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Evaluation of Thiol Borth for the Culture of *Salmonella typhi* and Other Bacteria from Blood

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Thiol broth is known to neutralize various antimicrobial agents. Positivity of growth of various species of bacteria from blood in thiol broth was reported as similar to that in tryptic soy broth (TSB). As blood cultures are often used for the diagnosis of typhoid fever, and as patients may receive antimicrobial therapy before blood culture, the positivity and rapidity of growth of Salmonella typhi in thiol broth were compared to those in TSB. Routine blood culture samples from Yonsei Medical Center patients were inoculated in 50-ml amounts of TSB and thiol broth. The media were prepared from dehydrated products and did not contain CO_2, but TSB contained 0.025% sodium polyanethol sulfonate (SPS). Growth of S. paratyphi-A, Klebsiella pneumoniae, Enterobacter sp., Serratia marcescens and α-hemolytic Streptococcus were similar in both media. However, greater positivity and shorter incuabtion time for macroscopic detection were noted in TSB with S. typhi, Escherichia coli and Staphylococcus aureus. It is concluded that thiol broth is inferior to TSB plus SPS for the culture of S. typhi from blood.

Key Words: Blood culture, thiol broth, *S. typhi*

Septicemia represents a medical emergency that requires the prompt initiation of therapy. The laboratory's role is to isolate and identify the etiologic agent as rapidly as possible (Washington 1978). In Korea, blood culture is often used for the diagnosis of typhoid fever (Kim *et al.* 1985).

Various methods and media are used for blood culture to obtain more rapid results and higher positivity (Reller *et al.* 1982). Thiol broth was described by its manufacturer to inactivate penicillin, streptomycin, sulfonamides, cephalosporins, lincomycins, aminoglycosides, tetracycline and chloramphenicol.

In an actual blood culture study, the positivities with thiol broth were reported to be similar to those with tryptic soy broth (TSB), but the effect on the isolation of *Salmonella typhi* remains uncertain (Washington 1971; Hall *et al.* 1974).

S. typhi is susceptible to various antimicrobial agents in vitro. Although in vitro studies showed that *S. typhi* or *S. paratyphi-A* grew better in thiol borth than in TSB when both of the media contained ampicillin, cotrimoxazole, carbenicillin, piperacillin, ticarcillin, moxalactam, or gentamicin, it was also shown in a limited number of actual blood cultures that longer incubation time was required with thiol broth (Lee *et al.* 1983; Lee, 1984). The beneficial effect of thiol broth may possibly be expected only in patients with antimicrobial therapy before blood culture.

In this study, the positivity and rapidity of growth detection, especially of *S. typhi*, with thiol broth were compared to those with TSB to determine the usefulness of the medium for routine blood culture.

MATERIALS AND METHODS

Thiol broth and TSB were prepared from dehydrated products (Difco) in 50-ml amounts in 120-ml bottles with rubber stoppers. Carbon dioxide was not added, but sodium polyanethol sulfonate (SPS, American Research Products Co., South Euclid, Ohio) was added to TSB to a final concentration of 0.025% (Reller *et al.* 1982).

Routine blood culture samples were collected from both in- and outpatients of Yonsei Medical Center during the two prevalent typhoid fever seasons,

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December 1984 to April 1985 and December 1985 to February 1986 (Kim *et al.* 1985).

The routine method of blood culture was to collect 10-ml amounts from adult and 1-3ml from pediatric patients and inoculate equally divided amounts into thiol broth and TSB. The bottles were incubated at 35°C and macroscopic observations were made once a day for up to 10 days. We did not do routine blind subcultures.

Positivity and rapidity of growth, by medium, of *S. typhi* and other bacterial species with appreciable numbers of isolates were analysed. Chi-square analysis was used to test for statistical significance of positivity and rapidity of growth. The statistical analysis of incubation time was performed by Student's *t* test (Ilstrup, 1978).

RESULTS

Altogether, 7493 blood samples were cultured and 287, with growth of *S. typhi, S. paratyphi-A, Escherichia coli, Klebsiella pneumoniae, Enterobacter* sp., *Serratia marcescens, Staphylococcus aureus* and α-hemolytic *Streptococcus,* were used to compare the positivity and rapidity of growth in TSB and thiol borth (Table 1).

Among the 82 *S. typhi* positive specimens, 44 (53.7%) had growth in both TSB and thiol broth. Thirty-three (40.2%) were positive only in TSB, while 5 (6.1%) were only in thiol broth. Among the 13 *S. pratyphi-A* positive samples, 7 were positive in both media and remaining 6 were only in TSB.

E. coli was isolated from 56 specimens. Thirty-four specimens (60.7%) were positive from both media, while 17 (30.4%) and 5 (8.9%) were from TSB and thiol broth, respectively. In *S. aureus,* 31 of 62 samples (50.0%) had growth in both media, while 26 (41.9%) and 5 (8.1%) had growth only in TSB and thiol broth, respectively. α-hemolytic *Streptococcus* also showed more positivity in TSB with 9 samples (45.0%), than in thiol broth with 1 (5.0%). In these species, the greater positivity in TSB were statistically significant.

The positivities of *K. pneumoniae, Enteobacter* sp. (17 *E. cloacae* and 1 *E. aerogenes*) and *S. marcescens* were slightly higher in TSB than in thiol broth, but the differences were not statistically significant.

The samples with positivity in both TSB and thiol broth were used to compare the growth detection time depending on the media (Table 2, Fig. 1). The growth of *S. typhi* was detected after 1-6 days (mean 2.7 days) incubation in TSB, while after 2-8 days (mean 4.2 days) in thiol borth. The difference in mean incubation times was statistically significant ($p<0.01$). In *E. coli,* the growth was detected after 1-4 days (mean 1.9 days) incubation in TSB and after 1-7 days (mean 2.4 days) in thiol broth. The difference in mean incubation times was statistically significant ($p<0.05$). Also, *in S. aureus,* the growth was detected earlier in TSB, after 1-6 days (mean 2.9 days), than in thiol broth, after 2-8 days (mean 3.9 days). Again the difference was statistically significant ($p<0.01$).

In *S. paratyphi-A, K. pneumoniae, Enterobacter* sp., *S. marcescens* and α-hemolytic *Streptococcus,* the mean incubation times were the same with both media or were insignificantly different. Overall, the mean incubation time for the macroscopic detection of growth was 2.4 days in TSB and 3.1 days in thiol broth.

Rapidity of growth by medium, in samples with positivity in both media, was compared (Table 3).

Table 1. Comparison of positivity of bacteria in TSB and thiol broth

Species	No. (%) positive with				*p*-value
	Both media	TSB only	Thiol only	Total	
S. typhi	44	33	5	82	<0.01
S. paratyphi-A	7	6	0	13	<0.02
E. coli	34	17	5	56	<0.02
K. pneumoniae	7	7	3	17	NS*
Enterobacter sp.	12	5	1	18	NS
S. marcescens	14	4	1	19	NS
S. aureus	31	26	5	62	<0.01
α-hemolytic *Streptococcus*	10	9	1	20	<0.02
Total	159 (55.4)	107 (37.3)	21 (7.3)	287 (100)	

* NT: not significant.

Table 2. Comparison of incubation time for the macroscopic detection of growth in samples with growth in both media

Species (No. positive)	Media	No. positive after incubation (day)									*p*-value
		1	2	3	4	5	6	7	8	Mean	
S. typhi (44)	TSB	2	23	9	8	1	1			2.7	<0.01
	Thiol		7	6	17	8	1	3	2	4.2	
S. paratyphi-A (7)	TSB		2	3	2					3.0	NS*
	Thiol		2	3	2					3.0	
E. coli (34)	TSB	11	18	4	1					1.9	<0.05
	Thiol	9	14	6	2		1	2		2.4	
K. pneumoniae (7)	TSB	5	2							1.3	NS
	Thiol	5	2							1.3	
Enterobacter sp. (12)	TSB	5	5	1				1		2.1	NS
	Thiol	5	5	1				1		2.1	
S. marcescens (14)	TSB	8	4			1	1			1.9	NS
	Thiol	7	4	2				1		2.0	
S. aureus (31)	TSB	2	11	10	5	1	2			2.9	<0.01
	Thiol	2	6	5	8	3	4	2	1	3.9	
α-hemolytic *Streptococcus* (10)	TSB	2	7	1						1.9	NS
	Thiol	3	4	3						2.0	
Total (159)	TSB	35	72	28	16	3	4	1	0	2.4	
	Thiol	31	44	26	29	11	6	9	3	3.1	

* NT: not significant.

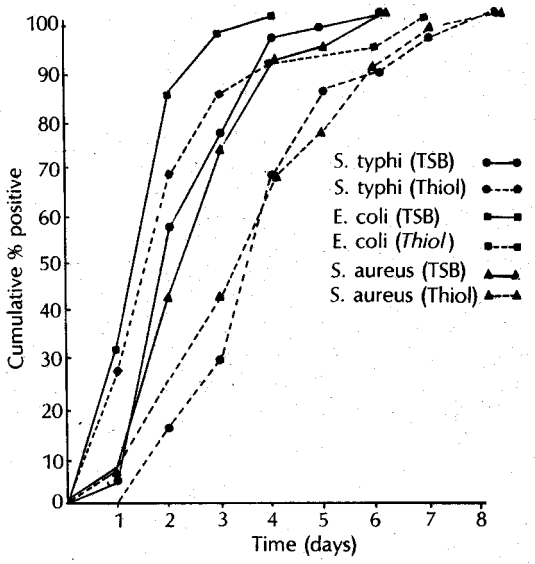

Fig. 1. *Cumulative percentage positive by day of incubation of S. typi, E. coli and S. aureus in TSB and thiol broth.*

Overall, 67.3% of 159 specimens had growth in both media at the same time, while 30.2% had earlier growth in TSB and 2.5% in thiol broth. The growth of *S. typhi* was detected at the same time in both media in 17 samples (38.6%), while 25 (56.8%) were detected earlier in TSB and 2 (4.5%) in thiol broth. The growth of *E. coli* was detected at the same time in 27 (79.4%) samples, while 7 (20.6%) were detected earlier in TSB and none earlier in thiol broth. Twenty-one of 31 (67.7%) blood culture samples showed growth of *S. aureus* at the same time in both media, while 10 (32.3%) showed earlier growth in TSB and none in thiol borth. In these species, the differences in rapidity of growth were statistically significant.

In *S. paratyphi-A, K. pneumoniae* and *Enterobacter* sp., the growth was detected at the same time. Although more specimens showed earlier growth of *S. marcescens* and α-hemolytic *Streptococcus* in TSB, the differences were not significant.

DISCUSSION

Antimicrobial agents are so commonly administered, even in countries where their prescription is controlled, that it is not always possible to take blood for culture before their administration (Kunin *et al.* 1973). Quite a variety of methods and media are used to obtain greater positivity and rapid results

Table 3. Comparison of rapidity of detection of growth in samples with growth in both media

Species	No. (%) positive				*p*-vlaue
	Same time	Earlier in TSB	Earlier in thiol broth	Total	
S. typhi	17	25	2	44	<0.01
S. paratyphi-A	7	0	0	7	NS*
E. coli	27	7	0	34	<0.01
K. pneumoniae	7	0	0	7	NS
Enterobacter sp.	12	0	0	12	NS
S. marcescens	9	4	1	14	NS
S. aureus	21	10	0	31	<0.01
α-hemolytic *Streptococcus*	7	2	1	10	NS
Total	107 (67.3)	48 (30.2)	4 (2.5)	159 (100)	

* NT: not significant.

of blood culture even from patients who were treated with antimicrobial agents (Appelbaum *et al.* 1982; Henry *et al.* 1984). Blood culture media with constituents which neutralize antimicrobial agents are most attractive as their use neither requires complicated procedures (Tilton, 1982) nor has possibilities of introducing contaminants (Henry *et al.* 1984). Thiol broth, a medium with a low oxidation-reduction potential and suitable for anaerobic culture, was known to inactivate various antimicrobial agents. Another commonly used anaerobic culture medium is thioglycollate broth.

At the Yonsei Medical Center, sets of TSB and thioglycollate broth are used for blood culture. However, the growth of *S. typhi*, which is often the purpose of the culture, is slow in thioglycollate broth (Chong 1974). In comparative studies of TSB and thioglycollate medium, or thiol broth, it was shown that the media were similar, except there was less positivity of *Actinobacillus, Pseudomonas* or *Corynebacterium*, in thiol broth (Washington 1971; Hall *et al.* 1974), and in thioglycollate broth (Washington 1972). Media with or without SPS in unvented bottles with CO_2 was used and blind subcultures were done.

It was felt that thiol broth would be very attractive if it could give comparable results with TSB in a room air environment and without a blind subculture, as our laboratory-prepared blood culture bottles did not contain additional CO_2 and as 1-day blind subculture is a time-consuming but less rewarding work for the rapid detection of *S: typhi* (Chong *et al.* 1979). Another advantage seemed to be similar results with or without SPS. As SPS is inhibitory to *Neisseria* and *Peptostreptococcus anaerobius* (Wilkins and West 1976; Pai and Sorger 1981), we routinely add it only to TSB, but not to thioglycollate broth.

The present study showed significantly less positivity of *S. typhi, S. paratyphi-A, E. coli, S. aureus* and α-hemolytic *Streptococcus* in thiol broth (Table 1). It can be assumed that because of the anaerobic condition of thiol broth, it did not provide optimal condition for the growth of facultatively anaerobic bacteria. It is well recognized that growth of aerobic bacteria is not optimal in anaerobic broth (Reller *et al.* 1982). It can also be assumed that the dilution of blood with 10 volumes of TSB was sufficient to allow growth of bacteria even if patients' blood contained antimicrobial agents.

Comparison of the incubation time required before macroscopic detection (Table 2) showed that the time for *S. typhi* was longer than that for other gram-negative bacilli. The mean incubation time of 2.7 days with TSB plus SPS was the same as the previous result with brain heart infusion (BHI) plus SPS, and 4.2 days with thiol broth was similar to 3.8 days with fluid thioglycollate medium (FTM) (Chong 1974). Considering the fact tha t SPS can shorten the incubation time both in BHI and FTM (Chong 1974), the shorterr time with TSB in the present study may partly be due to the presence of SPS in TSB. Significantly shorter incubation times were also noted in TSB in the detection of growth of *E. coli* and *S. aureus*.

In the comparison of rapidity of detection of growth in paired media (Table 3), significantly rapid growths of *S. typhi, E. coli,* and *S. aureus* were observed in TSB. These results were contrary to the expectation, as thiol broth was able to neutralize various antibiotics (Lee *et al.* 1983; Lee 1985), and as quite a high proportion of patients may have received an-

timicrobial therapy before blood culture. Further comparative study of thiol broth and thiol broth puls SPS may be required to reveal the cause.

It is concluded from the analysis of routine blood culture results that thiol broth is inferior to TSB plus SPS in the positivity and rapid detection of growth of *S. typhi, E. coli* and *S. aureus,* but is similar in that of *S. paratyphi-A, K. pneumoniae, Enterobacter* sp., *S. marcescens* and α-hemolytic *Streptococcus.*

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