PRODUCTION OF HIGH POTENT BOTULINAL TYPE E TOXIN FROM RESTING CELLS WITH ENZYMIC DIGESTION

Tokiya ITÔ and Yûji SATÔ

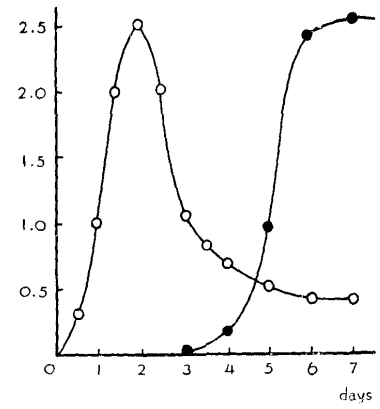
Department of Biochemistry, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

(Received for publication, Jan. 15, 1957)

Clostridium botulinum type E strain "Iwanai", which was isolated from herring-izushi, the food which caused an outbreak of fatal botulism in Japan,[1] could hardly produce toxin in culture media of routine use. Only little toxin (10 to 20 mouse MLD per ml) was found in the culture filtrates of liver infusion broth and casein hydrolysate media. At the most, toxin to the order of 20,000 to 30,000 MLD per ml was found in the filtrates, inoculated with the organism in flatfish infusion media and oyster infusion media at 27°C for 7 days.[1]

The growth curve of the organism in the media was characterized by a sharp peak, followed by rapid decline presumably owing to autolysis as was shown by KINDLER et al. with *Clostridium parabotulinum* type A. Little toxin (10 to 20 MLD per ml) was found in the filtrates obtained in 72 to 96 hours inoculation. The toxic titer increased steeply and reached a maximum at the completion of the lysis in 7 days (Fig. 1).

FIG. 1. *Growth and Toxin Production by Cl. botulinum Type E Strain "Iwanai"*



● - - - ● Toxic titer (MLD $\times 10^4$ per ml)
○ - - - ○ Bacterial nitrogen (mg per ml)

It was suggested by MATANO, who obtained high potent botulinal type E strain "Tenno" toxin with tryptic digestion of the bacteria, that the enzymic digestion of the cells might bring out the appearance of the toxin in the filtrates.

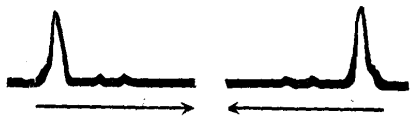
One liter of oyster infusion medium (pH 7.2) was inoculated with the organism at 27°C for 36 hours by routine anaerobic method. The cells were collected by centrifugation, washed with saline three times, and were suspended in 40 ml of saline. The suspension was incubated at 30°C for 30 minutes with equivalent volume of 0.1 % trypsin solution at pH 7.8.

The toxic titer of the filtrate, which was obtained by SEITZ's filter, was titrated by intraperitoneal injection into mice (15 to 20 g weight) with half ml of the dilutions of the filtrate.

The titer was found to be 2,000,000,000 to 3,000,000,000 MLD per ml. Content of protein nitrogen was 4.3 mg per ml.

Electrophoretic patterns showed that it contains approximately single protein, presumably gamma globulin.

FIG. 2. *Electrophoretic Patterns of Filtrate of Trypsin-Digested Resting Cells of Cl. botulinum Type E Strain "Iwanai" in pH 7.0 Phosphate Buffer, Ionic Strength of 0.146, for 40 Minutes*



REFERENCES

1) ITÔ, T., H. SHIOKAWA & Y. SATÔ (1955): *Med. & Biol.*, **39**, 77 (in Japanese).
2) KINDLER, S. H., J. MAGER & N. GROSSOWICZ (1955): *Science*, **122**, 926.
3) MATANO, K. (1956): Private Communication.
4) NAKAMURA, Y. (1952): Special Report Hokkaido Inst. Pub. Health (in Japanese).