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Evaluation of Sodium Polyanethanol Sulfonate as a Blood Culture Additive for Recovery of *Salmonella typhi* and *Salmonella paratyphi* A

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A total of 640 blood specimens from patients in an area endemic for enteric fever were cultured in parallel in tryptic soy broth with and without sodium polyanethanol sulfonate (SPS). A total of 95 specimens were positive for *Salmonella* spp., 54 for *Salmonella typhi*, and 41 for *Salmonella paratyphi* A in one or both bottles of a set. Significantly higher rates of recovery were obtained from the SPS-containing medium ($P < 0.01$) upon subculturing blindly at 24 h and 3 days of incubation. Subcultures performed at 7, 14, and 21 days also yielded a greater number of positive cultures with SPS than without it, although the differences between the two media were not significant ($P > 0.05$). Neither of the media yielded 100% of the positive cultures. Moreover, even if the results of the two media were combined, 34 and 19% of the isolates would have been missed if the specimens had not been incubated to 14 and 21 days, respectively. The data indicate that SPS aids in early recovery of *S. typhi* and *S. paratyphi* A from blood cultures, and additionally, that under the conditions used in the study, incubation beyond a 1-week period is required for efficient isolation of these organisms from blood.

Von Haebler and Miles (17) and Watson (18) observed that sodium polyanethanol sulfonate (SPS) was beneficial to the growth of *Salmonella typhi* in model blood culture systems. The possibility exists, however, that results of such experimental cultures may not accurately reflect those of routine clinical practice, since the models involved the use of only one or a small number of bacterial strains. Furthermore, some of the strains were laboratory adapted, and growth was assessed either in a single specimen or a largely uncharacterized pool of blood from patients (17, 19). A search of the literature failed to reveal references clearly documenting the value of the additive for recovery of typhoid and paratyphoid (enteric fever) *Salmonella* spp. in a clinical setting.

S. typhi and *Salmonella paratyphi* A are often isolated from blood cultures at San Lazaro Hospital (SLH), Manila, Republic of the Philippines. Prompt and efficient isolation of these agents of enteric fever is important since the disease produces protean manifestations (11, 20). For several years, the laboratory of SLH has used tryptic soy broth (TSB) for routine hemocultures. The present study was conducted to determine whether the addition of SPS would increase the yields or expedite the recovery or both of enteric fever *Salmonella* from our patient population. The study was conducted in a prospective manner using duplicate, parallel cultures of blood from patients, in TSB with and without SPS.

MATERIALS AND METHODS

Preparation of media. TSB (Difco Laboratories, Detroit, Mich.) was prepared as recommended by the manufacturer; TSB-SPS was prepared by adding sufficient SPS (Grobax; Hoffmann-La Roche Inc., Nutley, N.J.) to yield a 0.05% concentration of the compound in TSB. The broth media were dispensed in 30-ml samples into 100-ml serum bottles, capped loosely with rubber stoppers, and autoclaved for 15 min at 121°C and 15 pounds of pressure. The broth bottles were cooled to about 60°C and capped firmly with aluminum rings and a seal crimper. When the broth had cooled to room temperature, the vacuum in the sealed bottles was removed by puncturing the rubber stoppers with a 26-gauge hypodermic needle attached to a syringe barrel (syringe minus plunger) equipped with a disposable, 0.22-μm membrane filter (Millex; Millipore Corp., Bedford, Mass.) affixed between the needle and hub of the barrel. All broth bottles were preincubated at 37°C for 24 h and visually checked for contamination. The broth culture bottles were stored at room temperature and used within 10 days after preparation. No gaseous carbon dioxide was added to the bottles.

Inoculation of blood samples. Blood was collected at bedside from each patient by venipuncture with a 10-ml disposable syringe. The venipuncture needle was replaced with a sterile needle, and 3-ml samples of blood were aseptically added to each of the two types of broth culture bottles by injection through the rubber cap. The remaining blood was dispensed into test tubes for other laboratory determinations. The inoculated culture bottles were mixed by continuously inverting for 5 to 10 s and sent immediately to the hemoculture laboratory.

Incubation and subcultures. All culture bottles were incubated continuously at 36°C without further venting. Blind subcultures were done after 1, 3, 7, 14, and 21 days of incubation. Before subculturing, the bottles were shaken gently, and a 0.5-ml sample was aseptically withdrawn with a tuberculin syringe and a 26-gauge needle. Drops (2 or 3) of the sample were inoculated and streak diluted into sections of MacConkey agar (BBL Microbiology Systems, Cockeysville, Md.) and either tryptic soy agar (Difco) or chocolate agar plates; the former plates were incubated aerobically and the latter in a candle-extinction jar at 36°C.

Identification of bacterial growth. Inoculated plates were examined after 18 to 24 h of incubation. Those presenting no growth were incubated an additional 24 h and reexamined. Bacterial growth was identified by standard methods (6) and by use of the API 20-E system (Analytab Products, Plainview, N.Y.). *Salmonella* antisera (Difco) were used to confirm the *Salmonella* species.

Statistical analysis. The recovery rates of *S. typhi* and *S. paratyphi* A were evaluated by chi-square tests for comparison of proportions in paired samples. All specimens with *Salmonella* spp. in both bottles at any time during the 21-day incubation period were further compared by paired Student's *t* tests to determine the average time to positivity in each medium (12).

RESULTS

A total of 640 blood specimens were inoculated into culture bottles containing TSB and TSB-SPS. Fifty-four specimens yielded *S. typhi* and 41 yielded *S. paratyphi* A in one or both culture bottles. There was no instance in which one organism was isolated from one bottle and the other organism in the second bottle of the set. The total of 95 *Salmonella* isolations represented blood cultures obtained from 91 different patients.

Of 54 specimens presenting *S. typhi*, 14 were positive in TSB upon subculture at 24 h of incubation, whereas all 14 plus an additional 13 were positive in TSB-SPS at this time ($P < 0.01$). All culture sets were subcultured blindly a second time after 3 full days of incubation, and the results again showed significantly better performance of TSB-SPS over plain TSB ($P < 0.05$). The three subsequent subcultures, however, showed similar or identical cumulative recovery rates in the two media. By day 21 of incubation, 12 (22%) of the 54 *S. typhi*-positive specimens had failed to show growth in TSB, whereas 8 (15%) failed to grow in TSB-SPS ($P > 0.05$).

TABLE 1. Effect of SPS on the recovery of 95 *Salmonella* isolates[a]

Incubation time (days)	Culture positive in TSB				P^b
	Without SPS		With SPS		
	No. (%)	Cumulative (%)	No. (%)	Cumulative (%)	
1	24 (25.3)	24 (25.3)	49 (51.6)	49 (51.6)	<0.01
3	19 (20.0)	43 (45.3)	7 (7.4)	56 (59.0)	<0.01
7	11 (11.6)	54 (56.8)	3 (3.2)	59 (62.1)	NS[c]
14	8 (8.4)	62 (65.3)	8 (8.4)	67 (70.5)	NS
21	9 (9.5)	71 (74.7)	14 (14.7)	81 (85.3)	NS
No growth	24 (25.3)		14 (14.7)		NS

[a] Number includes 54 *S. typhi* and 41 *S. paratyphi* A isolates.
[b] For the difference between media.
[c] NS, Not significant.

The recovery of *S. paratyphi* A was similar to that observed with *S. typhi* isolations. TSB-SPS was significantly better than plain TSB on both the first and second subculture periods only. Of 41 *S. paratyphi* A isolates, 12 (29%) and 6 (15%) failed to grow in TSB and TSB-SPS, respectively, by day 21 of incubation ($P > 0.05$).

The data showing recovery of all 95 *Salmonella* isolations are shown in Table 1. Although the cumulative yields of TSB-SPS were greater than those of TSB at all incubation periods, only those of subcultures performed on days 1 and 3 were statistically different. TSB failed in 24 (25.3%) cases, whereas TSB-SPS failed in 14 (14.7%) of the 95 *Salmonella*-positive specimens upon 21 days of incubation.

The number of culture sets that were positive in either or both of the media at each of the various incubation periods is shown in Table 2. Subcultures performed after 7, 14, and 21 days of incubation yielded 6, 14, and 18 positive cultures, respectively, among sets that had failed to reveal growth on previous examinations.

A total of 34 *S. typhi* and 23 *S. paratyphi* A isolates were found in both TSB and TSB-SPS; among these specimens, the SPS-containing medium yielded *S. typhi* after a mean of 3.44 days of incubation compared to 4.68 days of incubation for plain TSB ($P < 0.05$). *S. paratyphi* A was also isolated more quickly in TSB-SPS (2.74 days) than in TSB (3.78 days), but this difference was not significant at a 5% confidence level.

DISCUSSION

Although SPS is a popular blood culture additive (1), relatively few trials, conducted in a clinical setting, in which culture media with and

TABLE 2. Incubation time required for earliest recovery of *S. typhi* and *S. paratyphi* A from blood cultures

Incubation time (days)	Positive blood culture set[a]	
	No. (%)	Cumulative (%)
1	49 (51.6)	49 (51.6)
3	8 (8.4)	57 (60.0)
7	6 (6.3)	63 (66.3)
14	14 (14.7)	77 (81.1)
21	18 (19.0)	95 (100.0)

[a] Culture set considered positive when either or both culture bottles (see text) presented growth.

without the compound were evaluated have been published. SPS, however, has been found to inactivate the natural bacterial inhibitors present in blood, such as complement, β-lysin, and lysozyme (4, 14, 17), to inhibit phagocytosis (3) and to inactivate certain antibiotics (5). It is largely these experimental data which have led to the widespread use of the compound in blood culture media.

The results of some clinical investigations on the use of SPS are conflicting. Rosner (16) reported that 0.05% SPS was beneficial for the recovery of gram-positive cocci but that gram-negative bacilli were unaffected. Finegold et al. (10) and Eng (8) demonstrated that SPS improved the recovery of both gram-positive cocci and gram-negative bacilli; the latter report noted that *Neisseria meningitidis* was adversely affected by the compound. Eng and Iveland (9) later reported that both *N. meningitidis* and *Neisseria gonorrhoeae* were inhibited by SPS. Minkus and Moffet (15) studied the effect of 0.05% SPS in blood cultures from infants and children and concluded that, with the possible exception of *Streptococcus pneumoniae*, SPS did not increase the efficiency of recovery of pathogenic bacteria. On the other hand, use of SPS was associated with an increased frequency of recovery of organisms judged to be contaminants, possibly because SPS enhanced the recovery of a very small inoculum of bacteria from the skin (15).

The data presented here show that the addition of SPS to TSB does not significantly affect the overall isolation frequencies of *S. typhi* and *S. paratyphi* A. Although a greater number of positive cultures was obtained in the SPS-containing broth (Table 1), the difference was not significant after 7 days or more of incubation.

Table 1 also shows that TSB-SPS yielded significantly more positive cultures than TSB during the early stages, i.e., 24 h and 3 days, of specimen incubation. These data thus indicate that the addition of SPS to TSB shortens the time required for isolation of the organisms. This fact was further substantiated by data which showed that mean time to positivity was shorter in TSB-SPS than in TSB. From a practical point of view, the use of SPS in blood culture media is clearly beneficial. At 24 h of incubation, for example, positive reports were available on 49 rather than 24 patients. Thus, more patients were given appropriate antimicrobial agents more quickly, and under routine conditions, the laboratory would have incurred fewer losses in terms of technician time and bacteriological media for the continued examination of the apparently negative specimens.

This report also demonstrates that, under the conditions of the study, a prolonged incubation period is required for the isolation of enteric fever *Salmonella* from a substantial number of blood specimens. If incubation had been allowed to proceed only to 7 days, as is often the case in routine blood cultures (1, 7), approximately one-third of the recoveries would have been missed even if the performance of the two media had been combined (Table 2). In this regard, it is important to note that this study was conducted in an area where enteric fever and other infectious diseases are endemic. A large number of the patients presented to the hospital beyond the second or third week of illness, and some had already received antimicrobial therapy through self medication or from private physicians who referred the patients to the hospital. An additional factor is that only 3 ml of blood was added to each bottle of broth medium. Although the small volume of blood sample is in keeping with common practice in many laboratories, it is probably inadequate for the efficient isolation of *S. typhi* and possibly other organisms, since the number of organisms in peripheral blood of typhoid fever patients is often very small (13, 18). A combination of these three factors: delay in presentation of patients to hospital, prior use of antimicrobial agents, and the small amount of blood inoculated into each culture bottle may account for the delayed growth in some of the specimens. These data are nevertheless in keeping with those reported by others. For example, Batty-Shaw and MacKay (2) reported that of 160 blood cultures positive for enteric fever *Salmonella* in a bile-broth medium, only 34 (21%) were positive at 24 h of incubation, and 47, 31, 21, 19, and 8 additional samples became positive on days 3, 5, 7, 9, and 11, respectively. The average period of incubation required to produce positive results in their study was between 4 and 5 days, with slightly over 50% being positive by day 3 (2). The data reported here are similar: about 3.5 to 4.5 days were the mean times required to produce positive cultures, depending on whether SPS was present or absent, respec-

tively, and about 60% of the positive cultures yielded growth by day 3 of incubation.

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