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Critical Analysis of Hypertonic Medium and Agitation in Detection of Bacteremia

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Over 18,000 clinical specimens collected in Vacutainer tubes with sodium polyanethol sulfonate were inoculated into modified Columbia broth (MCB) with and without 10% sucrose. The effects of venting and shaking on recovery were studied. The volume of the blood had a definite effect on the recovery rate. When inoculum size was held constant, recovery of aerobic and facultative organisms was maximal in vented and shaken bottles; the presence of sucrose had no demonstrable effect. Recovery of anaerobes was maximal using an unvented bottle incubated under stationary conditions; a significantly greater recovery of facultatives and a marginally greater recovery of anaerobes was obtained with the hypertonic formulation. We conclude that a hypertonic formulation of MCB offers no advantage in the recovery of anaerobes but is of value in the recovery of facultatives and anaerobes. It is recommended that blood cultures be routinely inoculated into isotonic MCB and then vented and shaken for at least 4 hours, and hypertonic MCB incubated without venting or shaking.

Several investigators (3, 11, 12, 14, 18, 19) have reported the isolation of cell wall-deficient forms of bacteria and fungi from the blood of patients with septicemia or endocarditis using hypertonic media after conventional blood cultures in isotonic broth revealed no growth. Other studies have claimed that increased numbers of bacterial isolates were obtained from blood (10, 13, 17, 20, 21, 25) and body fluids (24) when a hypertonic broth medium was routinely inoculated along with conventional broth. In many of these studies the variation in basal media formulation, inoculum, or other variables make it difficult to clearly ascribe the apparent increased efficiency of recovery to the osmotic stabilizer.

A preliminary study of 3,500 blood cultures (L. R. McCarthy and P. D. Ellner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, M48, p. 81) appeared to confirm the value of hypertonic media; it was recognized, however, that the conclusions were ambiguous.

The present study was undertaken to critically evaluate the role of osmotically stabilized media in the culturing of blood under controlled conditions.

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MATERIALS AND METHODS

Blood specimens for culture were obtained from patients admitted to the Columbia-Presbyterian Medical Center during the period 1972 to 1976. Skin preparation was either 2% tincture of iodine or povidone-iodine. Blood was drawn into sterile Vacutainer tubes (165 by 16 mm) containing approximately 3.2 ml of 0.35% sodium polyanethol sulfonate. Upon arrival at the laboratory, the blood was inoculated into Vacutainer bottles (Bioquest) (5) containing 50 ml of Columbia broth (15), modified by an increased cysteine concentration to 0.05% (MCB) plus 10% CO_2 as previously described (2). The hypertonic (HT) formulation of this medium used throughout this study contained 10% sucrose (MCB-HT) plus 10% CO_2. Aerobic bottles were vented by insertion of a double-ended needle through the stopper for several seconds. Anaerobic bottles were not vented. Shaken bottles were agitated on a table-top incubator shaker (New Brunswick Scientific Co.) at 150 rpm.

When the requisition accompanying the specimen indicated that the patient had received a penicillin or cephalosporin, penicillinase was added to all bottles.

All bottles were incubated at 35°C. Bottles were routinely subcultured at 24 h and at 5 days (experiments 1 through 5) or when becoming turbid. (In experiment 6, aerobic bottles were subcultured at 10 h and anaerobic bottles were cultured at 16 h rather than at 24 h.) Aerobic bottles were subcultured to sheep blood agar, chocolate agar, and, when appropriate, eosin-methylene blue agar. All plates except eosin-methylene blue were incubated for 48 h in 10%

CO_2. Anaerobic bottles were subcultured to anaerobic blood agar (6) and incubated for 48 h in GasPak (Bioquest) anaerobe jars. Rapid presumptive identification of isolates from turbid bottles was routinely performed whenever possible (29). All organisms were definitively identified by conventional procedures.

A series of six independent experiments was conducted. Experimental design was constrained by the necessity for maintaining minimum satisfactory cultural conditions for a variety of pathogens. Consequently, an anaerobic bottle was included in each experiment.

Experimental design. Experiment 1 was designed to determine whether the addition of a third bottle containing hypertonic medium would result in increased recovery. Approximately 5 ml of blood was inoculated into each of three bottles: MCB-aerobic, MCB-anaerobic, and MCB-HT-aerobic. All bottles were incubated under stationary conditions.

Experiment 2 was designed to compare the relative efficiency of isotonic and hypertonic formulations for the recovery of anaerobes. Approximately 5 ml of blood was inoculated into each of three bottles: MCB-aerobic, MCB-anaerobic, and MCB-HT-anaerobic. All bottles were incubated under stationary conditions.

Experiment 3 was designed to determine whether the results obtained in experiment 1 were due to the hypertonic medium or just to a third bottle. Approximately 5 ml of blood was inoculated into each of three bottles: two MCB-aerobic and an MCB-HT-anaerobic. All bottles were incubated under stationary conditions.

Although experiment 4 was originally designed to study the detection of bacteremia by the evolution of labeled carbon dioxide, it was possible to compare isotonic and hypertonic media under aerobic agitated conditions. Approximately 3 ml of blood was inoculated into each of three bottles. These bottles contained 30 ml of MCB or MCB-HT and a ^{14}C-labeled substrate. The MCB-aerobic bottle was stirred on a BACTEC 225 machine, the MCB-HT was agitated as described above, and the anaerobic bottle was incubated under stationary conditions. The details of this experiment have been previously described (2).

Experiment 5 was designed to test the effect of agitation of isotonic media. Approximately 5 ml of blood was inoculated into each of three bottles: MCB-aerobic (shaken), MCB-aerobic (stationary), and MCB-HT-anaerobic (stationary).

Experiment 6 was designed to compare an "optimal" two-bottle system with the three-bottle systems described in experiments 1 through 5. The volume of blood in the Vacutainer tube was measured and recorded before inoculation of the bottles. Approximately 8 ml of blood was inoculated into each of two bottles: an MCB-aerobic (shaken) and an MCB-HT-anaerobic (stationary). Aerobic bottles were shaken for 4 to 18 h (average, 10 h) and then subcultured to chocolate agar. The anaerobic bottles were incubated from 4 to 28 h (average, 16 h) and subcultured to anaerobic blood agar. After the first subculture, aerobic bottles were removed from the shaker and incubation was continued under stationary conditions. A second subculture was made on both bottles on the 5th day.

Statistical analysis. Experiments 1 through 5 were first evaluated with the Cochran Q test (4) to determine whether significant differences existed within each experiment. To evaluate the effect of a variable such as tonicity, agitation, or anaerobiosis, the sign test was applied. Chi-square values were calculated for each variable. Isolates occurring in combinations of bottles that simultaneously involved both aspects of the variable being evaluated were excluded, as were those from bottles unrelated to the variable. For the purpose of these comparisons, values greater than 3.84 (chi-square for 95% confidence limit with one degree of freedom) were considered significant.

Since each experiment was an independent entity involving different patients, statistical analysis could only be applied within that experiment.

RESULTS

The Cochran Q test was positive for experiments 1 through 5, indicating that differences existed within each experiment.

Experiment 1. Six thousand specimens were cultured. The positive isolates recovered in each of the three bottles are shown in Table 1. Also shown are the numbers of isolates recovered in isotonic and hypertonic sets of bottles that were used for statistical analysis. It can be seen that with the exception of *Pseudomonas* and *Propionibacterium acnes*, there was no difference in isolation rates between isotonic and HT media. In the case of *Pseudomonas*, isotonic medium was markedly superior to HT medium. In contrast, *P. acnes* was isolated with significantly greater frequency in HT medium.

Experiment 2. A total of 2,137 specimens were cultured. The positive isolates recovered in each of the three bottles are shown in Table 2. The isolates recovered in anaerobic isotonic and anaerobic hypertonic sets of bottles that were used for statistical analysis are also shown. No differences in the recovery of anaerobes were observed between isotonic and hypertonic media. The results also show that under anaerobic conditions, *Streptococcus pneumoniae* was isolated with greater frequency in isotonic medium and *Klebsiella* was isolated with greater frequency in the hypertonic medium. The total number of facultative organisms recovered was also greater in the hypertonic medium.

Experiment 3. A total of 3,580 specimens were cultured. There was little difference in the rates of recovery of aerobes or anaerobes when duplicate isotonic bottles were substituted for an isotonic and a hypertonic combination (Ta-

TABLE 1. *Comparison of isotonic and hypertonic media under aerobic stationary conditions; isolates by bottle*

Organism	Sets excluded from sign test comparison[a]			Isotonic sets		Hypertonic sets		Chi-square value
	All bottles	MCB-aer, MCB-HT-aer	MCB-anaer only	MCB-aer only	MCB-aer, MCB-anaer	MCB-HT-aer only	MCB-HT-aer, MCB-anaer	
Pseudomonas sp.	15	7	1	5	2	1	0	4.50[b]
Other aerobes[c]	6	13	2	17	0	14	0	0.29
Total aerobes	21	20	3	22	2	15	0	2.08
Gram-positive cocci	152	20	18	46	25	42	11	2.61
Gram-negative bacilli	87	12	9	14	10	22	8	0.66
Other facultatives[c]	9	3	2	5	0	8	2	1.66
Total facultatives	248	35	29	65	35	72	21	0.25
P. acnes	0	0	9	0	1	7	1	5.44[b]
Bacteroides sp.	9	1	9	0	4	4	1	0.11
Other anaerobes[c]	5	1	10	3	0	4	0	0.14
Total anaerobes	14	2	28	3	5	15	2	3.24
Total organisms	283	57	60	90	40	102	23	0.10

[a] Aer, aerobic; anaer, anaerobic.
[b] Significant.
[c] See Table 8.

TABLE 2. *Comparison of isotonic and hypertonic media under anaerobic stationary conditions; isolates by bottle*

Organism	Sets excluded from sign test comparison[a]			Isotonic sets		Hypertonic sets		Chi-square value
	All bottles	MCB-anaer, MCB-HT-anaer	MCB-aer only	MCB-anaer only	MCB-anaer, MCB-aer	MCB-HT-anaer only	MCB-HT-anaer, MCB-aer	
Total aerobes	3	0	9	0	0	1	1	
Streptococcus pneumoniae	2	0	0	1	3	0	0	4.00[b]
Other gram-positive cocci	53	2	15	8	1	15	4	3.57
Klebsiella pneumoniae	19	0	1	0	0	4	3	7.00[b]
Other gram-negative bacilli	33	2	13	5	2	7	3	0.52
Other facultatives[c]	1	0	1	1	1	3	0	0.20
Total facultatives	108	4	30	15	7	29	10	4.73[b]
Bacteroides sp.	5	3	1	3	0	4	0	0.14
Other anaerobes[c]	4	2	1	2	3	6	1	0.33
Total anaerobes	9	5	2	5	3	10	1	0.47
Total organisms	120	9	41	20	10	40	12	3.95[b]

[a]–[c] See Table 1.

ble 3). Isolates recovered solely in the anaerobic bottles were not considered in this comparison. The isotonic-hypertonic combination resulted in an apparent increase in the recovery of facultative organisms over the duplicate isotonic combination.

Experiment 4. One thousand specimens were cultured. The positive isolates recovered in each of the three bottles are shown in Table 4. Also shown are the isolates recovered in stirred isotonic and shaken hypertonic sets of bottles that were used for statistical analysis. The only difference observed was the greater isolation of *Bacillus* sp. in the shaken hypertonic bottles. Although not statistically significant, it should be noted that the two isolates of *Pseudomonas*

that occurred in comparable sets were recovered from isotonic medium.

Experiment 5. A total of 2,347 specimens were cultured. The positive isolates recovered in each of the three bottles used for statistical analysis are shown in Table 5. The isolates recovered in shaken isotonic and stationary isotonic bottles that were used for statistical analysis are also shown. The recovery of aerobes was markedly greater in the shaken bottle. The recovery of *Escherichia coli* was also enhanced by agitation. The overall recovery of bacteria was greater in the shaken bottle.

TABLE 3. *Comparison of isolation rates: duplicate isotonic versus isotonic-hypertonic bottles*

Organism	Rates/1,000 cultures	
	Duplicate isotonic bottles (expt 3, 3,580 cultures)	Combination of HT and isotonic (expt 1, 6,000 cultures)
Pseudomonas sp.	4.5	5.0
Other aerobes[a]	7.8	8.3
Total aerobes	12.3	13.3
Gram-positive cocci	36.0	49.3
Gram-negative bacilli	27.9	24.6
Other facultatives[a]	2.2	5.5
Total facultatives	66.2	79.3
Bacteroides sp.	1.7	3.1
Other anaerobes[a]	3.4	3.7
Total anaerobes	5.1	6.8
Total organisms	83.6	99.4

[a] See Table 8.

Experiment 6. A total of 3,016 specimens were cultured. The positive isolates recovered from both bottles are shown in Table 6. The numbers and types of organisms isolated approximated those obtained with the three-bottle systems of experiments 1 through 5. The recovery rates of bacteria obtained with this two-bottle system were compared with the recovery rates obtained in experiments 1 through 5 (Table 7). There was no significant change in the recovery rate of aerobes, facultatives, or anaerobes when a two-bottle system was substituted for a three-bottle system. The volumes of blood inoculated ranged from 3 to 36 ml (mean, 16.4 ± 3.4 standard deviations). Preliminary results suggest that specimens receiving greater amounts of blood resulted in higher recovery rates. The greatest recovery rates were obtained with specimens of approximately 20 ml, i.e., 10 ml per bottle.

A detailed listing of the isolates recovered in each of the six experiments is shown in Table 8. The percentages of positive cultures, contaminants, and polymicrobic cultures obtained in this study are summarized in Table 9.

TABLE 4. *Comparison of isotonic and hypertonic media under aerobic agitated conditions; isolates by bottle*

Organism	Sets excluded from sign test comparison[a]			Isotonic stirred sets		Hypertonic shaken sets		Chi-square value
	All bottles	MCB-aer,[b] MCB-HT-aer[c]	MCB-anaer only	MCB-aer[b] only	MCB-aer,[b] MCB-anaer	MCB-HT-aer[c] only	MCB-HT-aer,[c] MCB-anaer	
Pseudomonas sp.	3	4	0	1	1	0	0	
Bacillus sp.	0	0	3	1	0	7	0	4.5[d]
Other aerobes[e]	0	0	0	0	0	2	0	
Total aerobes	3	4	3	2	1	9	0	3.0
Gram-positive cocci	19	7	2	7	0	9	0	0.25
Gram-negative bacilli	19	3	0	5	0	1	0	2.7
Other facultatives[e]	1	1	0	1	0	1	0	
Total facultatives	39	11	2	13	0	11	0	0.16
Bacteroides sp.	0	0	4	0	0	0	0	
Other anaerobes[e]	1	0	0	0	0	1	0	
Total anaerobes	1	0	4	0	0	1	0	
Total organisms	43	15	9	15	1	21	0	0.67

[a] Aer, aerobic; anaer, anaerobic.
[b] Stirred.
[c] Shaken.
[d] Significant.
[e] See Table 8.

TABLE 5. *Comparison of effects of agitation with aerobic isotonic medium; isolates by bottle*

Organism	Sets excluded from sign test comparison[a]			Isotonic shaken sets		Isotonic stationary sets		Chi-square value
	All bottles	MCB-aer,[b] MCB-aer	MCB-HT-anaer only	MCB-aer only	MCB-aer, MCB-HT-anaer	MCB-aer only	MCB-aer, MCB-HT-anaer	
Pseudomonas sp.	5	1	0	3	1	2	0	0.66
Yeast	1	7	0	3	1	0	0	4.00[c]
Other aerobes[d]	0	4	1	6	0	2	0	2.00
Total aerobes	6	12	1	12	2	4	0	5.56[c]
Gram-positive cocci	40	13	12	23	3	16	5	0.09
Escherichia coli	23	6	4	8	5	3	1	4.80[c]
Other gram-negative bacilli	22	1	0	5	0	1	1	1.29
Other facultatives[d]	0	1	0	1	0	4	0	1.80
Total facultatives	85	21	16	37	8	24	7	2.58
Bacteroides sp.	0	0	6	2	0	1	2	0.20
Other anaerobes[d]	1	1	6	1	0	3	0	1.00
Total anaerobes	1	1	12	3	0	4	2	1.00
Total organisms	92	34	29	52	10	32	9	4.28[c]

[a] Aer, aerobic; anaer, anaerobic.
[b] Shaken.
[c] Significant.
[d] See Table 8.

TABLE 6. *Recovery of bacteria with an "optimal" two-bottle system; isolates by bottle*

Organism	Both bottles	MCB-aerobic shaken only	MCB-HT anaerobic stationary only
Pseudomonas sp.	6	8	3
Yeast	0	6	1
Other aerobes[a]	6	3	0
Total aerobes	12	17	4
Gram-positive cocci	98	47	13
Gram-negative bacilli	61	23	8
Other facultatives[a]	3	5	0
Total facultatives	162	75	21
Bacteroides sp.	1	0	13
Other anaerobes[a]	2	4	16
Total anaerobes	3	4	29
Total organisms	177	96	54

[a] See Table 8.

TABLE 7. *Recovery rates per 1,000 cultures of experiments 1 through 6*

Expt	Aerobes	Facultatives	Anaerobes
1	13.8	71.2	14.3
2	5.6	85.9	17.3
3	12.0	72.1	8.9
4	22.0	76.0	6.0
5	15.1	81.2	9.4
6	10.9	85.2	11.9
1-5 avg	13.7	77.3	11.2

DISCUSSION

The present study addresses itself to claims of increased recovery of bacteria with the use of hypertonic media in the routine culturing of blood. These claims are based in part upon the assumption that some of the bacteria in the circulating blood of a bacteremic patient exhibit a degree of cell damage and that the osmotic stabilization of hypertonic sucrose exerts a protective effect (21, 25). It has also been demonstrated that hypertonic sucrose is anticomplementary in blood cultures (16).

Rosner (20, 23, 24) has presented data suggesting the superiority of hypertonic media in the recovery of bacteria from blood and other body fluids. It is difficult to interpret the reported data with regard to the value of hypertonic media because of the simultaneous inclusion of other variables such as inoculum size, basal medium, and additives such as sodium polyanethol sulfonate. In addition, his rates of recovery of clinically significant isolates have varied from 5.4% (21) to 29% (22). Henrichsen and Bruun (10) found that the use of hypertonic media favored the recovery of certain types of bacteria but did not significantly increase the total recovery rate.

TABLE 8. *Total isolates, experiments 1 through 6*

Organism	Expt						
	1	2	3	4	5	6	Total
Pseudomonas	31	8	16	9	12	17	93
Bacillus sp.	23	2	10	11	13	4	63
Yeast	15	4	14	2	12	7	54
Acinetobacter	12		4			4	20
II-K			1				1
Aeromonas sp.	2					1	3
Staphylococcus aureus	85	26	27	11	25	58	232
S. epidermidis	104	27	62	13	43	34	283
Streptococci	93	45	52	15	35	45	285
Viridans group	27	19	21	3	22	27	119
Group A	6	1	1				8
Group B	4	4	4	1	1	3	17
Group C	1			3		2	6
Group D					1		1
Group F					2	1	3
Group G	1	5	2				8
S. faecalis	36	6	8	5		2	57
S. faecium	1		6	1	7	1	16
S. durans	1		1	1		3	6
S. bovis	3	11	2	1		6	23
S. equinus	2		6				8
Nonhemolytic	2				2	2	6
Beta, not A	9						9
Streptococcus pneumoniae	31	6	6	4	6	21	74
Escherichia coli	71	41	62	16	50	36	276
Klebsiella sp.	32	27	24	9	8	23	123
Enterobacter sp.	19	5	7		8	5	44
Proteus sp.	6	9	19	2	6	10	52
Corynebacterium sp.	17	2	9	3	5	5	41
Serratia marcescens	6	4	1	1		6	18
Neisseria meningitidis	5	1		1		3	10
Neisseria gonorrhoeae	1		1		1		3
N. perflava	1						1
Haemophilus influenzae	3					2	5
Salmonella sp.	14		2			3	19
Citrobacter sp.	3	3	1			5	12
Listeria monocytogenes	5	3					8
Lactobacillus sp.	5	1					6
Micrococcus sp.	1		2	1	3		7
Eikenella corrodens	1	1					2
Haemophilus aphrophilus	1	1			1		3
Pasteurella hemolytica	1						1
Providencia stuartii		1					1
Arizona sp.						2	2
Clostridium sp.	11	11	10	1	8	9	50
Propionibacterium acnes	18	3	1	1		9	32
Bacteroides sp.	28	16	16	4	11	14	89
Fusobacterium sp.	3	1	1		1		6
Peptostreptococcus sp.	4	2	3				9
Peptococcus sp.	3				1		4
Eubacterium sp.		1				1	2
Actinobacillus actinomycetemcomitans	2				1		3
Actinomyces bovis		1					1
Bifidobacterium eriksonii			1		1		2
Lactobacillus sp.			1			2	3
Arachnia sp.						1	1

TABLE 9. *Summary of positive cultures, contaminants, and polymicrobic cultures*

Expt	Total isolates	Total contaminants	Total polymicrobic cultures	% Positive cultures	Contamination (% of positive cultures)	Polymicrobic cultures (% of positive cultures)
1	657	139	38	10.2	21.2	5.8
2	252	28	7	11.4	11.1	2.8
3	353	78	27	9.0	22.0	12.3
4	104	28	7	9.7	26.9	6.7
5	258	58	12	10.1	22.5	4.7
6	326	45	14	10.3	13.8	4.3
1-6	1,950	376	105	10.1	19.3	5.8

The present study has confirmed the observations of Henrichsen and Bruun (10) that although the use of hypertonic medium does not result in an increase in total recovery, significant differences are seen in the recovery rates of individual species and groups of organisms.

Hypertonic medium is definitely inferior to isotonic medium when the recovery of *Pseudomonas*, and perhaps yeasts, is considered. Finegold (7) noted that the growth of *Pseudomonas aeruginosa* could only be obtained in anaerobic hypertonic medium with the addition of potassium nitrate, which can be metabolized anaerobically. We believe that this may be a reflection of the decreased oxygen tension in the hypertonic medium. Reduced methylene blue indicator added to bottles of media showed significant differences in the time required for a color change to the oxidized form: MCB-aerobic stationary required 15 min; MCB-aerobic shaken required 5 min; MCB-HT-aerobic stationary required 6 h; and MCB-HT-aerobic shaken required 45 min. We observed an increased recovery of *Bacillus* sp. in hypertonic media. Similar results were reported by Washington et al. (29). On the other hand, we did not observe the enhanced recovery of *Staphylococcus aureus* and *Bacteroides* in isotonic media reported by those authors.

No significant differences were observed in the total recovery of facultative organisms or any individual species between MCB-aerobic and MCB-HT-aerobic under stationary or agitated conditions. Under anaerobic conditions, however, MCB-HT was superior to MCB for the total recovery of facultatives.

The total recovery of anaerobes was greater in MCB-HT than in MCB under stationary conditions. Although this difference is not significant, the rate of recovery of *Bacteroides* sp. in hypertonic medium was significantly greater than in isotonic medium. Finegold (7) considered an anaerobic osmotically stabilized broth the ideal medium for the recovery of strict anaerobes.

Our studies confirm the observations of others (1, 8, 9, 28) of the importance of atmosphere in the isolation of aerobes and anaerobes. Shaking of isotonic medium resulted in a marked increase in the recovery of aerobes, facultatives, principally *E. coli*, and total organisms.

It became increasingly apparent to us that the volume of blood used was a significant determinant of the recovery rate. The high recovery of anaerobes and the concomitant low recovery of aerobes in experiment 2 reflect the fact that in this experiment two-thirds of the blood was inoculated into anaerobic bottles. In experiment 6, increased recovery was obtained when larger volumes of blood were used. Finally, there was no difference in total recovery when the volume of blood previously distributed into three bottles (experiments 1 through 5) was divided between two bottles (experiment 6). Henrichsen and Bruun (10) also recognized the importance of the volume of blood cultured on the recovery rate.

The results of a preliminary study (L. R. McCarthy and P. D. Ellner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, M48, p. 81) led us to propose a three-bottle system, which included a bottle of hypertonic medium. We soon questioned whether the apparent increase in recovery obtained with this system was due to the intrinsic effect of the hypertonic medium or merely the result of including a third bottle. This question was answered by comparing experiments 1 and 3, in which no differences in recovery rates were observed when duplicate isotonic bottles were substituted for an isotonic-hypertonic combination. Although these experiments were not performed concurrently, the uniformity of the methodology and patient population allows this comparison. The recovery rates of the various groups of organisms as well as the percentage of positive cultures, contaminants, and polymicrobic cultures have been remarkably constant in our laboratory for the past several years and compare favorably with those rates reported by others (9, 29).

The chi-square results obtained by the Cochran Q test for experiments 1 through 5 con-

firmed that significant differences existed within each experiment, but did not reveal the variable(s) responsible for that difference. In experiments 1 and 4, where subsequent analysis by the sign test failed to reveal a significant difference due to tonicity, the observed chi-square values were, in fact, due to the effect of venting. On the other hand, in experiments 2 and 5, the significant chi-square results were actually the result of the effects of tonicity and agitation.

This study demonstrates the importance of careful statistical evaluation of experimental results. Apparently significant increases in recovery reported by others to be due to the hypertonic medium could be as well explained by inoculum size, basal medium formulation, or other cultural variables.

The value of osmotically stabilized media has been clearly established in those relatively rare cases of endocarditis or septicemia when the only circulating organisms are cell wall-deficient forms that fail to grow in conventional isotonic broth.

The results of this study lead to the conclusion that optimal recovery of bacteria from blood is accomplished with an aerobic isotonic medium that is agitated and an anaerobic hypertonic medium that is incubated under stationary conditions. The former provides maximal recovery of aerobes, in particular *Pseudomonas* and facultative organisms; the latter also facilitates the isolation of facultatives and is at least as good if not slightly better for the recovery of strict anaerobes, in particular *Bacteroides*.

The recovery rates obtained with this two-bottle system compare favorably with those obtained by us in previous studies (5, 15) and by others (9, 27, 30). The change in the time of subculture as suggested by Todd and Roe (26) and Harkness et al. (9) resulted in earlier detection and reporting of positive blood cultures.

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