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The Rat as an Animal Model for Infant Botulism

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Susceptibility to intraintestinal *Clostridium botulinum* colonization of conventional infant and germfree adult rats is comparable to that of mice. *C. botulinum*-monoassociated rats pass *C. botulinum* toxin in their milk.

Infant botulism differs from the classical botulism food poisoning in that the causative toxin is acquired from an intraintestinal multiplication of *Clostridium botulinum* instead of from a contaminated food (1, 3). Although a toxicoinfection comparable to infant botulism very rarely develops when as many as 10^9 *C. botulinum* spores are fed to adult animals of species commonly used in laboratories, the intestinal colonization occurs when much fewer spores are administered intragastrically to 7- to 13-day-old mice. Mice 8 to 9 days old at the time of challenge are the most susceptible. The multiplication of the challenge organism does not cause overt botulism, but its occurrence is shown by *C. botulinum* toxin that is present in the cecum-colon during the first 1 to 5 days after the challenge (5).

Although conventional adult mice are not colonized when fed 10^5 spores, all germfree adults fed 10 spores become infected and show signs of botulism within 4 to 6 days. The contrasting susceptibilities of the two groups of mice are due to the difference in their intestinal microflora since the highly susceptible axenic mice become resistant to challenge with 10^5 spores within 3 days of exposure to a colony of normal mice (4).

The present work with rats was undertaken to eliminate the possibility that mice are unique in their receptiveness to colonization by *C. botulinum*. The results show that the response of rats is similar to that of mice and that either animal can be used to study certain aspects of botulism of human infants.

Rats of the Sprague Dawley strain were used; the conventional infants were raised in this laboratory, whereas the germfree rats were bred and maintained in the Gnotobiote Laboratory of the University of Wisconsin. Experimental procedures with the spore suspension of *C. botulinum* type A strain 62A were the same as those used with mice (4, 5) except that (i) adult rats to be fed spores were anesthetized with an intramuscular injection of ketamine hydrochloride (5 mg/kg of body weight; Vetalar; Parke, Davis & Co., Detroit, Mich.), and (ii) 2 ml of buffer was used to homogenize the intestinal segments to be tested for toxin. Qualitative and quantitative determinations of botulinum toxin were made by the intraperitoneal method with adult mice.

The age-related susceptibility of conventional infant rats was similar to that previously reported for infant mice. Only animals from 7 to less than 13 days old at time of intragastric challenge with 10^5 spores were colonized; 9-day-old rats were the most susceptible (Fig. 1). The colonized infants did not become ill, but *C. botulinum* toxin was present in the cecum-colon segment from 1 to 6 days postchallenge. The highest incidence of positives was obtained when the animals were tested for toxin on day 4 after administration of the inoculum (Fig. 2). The mean infective dose for 9-day-old rats (1,500 spores per animal with 95% confidence limits of 1,300 to 1,800) was not significantly different from that for infant mice of the most susceptible age (700 spores with 170 to 3,000 confidence limits).

The experiment to determine the susceptibility of axenic adult rats was performed with four isolators, each holding two separately caged animals. All eight rats fed 10 spores each were colonized and showed signs of botulism within 5 to 6 days. *C. botulinum* growth was limited to the lower ileum, cecum, and colon; at 4 to 7 days postchallenge, the total toxin in the gut ranged from 130,000 to 180,000 mouse 50% lethal doses (LD_{50}). Less than 1,500 LD_{50} was in the ileum, and the remainder was distributed so that the cecum had 4 to 20 times the amount in the corresponding colon.

Germfree adult rats were fed 10^5 spores at different times after their transfer from the sterile isolator into a room holding a colony of normal rats. The animal given spores at the time of its removal from the sterile environment was colonized by *C. botulinum* but had only 40 LD_{50} of toxin in its intestine 4 days after receiving the inoculum. The animals fed spores after 3 or 6 days of conventionalizing exposure were not col-

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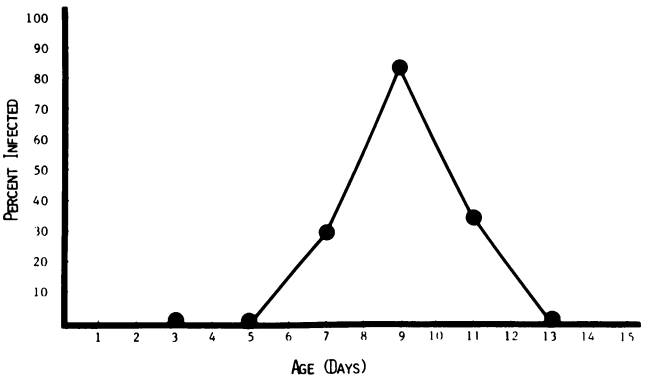

FIG. 1. *Infection rates among 20 conventional rats of different ages. Each animal challenged intragastrically with 10^5 C. botulinum spores and tested 4 days later for toxin.*

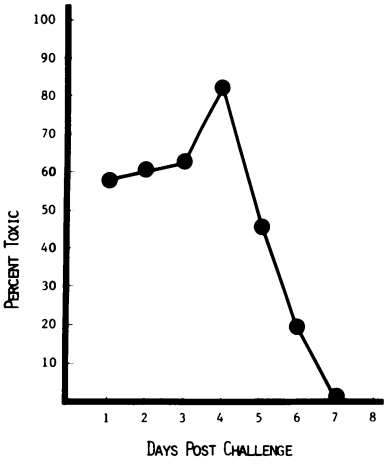

FIG. 2. *Presence of C. botulinum toxin in intestine at different days after intragastric challenge of 9-day-old rats with 10^5 spores; 20 animals to each test period.*

TABLE 1. *Influence of age of germfree infant rats on susceptibility to enteric C. botulinum colonization*

Age (days)[a]	No. of rats tested	No. of rats with toxin[b]	No. of rats with organisms[b]
2	12	12	0
3	10	9	0
4	13	13	0
5	10	10	6
7	10	10	10
8	10	10	10
9	12	12	12

[a] Age when given 10^5 spores intragastrically.

[b] Four days postchallenge; actual colonization occurs only when toxin and organisms are both present in the intestine.

onized as judged by absence of toxin in the gut.

Conventional infant mice (5) and rats (Fig. 1) less than 5 to 6 days old are resistant to intestinal *C. botulinum* colonization. This age restriction is also seen in the very young germfree mice (4) and rats (Table 1). *C. botulinum* toxin (<20 LD_{50}) was found in the intestines of rats challenged when 2, 3, or 4 days old, but it was probably not produced in the infants since smears of the intestinal contents showed no bacteria. Organisms were not present in four of the toxin-positive intestines of animals in the 5-day-old group, but the others had numerous *C. botulinum*-like rods in apparently pure culture. All infants 6 days of age or older were monoassociated with *C. botulinum*.

Since all mothers of the experiment had some degree of botulism by day 3 after administration of spores to their infants, it seemed likely that the toxin in infants not colonized by *C. botulinum* was that acquired with the milk of their sick mothers. Eight axenic rats with litters were fed 10^5 *C. botulinum* spores each. When they showed severe botulism on day 4, each was used to nurse 10 conventional, 2- to 3-day-old rats which had not been experimentally exposed to *C. botulinum* and which, immediately before use, were fasted for 4 h by being separated from their natural mothers. After the infants had suckled for 3 h on the surrogate mothers, they were sacrificed, and the stomach contents of those nursed by the same animal were pooled. All of these pools, none of which had more than 2 ml of coagulated milk, contained *C. botulinum* toxin, although the total did not exceed 5 LD_{50}.

The results obtained with rats parallel those found with mice (4, 5). The rat data confirm the earlier suggestions (2, 4) that the *C. botulinum* toxicoinfection of human infants is probably due to the intestinal microflora lacking certain microorganisms which can prevent multiplication of *C. botulinum*. Such anti-*C. botulinum* species would be present in older individuals and

would explain the age incidence of the disease. The resistance of very young animals is due to factors other than microbial competition.

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