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Controlled Evaluation of Hypertonic Sucrose Medium at a 1:5 Ratio of Blood to Broth for Detection of Bacteremia and Fungemia in Supplemented Peptone Broth

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The value of hypertonic media in the detection of bacteremia and fungemia is controversial, since prior clinical trials have yielded conflicting results with different media. Earlier, we showed that the addition of 10% sucrose to supplemented peptone broth at a 1:10 ratio of blood to broth yielded better recovery of *Staphylococcus epidermidis*, the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and yeasts. To evaluate the effect of 10% sucrose on blood cultured at a 1:5 ratio, we compared the yield and speed of detection of clinically important microorganisms from adult patients in 5,839 blood samples cultured in supplemented peptone broth with 0.03% sodium polyanetholesulfonate with and without 10% sucrose. The atmosphere of incubation (open venting units), 1:5 ratio of blood to broth, and methods of processing were the same for both bottles. Recovery of facultative gram-positive ($P < 0.02$) and gram-negative ($P < 0.02$) bacteria was improved, but the recovery of anaerobic gram-negative bacteria was both reduced ($P < 0.01$) and delayed ($P < 0.02$) by sucrose. The total yield of microorganisms including fungi, however, was increased with sucrose. The effect of sucrose on blood cultures appears to depend on the ratio of blood to broth as well as on the medium used and strains of microorganisms encountered.

The addition of 10 to 30% sucrose or sorbitol to blood culture media has been advocated to improve recovery of microorganisms, especially cell wall-defective bacteria (4–7, 9, 21–24). However, drawing conclusions from previous studies is difficult, since many included small numbers of isolates or multiple variables known to affect the yield of microorganisms from blood cultures (5, 7, 21–23). Well-controlled trials showed no benefit from 15% sucrose or sorbitol in tryptic soy broth (8, 30), questionable improvement from 10% sucrose in modified Columbia broth (5), and a deleterious effect on the recovery of yeasts from 15% sucrose in brain heart infusion broth (20).

In a previous study that evaluated the effect of added sucrose on the recovery of microorganisms from supplemented peptone broth (SPB), we showed that 10% sucrose in SPB at a 1:10 ratio of blood (5 ml) to broth improved the overall rate of recovery of bacteria ($P < 0.001$), including *Enterobacteriaceae* ($P < 0.05$) and *Bacteroidaceae* ($P < 0.02$), and enabled the detection of staphylococci ($P < 0.001$), *Enterobacteriaceae* ($P < 0.01$), *Pseudomonas aeruginosa* ($P < 0.01$), and yeasts ($P < 0.05$) ≥1 days sooner than did SPB without sucrose (32).

Considered together, these studies suggest that the effect of sucrose is medium dependent (29). To determine whether it also depends on the ratio of blood to broth, we compared SPB with and without 10% sucrose for the detection of bacteremia and fungemia at a 1:5 ratio of blood to broth in a controlled clinical trial.

MATERIALS AND METHODS

Collection of samples. For a 9-month period, a 50-ml bottle of SPB with 0.03% sodium polyanetholesulfonate (SPS) and a 50-ml bottle of SPB-SPS with 10% added sucrose (B D Vacutainer Systems, Rutherford, N.J.) were used for all blood cultures from adult patients at the University of Colorado Hospital and Denver Veterans Administration Medical Center, Denver, Colo. House staff and medical students obtained the cultures at the bedsides of the patients after preparation of the skin with a 10% povidone-iodine solution (1% available iodine), followed by isopropyl alcohol. Blood from each separate venipuncture was distributed by needle and syringe as follows: 10 ml of blood to a bottle with 40 ml of SPB and 10 ml of blood

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to a bottle with 40 ml of SPB with 10% sucrose. Thus, the volume of blood, concentration of SPS anticoagulant, and 1:5 ratio of blood to broth were the same for both culture bottles. The bottles were under vacuum and had been evacuated and back-flushed with 10% carbon dioxide in nitrogen at stoppering in the manufacturing process.

Processing of samples. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at both hospitals. When the paired samples were received, a sterile open venting unit that allowed continuous exchange of air was placed on each bottle (28). Both bottles were incubated in a CO_2 atmosphere at 35°C. Subcultures of both bottles were done through the needle of the open venting unit. Schedules and methods for macroscopic and microscopic examination of bottles and subcultures and identification of microorganisms from positive cultures have been presented in detail previously (27).

Data analysis. Paired comparisons of SPB and hypertonic SPB bottles were done only on adequately filled (≥8 ml of blood) bottles that grew microorganisms causing true bacteremia or fungemia. Criteria for volume standards and clinical assessments have been described previously (27, 31, 33). Significance testing was done with the modified chi-square test described by McNemar (16).

RESULTS

A total of 7,100 blood culture sets were obtained during the study: 5,839 (82.2%) sets had both 50-ml bottles adequately filled, 1,101 (15.5%) sets had one or both bottles inadequately filled, and 160 (2.3%) had one bottle missing. Of 5,839 adequate blood culture sets, 827 (14.2%) were positive, including 578 (9.9%) that grew microorganisms causing illness, 232 (4.0%) that grew contaminants, and 17 (0.3%) that grew at least one pathogen and one contaminant. A total of 679 microorganisms associated with sepsis were isolated from 5,839 adequately filled pairs of 50-ml bottles (Table 1). Of these 679 clinically important microorganisms, 468 (68.9%) grew in both the SPB and hypertonic SPB bottles, and 395 (58.1%) were detected on the same day in both bottles (Table 2).

Aerobic and facultative bacteria ($P < 0.001$) and clinically important microorganisms overall ($P < 0.05$) were recovered more often in the SPB bottle with 10% sucrose (Table 1). No single microorganism, however, was recovered significantly more often in the hypertonic SPB. In contrast, hypertonic SPB yielded fewer ($P < 0.01$) anaerobic gram-negative bacteria.

Supplemented peptone broth with 10% sucrose did not improve the speed of recovery for any group of microorganisms, but in fact delayed ($P < 0.01$) the detection of gram-negative anaerobic bacteria by ≥1 days.

TABLE 1. Comparison of yield of clinically important bacteria and fungi from 10 ml (50-ml bottle) samples of blood cultured in SPB with and without 10% sucrose

Microorganisms	No. of isolates recovered from:			P value
	SPB + SPB-sucrose	SPB only	SPB-sucrose only	
Aerobic and facultative bacteria	392	55	96	<0.001
Gram positive	174	22	41	<0.02
S. aureus	74	7	14	NS[a]
S. epidermidis	14	3	7	NS
Streptococci[b]	81	10	17	NS
Other[c]	5	2	3	NS
Gram negative	218	33	55	<0.02
E. coli	69	8	17	NS
Other *Enterobacteriaceae*	104	14	23	NS
P. aeruginosa	24	3	9	NS
Other[d]	21	8	6	NS
Anaerobic bacteria	58	32	14	<0.01
Gram positive[e]	14	18	10	NS
Gram negative[f]	44	14	4	<0.01
All bacteria	450	87	110	NS
Fungi[g]	18	4	10	NS
All microorganisms	468	91	120	<0.05

[a] NS, $P \geq 0.05$.

[b] Group A streptococci (16), group B streptococci (9), enterococci (18), *Streptococcus pneumoniae* (37), viridans streptococci (11), and other streptococci (17).

[c] *Listeria* spp. (5), *Lactobacillus* spp. (3), and unidentified, gram-positive rods (2).

[d] *Aeromonas* spp. (4), *Acinetobacter* spp. (1), *Pseudomonas maltophilia* (2), *N. gonorrhoeae* (2), *Neisseria meningitidis* (1), *Haemophilus influenzae* (14), *Campylobacter* spp. (1), *Gardnerella* spp. (9), and unidentified gram-negative rods (1).

[e] *Clostridium* spp. (10), *Eubacterium* spp. (1), peptococci (10), peptostreptococci (10), *Propionibacterium* spp. (7), *Actinomyces* spp. (2), *Bifidobacterium* spp. (1), and unidentified, gram-positive cocci (1).

[f] *Bacteroides* spp. (51), *Fusobacterium* spp. (9), and *Veillonella* spp. (2).

[g] *Candida albicans* (15), *Candida tropicalis* (10), *Candida parapsilosis* (3), *Cryptococcus neoformans* (1), and *Torulopsis glabrata* (3).

DISCUSSION

In this controlled evaluation, the addition of 10% sucrose to SPB at a 1:5 ratio of blood (10 ml) to broth resulted in an overall greater recovery of clinically important microorganisms. The beneficial effect, however, was not as pronounced as in an earlier comparison at a 1:10 ratio of blood (5 ml) to broth (32). Moreover, in the present trial, the speed of recovery at the 1:5 ratio was not enhanced by sucrose, in contrast

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi grown from both 10-ml (50-ml bottle) samples of blood cultured in SPB with and without 10% sucrose

Microorganisms	No. of isolates recovered from:			P value
	SPB + SPB-sucrose positive (same time)	SPB positive (>1 day earlier)	SPB-sucrose positive (>1 day earlier)	
Aerobic and facultative bacteria	334	26	32	NS[a]
Gram positive	140	14	20	NS
S. aureus	56	8	10	NS
S. epidermidis	12	0	2	NS
Streptococci[b]	68	5	8	NS
Other[c]	4	1	0	NS
Gram negative	194	12	12	NS
E. coli	64	3	2	NS
Other *Enterobacteriaceae*	94	5	5	NS
P. aeruginosa	21	1	2	NS
Other[d]	15	3	3	NS
Anaerobic bacteria	47	10	1	<0.01
Gram positive[e]	12	2	0	NS
Gram negative[f]	35	8	1	<0.02
All bacteria	381	36	33	NS
Fungi	14	1	3	NS
All microorganisms	395	37	36	NS

[a] NS, $P \geq 0.05$.
[b] Group A streptococci (15), group B streptococci (8), enterococci (8), *Streptococcus pneumoniae* (33), viridans streptococci (9), and other streptococci (8).
[c] *Listeria* spp. (5).
[d] *Aeromonas* spp. (2), *N. gonorrhoeae* (1), *N. meningitidis* (1), *H. influenzae* (11), *Gardnerella* spp. (5), and unidentified, gram-negative rods (1).
[e] *Clostridium* spp. (3), *Eubacterium* spp. (1), peptococci (5), peptostreptococci (4), and *Actinomyces* spp. (1).
[f] *Bacteroides* spp. (35), *Fusobacterium* spp. (8), and *Veillonella* spp. (1).
[g] *C. albicans* (9), *C. tropicalis* (4), *C. parapsilosis* (3), and *Torulopsis glabrata* (2).

to the significantly earlier detection with sucrose in the previous trial at the 1:10 ratio. Recovery of gram-negative anaerobic organisms, in particular the *Bacteroidaceae*, was also markedly different for the two blood-to-broth ratios: at 1:10, sucrose improved recovery, whereas at 1:5, sucrose reduced and delayed recovery.

The effect of the ratio of blood to broth on the yield from blood cultures in general is controversial. A 10- to 20-fold dilution was formerly recommended as being necessary for best results (2) because of the need to overcome the antibacterial activity of human serum. Several investigators, however, have suggested that lower dilutions would be acceptable in media containing SPS. In artificial cultures, Lowrance and Traub found inactivation of the bactericidal effect of 50% human serum with 0.025% SPS (13), but they tested simulated cultures with only four bacterial strains. Salventi et al. recovered similar numbers of pathogenic microorganisms in directly compared clinical blood cultures in dilutions from 1:4 to 1:30, but their study was too small for adequate statistical analysis (25). Auckenthaler et al., in a controlled trial comparing a 1:5 to 1:10 ratio in tryptic soy broth with SPS, found better yield at a 1:10 ratio for gram-negative bacilli, *Staphylococcus aureus*, enterococci, and *Candida* spp.; better yield at the 1:5 ratio for *Streptococcus pneumoniae*; and an overall earlier detection of microorganisms at the 1:10 ratio (1). In contrast, in a controlled clinical trial at our own hospitals, we found similar recovery of organisms at a 1:5 and 1:10 ratio of blood to broth with SPB-SPS, except staphylococci were not always recovered at the 1:5 ratio when patients were receiving antibiotics. (L. B. Reller, K. A. Lichtenstein, S. Mirrett, and W.-L. L. Wang, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C177, p. 306).

Our different results for SPB-SPS with and without 10% sucrose at the two ratios of blood to

broth need not be explained solely by the different ratios of blood to broth, since the comparisons were not done on simultaneously obtained blood cultures from the same patients with recovery of the same strains of microorganisms. Moreover, the 10-ml volume of blood cultured at a 1:5 ratio in each medium in this study would minimize the differences seen earlier with 5 ml at a 1:10 ratio, owing to the effect of volume itself (27).

Comparison of our findings in the present study with those of other trials of hypertonic media are also difficult but nonetheless important. In a radiometric system, 10% sucrose in tryptic soy broth improved recovery of streptococci and *Staphylococcus aureus* (4). In conventional systems, 15% sucrose in tryptic soy broth inhibited recovery of *Haemophilus* spp., *Staphylococcus aureus*, and *Bacteroidaceae* (30), whereas the effect of 15% sorbitol in tryptic soy broth was similar to that of tryptic soy broth alone (8). Brain heart infusion broth with gelatin and 20% sucrose enabled the recovery of more staphylococci and *Enterobacteriaceae* sooner than did brain heart infusion broth alone (5). In another study, brain heart infusion broth with 15% sucrose decreased the yield of fungi (12). Brucella broth enabled the recovery of similar numbers of microorganisms with and without 30% sucrose (21).

These studies suggest that differences in the value of added sucrose or sorbitol are partially explained by the medium used. In our two studies with SPB, 10% sucrose improved the overall recovery of microorganisms but, in our earlier study, was detrimental (32) to the recovery of *Neisseria gonorrhoeae* and, in the present study, to *Bacteroides* spp.

Why sucrose should be beneficial in some media or some ratios of blood to broth is unclear. One explanation is that sucrose protects cell wall-defective bacteria from osmotic lysis (14, 15, 30), but evidence for a pathogenic role of these bacteria is lacking (11, 19). Hypertonic media have been helpful in a small number of cases in recovering bacteria from patients receiving cell wall-active antimicrobial agents (12). In simulated blood cultures, hypertonic media have been shown to protect bacteria from the action of penicillin (10). Another possible explanation is that antimicrobial agents, particularly penicillins, are inactivated by sucrose (26), but the presence of penicillinase in our SPB and in the brain heart infusion broth used in previous studies makes the possibility of direct inactivation of antibiotics less likely. Sucrose inhibits serum bactericidal activity through an anticomplementary effect (18), but all the media studied with or without sucrose have also contained SPS, which inactivates complement (30). Finally, sucrose may provide an important nutrient for bacterial growth, although improved recovery rates have not been limited to or associated with bacteria known to utilize sucrose.

In contrast, added sucrose may be harmful in some situations. Studies already cited have found reduced recovery of *N. gonorrhoeae*, *Bacteroides* and *Haemophilus* spp., *Staphylococcus aureus*, and fungi in sucrose-containing media, and an additional study showed delayed growth of *Salmonella typhi* in brain heart infusion broth or thioglycolate broth containing 15 to 30% sucrose (3). In hypertonic media, erythrocytes lyse within 72 h, the broth thickens, and macroscopic examination of culture bottles and microscopic examination by Gram stain becomes difficult or impossible (6, 23, 34), although microscopic examination with acridine orange staining may still be adequate (17). Hypertonic sucrose media also shorten the survival of bacteria and make early, routine subcultures essential (24).

In summary, the addition of 10% sucrose to SPB at either a 1:10 or a 1:5 ratio of blood to broth appears to be beneficial. However, the benefit of sucrose is likely to be dependent on the medium employed, the bacteria strains encountered, and possibly, the volume of blood cultured. A hypertonic medium, regardless of the broth or ratio of blood to broth, should not be used as the only medium for detection of bacteremia and fungemia, since some specific microorganisms may be missed even though overall recovery may be enhanced.

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