Histopathological Effect of *Clostridium perfringens* Enterotoxin in the Rabbit Ileum

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Received for publication 20 May 1975

Highly purified enterotoxin from *Clostridium perfringens* was found to have histopathological activity in the rabbit ileum. Unlike the action of cholera, *Escherichia coli*, and Shigella enterotoxins, epithelium was denuded from the tips of ileal villi at concentrations of the enterotoxin necessary to induce fluid accumulation in the rabbit. Whether or not this observed histopathology is essential for the diarrheal syndrome associated with *Clostridium perfringens* food poisoning remains unclear. The physiology and histopathology of experimental diarrhea induced in the rat by *Clostridium perfringens* recently have been studied and described (8, 9). One aspect of the pathology seen, villus epithelial desquamation, is important both in understanding the mode of action of the enterotoxin and comparing it to other enterotoxins currently under study (cholera, *Escherichia coli*, staphylococcal, and *Shigella* enterotoxins). Duncan et al. (1) first reported epithelial damage in rabbits in association with diarrhea induced by whole cells of enteropathogenic strains of *C. perfringens*, whereas Hauschild et al. (5) reported no significant lesions in lambs challenged by cells or culture filtrates. Niilo (11, 12) then reported that intravenous administration of crude cell extracts from sporulating cells of enteropathogenic strains of *C. perfringens* into lambs caused partial loss of villus epithelium in association with diarrhea and other systemic reactions. He also reported variable damage in ligated loops of lambs when the enterotoxin was placed in the lumen of the intestine. Some sections showed sloughing of epithelial cells and some did not, depending upon the dosages and the susceptibility of each animal. Rabbits that developed diarrhea after intravenous injection with crude cell extracts displayed an intact villus epithelial membrane. Guinea pigs treated similarly to the rabbits also showed intact epithelium but did not develop diarrhea.

McDonel (8) showed that diarrhea in the rat due to highly purified *C. perfringens* enterotoxin is associated with epithelial desquamation, with the degree of damage being relative to the dosage of enterotoxin. Though diarrhea could be developed with low doses of enterotoxin that caused only slight damage to the epithelium (8, 9), it is uncertain if damage is an essential part of the mechanism of diarrhea in the case of this enterotoxin.

In the present study ileal loops of five adult white New Zealand rabbits were exposed in duplicate to various dosages of purified enterotoxin (16) for histology studies and four rabbits were treated similarly for fluid transport and protein determinations. After anesthesia with sodium pentobarbital (Nembutal) and subsequent cannulation and washing of the ileum with warm oxygenated Ringer glucose solution (8), loops were tied in 4- to 5-cm sections and excess fluid was removed by withdrawal with a syringe. Duplicate loops were then filled with 2 ml of Ringer solution containing 1,000, 500, 250, 100, or 50 erythemal units (EU) of enterotoxin. Controls consisted of loops containing 2 ml of solution without enterotoxin and untreated portions of intestine. The loops were carefully placed back into the abdominal cavity and the animal was kept covered and warm throughout the 90-min incubation period. At timed intervals the loops were removed, cut open lengthwise, and placed into phosphate buffered 4% formaldehyde solution prior to paraffin embedding and thin sectioning. Sections were stained with hematoxylin and eosin and with mucin stains. Fluid contents were withdrawn from loops prior to removal from rabbits studied for fluid production and protein release. The volumes were measured and total protein was determined from trichloroacetic acid precipitates by the method of Lowry et al. (7).

All doses of enterotoxin caused some degree

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**Fig. 1.** Histopathological effect of *C. perfringens* enterotoxin in the rabbit ileum. (A) Control; (B) portion of ileum exposed to 50 EU for 90 min; (C) 100 EU; (D) 250 EU; (E) 500 EU; (F) 1,000 EU. Magnification ×100. Strain, hematoxylin, and eosin.
of damage to the intestinal tissue in each sac studied. Although variations in response did occur, the severity of reaction increased with the dose in each animal. The sections shown in Fig. 1 are representative of the damage at each dosage. It was found that the response was dose dependent up to 500 EU, beyond which excess enterotoxin had no noticeable augmenting effect upon the histopathology described. Figure 1A shows control tissue exposed to solution without enterotoxin. It appeared normal, as did untreated sections of the ileum. Figure 1B shows slight but noticeable epithelial damage with exposure to 50 EU of enterotoxin. Congested submucosa was also evident (not shown). In Fig. 1C (100 EU) a few epithelial cells are present in the lumen from the partially desquamated epithelium. Mucin was also present that had been expelled from the goblet cells. Figure 1D (250 EU) is progressive and shows further desquamation. Intense desquamation, inflammatory cells (mostly lymphocytes), and slight hyperemia in the mucosa are seen in Fig. 1E (500 EU). Figure 1F (1,000 EU) shows a degree of damage similar to that in Fig. 1E.

It can be seen (Table 1) that net fluid accumulation in the lumen was the characteristic response to the enterotoxin as compared to a decrease in fluid content in control loops. Each dose of toxin caused a significant difference from controls in fluid transport (P < 0.001) as determined by Student's t test. However, there was no significant difference between amounts of fluid secreted at the doses studied. This suggests a possible threshold of response for fluid transport above which excess stimulation has little or no augmenting effect. That, however, is not the case for tissue damage. The data in Table 2 support those seen in Fig. 1, in that luminal fluid protein content increased with the toxin dose. This would be expected since increased desquamation seen with increased dosages (Fig. 1) and subsequent leakage of fluids into the lumen would cause an increase in protein to be found there. The differences between protein release induced by 50 and 250 EU and by 100 and 500 EU were significant (P < 0.05). It seems that an increase of nearly 200 EU was needed at the time interval studied to induce a significant increase in fluid protein content. The addition of more than 500 EU caused no significant increase in protein.

Stark and Duncan (16) found the minimal dose of enterotoxin needed to induce measurable fluid accumulation by the rabbit ileal loop test, as measured after an incubation period of several hours, to be about 140 to 200 EU of

<table>
<thead>
<tr>
<th>n</th>
<th>Erythemal activity/loop</th>
<th>Total fluid transport (µl/cm of intestine)</th>
<th>Standard error Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0 (Control)</td>
<td>72.9</td>
<td>±15.9</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>−50.1</td>
<td>±22.1</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>−40.0</td>
<td>±7.0</td>
</tr>
<tr>
<td>8</td>
<td>250</td>
<td>−61.8</td>
<td>±16.6</td>
</tr>
<tr>
<td>8</td>
<td>500</td>
<td>−57.7</td>
<td>±9.2</td>
</tr>
<tr>
<td>3</td>
<td>1,000</td>
<td>−66.7</td>
<td>±25.5</td>
</tr>
</tbody>
</table>

* All loops were exposed for 90 min.

** n, Number of loops.

* Negative values indicate secretion into the lumen.

<table>
<thead>
<tr>
<th>n</th>
<th>Erythemal activity/loop</th>
<th>Total protein content (mg/cm)</th>
<th>Standard error Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0 (control)</td>
<td>0.31</td>
<td>0.03</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>1.46</td>
<td>0.10</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>1.79</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>250</td>
<td>2.15</td>
<td>0.33</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
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<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>1,000</td>
<td>2.20</td>
<td>0.55</td>
</tr>
</tbody>
</table>

* All loops were exposed for 90 min.

** n, Number of loops.

activity. Hauschild et al., by using a rapid detection procedure using a 90-min incubation period (4), found that as little as 2.5 EU of enterotoxin was sufficient to prevent absorption of fluid whereas between 40 and 160 EU was needed to cause a net increase in fluid volume of loops tested. It can be seen from the evidence presented here that the minimal range of toxin needed to induce fluid production is within the range needed to induce tissue damage in the rabbit.

This report establishes that a distinct histopathology is produced in the rabbit intestine by the intraluminal administration of C. perfringens enterotoxin at doses needed to induce fluid accumulation in ileal loops. This is similar to results obtained in the rat (8). How the desquamation of villus epithelium is related to the transport reversals associated with diarrhea due to this enterotoxin is not yet clear. Certainly if tissue disruption is a part of the pathology in human cases it would contribute to fluid and electrolyte loss. However, it would not be expected that the damage in human cases could be very severe as apparent recovery is often complete in 24 h. The susceptibility of human
epithelial tissue to the desquamating activity of the enterotoxin could be very different from that seen in these experimental models.

The action of *C. perfringens* enterotoxin in the rabbit model is in contrast to that of cholera toxin in the rabbit (13), which causes no apparent change in the villus epithelium. Cholera toxin does not cause noticeable damage in the canine model (2) or human cases of the disease (3). *E. coli* enterotoxin also acts very similarly to cholera toxin in the rabbit (10) and therefore is contrasted to this enterotoxin. Although *Shigella* enterotoxin alone has no effect upon villus morphology (6), staphylococcal enterotoxin has been shown to be capable of denuding villus epithelium in cats (15) and dogs (12).

This research was supported by the College of Agriculture and Life Sciences, University of Wisconsin, Madison, Public Health Service research grant AI-11865-05 from the National Institute of Allergy and Infectious Diseases, Public Health Service research grant FD-00203-05 from the Food and Drug Administration, and by Contributors to the Food Research Institute by member industries. J.L.M. is the recipient of a post-doctoral award from Public Health Service grant T32-EF0715-01 of the National Institute of Environmental Health Sciences. C.L.D. is the recipient of a Public Health Service Research Career Development award AI-70721-02 from the National Institute of Allergy and Infectious Diseases.

We thank Joseph Lalich for assistance in interpreting histopathological data.

**LITERATURE CITED**