Susceptibility of the Mouse Intestine to Heat-Stable Enterotoxin Produced by Enteropathogenic *Escherichia coli* of Porcine Origin

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Ligated intestinal loops of mice were found suitable for the assay of heat-stable enterotoxin produced by enteropathogenic *Escherichia coli* strains of porcine origin; loops inoculated with heat-labile enterotoxin failed to react.

In recent studies, the mouse intestine was shown to be susceptible to the enteropathogenic action of *Escherichia coli* of human and porcine origin (1, 3). Porcine *E. coli* are either producers of heat-labile (LT) and heat-stable (ST) toxin or producers of ST alone (4). The rabbit intestine is very sensitive to LT, but is unsatisfactory for the assay of ST (2). This explains the irregular response obtained in ligated intestinal loops of rabbits to strains of enteropathogenic *E. coli* of porcine origin. It may also explain the complete lack of reactivity of the rabbit intestinal loop to strains of bovine or ovine origin, since the enterotoxins produced by these latter strains show a close resemblance to ST (5, 7).

The purpose of the present study was to investigate the sensitivity of the ligated intestinal loop of the mouse to ST and LT and to evaluate its suitability for the assay of ST.

LT and ST were prepared according to the methods described by Smith and Halls (6) and Smith and Gyles (4). The toxins were lyophilized and rehydrated just before use. They were obtained from three enteropathogenic strains of porcine origin: strain CPU 291 (0 group 8), which produces LT and ST, and strains CPU 330 (0 group 141) and CPU 41 (0 group 147), which produce ST only. Mice weighing 20 to 25 g were fasted for 18 h; they were then anesthetized with urethane and the small intestine was ligated. In each mouse three loops were prepared corresponding to the proximal (I), median (II), and distal (III) parts of the small intestine.

In one experiment three inocula were used: LT (1 mg), ST (20 mg), and ST (40 mg); culture medium concentrated 10 times by lyophilization was used as a negative control. All three inocula, LT, ST, and culture medium, were tested simultaneously in each mouse. All three preparations were tested in the three different locations, because in other species differences in sensitivity have been reported between the different parts of the small intestine. In another test two inocula were used: LT (25 mg) and ST (20 mg). The intensity of the reaction was measured after 4 h and expressed as the ratio of the volume of liquid in microliters and the length of the loop (in centimeters).

All three preparations of ST produced the accumulation of fluid in loops inoculated with 20 mg or more. Lower doses were not tested. These same preparations when injected into ligated loops of pigs produced dilatation at a dose of 160 mg or more; 40 or 80 mg had no effect.

Intestinal loops of mice inoculated with 25 mg of LT failed to react; a similar amount of the same LT preparation produced a positive reaction in intestinal loop of pigs. One milligram of this LT preparation was negative both in the mouse and pig intestines. Very small amounts of this and other LT preparations produced positive reactions in the rabbit intestine; 0.05 mg produced dilatation in 44% of the rabbit intestinal loops tested and the percentages of positive reactions increased to 95 and 100, respectively, when 0.5 or 1 mg was given. This confirms previous observations on the sensitivity of the rabbit intestine to LT (2).

Our data (Table 1) demonstrate that the
Table 1. Effect of ST and LT on ligated intestinal loops of mice

| Inoculum   | Dose (mg) | Segment of intestine | No. of positive animals/No. of animals tested* | Intensity of reaction (mean) | Variation |
|------------|-----------|----------------------|-----------------------------------------------|------------------------------|-----------|
| ST 330 (O 141) | 20        | I                    | 5/5                                           | 67                           | 21-130    |
|            |           | II                   | 6/6                                           | 51                           | 37-85     |
|            |           | III                  | 5/5                                           | 37                           | 29-66     |
| ST 291 (O 8)   | 20        | I                    | 4/4                                           | 31                           | 21-50     |
|            |           | II                   | 3/3                                           | 54                           | 50-61     |
|            |           | III                  | 4/4                                           | 57                           | 50-68     |
| ST 41 (O 147)  | 20        | I                    | 3/5                                           | 26                           | 0-60      |
|            |           | II                   | 4/4                                           | 50                           | 33-80     |
|            |           | III                  | 2/4                                           | 27                           | 0-60      |
| ST 41 (O 147)  | 40        | I                    | 4/4                                           | 170                          | 88-200    |
|            |           | II                   | 3/3                                           | 126                          | 100-140   |
|            |           | III                  | 3/3                                           | 80                           | 55-100    |
| ST 330 (O 141) | 20        | I                    | 2/4                                           | 26                           | 0-61      |
|            |           | II                   | 4/4                                           | 51                           | 20-88     |
|            |           | III                  | 4/4                                           | 64                           | 41-100    |
| LT 291 (O 8)   | 1         | I                    | 0/14                                          | 0                            |           |
|            |           | II                   | 0/29                                          | 0                            |           |
|            |           | III                  | 0/7                                           | 0                            |           |
| LT 291 (O 8)   | 25        | I                    | 0/4                                           | 0                            |           |
|            |           | II                   | 0/4                                           | 0                            |           |
|            |           | III                  | 0/4                                           | 0                            |           |
| Culture medium concentrated 10× | I        | 0/22                                          | 0                            |           |
|            |           | II                   | 0/9                                           | 0                            |           |
|            |           | III                  | 0/91                                          | 0                            |           |

*Loops showing a reaction of ≥ 20 were considered positive.

Mouse is to be preferred to the rabbit for the assay of ST, but that the rabbit remains the animal of choice for the assay of LT.

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