Identification of Enterotoxin E

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Identification of a new enterotoxin was accomplished by purification of the enterotoxin produced by staphylococcal strain FRI-326 and by preparation of specific antitoxin to the enterotoxin. Toxicity of the preparations was determined in rhesus monkeys, and specificity of the enterotoxin-antitoxin reaction was determined in gel diffusion plates. The enterotoxin was designated enterotoxin E.

The staphylococcal enterotoxins, major causes of food poisoning, are classified according to their reactions with specific antibodies as enterotoxins A, B, C, etc. (7). Four enterotoxins have been identified as immunologically distinct entities (2, 3, 5, 6). Two type C enterotoxins (C₁, reference 4; C₂, reference 1) that differ in some of their physicochemical properties have been purified; however, they react with antibodies prepared to either of the enterotoxins. One of the primary objectives of our investigations is to continue the search for antigenically different enterotoxins, because the only practical methods for their detection are based on the use of specific antibodies. A new enterotoxin, distinct from enterotoxins A, B, C, and D, was isolated in our laboratory. This communication presents evidence for identification of the new enterotoxin as enterotoxin E.

MATERIALS AND METHODS

Enterotoxin. The enterotoxin (enterotoxin E) used in this investigation was obtained from Staphylococcus aureus FRI-326 (from the Food Research Institute). This strain was originally isolated in 1960 by the Chicago Health Department from chicken tetrastizini which was implicated in a food poisoning outbreak. The enterotoxin produced by this strain was shown to be different from enterotoxins A, B, C, and D by a modification of the Ouchterlony plate technique (2).

Enterotoxin E was produced in the same way as described previously for enterotoxin C (2) and was purified by chromatography on carboxymethyl cellulose and gel filtration on Sephadex (Borja et al., manuscript in preparation). The purity of the enterotoxin was estimated with the double gel diffusion tube technique (2) and by polyacrylamide gel electrophoresis.

Enterotoxin antiserum. The antiserum to enterotoxin E was prepared as described previously for enterotoxin C (2). Antiserum prepared with crude enterotoxin was used to follow the purification of enterotoxin E, because the presence of antibodies to the impurities was useful in establishing at what point the impurities were eliminated.

Enterotoxin detection. The enterotoxin in the various samples was detected by intragastric administration of solutions by catheter to young rhesus monkeys (9) or by intravenous injection in a leg vein. Emesis within a 5-hr period was accepted as a positive reaction for enterotoxin.

Neutralization of the enterotoxin. The effect of the antibodies specific to the various enterotoxins on the toxicity of enterotoxin E in monkeys was determined as follows. One milliliter of an enterotoxin E solution (2.5 μg/ml) was mixed with an equal volume of diluted specific antiserum (1 volume of antiserum plus 3 volumes of pyrogen-free physiological saline) 30 min before intravenous injection into previously untreated monkeys (2).

RESULTS AND DISCUSSION

S. aureus FRI-326 was selected for investigation because it produced an enterotoxin that did not react with the specific antibodies to enterotoxins A, B, C, and D. Also, this particular organism was implicated in staphylococcal food poisoning, which indicated that the enterotoxin it synthesizes might be an important cause of such illnesses.

The identity of a new biological substance is difficult to establish unless the substance can be obtained in a high degree of purity. The enterotoxin E preparations used in this investigation were estimated to be at least 95% pure by polyacrylamide gel electrophoresis. The purified enterotoxin caused emesis in monkeys when injected intragastrically or intravenously in amounts equivalent to those required to cause emesis by enterotoxins A, B, and C (Table 1). (Purified enterotoxin D was not available.) These results verify the identity of the enterotoxin, since much larger doses would be required to provoke vomit-
ing if the enterotoxin were present as a minor contaminant of the purified material.

One proof that the antigen-antibody system identified resulted from the interaction of enterotoxin with its specific antibody is the neutralization of the toxic action of the antigen with its antibody. Neutralization was demonstrated when enterotoxin E was mixed with its specific antibody before injection into monkeys (Table 2).

One test that has been used to determine the specificity of the enterotoxins is to treat them with heterologous enterotoxin antisera. Failure of the heterologous antibodies to neutralize the enterotoxin in question was used as proof that the enterotoxin was antigenically different (2, 6). Results obtained in this investigation and earlier work done in this laboratory showed that neutralization apparently does occur in some instances (Table 2). The specific antibodies to enterotoxin C did not neutralize enterotoxin E, whereas enterotoxin B antibodies partially neutralized E, and enterotoxin A antibodies appeared to neutralize E completely. This was unexpected because, initially, no cross-reaction of enterotoxin E with enterotoxin A-specific antisera was observed by double gel diffusion techniques. On closer examination, however, it was noted that cross-reactions did occur when high concentrations of the heterologous antisera were used (Fig. 1). This indicated that a common antibody was present in the heterologous antisera in relatively small amounts in comparison to the concentration of the antibodies specific for each of

### Table 1. Response of monkeys to enterotoxins A, B, C, and E

<table>
<thead>
<tr>
<th>Enterotoxin (purified)</th>
<th>Intragastric administration</th>
<th>Intravenous injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amnt (µg) Results³</td>
<td>Amnt (µg) Results³</td>
</tr>
<tr>
<td>A</td>
<td>10  2/6</td>
<td>0.5  5/6</td>
</tr>
<tr>
<td>B</td>
<td>10  3/6</td>
<td>0.5  3/6</td>
</tr>
<tr>
<td>Cₙ</td>
<td>10  2/6</td>
<td>0.5  4/6</td>
</tr>
<tr>
<td>E</td>
<td>10  2/6</td>
<td>0.5  3/6</td>
</tr>
</tbody>
</table>

³ Monkeys vomiting/monkeys challenged.

### Table 2. Neutralization of enterotoxin E

<table>
<thead>
<tr>
<th>Enterotoxin E (µg)</th>
<th>Antiserum</th>
<th>Results³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>None</td>
<td>3/3</td>
</tr>
<tr>
<td>2.5</td>
<td>E</td>
<td>0/6</td>
</tr>
<tr>
<td>2.5</td>
<td>A</td>
<td>0/6</td>
</tr>
<tr>
<td>2.5</td>
<td>B</td>
<td>1/6</td>
</tr>
<tr>
<td>2.5</td>
<td>C</td>
<td>2/3</td>
</tr>
</tbody>
</table>

³ Monkeys vomiting/monkeys challenged (intravenous injection).

**Fig. 1.** Reaction of enterotoxins A and E with enterotoxin A- and E-specific antisera in the plate double gel diffusion test. (a) Enterotoxin E against normal concentrations of E antisemur (1 part antisemur plus 11 parts buffer) and normal (1 part antisemur plus 11 parts buffer) and high (1 part antisemur plus 2 parts buffer) concentrations of A antisemur. (b) Enterotoxin A against normal concentrations of A antisemur (1 part antisemur plus 11 parts buffer) and normal (1 part antisemur plus 11 parts buffer) and high (undiluted) concentrations of E antisemur.

**Fig. 2.** Reaction of enterotoxins A and E with enterotoxin A- and E-specific antisera in the plate double gel diffusion test. (a) Enterotoxins A and E against normal (1 part antisemur plus 11 parts buffer) and high (1 part antisemur plus 2 parts buffer) concentrations of enterotoxin A antisemur. (b) Enterotoxins E and A against normal (1 part antisemur plus 11 parts buffer) and high (undiluted) concentrations of enterotoxin E antisemur. (c) Enterotoxins A and E against high concentrations of enterotoxins A and E antisera (2 parts antisemur A plus 3 parts antisemur E plus 1 part buffer).
the enterotoxins. Further evidence for the presence of two antibodies in each antiserum was obtained when both enterotoxins were reacted with either of the specific antisera. A spur was formed with the homologous toxin, indicating the presence of an antibody which did not react with the heterologous toxin (Fig. 2a, b). Both enterotoxins formed spurs when a mixture of the A and E antisera was used (Fig. 2c). It was concluded that enterotoxin A contains an antigenic site specific for enterotoxin A, enterotoxin E contains an antigenic site specific for enterotoxin E, and both enterotoxins contain a minor common antigenic site. No evidence could be obtained which indicated a cross-contamination of the enterotoxins. When relatively high concentrations of the purified enterotoxins were used against the heterologous antisera, no cross-reactions were observed. Earlier it was observed that enterotoxin B antiserum neutralized enterotoxin C (unpublished data). Studies by Gruber and Wright (8) with the ammonium sulfate co-precipitation antibody determination indicated a definite cross-relationship between enterotoxins B and C, although this was not apparent in the double gel diffusion tests (2). Enterotoxin E appeared to be partially neutralized with enterotoxin B antiserum. Gruber and Wright (8) reported a slight cross-reaction between enterotoxins A and B. These cross-reactions indicate that an antigenic relationship may exist among all of the enterotoxins, but it is too weak to be employed in the detection of the various enterotoxins. It does indicate, however, that further studies in this area may reveal a relationship which can be utilized in the detection of all of the enterotoxins.

The staphylococci produce many antigenic substances other than enterotoxin, and not all staphylococci produce enterotoxin. Enterotoxin E was not produced by any cultures which had been identified as nonenterotoxigenic by the monkey feeding test.

At the time an unidentified enterotoxin is selected for study, there is no way to determine its prevalence. It is hoped that many of the cultures which produce unidentified enterotoxins will produce the new enterotoxin, but this is not always the case. In the case of enterotoxin E, only about six cultures have been found to produce it. However, its importance cannot be minimized because, in the summer of 1970, a food poisoning outbreak in Wisconsin involving more than 50 people, half of whom were hospitalized, was a result of the food ingested being contaminated with enterotoxin E.

ACKNOWLEDGMENTS

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LITERATURE CITED