JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1975, p. 119-123

Vol. 1, No. 2
Printed in U.S.A.

Effect of Sodium Polyanethol Sulfonate in Blood Cultures

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Received for publication 7 October 1974

Fifteen-hundred hospital blood cultures were made in duplicate, with and without 0.05% sodium polyanethol sulfonate in the broth medium. A significantly higher rate and speed of recovery of both gram-positive cocci and gram-negative bacilli was accomplished in sodium polyanethol sulfonate broth. The effect was independent of the content of 0.1% agar in the growth medium. In the cases of *Neisseria meningitidis* septicemia examined, however, a detrimental result on recoveries was observed. The addition of sodium polyanethol sulfonate also resulted in an increased frequency of recoveries of contaminating organisms.

Sodium polyanethol sulfonate (SPS; Liquoid, Hoffman-La Roche) is an anticoagulant and a surface-active agent which is widely employed as an additive to fluid blood culture media. It is generally considered to enhance the rate and speed of bacterial isolations by counter-acting the bacterial inhibitors of human blood. The latter effect has been clearly demonstrated in artificial cultures (2, 3, 5, 6, 11). SPS is known to neutralize the bactericidal activity of fresh human serum (6) and to inhibit phagocytosis (1). However, relatively few prospective clinical trials using duplicate cultures with and without SPS have been published, and some of their results are conflicting. Rosner reported that the addition of 0.05% SPS had a beneficial effect on the recovery of gram-positive cocci, whereas no effect was demonstrated on the recovery of gram-negative bacilli (8, 9, 10). Finegold et al. found a favorable effect of 0.5% SPS in a small study comprising patients with both gram-positive and gram-negative bacteremia (4). Minkus and Moffet, working with blood cultures from infants and children, concluded that the addition of 0.05% SPS did not increase the recovery rate of any group of pathogenic bacteria and that it led to an increased frequency of contaminations (7). In the present paper, the results of a prospective clinical study comprising 1,500 duplicate blood cultures are presented. A significant favorable effect of 0.05% SPS upon the rate and speed of isolations of both gram-positive and gram-negative pathogenic bacteria was clearly demonstrated, with the exception of *Neisseria meningitidis* septicemias, in which an adverse effect was observed. The addition of SPS also resulted in an increased frequency of recoveries of typical contaminating organisms.

MATERIALS AND METHODS

Blood cultures were prepared in this hospital by inoculating 5 ml of venous blood into 50 ml of broth medium contained in 100-ml screw-top bottles. The medium is a nutrient broth with 0.1% dextrose produced in this laboratory. The broth bottles did not contain CO_2 and were not under vacuum. Broth with SPS was made by adding 0.05% SPS to the nutrient broth before autoclaving. With a view to assessing the effect of SPS, Medical Departments I and VII and Paediatric Department XVII of this hospital used parallel tests in their blood culture routines during a 1-year period. Ten milliliters of venous blood were withdrawn in a syringe, and a 5-ml sample was immediately inoculated into each of two flasks, one with broth medium to which 0.05% SPS had been added and one with exactly the same medium but without SPS. (The two media were inoculated in random order.) The bottles were kept at 37 C and sent to the Microbiological Laboratory as soon as possible. The time which elapsed between inoculation of the bottles at the bedside and the arrival of the cultures at the laboratory varied from a few hours to 2 days.

Immediately upon the receipt in the laboratory, all culture bottles were subcultured onto solid media. The bottles were then incubated at 37 C and inspected visually once a day. If both bottles remained clear, apart from the characteristic, faint clouding which regularly appeared in the SPS bottles, they were subcultured after 4 days and then discarded if negative. Cultures from patients with a clinical diagnosis of endocarditis, however, were kept for 7 days. If one of the bottles showed macroscopic signs of bacterial growth, subcultures and Gram stains were made immediately from both bottles in parallel. If the subculture from one bottle yielded colony formation, whereas the other bottle was negative, the latter was subcultured daily until growth appeared or up to 7 days, when the bottle was discarded as negative. All subcultures were made onto chocolate agar plates by means of a calibrated wire loop. The subculture plates were incubated in air at 37 C, except that plates from

cultures suspected of containing *N. meningitidis* were incubated in air with an increased content of carbon dioxide. Anaerobic platings were performed from turbid culture bottles yielding negative aerobic subcultures. The day when growth first appeared in the subcultures was noted, as well as the approximate number of colonies. The bacterial strains isolated were identified by standard methods.

RESULTS

During a 1-year period, 1,500 blood cultures sets were examined. Two-hundred and sixty-five sets of cultures were positive, i.e., organisms were recovered from one or the other, or both, bottles in the pair. Of these, 151 cultures obtained from 66 patients yielded growth of potentially pathogenic organisms. From the remaining 114 positive cultures were isolated *Staphylococcus epidermidis*, diphtheroid bacilli, *Bacillus* sp., or yeasts. Cases in which a real *S. epidermidis* bacteremia could be suspected on clinical and bacteriological grounds were not encountered during the study period. The two groups of positive duplicate cultures will be treated separately in the following. No anaerobic organisms were recovered.

During the last one-third of the study, 0.1% agar was added to all broth medium bottles for blood cultures in this hospital, whereas during the first two-thirds of the period only broth medium without agar was used.

Growth of potentially pathogenic organisms from 151 duplicate blood cultures. One hundred of these cultures were made in broth medium without added agar; their growth patterns in gram-positive and gram-negative bacteremias are shown in Table 1. Table 2 shows the corresponding findings in the remaining 51 culture pairs, positive in broth medium with 0.1% agar added. The central columns of both tables show the number of culture pairs from which an approximately equal number of colonies were formed from both bottles at the same time. The neighboring columns show the number of pairs from which colony formation arose from both bottles in simultaneous platings, but with a higher colony count from one bottle than from the other. In the second columns from the left and right are given the number of pairs of bottles in which growth in the subcultures differed by 1 or more days between the two bottles in the pair. The outside columns show the number of pairs in which only one bottle yielded growth, the other bottle in the pair remaining negative throughout 7 days of incubation. In both tables an asymmetrical distribution is evident, in that the number of culture pairs showing earlier growth from SPS broth, or growth exclusively from SPS broth (i.e., the totals of the figures in the two outermost left columns), clearly outweighs the number of paired cultures giving the reverse growth patterns. The effect is present in both gram-positive and gram-negative bacteremias, and in broth medium with, as well as without, 0.1% agar added. In *N. meningitidis* septicemias, however, an adverse effect was observed. Eleven pairs of cultures from seven patients were positive for this organism; 10 of these pairs yielded better growth, earlier growth, or growth exclusively from the broth medium without SPS.

The two subgroups of the data which are given in Table 1 and 2 are combined in Table 3, which also shows the various organisms isolated. Out of a total of 88 paired cultures yielding growth of gram-negative bacilli, earlier growth or growth exclusively from SPS broth was found in 46 (52.3%), whereas the same growth patterns were observed in 17 out of 52 paired cultures yielding growth of gram-positive organisms (32.7%).

In the extreme right column of Table 3 is given the number of patients in whom the organism listed was isolated from one paired culture only. Of the three cases of single isolations of *Diplococcus pneumoniae*, two patients suffered from pneumonia and the third one from meningitis; in the latter case, *D. pneumoniae* was isolated from cerebrospinal fluid as well. *Haemophilus influenzae* was isolated from one paired blood culture only in four cases, all of

TABLE 1. *Effect of 0.05% SPS on recovery of gram-positive and gram-negative organisms from 100 paired blood cultures in broth medium without agar*

Organisms	No. of paired blood cultures						
	+, −[a]	Earlier +, +	Heavier +, +	+, + (equal)	+, heavier +	+, earlier +	−, +
Gram-positive organisms	5	1	4	8	2	1	3
Gram-negative bacilli	21	17	1	20	1	4	3
N. meningitidis					3	4	2

[a] Growth in SPS and plain broth, respectively.

TABLE 2. *Effect of 0.05% SPS on recovery of gram-positive and gram-negative organisms from 51 paired blood cultures in broth medium with 0.1% agar added*

Organisms	No. of paired blood cultures						
	+, –[a]	Earlier +, +	Heavier +, +	+, + (equal)	+, heavier +	+, earlier +	–, +
Gram-positive organisms	3	8	5	9	1		2
Gram-negative bacilli	6	2		9	2		2
N. meningitidis				1	1		

[a] Growth in SPS and plain broth, respectively.

TABLE 3. *Effect of 0.05% SPS on recovery of various organisms isolated from 151 paired blood cultures*

Organisms	No. of paired blood cultures							
	+, –[a]	Earlier +, +	Heavier +, +	+, + (equal)	+, heavier +	+, earlier +	–, +	Single isolations
Gram-positive organisms								
Staphylococcus aureus	3	6	4	3			1	2
Diplococcus pneumoniae	1		1	2			1	3
β-Hemolytic streptococci	1		1	2	1			0
α-Hemolytic streptococci	2	3	3	3	2	1	1	3
Enterococci				7			1	3
Listeria monocytogenes	1						1	0
Gram-negative bacilli								
Escherichia coli	12	4	1	12	1	4	2	3
Klebsiella sp.	3			5			1	4
Proteus mirabilis	2	5		1	1			1
Salmonella sp.[b]	3	9		6			1	1
Alcaligenes faecalis	1							1
Acinetobacter sp.	2			3				1
Haemophilus influenzae		1		2	1			4
No genus determined	4						1	3
Neisseria meningitidis				1	4	4	2	5

[a] Growth in SPS and plain broth, respectively.
[b] *S. paratyphi* A, two patients; *S. paratyphi* B, two patients; *S. heidelberg*, one patient.

whom were meningitis patients in whom the same organism was cultivated from cerebrospinal fluid. Of the seven patients whose blood cultures yielded growth of *N. meningitidis*, five suffered from meningitis; in four of them, *N. meningitidis* was isolated from cerebrospinal fluid and in one case attempts at spinal puncture were unsuccessful. Two patients suffered from septicemia with an exanthema, but meningitis was not present.

In the paired cultures showing a time difference in the growth from individual bottles, a difference of 1 day was observed most frequently (Table 4). Of the cultures yielding earlier growth from SPS broth, however, an equal number differed by 2 or more days, 5 days being the highest difference observed.

In five patients, different organisms were isolated from different duplicate cultures. In one bottle pair, two organisms (*Escherichia coli* and *Proteus mirabilis*) were isolated from both bottles in the pair. In the remaining 61 patients, only one species was isolated.

In the patient material under study elderly people predominated; thus, half of the patients were 60 years or more.

Growth of S. epidermidis, diphtheroid bacilli, Bacillus sp., or yeasts from 114 duplicate cultures. An enhancing effect of 0.05% SPS on the recovery of the above-mentioned organisms is evident from Table 5. Sixty-two paired cultures yielded earlier growth or growth exclusively from SPS broth, whereas only 37 bottle pairs exhibited the reverse growth patterns. In only 23 of the 114 duplicate cultures (20.2%), the organisms listed were isolated from both bottles in the pair. This is in sharp contrast to the corresponding finding in the

group of isolations of potentially pathogenic organisms, in which recovery was accomplished from both bottles in 104 (68.9%) of 151 bottle pairs (Table 3).

DISCUSSION

The group of recoveries of potentially pathogenic organisms may contain some cases in which the organisms recovered were introduced as contaminants during inoculation of patient's blood into the culture bottles, or during the subsequent processing of the cultures in the laboratory. As an aid in the evaluation of the occurrence of possible contaminations, the number of single isolations is given in Table 3 for each organism recovered. All single isolations of *D. pneumoniae*, *H. influenzae*, and *N. meningitidis* were proven to be real bacteremias according to the clinical data given above. If the number of cases of possible contamination is sought, in accordance with generally accepted rules, among the remaining single isolations, the frequency of contamination will probably be low, most likely below 10% of the 151 positive duplicate cultures. Hence, it is inferred that the conclusions which can be drawn from the material with regard to the effect of SPS are valid for true bacteremias. These conclusions are: (i) a significantly higher rate and speed of recovery of both gram-positive cocci and gram-negative bacilli were accomplished in broth medium with 0.05% SPS, as compared with control broth medium without SPS; (ii) although present in both main groups of bacteria, the effect was more pronounced on gram-negative rods than on gram-positive cocci; (iii) the effect was independent of the content of 0.1% agar in the broth medium; and (iv) *N. meningitidis* septicemias constituted an exception to the general favorable effect of 0.05% SPS, in that the cultures showed better growth, earlier growth, or growth exclusively from the broth medium without SPS.

In accordance with the findings of Minkus and Moffet (7), the inclusion of 0.05% SPS in the broth medium resulted in an increased recovery rate of typical contaminating organisms from blood cultures (Table 5). The effect must be explained by the antagonizing activity of SPS against the nonspecific bacterial inhibitors of fresh human blood. I do not consider that the increased recovery rate of contaminating organisms interferes seriously with the usefulness of SPS in practical work.

The repressed growth of *N. meningitidis* from clinical blood cultures in the presence of 0.05% SPS observed in the present study (Table 3) provided the impetus for an investigation in the present laboratory of the sensitivity of a number of *N. meningitidis* strains to 0.05% SPS. The study was performed using a broth titration technique; it showed that several of the strains were markedly inhibited. Also, a paper disk method was evolved for screening the sensitivity of strains of various species to SPS; the highest formation of zones of inhibition was seen in *N. meningitidis* and *N. gonorrhoeae*. When viewing the results of these experiments, which will be published separately, together with those of the present study, it is considered likely that the addition of 0.05% SPS to blood culture broth may exert a toxic effect on *N. meningitidis* outweighing the favorable effect on the bactericidal components of patients' blood, thus yielding a detrimental total result on the rate and speed of recovery.

TABLE 4. *Time difference between growth from individual bottles in 37 paired blood cultures*

Difference (days)	No. of paired blood cultures	
	Earlier +, +[a]	+, earlier +
1	14	7
2	5	1
3	4	
4	4	1
5	1	

[a] Growth in SPS and plain broth, respectively.

TABLE 5. *Recovery of Staphylococcus epidermidis, diphtheroid bacilli, Bacillus sp., and yeasts from 114 paired blood cultures*

Organisms	No. of paired blood cultures						
	+, −[a]	Earlier +, +	Heavier +, +	+, + (equal)	+, heavier +	+, earlier +	−, +
S. epidermidis	46	7	7	7	1		31
Diphtheroid bacilli	3					1	1
Bacillus sp.	6						1
Yeasts							3

[a] Growth in SPS and plain broth, respectively.

The beneficial overall effect of 0.05% SPS on recovery from clinical blood cultures, including the striking effect in gram-negative rod bacteremias on the one hand, and the strong indications of a detrimental effect in *N. meningitidis* bacteremias on the other, naturally lead to the conclusion that duplicate blood cultures with and without SPS should be used routinely. The possibility that a lowering of the SPS concentration below 0.05% could eliminate toxic effects with preservation of the growth-promoting capacity might also be considered. Lowrance and Traub, however, examined the effect of varying SPS concentrations below 0.05% on the killing of serum-sensitive strains of *E. coli* in fresh human serum and concluded that at least 0.025% SPS should be added to broth medium for blood cultures to improve the chances of isolating pathogens present in small numbers (6).

Where routine duplicate blood cultures with and without SPS are not considered practicable and the use of one blood culture medium only is preferred, it is considered that this medium should contain 0.05% SPS, whose beneficial effect in most clinical bacteremias has been clearly demonstrated. It should be recognized, however, that this practice involves a danger of delaying or repressing the isolation of the causative agent in *N. meningitidis* bacteremias. *N. meningitidis* septicemias are often recognizable or suspected on clinical grounds. When blood cultures are made from such patients to verify the diagnosis bacteriologically, broth medium without SPS should be used.

ACKNOWLEDGMENTS

I am indebted to the staffs of Departments I, VII, and XVII of Ullevål Hospital for their ready cooperation, and to Inger Bjerke for skillful work in the laboratory.

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