Detection of Heat-Labile Enterotoxin-Like Activity in Stools of Patients with Cholera and *Escherichia coli* Diarrhea†

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The Y1 adrenal cell tissue culture assay was used to detect heat-labile enterotoxin-like activity in the stools of 14 of 74 patients with diarrhea. A positive effect of the stool on the adrenal cells was heat-labile and neutralized by cholera antitoxin. Enterotoxin-like activity was detected in the stools of 10 of 30 patients with cholera and in those of 2 of 4 from whom heat-labile *Escherichia coli* were isolated. None of the stools from nine individuals with *Vibrio parahaemolyticus*, *Salmonella*, or *Shigella* infections were positive. Two of 31 individuals from whom no pathogens were isolated had detectable toxin-like activity in their stools. The Y1 adrenal cell assay provides a rapid method of diagnosing heat-labile enterotoxigenic diarrhea and could be an adjunct in epidemiological studies of gastroenteritis.

Stool filtrates derived from patients with cholera produce an increase in capillary permeability after intracutaneous injection in guinea pigs (2). This response is similar to that obtained with *Vibrio cholerae* culture filtrates and is neutralized by convalescent sera. Donata et al. detected heat-labile enterotoxin (LT) with the sensitive Y1 adrenal tissue culture assay (2) in pigs experimentally infected with *Escherichia coli* (LT *E. coli*). We used this sensitive technique to identify enterotoxin-like activity in human stools.

Between May and October 1976, 74 children and adults admitted to San Lazaro Hospital, Manila, Republic of the Philippines, with gastroenteritis were studied. Stools were collected upon their admission to the hospital and were immediately frozen at −50°C until tested within 3 months for toxin. Stools were cultured daily for *Vibrio* species before and after enrichment in alkaline peptone water, on thiosulfate-citrate bile sucrose media. Stools were also cultured on MacConkey and Heektoen agar before and after enrichment in GN broth (Difco Laboratories, Detroit, Mich.). *Vibrio*, *Salmonella*, and *Shigella* species were identified by standard methods (1).

Ten lactose-positive colonies were tested for LT in the Y1 adrenal cell assay (6). All isolates producing toxin were identified as *E. coli* by standard criteria (4).

A 0.1-ml amount of stool was tested for LT in the Y1 adrenal cell miniculture assay (6) on four separate occasions. Positive (crude *E. coli* LT) and negative phosphate-buffered saline controls were included in each 96-well plate. The cell assay was read after 4 and 24 h of incubation. Stools that produced the characteristic rounding were retested after heating to 100°C for 30 min and after incubation with a 1:5 dilution of purified cholera antitoxin (Swiss Serum and Vaccine Institute) in phosphate-buffered saline at 37°C for 1 h. The description of stool and the result of the Y1 adrenal cell assay were recorded without knowledge of the bacteriological results.

Of the 74 stools, 14 were positive in the Y1 adrenal cell assay. Positive specimens failed to produce the same response after heating or incubation with cholera antitoxin. In 7 of the 74 stools, the results of the cell assay were uninterpretable after 24 h, but were consistently reproducible after a 4-h incubation.

Enterotoxin-like activity was detected in the stools of 10 of 30 patients with cholera and in those of 2 of 4 patients with LT *E. coli* disease (Table 1). None of the stools from nine patients with *V. parahaemolyticus*, *Salmonella*, or *Shigella* infections were positive in the adrenal cell assay. Two of 31 individuals from whom no pathogens were isolated had detectable toxin in their stools.

Toxin was present in the stools of 9 of 19 patients with cholera and in those of 2 of 3 patients with LT *E. coli* with rice water stools (almost clear with a characteristic odor of rotten fish). Toxin was detectable less often in semiliquid stools (brown liquid with particulate matter.
TABLE 1. Results of adrenal cell assay in relation to enteric pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Adrenal cell assay</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>V. cholera</em></td>
<td>10</td>
<td>20b</td>
</tr>
<tr>
<td>Toxigenic <em>E. coli</em></td>
<td>2</td>
<td>2b</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Shigella</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td><strong>None</strong></td>
<td>2</td>
<td>29</td>
</tr>
</tbody>
</table>

a Eight strains of serotype Ogawa and 2 Inaba were presumably biotype El Tor because of resistance to polymyxin B and hemolysis of sheep erythrocytes. b Cholera biotype Inaba and LT *E. coli* were isolated from acute stools. c Toxigenic *E. coli* was not isolated until Day 4 and 5 of hospitalization.

and strands of mucus) (Table 2). There was no relation between the duration of diarrhea before admission or the age of the patient and our ability to detect toxin in the stools.

The Y1 adrenal cell tissue culture assay is a rapid and specific test to detect LT-like activity of *V. cholerae or E. coli*. The effect on the adrenal cells was heat-labile and neutralized by cholera antitoxin. Two of 31 patients from whom we were unable to isolate *V. cholera* or LT *E. coli* had detectable toxin in their stools. Although we attempted to exclude all patients who had received antibiotics before admission, we speculate that these two individuals had received some antimicrobial agent, which made it impossible to isolate the toxigenic organisms.

The assay can be read after 4 h of incubation, which is preferable because of the destructive effect stools occasionally have on cells incubated for 24 h. LT-like activity was detectable more frequently in rice water stools than in liquid stools containing fecal particulate matter. This may be due to nonspecific absorption of the toxin by debris or mucin that contain gangliosides known to bind the *V. cholera* and LT *E. coli* (5, 7). Despite these difficulties, the Y1 adrenal cell assay provides a rapid method of diagnosing enterotoxigenic diarrhea and can be an important adjunct in epidemiological studies of diarrhea.

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LITERATURE CITED