

Isolation of *Clostridium perfringens* Type D from a Case of Gas Gangrene

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A workman who had heavy tissue congestion in the upper part of the left arm and fractures of the left humerus and ribs due to accident was admitted to the Toyama Rosai Hospital on 24 September 1964. (The work of the patient involved no particular contact with animals.) Amputation of the left arm was carried out on the day of admission. An onset of symptoms indicating gas gangrene appeared on 23 September. A bloody gauze soaked in the emphysematic site of amputation was sent for microbiological examination on that day. Gas-gangrene antiserum was not given before the microbiological examination.

Upon examination, plump, large gram-positive rods (5 to 10 per microscopic field) were seen among spores, cocci, and rods. Isolation of the gram-positive rod was performed by (i) directly streaking the specimen onto a 10% blood-agar plate containing 1% glucose and (ii) by shaking a piece of the bloody gauze in 3 ml of sterilized saline, distributing 0.5-ml portions of the suspension into small test tubes, and heating them at 60, 80, or 100 C for 10 min and 100 C for 60 min; the heated samples were transferred into chopped-meat broth for further cultivation.

Clostridium perfringens was obtained only from the unheated specimen and the one heated at 60 C for 10 min. A few colonies resembling those of *C. bifermentans* and *C. sporogenes* were

also found, together with a number of colonies of *Staphylococcus*. From the plate culture directly inoculated, two strains of *C. perfringens* were isolated and toxigenically examined for typing according to the description issued with the sera supplied by Wellcome Research Laboratories, Beckenham, Kent, England.

Production of α -, β -, and ϵ -toxins was carried out by use of cooked-meat broth previously described (Nishida, Murakami, and Yamagishi, Japan. J. Microbiol. 6:33, 1962). The concentration of fructose added to the medium for α - and β -toxin production was 1% and for ϵ -toxin production was 0.5%. Cultures were harvested in 9 hr for determination of α and β toxigenicity and in 24 hr for assay of ϵ toxigenicity. The result showed that α toxigenicity of the cultures was weak, corresponding to 0.1 α -antitoxin units-equivalent; no β -toxin could be demonstrated. When the cultural filtrate was activated with trypsin, ϵ toxigenicity was found to be as high as 4,000 minimal lethal doses for mice per ml. The toxicity was neutralized only by the antisera containing ϵ -antitoxin. We believe that this is the first instance of isolation of *C. perfringens* type D strain from a case of gas gangrene.

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