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Gelatin Neutralization of the Inhibitory Effect of Sodium Polyanethol Sulfonate on *Neisseria meningitidis* in Blood Culture Media

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The inhibitory effect of sodium polyanethol sulfonate (0.05%) upon growth of *Neisseria meningitidis* was found to be neutralized by adding gelatin (1.2%) to the growth medium. The neutralizing effect was demonstrated in solid medium, as well as in nutrient broth for blood cultures. The findings parallel those of Wilkins and West (6) regarding gelatin neutralization of the inhibitory effect of sodium polyanethol sulfonate on *Peptostreptococcus anaerobius*.

Addition of sodium polyanethol sulfonate (SPS) to fluid media for clinical blood cultures has a significant, favorable effect upon the rate and speed of isolations of a wide range of pathogenic bacteria (1). The compound operates by counteracting the bacterial inhibitors of fresh human blood (5). The main disadvantage of the routine use of SPS in blood culture media has been the inhibitory effect exerted by the compound upon certain bacteria, notably *Peptostreptococcus anaerobius* (3) and *Neisseria meningitidis* (2). As shown by Wilkins and West (6), the inhibition of *P. anaerobius* by SPS is medium dependent and may be abolished by the inclusion of 1.2% gelatin in solid medium. The present study was undertaken to examine whether the content of gelatin in solid and fluid media interacts with the toxic effect of SPS on *N. meningitidis*.

MATERIALS AND METHODS

Test organisms. Fifty-nine freeze-dried strains of *N. meningitidis*, all of which had been isolated from patients with meningococcal infections, were examined.

Media. Three solid and three fluid media were used. Solid media were: (i) plain blood agar made up of PDM agar medium (paper disc method, antibiotic sensitivity medium [AB Biodisk; Stockholm, Sweden]) supplemented with 5% defibrinated horse blood; (ii) blood agar with the addition of 0.05% SPS (Liquoid; Hoffman-La Roche, Inc., Nutley, N.J.); and (iii) blood agar with 0.05% SPS and 1.2% gelatin (Difco Laboratories, Detroit, Mich.). Fluid media were: (i) serum broth made up of nutrient broth with 0.1% dextrose (prepared in this laboratory for blood culture work) and supplemented with 5% horse serum inactivated at 56°C for 30 min; (ii) serum broth with the addition of 0.05% SPS; and (iii) serum broth containing both 0.05% SPS and 1.2% gelatin.

Cultivations on solid media. Suspensions of test organisms in serum broth were poured over the surface of the three solid media under study, and the surplus fluid was removed by suction. The density of the suspension was adjusted to yield a dense but not confluent growth on blood agar plates. The plates were dried for 30 min at room temperature on a flat surface. An SPS paper disk containing 10 μl of a 10% autoclaved, aqueous solution of SPS was applied to the plain blood agar plates (2). The plates were incubated at 37°C overnight. The number and size of the colonies on the three agar media were recorded, with growth on plain blood agar as a reference standard of full growth. Zones of growth inhibition around the SPS paper disks were measured.

Cultivations in fluid media. A suspension of test organisms, obtained by homogenizing two colonies in 2.7 ml of serum broth, was titrated in serum broth in a $\log_{10}$ serial dilution comprising 10 tubes. From each tube in the titration row, 0.05 ml was transferred to each of three tubes containing, respectively, 2.7 ml of serum broth, serum broth with 0.05% SPS, and serum broth with 0.05% SPS and 1.2% gelatin. The three rows of test tubes were incubated at 37°C overnight. Subcultures were then made by spreading 0.001 ml from each test tube on chocolate agar plates by means of a calibrated wire loop. The plates were incubated at 37°C overnight. The end point of growth in the three broth media under investigation was read as the highest dilution of inoculum that yielded colony formation.

All incubations were performed in an atmosphere containing 5% carbon dioxide.

RESULTS

As shown in Table 1, 30 of the 59 strains of *N. meningitidis* were resistant to 0.05% SPS when tested on solid medium, the growth on blood agar with SPS being equal to that on plain blood agar. Fifteen strains showed significantly reduced growth, and 14 strains failed to grow at

all on blood agar with SPS. Out of these 29 SPS-sensitive strains, 24 showed full, normal growth on blood agar that in addition to SPS also contained 1.2% gelatin. Table 2 shows growth patterns of the test organisms on blood agar containing SPS compared with the zones of growth inhibition around the SPS paper disk on plain blood agar. A good correlation is seen between these two parameters of SPS sensitivity in *N. meningitidis*. The five strains showing reduced growth on blood agar with SPS and gelatin (Table 1) showed zones of growth inhibition around the SPS paper disk (Table 2) of 13, 15, 16, 17, and 18 mm, respectively. It is concluded that the inhibitory effect of 0.05% SPS upon the growth of *N. meningitidis* on blood agar was abolished by the addition of 1.2% gelatin to the medium, with the exception of a few highly SPS-sensitive strains, which did, however, show some growth, although reduced, on medium with SPS and gelatin.

Twenty-three of the meningococcal strains were also tested in broth media. Eight strains proved SPS resistant, with the end point of growth in serum broth with 0.05% SPS equal to that in plain serum broth, or else the end points of growth in the two media differed by no more than 1 $\log_{10}$ dilution step. The growth of 14 strains was clearly inhibited, the end points of growth being 4 to 6 logs lower in serum broth with 0.05% SPS than in serum broth without the additive (difference of end points: 4 logs in seven strains, 5 logs in five strains, and 6 logs in two strains). In the one remaining strain, the result was considered dubious (difference of end points, 2 logs). In all the 14 strains significantly inhibited in broth with SPS, growth in broth containing gelatin together with SPS took place up to the same end point as that in plain serum broth, or the endpoints differed by no more than 1 log. The overall distribution of growth end points from the 14 SPS-sensitive meningococcal strains in the three broth media examined is given in Table 3. The inhibition of growth exerted by SPS was, thus, completely neutralized by the addition of 1.2% gelatin to the broth medium.

TABLE 1. *Gelatin neutralization of SPS inhibition of N. meningitidis on blood agar*

Growth on blood agar with 0.05% SPS and 1.2% gelatin	Growth on blood agar with 0.05% SPS[a]			Total
	Full growth	Reduced growth	No growth	
Full growth	30	14	10	54
Reduced growth	0	1	4	5
No growth	0	0	0	0
Total	30	15	14	59

[a] Figures in columns show number of strains giving indicated growth pattern on two test media.

DISCUSSION

Graves et al. (3) demonstrated that growth of *P. anaerobius* is inhibited by SPS at a concentration of 0.05%, which is widely used in fluid blood culture media to neutralize the bactericidal properties of fresh human blood. Wilkins and West (6) showed that the SPS inhibition of this species is neutralized in solid medium by the addition of 1.2% gelatin. In the present study, a similar protecting effect of gelatin has been demonstrated with regard to the toxic influence of SPS against *N. meningitidis*. The inclusion of 1.2% gelatin allows SPS-sensitive meningococcal strains to grow freely both on solid medium and in broth medium despite the presence of SPS. The gelatin protection might be mediated through the protective colloid effect (4), or through a stabilization of the cytoplasmic membrane. Wilkins and West (6) found that the inclusion of gelatin in the growth medium does not interfere with the ability of 0.05% SPS to abolish the growth-inhibiting effect of fresh human serum against two serum-sensitive strains of gram-negative rods, thus

TABLE 2. *Correlation between growth on blood agar with 0.05% SPS and diameter of growth inhibition zone around SPS paper disk on plain blood agar in 59 strains of N. meningitidis*

Growth on blood agar with 0.05% SPS	Zone of growth inhibition around SPS paper disk (mm)[a]													Total
	No zone	7	8	9	10	11	12	13	14	15	16	17	18	
Full growth	5	4	3	9	4	4	1							30
Reduced growth				1	3	7	3			1				15
No growth						1	1	2		1	3	4	2	14
Total	5	4	3	10	7	12	5	2	0	2	3	4	2	59

[a] Figures in the columns show number of strains.

TABLE 3. *Gelatin neutralization of SPS inhibition of N. meningitidis in serum broth*

End point of growth in:	Log_{10} dilution steps of inoculum suspensions of test strains[a]								
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Serum broth						1	7	6	
Serum broth with 0.05% SPS		6	6	2					
Serum broth with 0.05% SPS and 1.2% gelatin						3	6	5	

[a] Figures in columns show number of strains giving indicated end points of growth in three test media from a log_{10} dilution series of inoculum suspension of 14 SPS-sensitive meningococcal strains.

indicating that gelatin does not prevent SPS from neutralizing the bactericidal effect of blood. As pointed out by these authors, additional studies are desirable to investigate this point in more detail. At present, it seems reasonable to use the combination of 0.05% SPS and 1.2% gelatin in blood culture media, since the highly valuable neutralizing effect of SPS upon the bacterial inhibitors in human blood seems to be retained, whereas the toxic effect of SPS upon two important bacteremia agents, *P. anaerobius* and *N. meningitidis*, is abolished.

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