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Evaluation of Blood Culture Media Supplemented with Sucrose or with Cysteine[1]

JOHN A. WASHINGTON II, M. MARSHA HALL, AND EDWARD WARREN

Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

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A total of 5,883 blood samples from patients with suspected bacteremia were inoculated concurrently into each of three media under vacuum with CO_2: tryptic soy broth (TSB) with sodium polyanetholesulfonate (SPS), TSB with SPS and cysteine, and TSB with SPS and sucrose. There were 395 positive cultures, excluding presumed contaminants. No significant differences were noted with the addition of cysteine to TSB with SPS, and no streptococcal mutants requiring thiol groups were isolated. *Haemophilus*, *Staphylococcus aureus*, and bacteriodaceae were isolated more frequently ($P < 0.05$) in the absence of sucrose. The addition of sucrose to TSB containing SPS did not significantly increase the rate of positivity or the time interval to detection of positivity of any group of bacteria.

Studies by Rosner (9), Henrichsen and Bruun (6), L. R. McCarthy and P. D. Ellner (Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, M48, p. 81), and Sullivan et al. (10) have shown increased recovery of bacteria from media supplemented with sucrose. Because of methodologic differences in these studies, it was decided to compare tryptic soy broth (TSB) containing sodium polyanetholesulfonate (SPS) with TSB containing SPS and 15% sucrose. Concurrently, it was decided to evaluate TSB containing SPS and cysteine to ascertain whether or not streptococcal mutants requiring thiol groups (1, 4, 7) could be isolated from blood cultures.

MATERIALS AND METHODS

Blood was collected with a sterile syringe and needle, from patients suspected of having bacteremia, by members of a venipuncture team. The intended venipuncture site on the skin was vigorously swabbed concentrically with povidone-iodine (Swabsticks, Purdue Frederick Co.). Blood samples were inoculated (10% [vol/vol]) into one bottle each of 100 ml of TSB (Difco) containing 0.025% SPS, 100 ml of TSB containing 0.025% SPS and 0.05% cysteine, and 100 ml of TSB containing 0.025% SPS and 15% sucrose. All three media were bottled by the manufacturer under vacuum with 10% CO_2, and none was vented during incubation. Cultures were incubated at 35 C and were inspected daily for 7 days; they were then reincubated for an additional 7 days, inspected again, and discarded if macroscopically negative.

All units without visible evidence of growth were subcultured within 24 h and after 5 days of blood collection by sampling with a sterile syringe and needle through the stopper, inoculation of quadrants of chocolate blood agar plates, and incubation in 10% CO_2 for 48 h. Media with macroscopic or microscopic evidence of growth were subcultured aerobically and anaerobically to isolate and identify any bacteria present.

Methods of statistical analysis were those previously described (11) and are based on those described by Cochran (2). To facilitate analysis, TSB containing SPS was compared separately with TSB containing SPS and sucrose, and with TSB containing SPS and cysteine. In a few instances, one of the media with added sucrose or cysteine was not inoculated because an insufficient amount of blood was obtained.

RESULTS

Between 26 January 1974 and 30 April 1974, 5,883 blood specimens were received. There were 567 (9.6%) positive cultures, of which 172 represented single cultures containing *Bacillus*, *Corynebacterium* (including *Propionibacterium*), or *Staphylococcus epidermidis*. The adjusted positive rate, excluding these presumed contaminants, was 6.7%.

The numbers of isolates in positive cultures in TSB containing SPS with and without added sucrose are listed in Table 1. *Bacillus* was isolated more frequently ($P < 0.01$) from this medium with sucrose, representing 19.5% of the isolates in this medium. *Haemophilus*, *S. aureus*, and the bacteroidaceae were isolated more frequently ($P < 0.05$) from the medium without sucrose; otherwise, there were no significant differences in rates of positivity between these two media. The time intervals to detection of positivity were significantly shorter (by analysis by the delta t test) in TSB containing SPS only

[1] All correspondence regarding this paper should be addressed to: Section of publications, Mayo Clinic, Rochester, Minn. 55901.

TABLE 1. *Numbers of isolates in positive cultures by medium*[a]

Organism	Both media	TSB with SPS only	TSB with SPS and sucrose only	*P*
Bacillus	1	7	23	<0.01
Clostridium	0	2	0	NS
Corynebacterium	17	32	37	NS
Escherichia	37	7	13	NS
Klebsiella	13	4	4	NS
Enterobacter	2	1	0	NS
Serratia	7	0	2	NS
Proteus	13	3	3	NS
Cardiobacterium	4	3	1	NS
Haemophilus	3	7	0	<0.05
Streptococcus				
S. pneumoniae	9	1	0	NS
Viridans group	22	5	5	NS
Group A	5	4	0	NS
Group D	6	1	3	NS
Other groups	0	0	1	NS
Alcaligenes	0	3	9	NS
Neisseria	0	1	0	NS
Bacteroidaceae	13	7	0	<0.05
Staphylococcus				
S. aureus	36	13	3	<0.05
S. epidermidis	13	22	11	NS
Peptostreptococcus	0	0	1	NS
Veillonella	0	1	0	NS
Pseudomonas	14	5	2	NS

[a] NS, Not significant.

for viridans streptococci ($P < 0.02$), Bacteroidaceae ($P < 0.05$), *S. aureus* ($P < 0.001$), and *S. epidermidis* ($P < 0.05$); however, these differences, on the average, were between 0.85 and 1.54 days, which is small from a practical standpoint. Otherwise, the time intervals to detection of positivity closely resembled those reported in a previous study (5).

The numbers of isolates in positive cultures in TSB containing SPS and in TSB containing SPS and cysteine are listed in Table 2. There were no significant differences in isolation rates between the two media, and there were no streptococci isolated from the medium with added cysteine that could not be readily subcultured to sheep blood agar plates incubated at 35 C in air or in 10% CO_2.

The medium with added sucrose was generally more difficult to examine because of the degree of hemolysis that occurred within 48 h after its inoculation with blood. Therefore, this medium was more frequently tested by Gram stain or subculture, to rule out the presence of microorganisms, than were the other two media.

DISCUSSION

Previous studies (6, 9, 10; McCarthy and Ellner, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, M48, p. 81) indicated that liquid media supplemented with hypertonic sucrose were superior to media without sucrose. Rosner (9) studied flasks of brucella broth in parallel with two flasks of the same broth supplemented with SPS alone and in combination with 30% sucrose; 74, 106, and 121 organisms, respectively, were recovered from these three media. The value of SPS alone was notable. The organisms isolated more frequently from brucella broth containing SPS and sucrose were bacteroidaceae, anaerobic streptococci, meningococci, *Haemophilus* spp., and *Pasteurella*-like organisms; however, the total numbers of each of these groups of organisms were quite small and preclude statistical analysis.

In the study reported by Henrichsen and Bruun (6), 8 ml of blood was transported to the laboratory in an evacuated tube containing SPS and subsequently inoculated into 12 culture tubes, four of which contained nutrient broth, four of which contained semisolid nutrient agar, and four of which contained semisolid thio-

TABLE 2. *Numbers of isolates in positive cultures by medium*[a]

Organism	Both media	TSB with SPS only	TSB with SPS and cysteine only	*P*
Bacillus	1	7	1	NS
Clostridium	0	2	0	NS
Corynebacterium	14	35	29	NS
Escherichia	37	7	10	NS
Klebsiella	10	7	7	NS
Enterobacter	1	2	0	NS
Serratia	6	1	1	NS
Proteus	14	2	2	NS
Cardiobacterium	4	3	0	NS
Haemophilus	9	1	0	NS
Streptococcus				
S. pneumoniae	8	2	0	NS
Viridans group	24	3	4	NS
Group A	7	2	0	NS
Group D	5	2	4	NS
Alcaligenes	0	3	0	NS
Neisseria	0	1	0	NS
Bacteroidaceae	16	4	1	NS
Staphylococcus				
S. aureus	38	11	3	NS
S. epidermidis	15	20	16	NS
Veillonella	1	0	0	NS
Pseudomonas	17	2	2	NS

[a] NS, Not significant.

glycollate agar; two of each set of four tubes were supplemented with 10% sucrose. In 90 samples, enterobacteriaceae were recovered more frequently in the media with sucrose; the reverse occurred in only 57 samples ($P < 0.01$). A similar and significant ($P < 0.05$) trend occurred with *S. aureus*.

McCarthy and Ellner (Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, M48, p. 81) recovered 12% of their positive cultures sooner in Columbia broth with 10% sucrose than in the same broth without sucrose. Moreover, they detected 55 of 337 isolates in the hypertonic medium only. It is assumed that the blood was collected into and transported to the laboratory in an evacuated tube containing SPS (3). Sullivan et al. (10) recovered 20 isolates, of which eight were anaerobes and two were protoplasts of *Propionibacterium acne*, from an osmotically stabilized anaerobic broth containing SPS; neither of the two other liquid media concurrently inoculated with blood contained SPS.

In this study the same tryptic digest of casein soy broth base containing 0.025% SPS was used throughout. All media were bottled under vacuum with CO_2, were inoculated at the bedside on a 10% (vol/vol) basis, and remained unvented during their period of incubation. One might speculate about whether or not the differences between our results and those reported by Henrichsen and Bruun (6) and by McCarthy and Ellner (Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, M48, p. 81) might be attributable, at least in part, to the differences in specimen handling.

The assumption that growth of classic or typical bacteria in hypertonic media but not in isotonic media represents isolation of cell wall-defective bacterial variants may not be valid, because the addition of sucrose to stabilize the medium may simply inactivate any penicillins present in the medium and enable the classic bacteria to grow (8). The significance of isolation of classic bacteria from blood inoculated into hypertonic media requires further scrutiny.

The trial of a medium with added cysteine was unrevealing. The negative results may indicate that the blood of patients with streptococcal mutants requiring thiol groups was not cultured during the period of observation, that these mutants are rarely encountered, or that the media used were satisfactory for their isolation.

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