ACUTE AND CHRONIC ENTEROTOXIN ENTERITIS*

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PLATES 49 TO 52

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Studies of human enteritis and colitis have been difficult because it has not been possible to reproduce the disease in a suitable experimental animal by biological means. It has been shown by well controlled experiments that enterocolitis can be induced in chinchillas by oral administration of pure cultures of *Staphylococcus aureus* when the colonic flora of these rodents is altered by antibiotics (11). Subsequent experiments have shown that enterocolitis develops in untreated chinchillas in response to oral feeding of staphylococcal enterotoxin free of the microorganism from which it was extracted (9). Moderate to severe jejunitis has been observed in cats and kittens after repeated, daily feedings of staphylococcal enterotoxin (11). Microbiologists have known for a long time that enterotoxin induces vomiting in the monkey and food poisoning in man. These several observations led us to postulate that enterotoxin may exert its toxic effect in many animal species and that the responses may be quantitative. The review of the literature did not make these assumptions and prospects of discovery very attractive. Be that as it may, an experimental model of enteritis was needed in a suitable laboratory animal such as the dog.

The early attempts to induce enterocolitis in dogs by bacterial toxins were unsuccessful and led to the impression that the dog was remarkably resistant to this disease (6). Thus, Minett fed 20 to 40 ml of staphylococcal filtrate to five young dogs and was able to induce vomiting in one and diarrhea in another one; the other three animals showed no effect (7). Hagan and Bruner stated that with the exception of kittens and suckling pigs, domestic animals do not appear to be susceptible to staphylococcal enterotoxin (4). Elek, in reviewing the subject of species susceptibility to enterotoxin, indicated that the only good experimental animal was the monkey (3).

If our postulate was to be correct, then the previous failures could have been due to inadequate quantities of enterotoxin retained by the experimental animal. In our own laboratory, the dogs could not be induced to drink crude toxin, and, when the material was given by gavage, they promptly regurgitated it.

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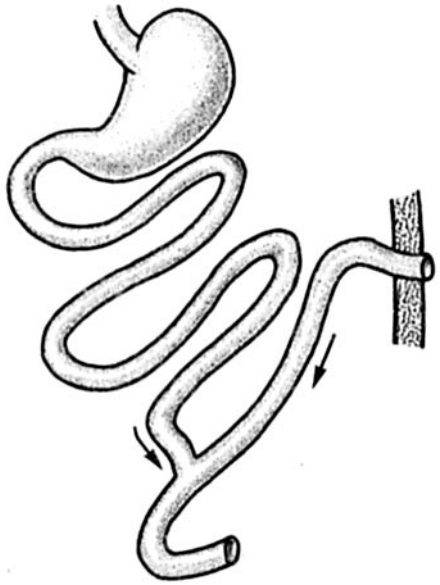
These considerations led us to devise a preparation in dogs which would enable us to introduce large quantities of enterotoxin directly into the alimentary tract. The preparation finally adapted was a Maydl enterostomy (Text-fig. 1) with the open end of the ileum forming an enterocutaneous feeding stoma (5). With this preparation the animals could not regurgitate the substance. Thus, the quantity given was controlled.



TEXT-FIG. 1. Maydl enterostomy. Material introduced into the enterocutaneous stoma is carried directly into the intestine.

Materials and Methods

Twenty-seven healthy, mongrel dogs, ranging in weight from 10 to 17 kg, were used. In each dog a cutaneous feeding enterostomy was constructed. The intestine was transected at levels varying from 30 cm distal to the pylorus to 60 cm proximal to the cecum. The open proximal end was anastomosed to the intestine, approximately 20 cm below the cutaneous stoma, in end-to-side fasion. This restored the enteric continuity and provided a feeding fistula with peristalsis directed toward the intestine (Text-fig. 1). The distal divided end was brought out through a small round incision lateral to the laparotomy wound. The open end of the intestine was sutured to the circular edge of the skin with chromic cat-gut 000. Material introduced into the enteric fistula was carried in most animals into the mid-ileum; in some the injected substance entered the jejunum. At least 2 weeks were allowed for recovery from surgery before the animals were used for an enterotoxin experiment. A No. 12 French catheter was inserted into the enterostomy and the enterotoxin was introduced slowly into the intestine by means of gravity. The infusion lasted 10 to 15 minutes.

The enterotoxin used was a crude staphylococcal extract prepared for maximum enterotoxin

content by Dr. M. S. Bergdoll of the Food Research Institute of the University of Chicago according to the method of Sugiyama, Bergdoll and Dack (10). The dose selected was 15 ml per kg of body weight with a maximum total dose of 200 ml per animal twice daily. On weight basis, this is about twice the dosage used in chinchillas by Prohaska, Drake, and Tan (9). This quantity was selected because of our assumption that dogs were resistant to the enterotoxin.

Two experiments were performed. In the first experiment 11 animals were observed during and immediately after the introduction of enterotoxin for salivation, retching, vomiting, diarrhea, and color of the stools. Gross and histological studies of the gastrointestinal tracts were made of animals that died from lethal doses of the enterotoxin and of those that were sacrificed. In the second experiment 10 dogs with Maydl fistulae were placed on sublethal doses of enterotoxin given once daily for periods ranging from 16 to 153 days. This dose was selected to be 5 ml per kg of body weight per day. The purpose of this experiment was to observe any possible cumulative or chronic changes in the intestine that could result from daily introduction of the enterotoxin over a long period of time. These animals were sacrificed at different intervals of time from the 1st day of injection, and necropsies were performed for investigation of gross and histological changes in the bowel.

RESULTS

All 11 animals used in the first experiment exhibited severe reaction after one or two doses of enterotoxin (Table I). The initial reaction consisted of salivation, retching, vomiting, and diarrhea. The diarrhea was often explosive and, when severe or prolonged, the stools became green and sometimes bloody. Repeated doses of enterotoxin caused repeated symptoms until the animals became dehydrated, prostrate, and died in a shock-like condition. Of the 11 dogs used in the first experiment, two died in shock-like condition 3 to 6 hours after

TABLE I

Experiment No. 1: Acute Response Provoked by Staphylococcal Enterotoxin Introduced into the Intestine through Maydl Enteroenterostomy

Dog	Dose	No. of doses given	Vomiting after dose No.	Diarrhea after dose No.	Response
	ml				
1	150	4	2	3	Severe
2	200	4	1	2	Lethal
3	200	3	1	3	Severe
4	200	2	None	1	Sacrificed
5	150	4	1	1	Sacrificed
6	200	5	1	2	Lethal
7	200	3	1	3	Severe
8	200	2	2	2	Severe
9	200	1	1	1	Severe
1-0	150	2	2	1	Severe
1-1	25	8	1	None	Mild
1-1	150	2	2	2	Severe

the injection of the fourth and fifth doses of enterotoxin respectively. Two other animals were sacrificed 2 to 3 hours after the infusion of their effective doses. At necropsy the liver, kidneys, and spleen were normal in most animals, hyperemic in some. The gastrointestinal tracts were variously dilated, edematous, and hyperemic. The greatest gross changes were noted immediately distal to the enterostomy. The small and large bowel contained liquid green stools. The mucosa was reddened, hyperemic, and edematous. This type of inflammatory reaction decreased in intensity as the intestine was examined distally from the feeding enteroenterostomy. No frank mucosal ulcerations were observed on gross inspection of the intestine.

TABLE II

Experiment No. 2: The Effect of Prolonged Daily Intraintestinal Administration of Staphylococcal Enterotoxin on the Dog's Intestine

Dog	Dose	Days given	Gross changes	Microscopic changes
	ml			
1-2	50	16	None	None
1-3	50	48	Enteritis	Enteritis, lymphoid hyperplasia
1-4	50	66	Enteritis	Enteritis, lymphoid hyperplasia
1-5	50	75	Enteritis	Severe enteritis
1-6	50	83	Enteritis	Severe enteritis, lymphoid hyperplasia
1-7	50	90	Enteritis	Enteritis
1-8	50	102	Enteritis	Enteritis
1-9	60	109	Enteritis	Severe enteritis, lymphoid hyperplasia
2-0	70	113	Enteritis	Enteritis lymphoid hyperplasia
2-1	80	153	Enteritis	Severe enteritis, lymphoid hyperplasia

Microscopically, the involved intestine showed considerable hyperemia, edema, inflammatory changes consisting of round cell infiltration, breakdown of the villous structures of the intestine, exudation, and ulcerations (Fig. 1). In some animals there was hyperemia of the liver and spleen.

In the second experiment, in which the dogs received small daily doses of enterotoxin for a long period of time, all the animals experienced salivation, vomiting, diarrhea, and loss of weight during the 1st week of experimentation. Thereupon they returned to normal behavior and tolerated the established dose of enterotoxin. At necropsy, enteritis was found in all animals receiving enterotoxin longer than 16 days (Table II). The most severe gross changes were observed immediately distal to the feeding enteroenterostomy. These changes extended in a skip-like fashion downward along the intestine and often presented severe enteritis at the terminal ileum (Fig. 2). The gross changes were edema, hyperemia, and submucosal thickening appearing as exaggerations of the longitudinal ridges or patches of the intestinal mucosa (Fig. 3). In some specimens these ridges measured 10 to 20 cm in length. Mucosal ulcerations

were not noted on gross inspection. In six of the ten animals, hypertrophy of mesenteric intestinal lymph nodes was easily discernible.

The histological study revealed round cell infiltration of the mucosa, submucosa, and, in many instances, of the muscularis. In some areas large lymphoid aggregates extended from the mucosa and submucosa into the muscularis (Figs. 4 *a* and 4 *b*). These lymphoid nodules or aggregates of nodules corresponded to the exaggerated mucosal ridges described under gross changes and illustrated in Fig. 3. Clubbing of the villi was observed in some areas. An increased number of cells showing mitotic activity were noted at the bottom of mucosal crypts.

TABLE III

Controls: Extracts, Given Intraluminally, Prepared from Non-Enterotoxigenic Staphylococci Containing No Enterotoxin

Dog	Dose	Doses given	Response	Enteritis gross-microscopic
	ml			
2-2	100	2	None	None
2-3	100	2	None	None
2-4	175	5	None	None
2-5	200	1	None	None
2-6	200	30	None	None
2-7	200	40	None	None

Non-enterotoxigenic *Staphylococcus aureus* extracts induced no response in control animals.

Controls.—The discovery of such profound symptoms and signs induced in the dog following the intraluminal administration of staphylococcal enterotoxin, coupled with the earlier belief of investigators that dogs were insensitive to enterotoxin, made it imperative that proper controls be devised. Accordingly, controls were established by administering the same quantity of staphylococcal extract prepared from non-enterotoxigenic staphylococci. Such extracts contain all the substances found in extracts made from enterotoxigenic staphylococci except the enterotoxin. The validity and effectiveness of this kind of control have been recently worked out by Warren, Sugiyama, and Prohaska (12). In addition, each harvested extract was tested for the presence of enterotoxin by the monkey test and, whenever applicable, by the precipitin test of Oudin (8).[1]

The animals receiving controlled doses of non-enterotoxigenic staphylococcal extract showed no signs of salivation, vomiting, or diarrhea. These same animals responded violently to the introduction of equal amounts of enterotoxigenic extract (enterotoxin). Dog 1 (Table I) received one full dose of non-

[1] Kindly performed for us by H. Sugiyama of the Food Research Institute.

enterotoxigenic staphylococcal extract and manifested no symptoms. Thirty days later this animal received enterotoxigenic staphylococcal extract. It reacted violently and again reacted to enterotoxin containing extract on day 31. On day 32 no response was obtained in this animal following the administration of non-enterotoxigenic extract. In similar fashion, dog 5 (Table I) received five successive doses of non-enterotoxigenic staphylococcal extract without effect. Three weeks later this animal responded to enterotoxin by developing severe toxic symptoms. Six additional animals received extracts from non-enterotoxigenic staphylococci in the same manner as the experimental dogs. No toxic reactions were noted (Table III). Thus, the reactions observed in the experimental dogs indeed were due to staphylococcal enterotoxin alone and not to other substances or impurities normally present in a crude staphylococcal extract.

DISCUSSION

The series of experiments demonstrate that the dog responds to staphylococcal enterotoxin administered directly into the gastrointestinal tract. Eleven of the 11 dogs used in the first experiment responded with salivation, vomiting, and diarrhea. No dog thus far tested has failed to respond to 15 ml per kg of body weight of enterotoxigenic staphylococcal enterotoxin or to 200 mg of this substance, when such a substance is administered directly into the intestinal tract.

The normal arrangement of the intestine was modified in these experiments in such a way as to by-pass the stomach so that large quantities of enterotoxin could be dripped directly into the lumen of the intestine. This seemed to be not only convenient, but also essential in order to obtain enterotoxigenic response. Borthwick has shown that staphylococcal enterotoxin is inactivated in the stomach of rabbits when the pH is either acid or alkaline (1). Thus, the introduction of enterotoxin into the intestine, which is approximately neutral and where acid pepsin has been diluted and inactivated, may prevent its degradation and may account for the susceptibility shown in these experiments. The dose of 25 ml (2.5 mg per kg of body weight) which induced vomiting as an initial dose for dog 1 is comparable with the initial dose which induces vomiting in the susceptible Rhesus monkey (3). Thus, the dog is about as sensitive to staphylococcal enterotoxin as is the Rhesus monkey, which Dack considers to be one of the most sensitive laboratory animals and the most useful for enterotoxin essay (2). Nevertheless, considerable individual variation in sensitivity to an initial dose of enterotoxin is demonstrated in the canine experiments.

Dog 1 did not respond to an initial dose of 15 ml per kg. of body weight (150 ml). It responded by vomiting 30 minutes after a second similar dose, and, after 50 ml of a third dose was introduced, it again vomited. After the rest of

the third dose was introduced, it vomited several times and had severe diarrhea. It displayed similar sensitivity to a fourth dose given later that same day.

The reaction of enterotoxin appears to be somewhat quantitative. This is illustrated in the behavior of dog 1-1 (Table I). This animal was initially given eight separate doses of 25 ml of crude extract containing staphylococcal enterotoxin. The dog responded to the first dose by salivation, vomiting, and diarrhea. No toxic response was noted following the introduction of the subsequent seven doses of the enterotoxin. When the dose of enterotoxin was increased to 150 ml, vomiting and diarrhea occurred in response to two consecutive doses.

The animals receiving control doses of non-enterotoxigenic staphylococcal extracts showed no signs of salivation, vomiting, or diarrhea. These same animals responded violently to the introduction of equal amounts of enterotoxigenic extracts. The reactions observed in the experimental dogs were due to the staphylococcal enterotoxin alone and not to other substances or impurities present in a crude extract.

CONCLUSIONS AND SUMMARY

1. Dogs develop severe gastrointestinal symptoms in response to intraintestinal administration of staphylococcal enterotoxin. These symptoms are salivation, vomiting, and diarrhea.

2. Staphylococcal enterotoxin induces acute enteritis marked by edema, hyperemia, round cell infiltration, mucosal exudation, and destruction of intestinal villi.

3. Prolonged administration of enterotoxin into the lumen of the intestine produced thickening of the entire bowel wall, edema, dilatation of the lymphatics, and exaggeration of submucous lymphoid nodules. The hypertrophy of the lymphoid nodules is visible, in the gross, as enlarged longitudinal mucosal ridges. This abnormality is arranged in skip areas, not dissimilar to those observed in human regional enteritis.

4. Chronic enterotoxin enteritis is associated with mesenteric lymph node hypertrophy.

5. The intestinal mucosa shows minute ulcerations, loss of villi, submucous fibrosis, and evidence of chronic inflammation.

BIBLIOGRAPHY

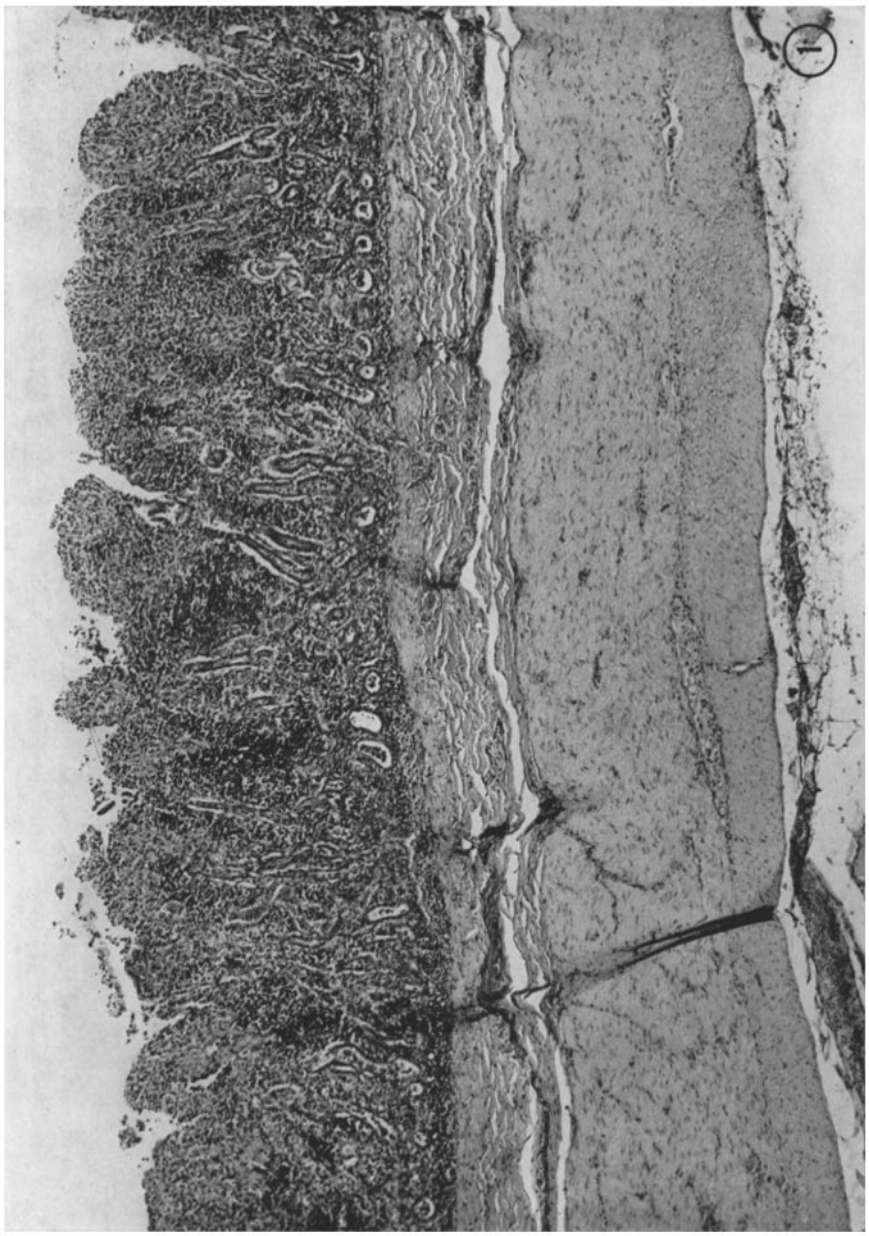
1. Borthwick, G. R., Experimental observations on the toxic effect of staphylococcal filtrates introduced enterally in laboratory animals, *Brit. J. Exp. Path.*, 1933, **14,** 236.
2. Dack, G. M., Food Poisoning, Chicago, University of Chicago Press, 3rd edition, 1956, 109–158.
3. Elek, S. D., *Staphylococcus pyogenes* and Its Relation to Disease, Edinburgh, E. & S. Livingstone Ltd., 1959, 304–308.

4. Hagan, W. A., and Bruner, D. W., The Infectious Diseases of Domestic Animals, Ithaca, Comstock Publishing Associates, 4th edition, 1961, 135–137.
5. Markowitz, J., Archibald, J., and Downie, H. G., Experimental Surgery, Baltimore, Williams & Wilkins Co., 4th edition, 1959, 228–230.
6. McNeil, E., Poisonings by organic toxins, *in* Canine Medicine, (H. P. Hoskins, J. V. Lacroix, and K. Mayer, editors), Santa Barbara, American Veterinary Publications, Inc., 2nd edition, 1959, 718.
7. Minett, F. C., Experiments on staphylococcus food poisoning, *J. Hyg.*, 1938, **38,** 623.
8. Oudin, J., Specific precipitation in gels and its application to immunochemical analysis, *Methods Med. Research,* 1952, **5,** 335.
9. Prohaska, J. V., Jacobson, M. J., Drake, C. T., and Tan, T. L., Staphylococcus enterotoxin enteritis, *Surg., Gynec. and Obst.*, 1959, **109,** 73.
10. Sugiyama, H., Bergdoll, M. S., and Dack, G. M., *In vitro* studies on staphylococcal enterotoxin production, *J. Bact.*, 1960, **80,** 265.
11. Tan, T. L., Drake, C. T., Jacobson, M. J., and Prohaska, J. V., The experimental development of pseudomembranous enterocolitis, *Surg., Gynec. and Obst.*, 1959, **108,** 415.
12. Warren, S. E., Sugiyama, H., and Prohaska, J. V., Correlation of staphylococcal enterotoxins with experimentally induced enterocolitis, *Surg., Gynec. and Obst.*, 1963, **116,** 29.

EXPLANATION OF PLATES

Plate 49

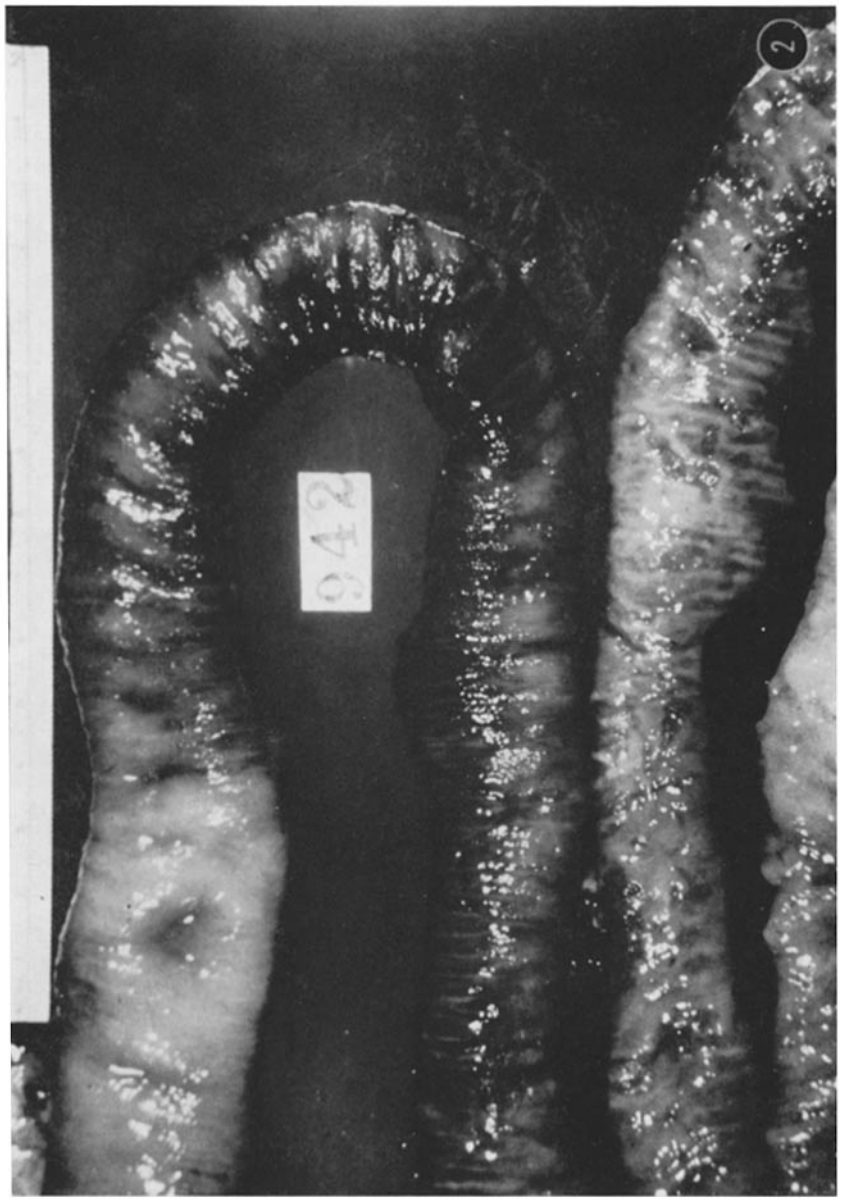
Fig. 1. Response to intraluminal infusion of staphylococcal enterotoxin. Photomicrograph of jejunum showing edema, round cell infiltration, and destruction of villi. × 34.



(Warren *et al.*: Acute and chronic enterotoxin enteritis)

PLATE 50

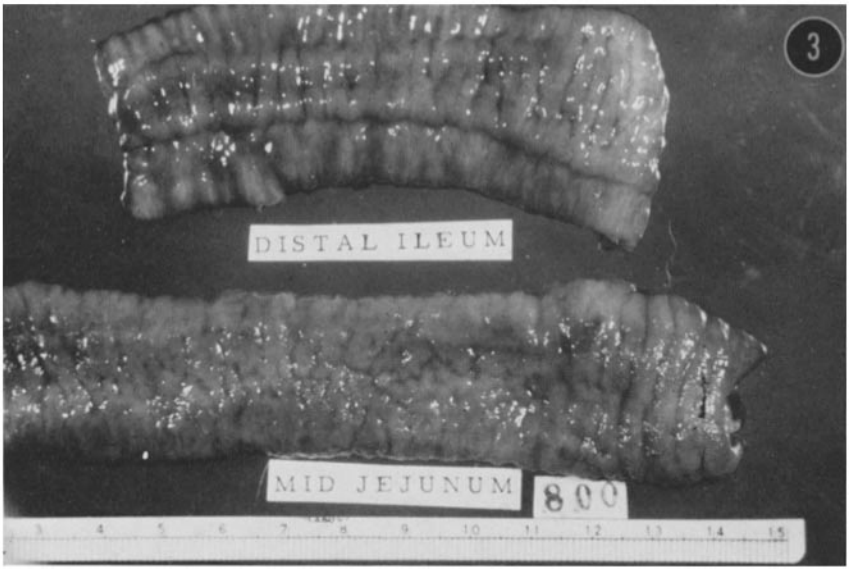
FIG. 2. Prolonged intraluminal infusion of staphylococcal enterotoxin induces severe enteritis which appears in skip-like fashion similar to human regional enteritis. × 1.



(Warren *et al.*: Acute and chronic enterotoxin enteritis)

PLATE 51

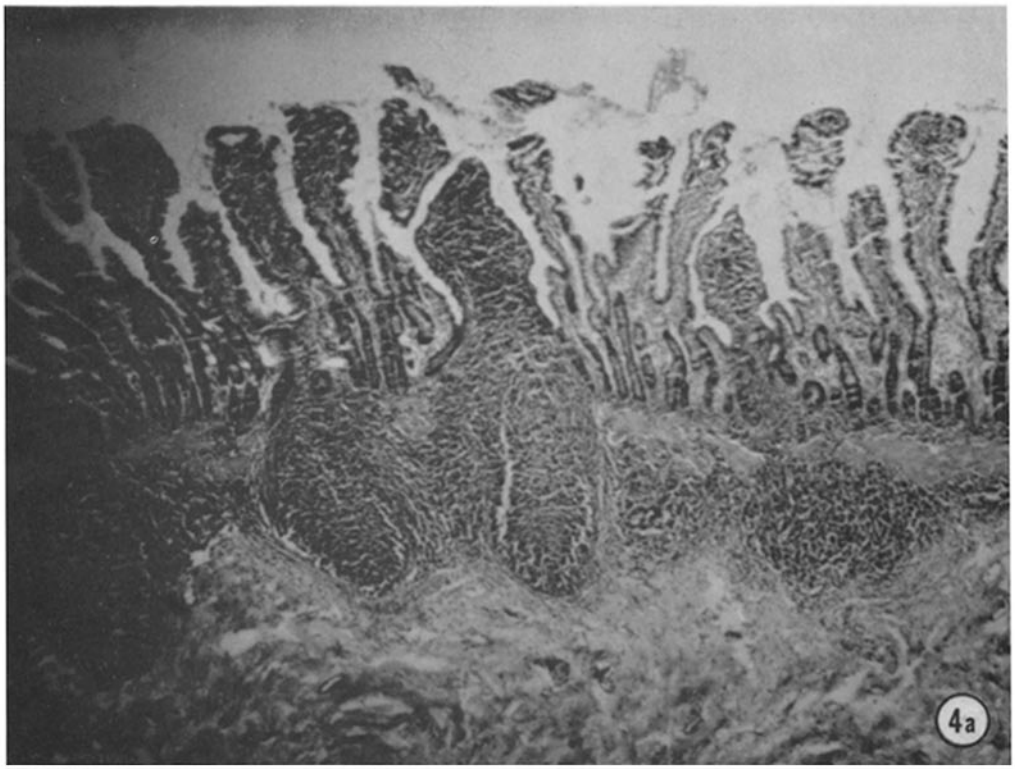
FIG. 3. Repeated daily, intraluminal infusion of enterotoxin induces exaggeration of longitudinal ridges due to submucosal fibrosis and lymphoid hypertrophy. × 1.

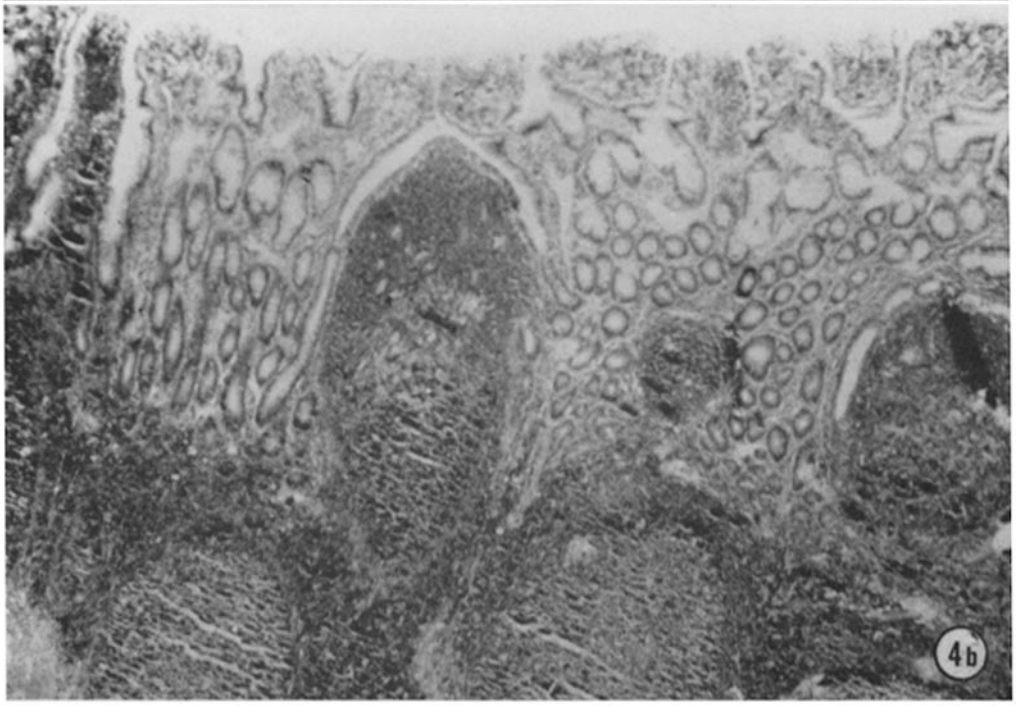


(Warren *et al.*: Acute and chronic enterotoxin enteritis)

PLATE 52

FIGS. 4 *a* and 4 *b*. Fig. 4 *a*. Early lymphoid hyperplasia with beginning destruction of villi. Fig. 4 *b*. Advanced development of chronic enterotoxin enteritis. × 44.





(Warren *et al.*: Acute and chronic enterotoxin enteritis)