APPLIED MICROBIOLOGY, Jan. 1972, p. 196-197

Vol. 23, No. 1
Printed in U.S.A.

Use of Dogs as an Assay for *Clostridium perfringens* Enterotoxin[1]

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Received for publication 16 September 1971

Three techniques for using the dog as an assay organism for *Clostridium perfringens* enterotoxin are described. These are believed to be more convenient than ligated ileal-loop procedures.

Clostridium perfringens has been implicated in outbreaks of food poisoning characterized by diarrhea (5). Various investigators have attempted to determine the nature of the intoxicant produced by some strains of *C. perfringens* (3). Various assays have been used to follow the course of isolation of the enterotoxin, including a mouse assay (8), rabbit assay (3), and lamb assay (4). This note describes three methods for use of the dog as an organism for *C. perfringens* enterotoxins.

C. perfringens strains NCTC 8798, NCTC 10240, and FD1 were maintained in cooked meat medium (Difco) and transferred twice through fluid thioglycolate medium (Difco). Ten- to 30-ml volumes of the second transfer were incubated at 37 C for 4 hr and transferred to 1 liter of DS-sporulation medium (2), which was then incubated at 37 C for at least 18 hr, but frequently longer. The culture supernatant fluids were concentrated by lyophilization, evaporation, or dialysis against Carbowax 20 M. These concentrated "crude" enterotoxin preparations were administered to dogs in the following ways. (i) The enterotoxin, in powdered form, was placed in a gelatin capsule, which was given an enteric coating with 10% cellulose acetate phthalate solution in acetone (7), and this was given orally; the dog was observed for signs of diarrhea. (ii) The enterotoxin, 15 to 50 ml of the liquid form, was given directly into the intestine through a Zeman canula (9) or Mann-Bollman fistula (6); the dog was observed for signs of diarrhea. (iii) The enterotoxin, in liquid form, was placed into a Thiry-Vella fistula (TVF), and this segment was observed for fluid accumulation as described by Carpenter et al. (1).

The results of the enteric-coated capsule test are reported in Table 1. Occasionally, carmine dye, approximately 1 mg/lb, was included with the toxin; in these instances, the feces, diarrhetic or normal, developed a red color. Diarrhea occurred in approximately 4 hr. When the enterotoxin was administered through the Zeman canula or the Mann-Bollman fistula, diarrhea appeared within 1 hr of treatment. Saline, concentrated culture medium, and heat-inactivated toxin preparations did not induce diarrhea when administered throgh the Zeman canula, Mann-Bollman fistula, or TVF. Toxic preparations induced accumulation of at least 12 ml of fluid during the first 30 min after treatment in the TVF. This was in contrast to 0 to 2 ml for negative controls. Of 11 samples tested in TVF where the expected results would be fluid accumulation (based on rabbit ileal-loop response and on diarrhea production by portions of the sample), 3 were negative.

TABLE 1. *Oral administration of toxin and control material*

Treatment	Milligrams of lyophilized powder/lb of dog weight	No. of animals with diarrhea/no. challenged
NCTC 8798	15-21	8/9
NCTC 10240	17-21	0/2
FD1	17-21	0/2
50 to 75% $(NH_4)_2SO_4$ cut of NCTC 8798 supernatant fluid	6-13	4/4
DS sporulation medium	21	0/1
Gelatin + carmine dye	20 + 2	0/1

[1] Journal paper no. J-7003 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, project no. 1896.

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The assay with enteric-coated capsules is relatively easy, and the capsules can be tested in simulated gastric juice before administration to determine if they are sufficiently coated to pass intact through the stomach. Insertion of the Zeman canula, Mann-Bollman fistula, and TVF require surgery, but these methods have the advantage of permitting repeated use of the animal; surgery is required only for the initial preparation. The dog model has some advantages in studying the physiology of enterotoxin action. Diarrhea production may be correlated with fluid accumulation in the TVF loop and with changes in such physiological parameters as peristaltic rate and strength. The sensitivity of the dog as compared to the ileal-loop response has not yet been established.

We are indebted to F. Thompson and Ron Grier of the Iowa State University College of Veterinary Medicine for their aid and advice in performing much of the surgery and aftercare. We are also indebted to Charles Duncan for supplying the strains of *Clostridium perfringens*.

This investigation was supported by grants FD-00106 and FD-00444.

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