Changes in the cell wall of *Clostridium* species following passage in animals

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**Abstract.** Morphological changes in clostridial isolates after animal passage with other flora in mixed infections were studied by utilizing a subcutaneous abscess model in mice. We used 26 isolates of 7 clostridial species, and one isolate each of *Bacteroides fragilis* and *Klebsiella pneumoniae*. Abscesses were induced by all 7 *Clostridium perfringens* and 3 *Clostridium butyricum* isolates and by some of the other isolates. A thick granular wall prior to animal inoculation was shown only in *C. perfringens*, *C. butyricum*, and *C. difficile*. This structure was observed in other clostridia only following their animal passage alone or when co-inoculated with *K. pneumoniae* or *B. fragilis*.

**INTRODUCTION**

*Clostridium* species are involved in a variety of human diseases, including gas gangrene, botulism, and food poisoning. They are often isolated from infected sites mixed with other anaerobic, facultative, and aerobic flora (Brook 1983).

*Clostridia* are the most widely studied of the anaerobes, but no studies have investigated the changes in the cell walls of *Clostridium* sp. after passage in animals. In this study we used a subcutaneous (sc) abscess model to evaluate the changes in the clostridial cell wall following inoculation into mice.

**MATERIALS AND METHODS**

**Organisms.** All organisms were recent human clinical isolates and were not plated-

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The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. The experiments conducted herein were conducted according to the principles set forth in the 'Guide for the care and use of laboratory animals', Institutes of Laboratory Resources, National Research Council, DHEW, Publ. no. (NIH) 74-23.
ed onto new media in the laboratory after their initial isolation. They were kept frozen in skim milk at \(-70^\circ\text{C}\). The 26 clostridial isolates used were received mixed with other aerobic and/or anaerobic flora except those that were isolated from blood, which were recovered alone. They included 7 *Clostridium perfringens*, (4 isolated from wound and 3 recovered from blood), 6 *Clostridium difficile* (3 recovered as the only isolate from blood, and one each recovered with other flora from a lung aspirate, peritoneal fluid and stool), 5 *Clostridium clostridiiforme*, (3 isolated from peritoneal fluid and 2 from perirectal abscess), 3 *Clostridium butyricum* (2 isolated from peritoneal fluid, and one from the conjunctiva), 2 each of *Clostridium ramosum* (one recovered from blood and the other from the peritoneal fluid), and *Clostridium sporogenes* (from abdominal cavity) and one *Clostridium tertium* (from peritoneal fluid). The other organisms were one isolate each of *Bacteroides fragilis* and *Klebsiella pneumoniae*. The aerobic isolates were identified by conventional methods (Lennette et al. 1980), and the anaerobic organisms were studied by the Wadsworth method (Sutter et al. 1980). The presence of a capsule was established by the Hiss stain (Lennette et al. 1980) and confirmed by electron microscopy after staining with ruthenium red (Kasper 1976).

*Electron microscopy studies of clostridial species.* Thin sections preparations of all 26 clostridial isolates were examined before and after their inoculation into mice alone or in mixture with *B. fragilis* or *K. pneumoniae*. They were also examined after anaerobic incubation in brain heart infusion broth (Difco) for 16 and 48 h.

*Animals.* Male Swiss albino mice (20 to 25 g) were obtained from the Naval Medical Research Institute mouse colony (NMRI/NIH-CV), and were raised under conventional conditions.

*Abscess formation.* The bacteria were grown anaerobically or aerobically on blood agar plates with brain heart infusion base (BHI, Difco). Bacterial suspensions in saline were prepared from the agar media and mice were inoculated sc in the right groin with 0.1 ml of each of the appropriate suspension.

*Examination of the abscesses.* Animals were killed by cervical dislocation on the 5th day following inoculation, and the abscesses were removed aseptically. The tissue location and histology were confirmed by hematoxylin and eosin stained paraffin sections from 2 mice of each experimental group. The abscess contents were plated on enriched BHI and blood agar plates (Sutter et al. 1980), which were incubated for 48 h at 3°C in the anaerobic or aerobic environments. Characteristic colonies of all organisms were picked and identified by Gram stain and biochemical tests (Lennette et al. 1980; Sutter et al. 1980). Statistical