Response of Ligated Intestinal Loops in Chickens to the Enterotoxin of *Clostridium perfringens*

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Approximately 45-cm length of jejunoileum of 7-week-old chickens was found to be responsive and suitable for testing the enterotoxin of *Clostridium perfringens* by the ligated intestinal loop technique. Injections of 20 to 30 μg of enterotoxin per loop caused positive response of fluid accumulation. Chickens were found to be more convenient and economical for this purpose than other laboratory and domestic animals.

Enteropathogenic properties of the enterotoxin of *Clostridium perfringens* type A were demonstrated and first assayed by the ligated intestinal loop technique in sheep and rabbits (1, 4, 5, 10). This enterotoxin causes accumulation of fluid in the ligated loops into which it is injected in a laparotomy procedure. Although recently developed immunological in vitro methods have superseded the ligated intestinal loop technique for greater sensitivity and convenience of the enterotoxin assay (2, 3, 12), the biological method is still sometimes required for the study of pathogenesis, confirmation of enteropathogenic activity, and for research in therapeutics of enterotoxic enteritis. In an attempt to find an alternative small laboratory animal model more economical and practical for this method than rabbits and sheep, young chickens were investigated and found to be very suitable for this purpose.

White Leghorn chickens, 7 weeks old and weighing 320 to 360 g, of approximately equal numbers of both sexes, were used. They were starved for 36 to 42 h by withholding solid feed, but allowed free access to drinking water. Each bird was anesthetized by inhalation from a paper cup containing a piece of cotton soaked with methoxyflurane (Metofane, Pitman-Moore Inc., Washington Crossing, N.J.). The bird was then placed on its left side and restrained by 2 to 3 strips of adhesive tape over its legs and body, fastened to a bench top. A few feathers were plucked from its abdomen and an incision, about 2 cm long, was made on the right side postero-lateral to the posterior extremity of the sternal crest through the skin, muscle, and peritoneum. Precautions for asepsis were followed. The duodenum was extracted and reflected out of the way to allow access to an empty jejunoileum. The latter was gently manipulated through the incision and ligated with size 00 surgical silk. Four to five test loops, 5 to 6 cm long, were created in this part of the small intestine. Each test loop was separated by an interloop of 3- to 4-cm length. Care was taken to avoid occluding the blood vessels along the mesentery. Up to about 45 cm of the small intestine was available that could be manipulated conveniently and without trauma.

The test loops were injected with 0.2-ml volumes of preparations containing 5 to 100 μg of pure enterotoxin of *C. perfringens*, either in a purified form or as a crude preparation of equivalent dosage, nonenterotoxic cell extract of the same organism (6, 9), or 0.15 M NaCl (saline). Saline was also used as a diluent for the above cell products. The interloops were left empty. The intestines were then replaced in the abdominal cavity and the incision wound was sutured. The entire procedure lasted approximately 20 min per bird and required about 5 min of intermittent inhalation of the anesthetic. Most of the birds so treated recovered sufficiently to be able to stand up within 15 min after the completion of the surgical procedure.

The chickens were killed 3.5 to 4.5 h (mean = 4.0 h) later and the intestinal loops were examined. The reactions of fluid accumulation in 92 test loops of 19 chickens is summarized in Table 1. The volumes of fluid were mostly, but not always, proportional to the amount of enterotoxin injected (Fig. 1). The largest fluid volume was 4.8 ml in a loop injected with 100 μg of enterotoxin, yielding the highest volume-length ratio of 0.8 ml/cm. None of the control loops accumulated fluid, but two interloops in this series of tests were found to contain fluid which necessitated the rejection of three adjacent positive test loops. This reaction in the interloops was thought to have originated from either
perfringens. No difference was evident in response between the purified and crude preparations of the enterotoxin. Histological sections of the intestinal wall of the positive loops showed minimal pathological change; if present, the changes were a mild degree of hyperemia of the mucosa and some edematous areas in the thin submucosa. These findings were basically similar to those seen in the ligated intestinal loops of rabbits, lambs, and calves inoculated with C. perfringens enterotoxin (5, 6, 7, 9).

The minimal amount of enterotoxin required for a positive response in these chickens was 20 to 30 μg per loop which compares with 26 to 80 μg per loop for a similar response in the mammalian species tested (3, 5, 6, 8, 9, 11) in spite of differences in loop size and area of mucosal surface between these animals.

There were no false reactions encountered in the jejunileum, except the two interloops which served as adequate controls to warn against possible false positive reactions. However, in a pilot study before this experiment, it was found that duodenum consistently produced false positive response in ligated loops and was therefore considered unacceptable for ligation.

These results show that chickens are suitable for the ligated intestinal loop technique and may be used as an experimental model in certain aspects of research on enterotoxic enteritis. The operative procedure appears to be more convenient on chickens than on other laboratory and domestic mammals. Danger of bleeding from abdominal incision is minimal since the avian abdominal muscles and their blood supply are less extensive than those of mammals. Experience has shown that chickens tolerate this inhalation anesthetic quite well although care should be taken to prevent overdosage.

Chickens may be more readily available than rabbits at some laboratories and are certainly more economical than large farm animal models for research on C. perfringens enterotoxin. If necessary, gnotobiotic birds can be procured easier and in shorter time than mammals. It may be worthwhile to investigate whether or not chickens can be used as an experimental model.

TABLE 1. Reactions of 92 ligated intestinal loops of chickens to the enterotoxin of C. perfringens

| Enterotoxin (μg/loop) | No. of loops | No. of loops with fluid | > 1.0 ml/loop | 0.5–1.0 ml/loop | Empty |
|----------------------|-------------|------------------------|---------------|----------------|-------|
| 100                  | 6           | 6                      |               |                |       |
| 50                   | 10          | 10                     |               |                |       |
| 30                   | 10          | 8                      | 2             |                |       |
| 25                   | 12          | 5                      | 3             | 4              |       |
| 20                   | 8           | 2                      | 1             | 5              |       |
| 10                   | 10          | 2                      |               | 8              |       |
| 5                    | 6           | 6                      |               |                |       |
| Saline               | 14          | 14                     |               |                |       |
| Control*             | 18          | 18                     |               |                |       |

* Enterotoxin-free cell extract of C. perfringens.

Fig. 1. Reactions in ligated intestinal loops of two chickens. Symbols: micrograms of enterotoxin injected per fluid volume in milliliters (in brackets); (C) enterotoxin-free cell extract; (S) saline.
to study enterotoxins of *Escherichia coli* and *Vibrio cholerae*.

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