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Quantitation of *Clostridium botulinum* Organisms and Toxin in the Feces of an Infant with Botulism

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A 4-month-old boy presented with symptoms and signs characteristic of infant botulism. Examination of feces revealed *Clostridium botulinum* type B and type B toxin. The numbers of *C. botulinum* and the amount of toxin in feces were measured throughout the 4-week period in hospital. The maximum numbers and amounts were detected in a fecal specimen collected 16 days after admission: this contained 8.4×10^6 *C. botulinum* type B colony-forming units and 61,440 mouse 100% lethal doses of type B toxin per g (wet weight) of feces. This latter figure is the highest fecal toxin titer reported yet for a case of infant botulism. By day 16, however, substantial improvement in the patient's clinical condition had occurred. This suggests that initiation of recovery from infant botulism is not necessarily preceded by a reduction in the numbers of *C. botulinum* organisms and the quantity of toxin in the gut.

Infant botulism results from intestinal infection with *Clostridium botulinum* with concomitant in vivo production of toxin (11). Although the first laboratory-proven case is now known to have occurred in 1931 (3), infant botulism was not recognized until 1976 (8, 10). Since then over 100 cases have been reported, mainly from the United States (1). Recently we reported the first case of infant botulism to be recognized in South Australia (4). This case was unusual in that both *C. botulinum* type A and type B were isolated from the patient's feces.

Little is known of the relationship between the level of *C. botulinum* organisms and toxin in the gut and the clinical condition of afflicted patients. Recently, Wilcke et al. (11) determined the number of *C. botulinum* and other organisms in fecal samples from four cases of infant botulism, but the amount of toxin in these samples was not determined.

The present report deals with the second case of infant botulism to be recognized in South Australia, in which the quantity of *C. botulinum* organisms and toxin were determined in all fecal samples passed during the 4-week period that the patient was in hospital. Clinical improvement occurred before the levels of organisms and toxin in the feces reached a maximum. This suggests that recovery from infant botulism is not preceded by a reduction in the level of toxin in the gut.

CASE REPORT

A 4-month-old boy, who had been predominantly breast-fed, presented with a history of absolute constipation for 2 weeks. His previous history had been uneventful, apart from a cicatricial phimosis secondary to circumcision; the phimosis was surgically corrected at the age of 2 months. Five days before admission he became irritable and fed poorly. At that stage the mother took the infant to the family doctor, who suspected a respiratory infection and prescribed cotrimoxazole. Two days before admission the child developed increasing difficulty in swallowing and showed dehydration and generalized hypotonia. At that stage he was admitted to a regional hospital, where the results of investigations suggested the presence of a urinary tract infection. Blood was cultured but showed no growth, and lumbar puncture yielded normal cerebrospinal fluid. The child was treated initially with penicillin and gentamicin. His condition continued to worsen, with increasing generalized weakness, and respiratory difficulty became apparent. At this stage he was transferred to The Adelaide Children's Hospital for further management because infant botulism was suspected.

At the time of admission (day 1), examination revealed a very weak, severely hypotonic infant, with noisy gurgling breathing. External ocular movements were full, and the pupils showed a partial reaction to light. There was slight voluntary jaw-closure, but an absent jaw jerk. The face was mask-like and immobile, the gag reflex was absent, and there was a mild degree of pooling of secretions in the pharynx. The infant gave a weak cry in response to painful stimulation. There was complete head lag when the supine infant was lifted forward by the arms. Only slight tongue movements were observed. The limbs were weak and hypotonic, but weak voluntary movements of all extremities were present. The deep tendon reflexes were depressed, but a weak ankle jerk was preserved. The plantar responses were extensor.

On day 2 electromyography revealed a 500% in-

crease in the amplitude of the compound motor action potential following 50 cycles-per-s stimulation of the left ulnar nerve. On day 6 50 cycles-per-s stimulation of the right ulnar nerve resulted in a 300% augmentation of the compound motor action potential. These electromyographic findings indicated a defect in acetyl choline release at the neuromuscular junction and, in this clinical context, strongly suggested the diagnosis of botulism.

This diagnosis was confirmed by examination of feces which showed the presence of *C. botulinum* type B and type B toxin. Further details of microbiological findings are provided below.

Antibiotic therapy was discontinued on day 1. The child was intubated on day 2 and ventilated for 12 days. Nasogastric feeding was begun on day 4. From day 8 there was a gradual improvement in strength, with increasing voluntary activity of limbs, face, and bulbar musculature. On day 12, he was able to maintain adequate spontaneous respiration and on day 19 the endotracheal tube was removed. On day 29 tube feeding was fully replaced by breast and bottle feeding, and he was discharged from hospital. When reviewed on day 35 he still had a significant degree of generalized hypotonia, especially in the neck and trunk, with an open-hanging mouth. However, he was feeding well, with good tongue and palatal movements. He was able to use both hands to play with objects, and he was able to kick with both legs.

MATERIALS AND METHODS

Laboratory procedures. Laboratory procedures for the isolation of *C. botulinum* and the detection of toxin used in this study were modifications of the methods described by Hatheway (6).

Culture of *C. botulinum*: Fecal samples were plated on Centers for Disease Control modified McClung Toabe egg yolk agar (5) and incubated anaerobically for 3 days. Alternatively, samples were cultured in chopped-meat glucose broth (5) and incubated for 3 days at 30°C before plating. Typical lipase-positive colonies were subcultured, and isolates in pure culture were tested biochemically. The viable count of *C. botulinum* organisms was determined by plating serial dilutions of feces on Centers for Disease Control modified McClung Toabe egg yolk agar.

Preparation of cell-free extracts for toxin analysis. Isolates with the biochemical properties of *C. botulinum* were inoculated into chopped-meat glucose broth and incubated at 30°C for 3 days. The broths were then centrifuged (12,000 × *g*, 20 min, 4°C), and the supernatant was centrifuged again (12,000 × *g*, 20 min, 4°C). This supernatant was passed through a 0.22-μm cellulose acetate filter (Millex). Cell-free extracts of feces were prepared by homogenizing samples with 2 ml of phosphate-gelatin diluent (pH 6.2) per g of feces. The homogenates were left overnight at 4°C and then centrifuged and filtered as described above.

Toxin neutralization test. To detect *C. botulinum* toxin, samples of the cell-free extracts (1.0 ml) from feces or broth were incubated at 37°C for 30 min in the presence or absence of 0.25 ml of polyvalent *C. botulinum* antitoxin (types A, B, and E) (Connaught Laboratories Limited, Ontario, Canada). For typing of toxin, samples were incubated in the presence or absence of monospecific *C. botulinum* antitoxins (Centers for Disease Control, Atlanta, Ga.). Pairs of mice were inoculated by the intraperitoneal route with 0.5 ml of these samples, and survival of the "protected" mice, combined with death of the "unprotected" mice within 4 days, indicated the presence and type of *C. botulinum* toxin. The titer of *C. botulinum* toxin in feces was determined by testing the lethality to mice of fecal extracts serially diluted in phosphate-gelatin diluent.

Test for the presence of antibodies to type B toxin in serum. The cell-free extract of a pure 3-day culture of *C. botulinum* type B in chopped-meat glucose broth was used as a source of type B toxin. Two samples of this extract (each containing approximately 5 mouse 100% lethal doses of type B toxin) were incubated (37°C, 30 min) with 1 ml of serum or phosphate-gelatin diluent. Pairs of mice were injected intraperitoneally with 0.4 ml of either extract.

RESULTS

All fecal specimens passed during the 4 weeks that the infant was in hospital were weighed and analyzed for the presence of *C. botulinum* organisms and toxin. If more than one stool was passed on a given day, the specimens were pooled before analysis. *C. botulinum* type B and type B toxin were detected, confirming the diagnosis of infant botulism. This strain of *C. botulinum* type B was proteolytic. Quantitative determinations were performed on all samples, and the results are shown in Table 1.

A sample of serum collected on day 4 did not contain *C. botulinum* toxin in detectable amounts. A second serum sample collected during convalescence (day 47) was analyzed for the presence of antibody to type B toxin as described above. Small volumes of type B toxin were treated with the patient's serum or diluent. Two mice injected with toxin + diluent died within 36 h. Of two mice injected with toxin + serum, one died after 3.5 days and the other died after 7 days. Other serum specimens (from either the acute or convalescent stage of the illness) were not available in sufficient quantity for repetition of this test.

In an effort to determine the source of infection, garden soil and house dust samples were collected from the patient's home and examined for the presence of *C. botulinum*. *C. botulinum* type B was isolated from house dust collected by a vacuum cleaner.

DISCUSSION

The detection of *C. botulinum* type B and type B toxin in the fecal sample collected on day 1 confirmed the diagnosis of infant botulism in this case. Although the amounts of organisms and toxin in this sample were high, maximum levels were not attained until day 16, when feces contained 8.4×10^6 colony-forming units of *C. botulinum* and 61,440 mouse 100% lethal doses of type B toxin per g. We believe this latter

TABLE 1. Levels of *C. botulinum* type B organisms and toxin in fecal specimens[a]

Time of specimen collection (days after admission)	Wet wt of specimen (g)	No. of *C. botulinum* (CFU/g of feces)	Toxin titer (mouse LD_{100}/g of feces)
1	2.3	4.9×10^6	10,240
4	0.8	3.0×10^5	160
8	0.8	3.6×10^6	1,600
12	0.1	2.4×10^6	3,200
14	0.4	3.0×10^6	6,400
15	0.7	3.8×10^6	12,800
16	22.7	8.4×10^6	61,440
17	39.1	4.5×10^5	1,920
18	43.4	1.1×10^5	960
19	75.8	2.1×10^4	120
20	40.0	2.7×10^3	7.5
24	45.0	2.0×10^2	ND[b]
25	9.3	$<10^2$	ND
26	13.0	ND	ND
27	44.0	ND	ND

[a] The amounts of *C. botulinum* type B organisms (colony-forming units, CFU) and toxin (mouse 100% lethal doses, mouse LD_{100}) in fecal samples were determined as described in the text.
[b] ND, Not detected.

figure to be the highest fecal toxin level reported for a case of infant botulism (the previous maximum report was 8,192 mouse 100% lethal doses per g [2]). The transient decrease in the levels of toxin and organisms during the first week of hospitalization may or may not have been due to penicillin and gentamicin therapy before admission to this hospital. It is also possible that these antibiotics may have temporarily exacerbated the paralysis. Several aminoglycosides (including gentamicin) have been shown to induce myasthenia-like syndromes (7, 9), and penicillin also has a weak neuromuscular blocking effect (9). In this case, however, withdrawal of antibiotic therapy as soon as botulism was suspected (day 1) did not result in early clinical improvement.

After the peak on day 16, the levels of toxin and organisms in the feces decreased rapidly and approximately followed first-order decay kinetics. Toxin and organisms were not detected after days 24 and 26, respectively. Follow-up fecal specimens, collected on days 39 and 61, were also negative for *C. botulinum* and toxin (results not presented). The most probable source of infection in this case was the patient's home, where *C. botulinum* type B was isolated from house dust.

Interestingly, there had been a marked improvement in the child's clinical condition before the level of toxin or the number of *C. botulinum* organisms in the feces reached their maxima. Gradual improvement in strength commenced 8 days after admission, with increasing voluntary activity of limbs, face, and bulbar musculature. By day 12 the infant was able to maintain adequate spontaneous respiration, and his bowels were functioning normally by day 16. This suggests that initiation of recovery from infant botulism is not dependent upon reduction in the amounts of *C. botulinum* organisms and toxin in the gut.

The reason for clinical recovery in spite of the presence of large amounts of toxin is unclear. It has been suggested that recovery might be accounted for by the production of protective antibody or cessation of absorption of toxin (1). We were unable to demonstrate unequivocally the presence of antibody to *C. botulinum* type B toxin in the patient's serum. Considering the sensitivity of the technique employed, it is unlikely that significant amounts of circulating antibody were present. However, it is possible that secretory immunoglobulin A is produced in the gut and either inactivates *C. botulinum* toxin or prevents its absorption. Cessation of absorption of toxin in the latter stages of the illness might also result from a shift in the location of the colonizing *C. botulinum* to more distal regions of the gastrointestinal tract, which may be less efficient in the uptake of toxin. Comprehensive studies employing animal models of infant botulism would be required to resolve these issues.

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