**Clostridium botulinum** Type F: Isolation from Venison Jerky

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A *Clostridium botulinum* type F was isolated from the venison jerky responsible for the only type F botulism outbreak reported in the United States. The isolate differed from the prototype Langeland type F strain in being nonproteolytic.

*Clostridium botulinum* type F was first isolated in 1958 by Moller and Scheibel (10) from home-prepared liver paste involved in an outbreak of human botulism on the Danish Island of Langeland. The organism resembled proteolytic *C. botulinum* types A and B in cultural and biochemical characteristics, but was designated the prototype of type F because its toxin was immunologically distinct from the known toxin types (3). Thus far, both proteolytic and nonproteolytic strains have been characterized in the United States.

Type F strains have been isolated from such sources as marine sediment collected from the Pacific Coast (6), salmon taken from the Columbia River (2), crabs caught on the eastern coast of the United States (16), and soil samples collected in Argentina (7). Additionally, type F toxin has been identified in mud samples from a stream in North Dakota (15) and from a fish caught in Louisiana (14).

In 1966, the first and only reported outbreak of botulism involving type F in the United States occurred in California from home-prepared venison jerky. Among the 20 people who ate the jerky, 3 developed botulism, 2 developed mild gastro-enteritis and 15 remained asymptomatic (1, 13).

The purposes of this paper are (i) to describe some of the laboratory work performed in connection with the outbreak, (ii) to present the characteristics of the *C. botulinum* type F strain isolated, and (iii) to compare this strain with the Langeland type F and a type E strain.

**MATERIALS AND METHODS**

Stock cultures of *C. botulinum* type F (Langeland strain) and type E (KA-2) were obtained from the Center for Disease Control, Atlanta, Ga. The toxin from each strain was neutralized only by its type-specific antitoxin. Toxin in the dried venison jerky was extracted with gelatin-phosphate buffer (4). These extracts and enrichment cultures to be injected intraperitoneally into mice were clarified by centrifugation. Toxin determinations were made on these using portions which were trypsin treated and portions left untreated. Procedures of Duff et al. (9) were followed for trypsin activation. Toxin neutralization tests were made by injecting pairs of mice with 0.5 ml of a mixture composed of 0.4 ml of supernatant fluid and 0.1 ml of a monovalent antitoxin of *C. botulinum* types A, B, C, D, E, or F, respectively, obtained from the Center for Disease Control. Attempts were made to isolate *C. botulinum* from the venison jerky as well as the ingredients used in its preparation, i.e., seasoned salt, black pepper, curing sugar, and Bar-B-Q liquid smoke, following procedures previously described (11). Anaerobic incubation of plates was done in Brewer Gas Pak anaerobic jars at 30 C.

A direct fluorescent-antibody (FA) procedure which detects the presence of *C. botulinum* vegetative cells (8, 9) was used in an attempt to rapidly identify the type or types involved. Food samples and cooked meat enrichment cultures were examined by using fluorescein isothiocyanate conjugates of antisera which were specific for the vegetative form of *C. botulinum* types A, B, C, D, E, and F.

The saccharolytic, proteolytic, and other biochemical activities of the strains of *C. botulinum* were performed as described by Dowell and Hawkins (4). Since gas chromatography studies have provided useful information for identification of clostridia, an analysis of the volatile fatty acid production of the Langeland strain and of our isolate of *C. botulinum* type F was performed using procedures described in "Outline of Clinical Methods in Anaerobic Bacteriology" (12).

**RESULTS AND DISCUSSION**

Extracts prepared from the venison jerky repeatedly killed mice. In initial tests, mice injected with the extract were not protected by types A or B antitoxins, but those protected
with type E or F antitoxin survived for 24 hr. After 48 hr, only mice protected by type F antitoxin were still alive. This partial cross neutralization of type F toxin by type E antitoxin has been reported previously (6). In repeat tests, however, fresh extracts of the food killed all mice within 24 hr except those protected with type F antitoxin. Trypsinization of the extracts did not alter the results.

FA techniques based on somatic antigens have been used experimentally for identification of \( C. botulinum \) organisms. At the time of the outbreak, a FA technique was being investigated as a tool for screening food specimens and enrichment cultures to determine which ones should be subjected to additional isolation and toxin testing procedures. It was this FA test procedure that gave the first indication that a type F organism was involved, and helped explain the cross-protection observed in the toxin neutralization test. With our conjugates, no crossings had been observed previously between the types E and F organisms.

Experience with this food specimen illustrates the value of the FA technique in detecting \( C. botulinum \) organisms, and shows the need to have a working knowledge of the spectrum of reactivity of the reagents used.

Following confirmation of the presence and identity of the toxin in the jerky, efforts were focused on isolating the botulinum organism from the ingredients used in its preparation. No toxin or \( C. botulinum \) organisms could be detected in any of the ingredients or in enrichment cultures of these products. All FA tests on these ingredients were also negative for the presence of \( C. botulinum \) organisms.

Toxin could be demonstrated repeatedly in the jerky and in some enrichment cultures made of the jerky-gelatin-phosphate buffer mixture that had been treated with absolute ethanol (11).

Many unsuccessful attempts were made to isolate pure cultures of the toxigenic organism since a high proportion of subcultures also contained other sporeformers, namely, \( C. perfringens \), \( C. bifermentans \), \( C. septicum \), and \( Bacillus sp \).

One of the toxic enrichment cultures gave a very small colony that had a dewdrop appearance when streaked on blood agar. A subculture of this colony was composed of motile, gram-positive rods with subterminal, bulging spores and produced type F botulinum toxin. This isolate of \( C. botulinum \) has been designated strain 5436. After streaking the growth of this organism from a 24-hr deep meat culture onto blood agar plates and incubating them anaerobically for 48 hr at 30 C, two types of surface colonies were observed. One type was raised, opaque, and granular and the other type was translucent, flat, and spreading. Clear zones of hemolysis were present around both these colonies. Subcultures from these colony types were found to produce type F toxin and in turn to produce both types of colonies. On egg yolk agar plates, an iridescent sheen developed over the surface of the colonies and the area surrounding them.

Strain 5436 was compared to the prototype Langeland type F and a type E (KA-2) strain. The biochemical characteristics observed are summarized in Table 1. The most notable difference between these two type F cultures is their proteolytic activity. Strain 5436 resembles other type F strains isolated on the Pacific coast (6) and type E strains in its saccharolytic activities and inability to digest proteins. The volatile acids produced in tryptone yeast extract glucose medium (12) were acetic and butyric acids.

Interestingly, the various reports of detection and distribution of \( C. botulinum \) type F in nature suggest that the ecology of type F, like type E, is in some manner related to salt or fresh water environments, but neither of the two known human outbreaks was water-re-

### Table 1. Biochemical activities of \( C. botulinum \) type F and E strains

| Characteristics          | Type F Langeland | Type F strain 5436 | Type E strain KA-2 |
|--------------------------|------------------|--------------------|--------------------|
| Proteolytic              |                  |                    |                    |
| Cooked meat              | +                |                    |                    |
| Loeffler medium          | +                |                    |                    |
| Gelatin                  | +                |                    |                    |
| Saccharolytic            |                  |                    |                    |
| Glucose                  | A*G*             | AG                 | AG                 |
| Maltose                  | AG               | AG                 | AG                 |
| Lactose                  |                  |                    |                    |
| Sucrose                  | A                | AG                 | AG                 |
| Salicin                  |                  |                    |                    |
| Mannitol                 | AG               | AG                 | AG                 |
| Glycerol                 |                  |                    |                    |
| Raffinose                |                  |                    |                    |
| Dulcitol                 |                  |                    |                    |
| Arabinose                |                  |                    |                    |
| Xylose                   |                  |                    |                    |
| Inulin                   |                  |                    |                    |
| Catalase production      |                  |                    |                    |
| Nitrate reduction        |                  |                    |                    |
| Indole production        |                  |                    |                    |
| Urease production        |                  |                    |                    |

*a* Acid.  
*b* Gas.
lated. In the outbreak discussed here, the organisms or toxin could not be detected in any of the ingredients used in the preparation of the venison jerky. The opportunity for soil contamination of the dressed venison was evident (1) and was probably the source of the organisms. No intensified investigations have been performed to determine the incidence of type E and type F C. botulinum in the soils of California.

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