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Characterization of an Organism That Produces Type E Botulinal Toxin But Which Resembles *Clostridium butyricum* from the Feces of an Infant with Type E Botulism

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The apparent causative organism from the only reported case of type E infant botulism was isolated and characterized. Except for its ability to produce type E botulinal toxin, this organism (strain 5262) would be unquestionably identified as *Clostridium butyricum*. This is the second time an organism resembling a defined *Clostridium* species other than a member of the *C. botulinum* group has been implicated in infant botulism.

In the 9 years since infant botulism was first described, more than 400 cases have been reported. Almost all of the cases were caused by *Clostridium botulinum* types A and B, proteolytic group I. Several of the type B strains have produced toxins that were atypical, but the biochemical characteristics of the bacterial isolates were consistent with the proteolytic *C. botulinum* type B strains (L. M. McCroskey and C. L. Hatheway, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C159, p. 263). One case caused by type F botulinal toxin has been reported (3). The characteristics of the causative organism in that case were vastly different from those of group I or any of the other groups (II, III, and IV) of *C. botulinum* (8), and the phenotypic characteristics were most like those of *C. barati*, except for production of type F botulinal toxin (1).

This report describes the phenotypic characteristics of an organism isolated from the first case of type E infant botulism. The clinical aspects of the case are reported elsewhere (P. Aureli, L. Fenicia, P. Pasolini, L. M. McCroskey, and C. L. Hatheway, submitted for publication). The microorganism associated with this case is just as unusual as the organism from the case of type F infant botulism described by Hall et al. (1) and corresponds to yet another *Clostridium* species. The case occurred in Rome, Italy, in a 4-month-old, 7-pound girl.

The techniques used for performing the toxin assays and for isolating the organism from feces have been described previously (2). Type E botulinal toxin was identified in the feces of the infant by the mouse neutralization test. The specimen was cultured in chopped meat-glucose-starch with and without heat treatment (80°C, 10 min) and also in Trypticase (BBL Microbiology Systems, Cockeysville, Md.) glucose-yeast extract-peptone-trypsin medium (6). The enrichment cultures were incubated anaerobically. Samples of the liquid cultures were then streaked to modified McClung Toabe egg yolk agar and incubated anaerobically. Since all known *C. botulinum* type E strains produce lipase on egg yolk agar, lipase was used as a selective marker to screen for *C. botulinum*. However, in addition to many lipase-positive colonies, several lecithinase-positive colonies and several colonies negative for lipase and lecithinase were picked and inoculated into either Trypticase-glucose-yeast extract-peptone-trypsin or chopped meat-glucose-starch and incubated anaerobically.

Type E botulinal toxin was detected in the supernatants of all enrichment cultures. All of the chopped meat-glucose-starch cultures from lipase-positive and lecithinase-positive colonies were nontoxigenic. Three of the cultures from lecithinase-negative, lipase-negative colonies produced a toxin that was neuroparalytic and lethal for mice. This toxin was neutralized by monovalent *C. botulinum* type E botulinal antitoxin but not by monovalent antitoxin types A, B, C, D, F, and G. A chopped meat-glucose-starch culture had a toxin titer of 1:100, which increased to 1:10,000 after treatment with trypsin. Mice injected with this toxic culture extract showed typical signs of botulism, including labored breathing and flaccid paralysis. The isolated, toxigenic organism was a gram-positive, medium-sized, nonmotile rod with rounded ends and oval, subterminal spores. In tests for biochemical characterization, it produced acid from glucose, lactose, sucrose, maltose, salicin, glycerol, xylose, mannose, trehalose, and starch. Mannitol, rhamnose, and arabinose were not fermented. Nitrate was not reduced, nor was gelatin liquefied. Indole, catalase, lecithinase, and lipase were not produced. Esculin and starch were hydrolyzed. No growth occurred on blood agar incubated in a candle extinction jar. Milk was acidified and coagulated with stormy fermentation, but no digestion was observed. Growth occurred in glucose-minimal salts-biotin (4) through the third transfer. The volatile acids, acetic and butyric, were detected in peptone-yeast extract-glucose medium cultures by gas-liquid chromatography. These characteristics correspond closely to those of *C. butyricum* as listed by Holdeman et al. (4).

The key differential characteristics of the four groups of *C. botulinum* (8), the two organisms (lipase positive and lipase negative) isolated from the infant, and *C. butyricum* are listed in Table 1. Except for its toxigenicity, the lipase-negative isolate corresponded to *C. butyricum*. The lipase-positive isolate resembled *C. botulinum* group I, except that it was nontoxigenic and thus corresponded to *C. sporogenes*. This lipase-positive isolate did not resemble *C. botulinum* group II, to which all type E toxin strains belong, and thus we cannot consider it as a typical *C. botulinum* type E that might have initially caused the illness of the infant but subsequently lost its toxigenicity.

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TABLE 1. Characteristics of *C. botulinum* groups I, II, III, and IV; the lipase-positive and -negative isolates from the infant; and *C. butyricum*

Organism	Reaction in[a]:				Fermentation of[a]:			Volatile acide produced in PYG culture[b]	Toxin type
	Milk	Gelatin (liquefaction)	Lecithinase	Lipase	Glucose	Lactose	Mannose		
C. botulinum group									
I	D	+	−	+	+	−	−	A, iB, B, iV (V, iC)	A, B, F
II	−	− (+)	−	+	+	−	+	A, B	E, B, F
III	D (−)	+ (−)	− (+)	+	+	−	+	A, P, B	C, D
IV	D	+	−	−	−	−	−	A, iB, B, iV	G
Isolates from infant									
Lipase positive	D	+	−	+	+	−	−	A, iB, B, iV, iC	
Lipase negative (strain 5262)	St	−	−	−	+	+	+	A, B	E
C. butyricum	St	−	−	−	+	+	+	A, B	

[a] Reactions: D, digestion; St, stormy fermentation; +, positive; −, negative.
[b] Volatile fatty acids detected by gas-liquid chromatography: A, acetic; B, butyric; P, propionic; V, valeric; iB, isobutyric; iV, isovaleric; iC, isocaproic. PYG, Peptone-yeast extract-glucose.

As Smith pointed out (8), *C. botulinum* is not a well-defined species; it is a conglomerate of organisms, all of which produce potent neurotoxins with similar if not identical pharmacological action. The strains of *C. botulinum* associated with foodborne or wound botulism belong to groups I and II. Before this case was identified, all strains except one (1) isolated from infants with botulism were consistent with group I. This organism, which we designate as strain 5262, is similar to *C. butyricum*, except for its toxigenicity.

C. butyricum has been considered as a causative agent of necrotizing enterocolitis in infants (5, 10) but has also been found in stools of normal infants and infants hospitalized for other illness (7). In one study, seven of eight control infants (patients without necrotizing enterocolitis) had high counts of *C. butyricum*; five of them exceeded 10^6/g of feces (9). These results indicate that an organism with physiological characteristics such as those of *C. butyricum* might find the infant gut a suitable environment for multiplication. If this same organism were also capable of producing botulinal neurotoxin, there seems little doubt that it would cause the paralysis which we recognize as botulism.

This is the first organism isolated from the feces of an infant with botulism that produces *C. botulinum* type E toxin. The clinical features of the infant were typical of infant botulism (Aureli et al., submitted). This is the second gelatinase-negative *Clostridium* sp. isolated from the stool of an infant with botulism that produces toxin neutralized by monovalent *C. botulinum* antitoxin. These two cases indicate that botulism can be caused by botulinal toxin elaborated by organisms other than typical *C. botulinum*.

The organism described in this report appears to be another in the conglomerate of organisms capable of producing the potent neurotoxin associated with *C. botulinum*. This points out the need to look for nontypical organisms in toxin-positive cases when no typical *C. botulinum* is found.

ADDENDUM IN PROOF

A second case of type E infant botulism in Italy has been confirmed by the Istituto Superiore di Sanitá. This case also involved an infant residing in Rome. We have established that the causitive organism was similar if not identical to the organism described in this note.

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