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Quantities of *Clostridium botulinum* Organisms and Toxin in Feces and Presence of *Clostridium botulinum* Toxin in the Serum of an Infant with Botulism

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A 7-week-old boy presented with symptoms and signs characteristic of infant botulism, and the diagnosis was confirmed by the detection of *Clostridium botulinum* type A organisms and toxin in the feces. The levels of organisms and toxin in the feces were measured throughout the 81-day period in hospital. The maximum levels detected were 2.46×10^8 *C. botulinum* type A colony-forming units and 64,000 mouse 100% lethal doses of type A toxin per g (wet weight) of feces. *C. botulinum* toxin was also detected in two samples of the patient's serum, collected 3 and 10 days after admission. Improvement in the patient's clinical condition occurred before the levels of organisms and toxin in the feces reached their maxima. A slight improvement may also have occurred while toxin was still present in the serum.

Infant botulism results from intestinal colonization with *Clostridium botulinum* and concomitant in vivo production of toxin (11). Since its first recognition in 1976 (7, 9), nearly 200 cases of infant botulism have been reported (2), mainly from the United States.

Recently, we reported a case of infant botulism in which the fecal levels of *C. botulinum* organisms and toxin were monitored throughout the period that the patient was in hospital (8). Improvement in the patient's clinical condition occurred even before levels of organisms and toxin reached their maxima.

The relationship between these parameters was further investigated in the present case (the third case of infant botulism to be recognized in South Australia). Fecal levels of *C. botulinum* organisms and toxin were monitored throughout the 11-week period that the patient was in hospital. High levels of *C. botulinum* organisms and toxin persisted in the feces for over 2 months. Remarkably, *C. botulinum* toxin was also detected in the patient's serum, a finding reported in only one previous case of infant botulism (1).

CASE REPORT

A 7-week-old boy, who had been predominantly breast fed, was admitted (on day 1) to the Adelaide Children's Hospital, North Adelaide, South Australia, with a short history of symptoms consistent with a diagnosis of infant botulism (constipation, poor feeding, and respiratory distress). He was intubated and ventilated shortly after admission, and over the next 24 h he became severely and generally hypotonic, with very little spontaneous movement. Electromyography performed on day 2 was also suggestive of botulism, and the diagnosis was confirmed by examination of feces, which showed the presence of *C. botulinum* type A organisms and toxin.

From days 3 to 10, there was virtually no respiratory effort, but spontaneous movement of limbs was observed on day 5, and movement gradually increased thereafter. Bowel actions became regular after the start of daily glycerine suppositories and senekot on day 9, and from day 12 the patient showed increasing intermittent periods of spontaneous breathing. On day 31, he was placed on intermittent mandatory ventilation, and he was taken off the ventilator completely on day 44 (although the endotracheal tube was not removed until day 74). The nasogastric tube was removed on day 79, and the patient was discharged from hospital on day 81.

When reviewed on day 110, the patient still had some head lag, and deep tendon reflexes were sluggish, but at a second follow-up visit on day 175, recovery appeared complete.

MATERIALS AND METHODS

Quantitation of *C. botulinum* organisms and toxin. Isolation and quantitation of *C. botulinum* organisms in feces were carried out as described previously (8), except that samples were plated on *C. botulinum* isolation agar (4) as well as on Centers for Disease Control modified McClung-Toabe egg yolk agar (6). Specific typing and quantitation of *C. botulinum* toxin in cell-free extracts of feces were also carried out as previously described (8).

Test for presence of antibodies to type A toxin in serum. The cell-free extract of a pure 3-day culture of *C. botulinum* type A in chopped meat glucose broth (6) was used as a source of type A toxin. Samples of this

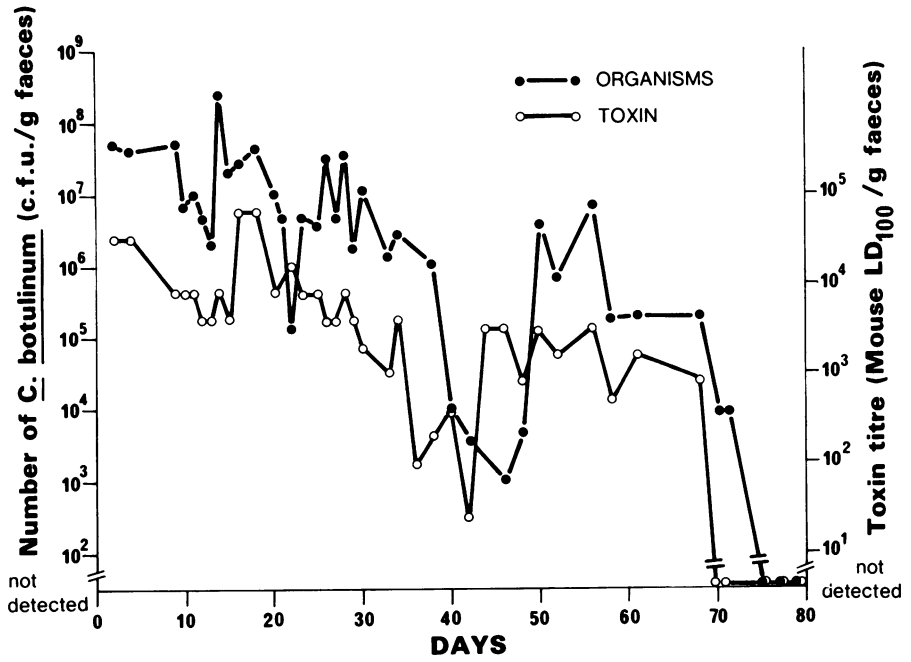

FIG. 1. Levels of *C. botulinum* type A organisms and toxin in fecal specimens. The amounts of *C. botulinum* type A organisms (●) and toxin (○) in fecal specimens collected on the indicated days were determined as described in the text. Results are expressed as colony-forming units (c.f.u.) and MLD_{100} per gram (wet weight) of feces, respectively.

extract containing various amounts of toxin (ranging from 2 to 40 mouse lethal doses [MLD_{100}]) were incubated (37°C for 30 min) with 1-ml amounts of the patient's serum, phosphate-gelatin diluent, or age-matched control serum. Pairs of mice were injected intraperitoneally with 0.4 ml of these extracts.

RESULTS

All fecal specimens passed during the first 5 weeks of hospitalization were 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. During the last 6 weeks of hospitalization, specimens collected on alternate days were examined (if available). *C. botulinum* type A and type A toxin were detected, confirming the diagnosis of infant botulism. Quantitative determinations were performed on all these specimens, and the results are shown in Fig. 1. The maximum number of *C. botulinum* organisms detected was 2.46×10^8 colony-forming units per g of feces on day 14, and the maximum toxin level was 64,000 MLD_{100}/g of feces on days 16 and 18.

C. botulinum toxin was also detected in two samples of serum, collected on days 3 and 10. In each case, the toxin titer was 2 MLD_{100}/ml. A third serum sample, collected on day 16, did not contain detectable amounts of toxin.

Serum collected on day 80 was analyzed for the presence of antibodies to type A toxin (see Materials and Methods). However, 1 ml of this serum was incapable of neutralizing even as little as 2 MLD_{100} of type A toxin.

In an effort to determine the probable source of infection in this case, garden soil, house dust, drinking water, and other environmental samples were collected from the patient's home and analyzed for the presence of *C. botulinum*. *C. botulinum* type A was isolated from house dust (collected by vacuum cleaner), but not from any other environmental sample.

DISCUSSION

The detection of *C. botulinum* type A and type A toxin in the fecal specimen collected on day 2 confirmed the diagnosis of infant botulism in this case. The maximum number of *C. botulinum* organisms detected in feces was 2.46×10^8 colony-forming units per g in the specimen collected on day 14, and the maximum toxin titer was 64,000 MLD_{100}/g on days 16 and 18. By this time, there had been a significant improvement in the patient's limb movements and spontaneous respiratory activity. High levels of organisms and toxin persisted in the feces for 8 weeks, and a specimen collected on day 56 still contained 2.2×10^7 colony-forming units of *C. botulinum* and 3,200 MLD_{100} of toxin per g.

There was a transient decrease in organisms and toxin in the vicinity of day 40, but the reason for this is not understood. The patient was being treated with oral co-trimoxazole at the time (for a urinary tract infection), but *C. botulinum* is not particularly sensitive to this combination of drugs (3). Indeed, the selective medium that we used for isolating *C. botulinum* from feces contained 76 μg of sulfamethoxazole and 4 μg of trimethoprim per ml. *C. botulinum* organisms and toxin were not detected in the feces after days 71 and 68, respectively. The most probable source of infection in this case was the patient's home, where *C. botulinum* type A was isolated from house dust. *C. botulinum* of the appropriate type was also isolated from house dust in the two previous cases of infant botulism recognized in South Australia (5, 8).

The most striking feature of the present case, however, was the detection of *C. botulinum* toxin in serum specimens collected on days 3 and 10. Toxin has been detected in serum in only one previous case of infant botulism (1). In the present case, there was virtually no respiratory effort by the patient between days 3 and 10, but from day 5 onward, spontaneous movement of the limbs was observed to gradually increase. This suggests that improvement (albeit only slight) in the clinical condition of the patient may have occurred despite the continued presence of toxin in the serum. The toxin acts by irreversibly blocking release of acetyl choline from cholinergic nerve terminals, and recovery occurs via budding and regrowth of the affected terminals (10). If the apparent improvement in the patient's clinical condition while toxin was still present in the serum was not artifactual, then one would have to speculate that regenerated nerve terminals are less susceptible to the toxin. Extensive in vitro studies would be required to further examine this possibility.

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