-ml bottles of blood-lysing solution, which were then placed in a water bath at 37°C for 1 h. The lysed blood samples were vacuum filtered through 0.45-μm, 47-mm-diameter cellulose filter membranes (HAWP 04700; Millipore Corp., Bedford, Mass.), and the membranes were transferred to 50-ml bottles of brain heart infusion broth, with incubation at 36°C. Blood (0.2 ml) was spread on horse blood agar plates.

Incubation and subculture. Culture bottles were examined daily and subcultured to blood agar plates if growth was evident. At 7 days all remaining negative bottles were cultured to blood agar plates.

Rabbits. Female SPF New Zealand white rabbits weighing 2.5 to 3.5 kg were obtained from Dutchland Farms, Denver, Pa.

RESULTS

Because a larger number (32) of bacteremic rabbits were used in culturing for *S. epidermidis*, these are presented separately in Table 1. When 5.0 ml of blood was cultured, the conventional culture was positive in 36 of 64 bottles, and the lysis-filtration culture was positive in 52 of 64 bottles. When 0.5 ml of blood was cultured, the conventional culture was positive in 8 bottles, and the lysis-filtration culture was positive in 31 bottles. When 0.1 ml of blood was cultured, the conventional culture was positive in 0 bottles, and the lysis-filtration culture was positive in 12 bottles. Overall, for the *S. epidermidis* series, 44 of 192 culture bottles were positive by conventional culture, and 95 of 192 were positive by lysis-filtration. The blood agar plates directly spread with 0.2 ml of heart blood were invariably negative.

TABLE 1. Comparison of positive blood cultures for three sample volumes of blood from 32 rabbits with *S. epidermidis* bacteremia by conventional culture and lysis-filtration culture (duplicate cultures)

Blood sample vol (ml)	No. positive/no. of cultures		Ratio of two methods[a]
	Conventional culture	Lysis-filtration culture	
5.0	36/64 (56%)	52/64 (81%)	1.0:1.4
0.5	8/64 (13%)	31/64 (48%)	1.0:3.9
0.1	0/64 (0%)	12/64 (19%)	0.0:12.0
Total	44	95	1.0:2.2

[a] Ratio of positive conventional cultures to positive lysis-filtration cultures.

Table 2 shows the results derived from 31 rabbits inoculated with other gram-positive, as well as gram-negative, organisms. Considering all of these organisms together, when 5.0 ml of blood was cultured, the conventional culture was positive in 47 of 62 bottles, and the lysis-filtration culture was positive in 57 of 62 bottles. When 0.5 ml of blood was cultured, the conventional culture was positive in 12 bottles, and the lysis-filtration culture was positive in 35 bottles. When 0.1 ml of blood was cultured, the conventional culture was positive in 2 bottles, and the lysis-filtration culture was positive in 18 bottles. Thus, as fewer organisms were available for culture, and the sensitivity of the culture system became more critical, lysis-filtration culture was far superior to conventional culture.

Superiority of the lysis-filtration procedure over a conventional bottle technique was more evident for gram-positive bacteria and yeasts, but the advantage was also noted for gram-negative organisms. Summarizing the data for gram-negative organisms, at the 5.0-ml blood inoculum level, 29 of 38 bottles were positive by conventional culture and 34 were positive by lysis-filtration culture. When 0.5 ml of blood was cultured, 8 bottles were positive by conventional culture and 19 were positive by lysis-filtration culture. When 0.1 ml of blood was cultured, 2 bottles were positive by conventional culture, and 10 bottles were positive by lysis-filtration culture.

Combined data for all cultures are presented in Table 3. At the 5.0-ml blood sample level, 66% (of 126 cultures) were positive by conventional culture and 87% were positive by lysis-filtration culture. At the 0.5-ml blood sample level, 16% were positive by conventional and 52% were positive by lysis-filtration culture. At the 0.1-ml blood sample level, 2% were positive by conventional and 24% by lysis-filtration culture. Overall, 27.8% (of 378 cultures) were positive by conventional and 54.2% by lysis-filtration culture.

The results noted in Table 3 are shown as a semilogarithmic plot in Fig. 1. A hypothetical line is drawn for the conventional method, which would suggest that the percentage of

TABLE 2. Comparison of conventional and lysis-filtration culture techniques for blood cultures from bacteremic rabbits inoculated with selected pathogenic bacteria

Organism	Rabbit	Lysis-filtration						Conventional					
		5.0 ml		0.5 ml		0.1 ml		5.0 ml		0.5 ml		0.1 ml	
		Bottle 1	Bottle 2	Bottle 1	Bottle 2	Bottle 1	Bottle 2	Bottle 1	Bottle 2	Bottle 1	Bottle 2	Bottle 1	Bottle 2
S. aureus	1	+	+	+	+	+	−	+	+	−	−	−	−
	2	+	+	+	+	+	+	+	+	+	+	−	−
	3	+	+	+	+	+	+	+	+	−	−	−	−
S. faecium	4	+	+	+	−	−	−	+	+	−	−	−	−
	5	+	+	+	+	−	−	+	+	−	−	−	−
	6	+	+	+	+	+	+	+	+	+	−	−	−
S. pyogenes	7	+	−	−	−	−	−	−	−	−	−	−	−
	8	+	+	+	−	−	−	+	+	−	−	−	−
	9	+	+	−	−	−	−	+	−	−	−	−	−
C. albicans	10	+	+	+	+	+	−	+	+	+	−	−	−
	11	+	+	+	+	−	−	−	−	−	−	−	−
	12	+	+	−	−	−	−	+	−	−	−	−	−
K. pneumoniae	13	+	+	−	−	−	−	+	−	−	−	−	−
	14	+	+	+	−	−	−	+	+	−	−	−	−
	15	+	+	+	+	+	+	+	+	+	+	+	−
	16	+	+	+	+	+	+	+	+	+	−	+	−
E. coli	17	+	+	−	−	−	−	+	−	−	−	−	−
	18	+	−	−	−	−	−	−	−	−	−	−	−
	19	+	+	+	+	+	−	+	+	+	−	−	−
S. typhi	20	+	+	−	−	−	−	+	−	−	−	−	−
A. hydrophila	21	+	+	+	+	+	−	+	+	+	−	−	−
	22	+	−	−	−	−	−	+	−	−	−	−	−
	23	+	−	−	−	−	−	+	−	−	−	−	−
P. aeruginosa	24	+	+	+	+	+	−	+	+	−	−	−	−
	25	+	+	+	+	−	−	+	+	−	−	−	−
	26	+	+	+	+	+	+	+	+	+	+	−	−
P. multocida	27	+	−	−	−	−	−	+	−	−	−	−	−
	28	+	+	+	+	+	−	+	+	−	−	−	−
	29	+	+	+	−	−	−	+	+	−	−	−	−
A. calcoaceticus	30	+	+	−	−	−	−	+	−	−	−	−	−
	31	+	+	+	−	−	−	+	+	+	−	−	−
Total cultures		62		62		62		62		62		62	
Total positive cultures		57		35		18		47		12		2	

positive bottles would increase by 15% if an additional 5 ml of blood were used and by 24% if an additional 10 ml were used.

At the blood volumes studied, and combining data from the two culture methods, increasing the inoculum from 0.1 ml to 0.5 ml, or fivefold, increased the number of positive cultures from 32 to 86, a 169% increase. Increasing the inoculum from 0.5 ml to 5.0 ml, or 10-fold, increased the number of positive cultures from 86 to 192, a 123% increase. Thus, there is a decreasing, but still significant, advantage to incremental increases in sample volume above the smallest sample volume that could yield a positive culture.

DISCUSSION

The probable advantage of the lysis-filtration culture technique in this rabbit model lies in its lysis of phagocytes, with release of intracellular bacteria that otherwise would be killed. Phagocytosis that had occurred in vivo was probably not completely reversed in vitro in the presence of 0.03% SPS. Although SPS-containing media have been shown to yield more positive cultures than media without SPS, the effect is far from absolute. Further phagocytosis may or may not

TABLE 3. Summary data of 378 conventional blood cultures and 378 lysis-filtration blood cultures in the rabbit bacteremia model

Blood sample vol (ml)	Positive cultures/total cultures	
	Conventional	Lysis-filtration
5.0	83/126 (66%)	109/126 (87%)
0.5	20/126 (16%)	66/126 (52%)
0.1	2/126 (2%)	30/126 (24%)
Total	105/378 (27.8%)	205/378 (54.2%)

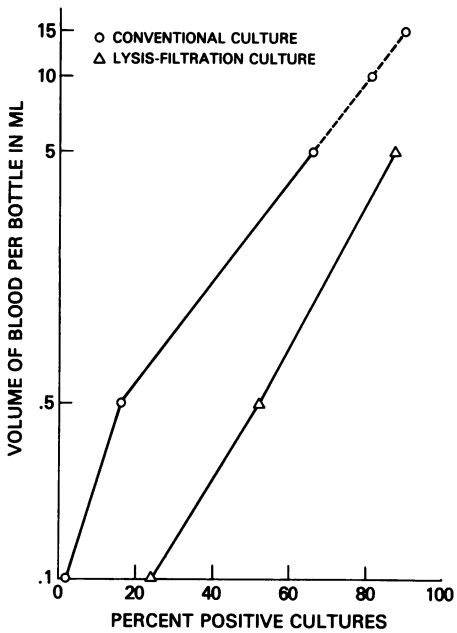

FIG. 1. Rabbit bacteremia. Effect of blood volume on percentage of positive cultures.

be blocked in culture by SPS, but those bacteria already engulfed continue to be destroyed. It is probable that SPS is not totally effective in blocking phagocytosis after culture is made.

It was noted that the increased sensitivity of the lysis-filtration culture became greatest as bacteremia was becoming undetectable. From the data provided, it is evident that the conventional culture may be negative even when microorganisms are present in the blood sample. It is not evident from the data that lysis-filtration culture is positive when only one microorganism is present, but these data do show that, when the level of bacteremia is so low that detection by culture is unlikely because of postculture phagocytic killing, lysis-filtration culture is much more sensitive than conventional culture. It is evident that attempts to quantitate the numbers of bacteria per ml of blood in septicemic patients, such as that of Werner et al. (3), are probably considerable underestimates of the actual magnitude of bacteremia.

Comment can be made about the data in Fig. 1. First, the data for the lysis-filtration technique yield a virtually straight line for the three volumes examined. Extrapolation to 0% of the bottles positive would suggest that 0.025 ml is the minimum volume that could yield detectable positive cultures. Information is not available to state with certainty that this would be 1 colony-forming unit. The straightness of this line may be related to the lack of inhibition from the blood and a very constant amount of nutrient. Second, the reasons for the shape of the conventional culture curve are not clear, but may be related to the decreasing amounts of blood added to the base medium, and hence a relative decrease in certain nutrients. An analogy may be drawn between the animal model presented and blood culture techniques in humans.

Washington has demonstrated convincingly that there is a direct relationship between the volume of blood cultured and the recovery of organisms (2). He showed that an increase in volume from 5 ml to 10 ml increased positive cultures 13% and an increase from 10 ml to 15 ml, an additional 13%, for a total of 26%. In our study, a hypothetical increase from 5 ml to 10 ml increased the yield by 15% and from 10 ml to 15 ml increased the yield 9%, for a total of 24%. This suggests that the rabbit model has validity when compared with the human situation.

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