Infant Botulism: Anticipating the Second Decade

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Ten years have passed since infant botulism was recognized as a distinct clinical and epidemiological entity [1, 2] that results from a novel pathogenesis: the germination, outgrowth, and production of botulinal toxin in the infant intestine by ingested spores of the obligate anaerobe *Clostridium botulinum* [3, 4]. In the ensuing decade, the illness has been reported from 41 of the 50 United States (including Alaska and Hawaii) and from eight countries on the four continents of Australia [5-7], Europe [8-10], North America [11], and South America [12, 13]. Undoubtedly, other countries and Africa and Asia will eventually report infant botulism, because *C. botulinum* spores are found in soils and on agricultural products worldwide [14].

The present paper from Italy [15] fittingly concludes the first decade and may presage the next. In a joint report from the Instituto Superiore di Sanità and the Centers for Disease Control, Aureli et al. [15] describe the first two cases of infant botulism in Italy, which are also the first cases anywhere to have been caused by type *E* botulinal toxin. What makes this contribution even more remarkable is that the bacterium that produced the type *E* botulinal toxin was not *C. botulinum*, but was instead (by biochemical and chromatographic criteria) *Clostridium butyricum* [16]. This discovery enhances the significance of the recent report [17] of isolation of a *Clostridium barati* strain that produced type *F* botulinal toxin from a patient with infant botulism who was hospitalized in New Mexico in 1979 [18].

Until the appearance of these two novel organisms, all cases of infant botulism had been caused by proteolytic *C. botulinum* strains that produced either type *A* or type *B* (or *Bf*) neurotoxin. Botulinal neurotoxin is the most potent poison known and exists in seven major antigenic forms that have arbitrarily been assigned the letters A–G [19]. All act by blocking peripheral cholinergic synapses, the most important of which is the neuromuscular junction [20]. The clinical result is flaccid paralysis, with death occurring from airway and respiratory muscle paralysis. With a rare exception, each spore of *C. botulinum*, on becoming a vegetative cell, produces only a single type of toxin molecule. Consequently, the various toxin types serve as convenient epidemiological and clinical markers.

The first two Italian cases of infant botulism occurred seven and one-half months apart in two 16-week-old girls whose families lived in Rome on opposite sides of the city [15]. Because the causative bacterium is so unusual and its source(s) remains a mystery, additional descriptive epidemiological information about the two families and their environments would be interesting. The first infant presented with a surgical abdomen (atypical for infant botulism), weakness, and hypotonicity. A slight ileocecal intussusception was corrected during laparotomy, but the patient became seemingly comatose after the operation. In this perplexing clinical setting, the individual who first suggested infant botulism was indeed perspicacious. The second patient had a presentation more typical of infant botulism [21], although she was part of the small minority of patients with botulism who are not constipated before admission. She also was considered to be comatose (for a time), because her profound paralysis made her appear to be unresponsive. With meticulous respiratory and nutritional supportive care, both babies recovered. Both patients had electrophysiological abnormalities consistent with botulism, again demonstrating the value of bedside electromyography in obtaining a rapid presumptive diagnosis [22]. Definitive diagnosis requires demonstration of botulinal toxin and organisms in the patient’s feces [21, 23], because *C. botulinum* is not part of the normal resident intestinal microflora of human infants [24-25a].

The diagnosis of botulism can also be established by identifying botulinal toxin in serum, a technique that has proved useful in foodborne and wound botulism. Until the present report, however, botulinal toxin had been found in the serum of only two pa-
patients with infant botulism [26–28]. One of the two
Italian patients with infant botulism had type E toxin
in her serum (five times the mouse LD
subscript 50/mL). In ad-
mendment, in Aureli et al. [15], investigators from the
CDC mention that they have found toxin in serum
from seven of 59 confirmed cases of infant botu-
lism—almost one patient in eight. This proportion
represents a substantial group of patients who might
benefit from receiving botulinal antitoxin, and the
proportion might have been higher if sera had been
obtained from all patients immediately at the onset
of illness. The presently available equine antitoxin
is not being used in infant botulism because of its
associated risk of anaphylaxis (which occurred in one
of the two patients with infant botulism to receive it)
and because experience has shown that its use was
not essential for successful recovery [29]. However,
the prevalence of botulinal toxemia found by Aureli
et al. [15] underscores the importance of comple-
ting development of a human-derived polyvalent bot-
ulism immune globulin [30].

Intestinal Pathophysiology

Ever since recognition of the pathophysiology of in-
fant botulism, the conditions that would permit or
prevent germination and outgrowth of C. botulinum
spores in the infant gut have been topics of interest.
The limited age of susceptibility to infant botulism
(94% of the patients are less than six months of age;
range, one-half to 11 months of age) directed atten-
tion to factors unique to infancy [29]. Much atten-
tion centered on the intestinal microflora against
which C. botulinum must compete to establish it-
self and how, in turn, the composition of the resis-
tant microflora might be influenced by dietary
factors.

By use of a mouse model system, Sugiyama and
colleagues have established that the intestinal
microflora of adult animals ordinarily prevents
C. botulinum colonization of the gut. Adult germ-
free mice could be intestinally colonized by just 10
C. botulinum type A spores, yet when the germfree
animals were placed in a room with conventional
mice, in three days the formerly germfree animals
became resistant to colonization with an inoculum
of 10
subscript 5 spores [31]. Administration of 10
subscript 5 type A spores failed to colonize the intestine of normal adult
mice, whereas after treatment for two and one-half
days with a mixture of oral erythromycin (350 mg/kg
per day) and kanamycin (300 mg/kg per day), 50%
of the mice could be intestinally colonized by 2 \times 10
superior spores. When the antibiotic-treated mice were
placed in cages with normal mice, they lost their sus-
ceptibility to intestinal colonization [32]. Treatment
with metronidazole (500 mg/kg per day for four
days), a drug selectively bactericidal for obligate
anaerobes, yielded similar results. Surprisingly, the
total number of anaerobes and of Clostridium spp.,
Bacteroides spp., and Fusobacterium spp. was no
different than that found in control mice [33]. In-
oculating germfree adult mice with a defined intes-
tinal flora consisting only of nine species also pro-
tected the animals against oral challenge with 10
spores [34]. In chickens and in mice, the ce-
cum has been identified as the initial site of spore
germination [35, 36].

Healthy infant mice, like human infants, were
susceptible to intestinal colonization with C. botulinum
spores [37], a result that contrasts with the exper-
imental findings in normal adult mice. Also like
human infants, the healthy infant mice were suscep-
tible to colonization only for a limited period (7–13
days of age), with susceptibility peaking between
days eight and 11 in a manner reminiscent of the
peaking of susceptibility seen between two and four
months of age in human infant botulism [25]. The
infective dose for infant mice was much smaller than
that for their antibiotic-treated adult counterparts:
the 50% infective dose for healthy infants was only
700 spores. In one experiment, just 10 spores colonized
a normal infant mouse [37]. The minimum infective
dose of C. botulinum spores for human infants is
not known, but from exposure to spore-containing
honeys, it has been estimated to be as low as 10–100
spores [38].

Diet, Intestinal Flora, and Host Susceptibility

The ability of the resident intestinal microflora to
block outgrowth and multiplication of C. botulinum
spores has directed attention to the determinants of
the flora’s composition. Because infants subsist on
a milk diet, the differences in the fecal flora of breast-
fed and formula-fed infants have long been of inter-
est [25a, 39–45]. Although findings have varied be-
tween countries, in general, infants fed human milk
have more acidic feces (pH 5.1–5.4) that contain large
numbers (∼10
superior/g) of Bifidobacterium spp., a few
other anaerobes, and small numbers of facultatively
anaerobic bacteria. Clostridia (as spores) are virtu-
ally absent. In contrast, formula-fed infants have less
acidic feces (pH 5.9–8.0) that contain Bifidobacterium spp. (of more variable number), more Bacteroides spp., clostridia, and anaerobic streptococci, and higher levels of facultative anaerobic bacteria than do the feces of breast-fed infants [41, 42]. The relative differences in fecal pH may be important, because multiplication of C. botulinum (and toxin production) declines with pH and usually stops below pH 4.6. In addition, breast-fed infants receive the immunologic components in human milk (secretory IgA antibody, unsaturated lactoferrin, lysozyme, leukocytes, etc. [46]) that formula-fed infants do not.

The cases in Aureli et al. [15] delineate the role of dietary issues in infant botulism. One infant was formula-fed for the first 15 weeks of life, at which time cow's milk, cereal grains, other solid foods, and honey were started. Within a week, the baby manifested the first signs of infant botulism. The second patient was entirely breast-fed for the first 15 weeks of life, at which time formula milk, cereal grains, and honey were introduced. Within a week, this child also displayed signs of infant botulism.

Are breast-fed infants or formula-fed infants more susceptible to infant botulism? Did the introduction of honey or solid foods (especially the nonsterile cereal grains) precipitate infant botulism in the patients? Honey is an obvious candidate for suspicion, having been identified both as a dietary risk factor for infant botulism [38] and as a natural reservoir of C. botulinum type A and type B spores [3, 38, 47, 48]. In several parts of the United States, appreciable proportions of patients have been fed honey before onset of illness: in Utah, 83.3% (10 of 12) [49]; in Southeastern Pennsylvania, 13.6% (6 of 44) [50]; and in California, 20.1% (56 of 272). However, no neurotoxigenic C. butyricum type E was found in the honeys fed to the two Italian infants [15].

The short interval between the introduction of solid foods and the onset of illness of the two Italian babies is striking. In southeastern Pennsylvania, Long et al. [50] reported that a majority of their 44 patients "... had first feedings of nonhuman milk or another food substance within 4 weeks of onset..." Also, all 44 patients were still receiving human milk at onset, a finding that prompted these authors to conclude that "... breast-feeding is a risk factor for infant botulism" [50].

Breast-feeding may perhaps be associated with hospitalized patients who have infant botulism because of changes in the intestinal flora that result from weaning. Stark and Lee [42] described these alterations as follows: "The introduction of solid food to the breast-fed infant causes a major perturbation in the gut ecosystem, with rapid rise in the number of enterobacteria and enterococci, followed by progressive colonization by Bacteroides spp., clostridia and anaerobic streptococci. The addition of solid food to the diet of the formula-fed infant does not have such an impact on the gastro-intestinal flora."

Unlike the patients studied in southeastern Pennsylvania, formula-fed infants as well as breast-fed infants in Italy and in California were susceptible to infant botulism. The formula-fed infants in California had a mean age at onset (7.6 ± 2.8 weeks) that was significantly less than that of their breast-fed counterparts (13.7 ± 8.4 weeks) [51]. The younger age at onset for formula-fed babies may reflect the earlier availability of suitable ecological niches for C. botulinum in the intestinal flora of the formula-fed infants [42, 50], as well as their lack of immune factors contained in human milk. Parenthetically, these factors include secretory IgA antibody that agglutinates vegetative cells of C. botulinum [51a]. The relative susceptibilities of formula-fed and breast-fed infants to infant botulism and the possible "precipitating effect" of cereals and other foods may become more clearly defined when the analysis, now underway, of a multi-year, prospective case-control study of infant botulism in California has been completed.

Clinical Spectrum and Newer Diagnostic Techniques

Before leaving the topic of milk and susceptibility, some mention may be made of a perspective that views the hospitalized patient as just one part of a broader clinical spectrum of illness. The mild end of this spectrum would comprise outpatients (documented in Alaska, California, and Utah), the middle portion would encompass hospitalized patients, and the severe end of the spectrum would include a modest portion of what is termed sudden infant death syndrome (SIDS, crib death) [25, 49, 52]. From this perspective, the association between breast-feeding and hospitalized patients reflects a more-gradual onset of illness and the relative (but not absolute) protection against intestinal illnesses conferred by the immune factors in human milk and its associated microflora. Because formula-fed infants lack these immune factors and harbor a different microflora, formula-fed infants would be expected
to be younger at onset of infant botulism and to have experienced more-severe illness, as was observed [51]. The virtual identity of the age distribution curves of infants hospitalized with infant botulism and of infants who die of SIDS also suggested that the two disorders may be connected [25]. The Italian case of sudden infant death that had laboratory evidence of type B infant botulism as its “probable cause” reiterates this possible connection [15].

How could the connection between infant botulism and sudden infant death be made? Demonstrating the presence of C. *botulinum* organisms in diagnostic specimens has traditionally relied on the use of enriched and selective culture media [53]. Briefly, the material (food, feces, tissue, soil) is innoculated into chopped-meat glucose medium (enrichment), from which a subculture to (selective) egg-yolk agar makes use of the ability of *C. botulinum* types A–F to produce the enzyme lipase. A positive lipase reaction on egg-yolk agar is the standard screening procedure for isolating *C. botulinum* from mixed cultures. Identification of botulinal toxin has traditionally relied on ip injection of suspect material into mice and the neutralization of its lethal effect by type-specific botulinal antitoxin and by heating.

The discovery of neurotoxigenic *C. butyricum* type E and neurotoxigenic *C. barati* type F leaves open the question of whether or not these organisms may be capable of causing fulminant illness, i.e., sudden infant death. Any search of intestinal contents or feces for these organisms should note that they, together with *C. botulinum* type G [54, 55], do not produce the enzyme lipase. Thus, earlier studies of intestinal contents of cases of SIDS [56, 57] might have missed these novel organisms. Also, further search for botulinic toxins in cases of SIDS should consider using improved selective agars [57a–57c] and ELISA assays [58, 59] that may be able to detect toxin antigens even though biological activity of botulinal toxin is not demonstrable. By use of ELISA, type A botulinal toxin antigen(s) was identified in one of six cases of SIDS, all of which had negative culture and mouse bioassay results [58].

ELISA might also be profitably applied to screening culture supernatants of existing strains of *C. barati* and *C. butyricum* for botulinal toxins to determine whether or not these unusual organisms presently exist in locations other than Rome and an Acoma Indian reservation in New Mexico. The more-cumbersome, mouse neutralization assay is the principal alternative for screening because botulinal neurotoxin is not cytotoxic [59a]. The existence of these novel *C. butyricum* and *C. barati* strains suggests that the gene for botulinal toxin, like the gene for tetanus toxin, might be carried on a plasmid [60]. Although a majority of *C. botulinum* types A–G contained plasmids, none of the plasmids carried the toxin gene [61]. It would be interesting to know whether or not these *C. butyricum* and *C. barati* organisms may have possibly acquired their toxin-producing capability from a bacteriophage or plasmid.

### A New Taxonomy?

Finally, as Aureli et al. [15] have noted, the discovery of neurotoxigenic *C. butyricum* brings to six the number of physiologically distinct groups of clostridia that can produce botulinal neurotoxin. Perhaps others await discovery. Yet even the present six groups of disparate species raises the vexing matter of appropriate taxonomy. Aureli et al. have proposed renaming each of the six groups to the species designation of its nontoxigenic type strain: “According to this concept, botulism could be caused by organisms of six or more species of clostridia” [15].

Although this proposal has logic and taxonomic purity to recommend it, it may be asked whether or not such a scheme would improve our clinical, microbiological, and epidemiological understanding of botulism. The name botulism derives from *botulus*, the Latin word for sausage. The term was applied to the disease early in the nineteenth century in Germany, where Kerner recognized certain kinds of sausages as the source of outbreaks of food-borne botulism [14]. For this reason, Van Ermengem named the causative bacterium *Bacillus botulinus* when he reported its discovery in 1897 [62]. Since then, the bacterium and the disease have shared the same name, and to separate them now might create confusion. Perhaps some time should be permitted to pass, in which it can be learned whether or not having six groups of neurotoxigenic *C. botulinum* proves to be cumbersome or confusing.

### Conclusion

Much has been learned about infant botulism in just ten years. The stimulating report by Aureli et al. [15] adds diversity and breadth to the present picture and raises topics and questions that deserve attention in
the decade to come. Additional knowledge should make possible the more effective treatment and prevention of this intriguing intestinal toxemia of infancy. The progress of the second decade may surpass that of the first.

References


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