Detection of Yersinia enterocolitica Heat-Stable Enterotoxin by Suckling Mouse Bioassay

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A comparison of heat-stable enterotoxins from Escherichia coli and Yersinia enterocolitica by the suckling mouse bioassay showed that whereas E. coli heat-stable enterotoxin evoked a maximum ratio of gut weight to remaining body weight after a 4-h incubation period, the maximum ratio for Y. enterocolitica was achieved at 2 h, decreasing thereafter to values regarded as negative (<0.083). This action of Y. enterocolitica heat-stable enterotoxin may give false-negative results for the standard suckling mouse bioassay.

Yersinia enterocolitica is currently recognized as an important human pathogen associated with a variety of clinical syndromes, particularly gastroenteritis (1). Recently, it was demonstrated by the suckling mouse bioassay that some strains produce a heat-stable enterotoxin (ST) which resembles the Escherichia coli ST (2, 8, 9, 11). Current reports indicate that among human-derived strains, the prevalence of enterotoxigenicity is high (10, 11). In our laboratory, however, the search for such a potential pathogenic property in some human isolates showed that frequently the ratios of gut weight to remaining body weight obtained by the suckling mouse bioassay were very close to the cutoff value between positive and negative results (0.083). Nevertheless, the mice often developed diarrhea by the end of the 4-h incubation period normally used in the assay (4). A similar observation was made by Robins-Browne et al. (11) when they tested concentrated Y. enterocolitica ST. These observations indicate that ST from some strains of this organism caused an early reaction in the mouse intestine which caused diarrhea. Thus, as a consequence of gut fluid loss, the true ratio of gut weight to remaining body weight would be altered.

In this communication, we report the results of experiments which suggest that the suckling mouse assay for Y. enterocolitica ST should be performed within a maximum incubation period of 2 h to avoid false results.

All bacteria used were human isolates. ST-producing E. coli (strain TR 22/4, serotype O128a, 128c:H12) was obtained from L. R. Trabulsi (Escola Paulista de Medicina, São Paulo, Brazil) and was originally isolated from a patient with acute diarrhea. Y. enterocolitica strain WA, serotype 8 (NCTC 10938), has been described previously (3, 6) and reported as ST positive (5). The remaining 15 Y. enterocolitica strains assayed were isolated in our laboratory from infants with diarrhea by the methodology described previously (12). Serotyping of these strains was kindly performed by H. H. Mollared by the Wauters scheme (13) at the Centre National des Yersinia, Institut Pasteur, Paris, France. Culture filtrates were obtained from these bacteria as described by Pai and Mors (9).

A sample of the culture filtrate obtained from Y. enterocolitica strain WA grown at 37°C was also assayed. For the ST assay, groups of four 2- to 4-day-old suckling mice randomly distributed by age were inoculated through the abdominal wall into the milk-filled stomach with 0.1 ml of culture filtrate from each sample. After incubation periods of 30 min and 1, 2, 3, and 4 h, the mice were killed, and the ratio of gut weight to remaining body weight was calculated. Values greater than 0.083 were considered positive.

Figure 1 shows the curves observed for the ratios of gut weight to remaining body weight calculated at regular intervals for ST from E. coli and Y. enterocolitica strain WA. E. coli ST gave the maximum ratio after 4 h of incubation. Y. enterocolitica ST, on the other hand, evoked a peak of fluid stimulation at between 1 and 2 h, decreasing thereafter. The culture filtrate of strain WA grown at 37°C gave a ratio curve identical to that seen with the non-inoculated culture filtrate and therefore was not included in Fig. 1. Figure 2 shows the curves of ST production by six human-derived strains. With the 4-h incubation, the ratios obtained were all similar, and the results would normally be considered negative. However, the ratios calculated at
2 h clearly showed the existence of both positive and negative strains. Those regarded as non-ST-producing showed curves similar to that for non-inoculated culture filtrate, never reaching the cutoff value of 0.083.

Table 1 lists a comparison between ratios obtained at 2 and 4 h in the 15 human-derived strains of *Y. enterocolitica* tested. Thirteen strains gave higher ratios at 2 h than at 4 h. Two strains (2/80 and AR) did not show the same pattern because the ratios at 4 h were higher. Nevertheless, at 2 h, these two strains had readings well above the cutoff value of 0.083.

Our findings clearly show that incubation for 4 h would give false-negative results for ST production by some strains of *Y. enterocolitica*. A question that remains to be answered is that of why a report from Canada (10) shows a high prevalence of ST-producing *Y. enterocolitica* as determined by the standard mouse bioassay. It may be possible that biological differences among our tested strains could be associated with a different pattern of ST production. The following evidence would support such a hypothesis. (i) One of the strains which did not produce ST in our study had the ability to invade HeLa cells and to evoke keratoconjunctivitis in guinea pigs. This pattern of potential pathogenicity was described as theoretically possible by Mors and Pai (7) but was never noted among their strains. (ii) These authors also reported that they did not find strains positive by the Sereny test in any serotype other than serotype 8. However, among our isolates, four belonging to serotypes 3 and 5 produced keratoconjunctivitis in guinea pigs. (iii) In the investigation cited above for the prevalence of ST-producing *Y. enterocolitica* in Canada, all of the strains of serotype 5 and almost all (99%) of those of serotype 3 were found to be ST producing, whereas in our study, 73% of the strains of these two serotypes failed to produce ST when the suckling mouse assay was based on the 4-h incubation period. The absence of ST production in human isolates of serotype 3 was also observed by Okamoto et al. (8), who reported that only 26% of the strains in Japan were ST positive.

The existence of strains with ratios below or bordering the cutoff value between positive and negative results indicates that the suckling

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**Fig. 1.** Ratios of gut weight to remaining body weight of suckling mice, calculated at regular intervals after the inoculation of *E. coli* ST and *Y. enterocolitica* strain WA ST. Non-inoculated culture filtrate ratios are also shown. Each value represents the mean of at least five tests. The horizontal line represents the cutoff value (0.083) between positive and negative results in the suckling mouse bioassay.
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Each value represents the mean of at least five tests. Ratios of 0.083 or greater are positive.
Strains included in Fig. 2 as ST negative.
Strains included in Fig. 2 as ST positive.

Fig. 2. Ratios of gut weight to remaining body weight of suckling mice, calculated at regular intervals after the inoculation of culture filtrates from six strains of Y. enterocolitica. Non-inoculated culture filtrate ratios are also shown. Figures in parentheses indicate the number of strains which gave similar curves. These were combined through mean values in single curves representing Y. enterocolitica strains regarded as ST producing and non-ST-producing. The horizontal line represents the cutoff value (0.083) between positive and negative results.

TABLE 1. Comparison between ratios of gut weight to remaining body weight obtained at 2 and 4 h of incubation in suckling mice inoculated with culture filtrates of Y. enterocolitica serotypes 3 and 5

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Ratio of gut wt to remaining body wt at incubation time of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>68b</td>
<td>5</td>
<td>0.75</td>
</tr>
<tr>
<td>70b</td>
<td>5</td>
<td>0.72</td>
</tr>
<tr>
<td>42712a</td>
<td>3</td>
<td>0.70</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>0.72</td>
</tr>
<tr>
<td>S</td>
<td>3</td>
<td>0.81</td>
</tr>
<tr>
<td>59a</td>
<td>5</td>
<td>0.88</td>
</tr>
<tr>
<td>69a</td>
<td>5</td>
<td>0.91</td>
</tr>
<tr>
<td>156a</td>
<td>5</td>
<td>0.91</td>
</tr>
<tr>
<td>275</td>
<td>3</td>
<td>0.86</td>
</tr>
<tr>
<td>FRA</td>
<td>3</td>
<td>0.99</td>
</tr>
<tr>
<td>FAB</td>
<td>3</td>
<td>0.84</td>
</tr>
<tr>
<td>R</td>
<td>3</td>
<td>0.90</td>
</tr>
<tr>
<td>281</td>
<td>5</td>
<td>0.93</td>
</tr>
<tr>
<td>2/80</td>
<td>3</td>
<td>0.11</td>
</tr>
<tr>
<td>AR</td>
<td>3</td>
<td>0.10</td>
</tr>
</tbody>
</table>

"Each value represents the mean of at least five tests. Ratios of 0.083 or greater are positive.
Strains included in Fig. 2 as ST negative.
Strains included in Fig. 2 as ST positive.

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LITERATURE CITED
