Clostridium perfringens Type A Food Poisoning

I. Response of the Rabbit Ileum as an Indication of Enteropathogenicity of Strains of *Clostridium perfringens* in Monkeys

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Diarrhea and vomiting have been experimentally produced in monkeys after oral challenge with viable cells or culture filtrates of certain strains of *Clostridium perfringens* that previously had been shown to produce either fluid accumulation in the ligated ileum or overt diarrhea in the nonligated ileum of the rabbit, or both. Strains (or their culture filtrates) which did not produce a response in the rabbit likewise produced no symptoms in monkeys.

Diarrhea, the most common symptom of human food poisoning caused by *Clostridium perfringens* type A, has been experimentally produced in both rabbits (3) and lambs (6) after oral challenge with strains of this organism. In addition, ligated ileal loops of these animals have been shown to respond by fluid accumulation after intraluminal injection of viable cells of *C. perfringens* (5, 7). By using the rabbit, we have shown that only certain strains of *C. perfringens* repeatedly produce these responses and that this ability is dependent, in part, on the method of preparation of the cells for challenge (3, 5). We have also obtained good correlation between the ability of viable cells or of an enteropathogenic factor present in extracts and concentrated culture filtrates of these cells to produce both fluid accumulation in ileal loops and diarrhea when injected into the normal ileum of the rabbit (4). The enteropathogenic factor was produced during growth in a sporogenic medium but not in an asporogenic medium and was heat-labile, non-dialyzable, and inactivated by Pronase and extremes of pH. Hauschild et al. have recently confirmed the existence of this factor and demonstrated its antigenicity by using the lamb as an assay animal (8, 9).

The use of rabbits or lambs as experimental animals for the study of *C. perfringens* food poisoning has obvious limitations. Therefore, it was of importance to extend the investigation of this problem to include as an assay animal a species whose digestive tract more nearly resembles that of human beings in structure and function. The monkey was chosen for this purpose, and the present paper presents the results of that study. A second purpose of the experimental

work was to gauge the potential sensitivity of human subjects to challenge preparations. The latter information was essential if the use of humans as test subjects was to be undertaken without undue hazard. The results of studies with human volunteers are presented in a separate communication (10).

MATERIALS AND METHODS

Monkeys. Forty adult rhesus monkeys of both sexes were used. Although some of the animals were challenged more than once with the various preparations, 2 weeks was allowed to elapse between challenges. The animals were maintained on their normal pelleted food and water. On the day of challenge, the animals were not given their regular morning food but instead were fed immediately after challenge in the late afternoon.

Cultures. In this paper, the term "rabbit-positive" is used to describe strains of *C. perfringens* type A, or products thereof, which repeatedly have been shown to produce in young rabbits either fluid accumulation in the ligated ileum or overt diarrhea subsequent to intraileal injection of the nonligated gut, or both. Conversely, "rabbit-negative" denotes the absence of such response in any regular pattern.

Seven rabbit-positive cultures were employed. These included the following: NCTC 8239, NCTC 8798, NCTC 10239, NCTC 10240, E13, 79394, and 027. Strain 027 was obtained from beef liver. All other strains were isolated from food or feces associated with human food poisoning.

Rabbit-negative strains included NCTC 8247, F42, FD1, and 215b. Strain 215b was isolated from beef liver. The other strains were isolated from food or feces associated with food poisoning. Details of the original isolation of these strains are given elsewhere (10).

Preparation and administration of challenge materials. For challenge preparations containing viable

cells, an active culture was obtained by overnight (16 to 20 hr) growth of each strain in 10 ml of Fluid Thioglycollate Medium (BBL). A 10-ml amount of this culture was then inoculated into 100 ml of D-S sporulation medium (2) and incubated for 3 hr. The entire culture was then inoculated into 1,000 ml of D-S medium and grown for 4 hr. All incubations were at 37 C. The cells were then removed from the culture by centrifugation and resuspended in 25 ml of sterile Skim Milk (Difco). A total of 20 ml of this suspension was fed to each monkey by stomach catheter. The remaining 5 ml was used in determining the total viable cell and spore count of the suspension. A separate preparation was made for each monkey.

Lyophilized 48-hr-old D-S culture filtrates (concentrated culture supernatant fluids) were prepared as previously described (4). The lyophilized preparations were reconstituted in 20 ml of distilled water for administration to the animals by the stomach catheter. With one exception, the reconstituted preparations were equivalent to 2.2 to 2.4 liters of unconcentrated supernatant fluid. Filtrate of strain NCTC 10240 was fed at an equivalent of 1.0 liter of unconcentrated filtrate.

Control challenge preparations consisted of 20 ml of sterile Skim Milk or uninoculated D-S culture medium that had been lyophilized and reconstituted to an equivalent of 2.2 liters of medium per 20 ml of distilled water.

Five monkeys were used to test each cell suspension or culture filtrate. Subsequent to challenge, the animals were observed for the occurrence of diarrhea or vomiting at approximately 1- to 2-hr intervals for the first 16 hr postchallenge and then at 4-hr intervals for a total of 24 hr. The intestinal passage time was determined in five control monkeys by feeding each

of them, via stomach catheter, 20 ml of sterile Skim Milk containing 350 mg of carmine dye.

Enumeration of *C. perfringens*. In most instances when viable cells were fed to the animals, fecal samples were collected at specified intervals and analyzed to determine the total viable cell and spore count of *C. perfringens* in the fecal material. Dilutions of unheated fecal suspensions and suspensions heated at 75 C for 20 min were prepared in 0.1% peptone water and plated in TSN (BBL) agar to obtain total viable cell and spore counts, respectively. Incubation was at 46 C for 24 hr under a 90% N₂-10% CO₂ mixture. Counts were expressed as numbers per gram (dry weight) of fecal material.

Fecal samples were collected at the following intervals: prechallenge sample collected during the day before challenge; a sample 11 to 15 hr after challenge unless diarrhea had previously occurred; a sample at the first appearance of overt diarrhea or loose stool as indicated by a change in appearance of the stool from firm and formed to soft and unformed; the first normal-appearing fecal sample occurring after diarrhea; and a sample at 40 to 45 hr postchallenge.

RESULTS AND DISCUSSION

The intestinal passage time in the control monkeys fed Skim Milk containing carmine dye was 3.75 hr (three monkeys), 13.5 hr (one monkey), and 14.5 hr (one monkey). These results make it obvious that the passage times of individual monkeys may vary greatly. Therefore, carmine was not included in the other experiments, since its use seemed of little value. Instead, obvious changes in the appearance of the fecal material and vomiting

TABLE 1. Comparison of the response of *Clostridium perfringens* in rabbits and monkeys

Strain	Rabbit reaction ^a	Viable cells for challenge ^b				Monkey response to culture filtrate challenge ^b	
		No. fed		Monkey response		No. vomiting/ no. at test	No. with diarrhea/ no. at test ^c
		Total	Spores	No. vomiting/ no. at test	No. with diarrhea/ no. at test ^c		
NCTC 8798	+	8.2 × 10 ⁹	1.8 × 10 ⁷	0/5	2/5 (LS)	5/5	0/5
NCTC 10239	+	3.0 × 10 ¹⁰	3.6 × 10 ⁸	0/5	4/5 (DS)	1/5	3/5 (LS)
NCTC 10240	+	3.5 × 10 ¹⁰	9.6 × 10 ⁸	0/5	3/5 (DS)	0/5	2/5 (LS)
NCTC 8239	+	9.2 × 10 ⁹	2.6 × 10 ⁸	1/5	3/5 (DS)	5/5	3/5 (DS)
E13	+	7.1 × 10 ¹⁰	3.2 × 10 ⁷	0/5	0/5	NT ^d	NT
027	+	1.1 × 10 ¹⁰	6.7 × 10 ⁶	0/5	3/5 (DS)	0/5	0/5
79394	+	5.0 × 10 ⁹	3.7 × 10 ⁴	1/5	5/5 (DS)	0/5	0/5
NCTC 8247	—	9.5 × 10 ⁹	1.2 × 10 ⁶	0/5	0/5	NT	NT
F42	—	1.2 × 10 ¹⁰	2.1 × 10 ⁷	0/5	0/5	0/5	0/5
215b	—	1.4 × 10 ¹⁰	5.4 × 10 ⁸	0/5	0/5	NT	NT
FD1	—	2.3 × 10 ¹⁰	2.7 × 10 ⁹	0/5	0/5	0/5	0/5

^a Symbols: +, rabbit-positive; —, rabbit-negative.

^b See Materials and Methods for details of preparation.

^c LS in parentheses indicates loose, unformed stool, whereas the DS indicates overt diarrhea.

^d Not tested.

^b Not tested.

counts of diarrheal and nondiarrheal samples or between fecal samples obtained after challenge with rabbit-positive or rabbit-negative strains. This would indicate that the failure of a strain to produce a response in the monkey is not due to failure of the organism to multiply or sporulate in the intestine. Similar results were found in the rabbit (3).

These results show that strains of *C. perfringens* capable of producing the enteropathogenic factor that induces fluid accumulation in the rabbit ileal loop or overt diarrhea in the rabbit will also produce diarrhea and vomiting in the monkey. Although diarrhea is the principal symptom of perfringens food poisoning, vomiting is often reported to occur in a limited number of cases (1). Whether the enteropathogenic factor responsible for diarrhea production is also responsible for vomiting is a question that must await further purification and study of the factor. The results of these studies are further evidence that the factor (or factors) active in the rabbit is also active in the monkey.

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