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Enhancement of Recovery of *Neisseria meningitidis* by Gelatin in Blood Culture Media

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The efficacy of gelatin for the recovery of *Neisseria meningitidis* from blood cultures was evaluated in a clinical setting. The organism was isolated from seven patients with meningococcal infections in blood culture media containing 1% gelatin. In contrast, only two blood cultures from these patients were positive in media without gelatin ($P < 0.05$). Gelatin did not influence the recovery of other organisms isolated during this study. Conventional blood culture media may be supplemented with gelatin when meningococcemia is suspected.

The difficulty of isolating *Neisseria meningitidis* from blood is well documented (2, 8). In 40 cases of meningococcal infection diagnosed at the Montreal Children's Hospital, Montreal, Quebec, Canada, during the 5-year period from 1975 through 1979, the organism was isolated from blood from only 21 patients (unpublished data). This may be due to intermittent release of the organism into the blood stream, which probably accounts for the recurring fever spikes frequently encountered in cases of meningococcemia. The difficulty may also arise from the fact that the growth of some strains of *N. meningitidis* is inhibited by sodium polyanethol sulfonate (SPS) at the concentrations (0.025 to 0.05%) used when it serves as an additive to commercially available blood culture media (6). Eng and Holten (5) reported that the inhibitory effect of SPS in artificially seeded cultures of *N. meningitidis* is neutralized by adding gelatin (1.2%) to culture media. However, the protective action of gelatin has not been documented in clinical settings.

Recently, we had seven cases of meningococcal infections; the organism was isolated from the blood of all seven patients in media containing gelatin but from only two in conventional blood culture media.

MATERIALS AND METHODS

During the 13-month period from December 1979 through December 1980, blood drawn from all patients seen at the emergency service of the Montreal Children's Hospital and suspected of having bacteremia was inoculated (2 ml each) into two bottles containing 50 ml of Columbia broth (Difco Laboratories, Detroit, Mich.) with SPS (0.03%); one of the two bottles also contained 1% gelatin (Difco). These blood culture media, with or without gelatin, were purchased from Institut Armand-Frappier, Laval, Quebec, Canada. All bottles were incubated at 37°C without venting and subcultured blindly on days 1 and 2 onto sheep blood agar with *Staphylococcus* streak and chocolate agar plates, both incubated at 37°C in an atmosphere of 5% CO_2 for 48 h. The blood culture bottles were examined visually every day until day 7 when a final subculture was made, including anaerobic incubation. The gelatin did not interfere in any way with the visual inspection procedure.

RESULTS

Blood cultures were submitted for 1,662 patients from 1 December 1979 to 31 December 1980, and *N. meningitidis* was isolated from seven patients with or without meningitis; the organism was isolated from the blood of all seven patients in gelatin-containing media but from only two patients in media without gelatin. The major clinical and laboratory findings on the seven children are summarized in Table 1. Case 1 illustrates the importance of adequate blood culture techniques for the diagnosis of benign meningococcal infections. The patient was seen at the emergency service with a 7-day history of cold and mild fever. Physical examination was unremarkable with the exception of fever. A blood culture was drawn, and the patient was sent home without antibiotic treatment. A subculture drawn from the gelatin-containing bottle on day 1 yielded a moderate growth of meningococci; a subculture drawn similarly from the bottle without gelatin was sterile. The patient was then admitted to the hospital only because the blood culture grew meningococci. She was afebrile, but a few scattered petechiae were present on the back and lower abdomen which had not been noted earlier when she was examined at the emergency service. The patient was

TABLE 1. *Clinical and laboratory findings in seven cases of meningococcal infection at the time that blood was drawn*

Case	Age	Sex	Temp (°C)	Symptoms		Initial diagnosis	CSF culture	Blood culture	
				Skin eruption	Menin-geal signs			With gelatin	Without gelatin
1	5 mo	Female	39.5	−	−	Fever of unknown origin	ND[a]	MC[b] group A (moderate growth[c])	Negative
2	4.5 yr	Male	39.0	+	−	Meningococcemia	MC group B	MC group B (one colony)	Negative
3	1.5 mo	Female	39.5	+	−	Sepsis	MC group C	MC group C (light growth)	Negative
4	6 mo	Male	40.0	−	+	Meningitis	Negative × 3	MC group B (light growth)	MC group B (two colonies)
5	5 mo	Male	39.3	−	−	Sepsis	Negative × 3	MC group B (moderate growth)	Negative
6	4 yr	Female	39.4	+	−	Meningococcemia	MC group B		Negative
7	5 yr	Male	39.5	+	+	Meningitis	MC[d]	MC[d] (heavy growth)	MC[d] (heavy growth)

[a] ND, Not done when blood cultures were drawn at the emergency service. CSF cultures obtained subsequently were negative.

[b] MC, Meningococcus.

[c] The amount of growth on subcultures (blood agar and chocolate agar plates) drawn on day 1 is described as light (10 to 30 colonies), moderate (30 to 50 colonies), or heavy (>50 colonies).

[d] Ungroupable due to autoagglutination.

treated for meningococcemia and discharged after 10 days in normal condition. The initial blood culture drawn at the emergency service was subcultured on days 2 and 7. The gelatin-containing media continued to yield a moderate growth of meningococci, but all subcultures of the media without gelatin remained sterile.

For cases 2, 3, 6, and 7, the results of blood cultures did not influence the management of the patients since the cerebrospinal fluid (CSF) also grew meningococci. However, it should be noted that the blood culture drawn from case 2 grew only one colony of meningococci upon a subculture (day 1) from the gelatin-containing bottle, suggesting that the bacteriological diagnosis of meningococcemia by positive blood culture may be difficult in some cases if only an extremely small number of organisms are present in the blood.

In case 4, CSF cultures were negative, although clinical symptoms and signs and other CSF findings were consistent with acute bacterial meningitis. The patient was treated for meningococcal meningitis because the organism was isolated from the blood.

The frequency of isolation of other significant pathogens from blood culture media with and without gelatin is shown in Table 2. The presence of gelatin in the blood culture media did not appear to interfere with or enhance the recovery of bacteria other than meningococci. For 31 sets of blood cultures yielding the same organism in media with and without gelatin, the time of isolation was the same for both media.

TABLE 2. *Comparison of blood culture media with or without gelatin in the recovery of pathogens from blood*[a]

Organism	Total no. isolated	No. of isolates in:			*P* value[c]
		Both	Media with gelatin only[b]	Media without gelatin only	
N. meningitidis	7	2	5	0	<0.05
Haemophilus influenzae	20	19	0	1	NS[d]
Streptococcus pneumoniae	8	5	0	3	NS
Escherichia coli	4	2	1	1	NS
Salmonella species	3	2	1	0	NS
Staphylococcus aureus	1	0	0	1	NS
Streptococcus group B	1	1	0	0	NS
Streptococcus group D	1	0	1	0	NS

[a] The isolates that were considered contaminants are not included in this table.

[b] Blood culture bottles contained 50 ml of Columbia medium (Difco) with SPS (0.03%). Gelatin, when present, was at a concentration of 1%. All blood cultures were incubated up to 7 days before they were discarded as negative.

[c] *P* values were obtained by chi-square analysis for the difference between media with and without gelatin.

[d] NS, Not significant.

DISCUSSION

To our knowledge, this is the first clinical study evaluating the effect of gelatin in blood culture media on the recovery of meningococci from blood. In seven cases of meningococcal infections, the organism was isolated successfully within 24 h of inoculation from blood culture media containing gelatin, whereas five of the seven blood cultures inoculated into media without gelatin remained sterile throughout a 7-day course of incubation. In one of the seven cases, meningococcal infection was not even suspected at the time of the emergency service visit, since the only physical sign was that of mild fever. The patient was recalled and admitted to the hospital only because meningococci had grown from the blood. The diagnosis of meningococcal infection might not have been established without the gelatin-containing media.

Presumptive diagnosis of meningococcemia may be made by the demonstration of the organism in skin eruptions by the immunofluorescent technique or by a rise in specific antibody titers (2, 8), but the confirmatory diagnosis is a positive blood culture. However, the bacteriological diagnosis is difficult and may require multiple blood cultures. In a review of 148 cases with chronic meningococcemia, Benoit (2) noted that positive bacteriological diagnoses were established by blood culture in 129 cases, but the first positive blood culture was not obtained, on the average, until the fifth blood sample (range, 1 to 9 samples) was drawn. In other studies where multiple blood cultures were not drawn, the organism was isolated from blood in only 33% (9 of 27) (4) and 48% (21 of 44) (8) of patients with meningococcemia.

The difficulty in obtaining meningococci from blood cultures may be due to the presence of SPS in conventional blood culture media. SPS is widely used as an additive to blood culture media because of its anticoagulant, anticomplementary, and antiphagocytic properties. However, the growth of certain bacteria, notably *N. meningitidis* (6) and *Peptostreptococcus anaerobius* (7), is inhibited by SPS at the concentrations commonly used (0.025% to 0.05%). Eng and Holten (5) examined 59 clinical isolates of *N. meningitidis* and found that more than 50%

of the strains were sensitive to SPS at a concentration of 0.05%. The amount of growth overnight was 4 to 6 logs lower in serum broth with 0.05% SPS than in serum broth without the additive. The toxic effect of SPS against *N. meningitidis* was completely neutralized by the addition of 1.2% gelatin in the culture media; the amount of growth in broth containing SPS and gelatin is the same as that in plain broth or differs by no more than 1 log. The mechanism of gelatin protection is not known. It might be mediated through a protective colloid effect or through a stabilization of the cell membrane.

SPS may also have an inhibitory effect on *N. gonorrhoeae* (6). Blood cultures were positive in only 13% of patients with cases of disseminated gonococcal infection (1). Two cases of disseminated gonococcal infection were reported recently in which the organism was isolated from blood by the lysis-centrifugation technique, but failed to grow in conventional blood culture media containing SPS (3).

The number of meningococcal infections we have studied is too small to draw any conclusions at this time, but the preliminary results suggest that the addition of gelatin to blood culture media may enhance the recovery of meningococci without interfering with the growth of other organisms. Additional clinical studies are warranted to establish the efficacy of gelatin for the recovery of *N. meningitidis* from blood cultures.

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