CLOSTRIDIA IN GAS GANGRENE AND LOCAL ANAEROBIC INFECTIONS DURING THE ITALIAN CAMPAIGN

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When World War II began, some of the experimental results obtained on animals indicated that sulfonamides applied locally and taken by mouth might prevent infections with anaerobes of the gas gangrene group. It became evident, even in the Western Desert and Tunisia, that anaerobic infections would occur in spite of sulfonamide prophylaxis. When the fighting took place on the more cultivated soil in Sicily and Italy, the incidence of gas gangrene increased. Antiserums made in the United States, usually containing antitoxin only for Clostridium perfringens (B. welchii) and Clostridium septicum, did not appear to prevent gas gangrene and were of limited value in the therapy of cases (Jergesen, 1944). The question arose as to the incidence of Clostridium novyi (B. oedematiens) in anaerobic infections; it was questioned if the poor results with serum could be attributed to the lack of C. novyi antitoxin in some American polyvalent gas gangrene antiserums.

In order to determine the incidence of C. novyi, the clostridial flora of 25 cases of gas gangrene that occurred in Italy was studied (Stock, 1944). In a second study, made while the fighting was in the Northern Apennines, 5 additional cases of gas gangrene and 7 of local anaerobic infections were cultured, and at the same time an effort was made to determine the incidence and significance of positive blood cultures. It appeared important to learn whether therapy could save a case of gas gangrene once the causative organisms had entered the blood stream. Although only the preliminary phase of the latter study was completed, it may be of value to record these results and to summarize our entire findings because so few reports on cultures in gas gangrene or other anaerobic infection in World War II have appeared (MacLennan, 1943, 1944; MacLennan and Macfarlane, 1944; Jeffrey and Thomson, 1944; Smith and George, 1946).

MATERIALS AND METHODS

Specimens of muscle or blood were placed in chopped meat medium at surgical operation and forwarded to the laboratory. Anaerobic jars of the McIntosh-Fildes type, but without a heating coil, were fashioned from 105-mm shell cases (see Smith and George, 1946). Two g of palladium-asbestos (Fildes, 1917) covered by a wire screen served as catalyst. Anaerobes were grown on the surface of thioglycolate blood agar plates from inoculums of unheated, heated (80 C), and enriched heated samples. Isolation and identification of clostridia were

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TABLE 1
Clostridia isolated from wound and blood cultures in gas gangrene and local anaerobic infections
(Second Series)

<table>
<thead>
<tr>
<th>CASE NO.</th>
<th>TIME OF BLOOD CULTURE</th>
<th>SOURCE OF CLOSTRIDIA</th>
<th>CLOSTRIDIA FOUND</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>perfringens</td>
<td>necy</td>
</tr>
<tr>
<td>Group I &quot;Gas Gangrene&quot;</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>Post mortem</td>
<td>M*</td>
<td>B*</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Post mortem</td>
<td>M</td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Antemortem</td>
<td>M</td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>At operation</td>
<td>M</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>At operation</td>
<td>M</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Group II &quot;Local Infections&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>At operation</td>
<td>M</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>At operation</td>
<td>M</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>At operation‡</td>
<td>M</td>
<td>+</td>
<td>+</td>
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<td>4</td>
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<td>M</td>
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</tr>
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<td>6</td>
<td>At operation</td>
<td>M</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>At operation</td>
<td>M</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* M—Muscle culture. B—Blood culture. Only positive blood cultures are tabulated.
All others were negative. (See case no. 3, local infections.)
‡ Not isolated in pure culture. Overgrown by C. tetani.
§ Blood culture positive but technique questionable.
‖ "Unidentified" strains differed in some respects from anaerobes described in literature.

accomplished by methods similar to those outlined by the Committee of London Sector Pathologists (1943) and by Reed and Orr (1941). The cultures when isolated were replated as often as necessary for purity. For fermentations, sugar

Some of the methods employed were suggested by Major J. D. MacLennan, R.A.M.C.
broths of lactose, glucose, sucrose, and salicin sufficed; sodium thioglycolate (BBL) and semisolid agar media were employed so that growth in an anaerobic jar was unnecessary (Reed and Orr, 1941). Proteolysis was determined from growth in chopped meat medium and reduced-iron milk medium. In contrast to the report of Reed and Orr (1941), strains of C. novyi were not proteolytic in our tests. In pathogenicity tests made by injecting guinea pigs with 18-hour cultures in thioglycolate broth, no calcium chloride was used. A few protection tests were made with monovalent antiserums obtained from Major G. H. Eagles, R.A.M.C.

RESULTS

The distribution of anaerobes in muscle and blood cultures in the two groups of cases studied in the present series is shown in table 1. Regardless of clinical diagnosis, all wound specimens on culture showed pathogenic species of clostridia. Table 2 summarizes the combined cultural results in our first series (Stock, 1944) and second series. In the cases of gas gangrene, C. perfringens and C. novyi were the most prevalent of the pathogenic clostridia. C. septicum was found only once.

In two of the three fatal cases of gas gangrene, blood cultures taken post mortem revealed pathogenic species of clostridia (see table 1). Only one of the other cases had a positive culture for pathogenic species of clostridia, but this finding was discounted because the blood culture bottle apparently had been contaminated with the tissue culture (case no. 3 of "local infections").

In pathogenicity tests, some strains of C. novyi were either nonpathogenic or of low pathogenicity. Pathogenic strains were neutralized by specific antitoxin. The strain of C. septicum was highly virulent. All strains of Clostridium bifermentans were tested but no strain produced more than tumefaction in guinea pigs. The three strains of Clostridium tetani were pathogenic. All strains of C. perfringens that were tested were virulent. The strain of Clostridium fallax was nonpathogenic for a guinea pig. This organism, which had the characteristics of a hemolytic Clostridium multifermentans, has been discussed elsewhere (Stock, 1944). It should be noted that in all these tests whole broth
cultures were injected and no calcium chloride was used (Bullock and Cramer, 1919).

**DISCUSSION**

In general, the distribution of clostridia found in cases of gas gangrene was similar to that described in published reports (Weinberg and Seguin, 1918; Medical Research Committee, Brit., 1919; Sordelli, 1923; Zeissler and Neller, 1928; MacLennan, 1943, 1944; MacLennan and Macfarlane, 1944; Smith and George, 1946). The presence of *Clostridium novyi* in about 50 per cent of cases of gas gangrene is confirmed. *C. novyi* was found in soil by Zeissler and Rassfeld (1928) in 64 per cent of samples, so that a high incidence is possible in wounds. If the prophylactic and therapeutic efficiency of gas gangrene antiserums is to be determined, it would seem necessary to include *C. novyi* antitoxin in the polyvalent serum (see Hall, 1946). Gas gangrene toxoids for immunization should contain *C. novyi* toxoid as a component (Robertson and Keppie, 1943).

*C. septicum* was isolated only in 30 cases of gas gangrene. In a large series of cases, Weinberg and Seguin (1918) found a 13 per cent and MacLennan (1943) a 19 per cent incidence for this species. On the other hand, Zeissler and Neller (1928) isolated only 1 strain of *C. septicum* from 22 cases of gas gangrene in German civilians, and Zeissler and Rassfeld (1928), in an examination of soil, found an incidence of 8 per cent.

None of the strains of *Clostridium bifermentans* isolated in our studies was pathogenic for guinea pigs by the methods employed. It is to be noted that Clark and Hall (1937) and Stewart (1938) have found *C. bifermentans* antiserum of protective value against the pathogenic variety of this species (*Clostridium sordellii*).

No strains could be identified culturally or by pathogenicity tests as *Clostridium histolyticum*. In soil, Zeissler and Rassfeld (1928) reported an incidence of 2 per cent for this species. Smith and George (1946) working in Italy did not find strains of *C. histolyticum*. In their series, Weinberg and Seguin (1918) isolated 8 strains from cases late in the investigation. In MacLennan's series (1943), all 9 patients with *C. histolyticum* in the wound flora succumbed.

Death from gas gangrene has been attributed generally to toxemia (MacLennan, 1946). Bacteremia which is known to occur has been considered a terminal event, although this conclusion, drawn from Weinberg and Seguin's paper (1918), may not be warranted. Further studies on blood cultures in gas gangrene are needed to determine whether bacteremia is an additional factor in the high mortality rate which still exists in spite of present therapeutic agents and surgical technique. There is experimental evidence that is suggestive, for once clostridia had entered the blood stream in infected mice, which occurred after 3 hours, McIntosh and Selbie (1943a, 1943b) found local chemotherapy to be less effective.

In 7 local anaerobic infections cultured in Italy, 7 strains of *Clostridium perfringens* and 5 of *C. novyi* were found. Thus, pathogenic species of clostridia were commonly found and were not less frequent than proteolytic nonpathogenic clostridia. This is the opposite of the findings in a small series of cases of "anaerobic
cellulitis" cultured by MacLennan (1943) in the Western Desert, but agrees with those of Weinberg and Seguin (1918) in the cases called "gaseous phlegmon" or "gaseous wounds." In our experience, "heavy local anaerobic infection" used by Robertson (1929) may be a more accurate description of the lesions seen in Italy than "anaerobic cellulitis." Beginning with local anaerobic infections in dead tissue, all gradations and degrees of infection resulted, with fulminating gas gangrene at the extreme. Debridement removed infected tissue and often prevented further spread. Prophylactic penicillin was used in wounded patients in the latter part of the Italian campaign, but no data are available on its effect on the cultural findings of the bacterial flora of the wounds or on its therapeutic value in the dosage used.

Numerous nonpathogenic species of clostridia and many aerobes (not listed in our tables), particularly nonhemolytic streptococci, were found in the specimens from gas gangrene and local anaerobic infections but were not investigated further. No information was obtained on their significance.

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SUMMARY

In 30 cases of gas gangrene cultured in Italy, 80 per cent of the cases showed Clostridium perfringens, 50 per cent Clostridium novyi, and 1 case Clostridium septicum. The high incidence of Clostridium novyi confirms the earlier reports of French and British investigators. Clostridium tetani and nonpathogenic Clostridium bifermentans were found. No strains of Clostridium histolyticum were identified. In a trial series, clostridia were recovered post mortem from blood cultures in 2 cases of gas gangrene. No data were obtained on the prognostic significance of a positive blood culture. Seven local anaerobic infections showed on culture 7 Clostridium perfringens and 5 Clostridium novyi.

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