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ENTEROTOXIGENICITY
AND INVASIVE
CAPACITY OF
"ENTEROPATHOGENIC"
SEROTYPES OF
ESCHERICHIA COLI

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Enterotoxigenicity and invasive capacity of "enteropathogenic" serotypes of *Escherichia coli*,

Forty-two strains of *Escherichia coli* that agglutinated in pools of antisera used to identify "enteropathogenic" serotypes were tested for heat-labile and heat-stable toxin production and for their ability to invade intestinal mucosa. None of the strains tested were enterotoxigenic or enteroinvasive as determined by the adrenal cell (heat-labile toxin), the suckling mouse (heat-stable toxin), or guinea pig eye (invasive capacity) assays. Our observations suggest that serotyping of *E. coli* is an unreliable method to identify isolates that are capable of causing gastroenteritis, at least as determined by available in vitro techniques.

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ESCHERICHIA COLI have been shown to cause diarrhea by two separate mechanisms: enterotoxin production and mucosal invasion.¹ Strains with invasive capacity produce the characteristic findings of bacterial (*Shigella*) dysentery: local inflammation with hyperemia, ulceration, and intraluminal exudate comprised of polymorphonuclear leukocytes.² Toxigenic *E. coli* causes diarrhea by two separate mechanisms: (1) production of a heat-labile, antigenic toxin which resembles cholera toxin in that it activates cellular adenyl cyclase, thereby increasing intra-

cellular cyclic adenosine monophosphate and promoting secretion of sodium and water,³ and (2) production of a nonantigenic heat-stable toxin, the exact action of which is undefined.^{4,5}

The association of certain serotypes of *E. coli* with infantile diarrhea is now widely accepted. Institutional as well as community epidemics have been well described.^{6,7}

See related articles, pp. 91 and 166.

Abbreviation used
CDC: Center for Disease Control

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Nonetheless, most cases of infantile diarrhea are of undetermined etiology, and there is still doubt about the pathogenicity of all *E. coli* isolates designated as enteropathogenic. Furthermore, the term "enteropathogenic *E. coli*" was developed before mechanisms of *E. coli* diarrhea were understood. This study was undertaken to evaluate the pathogenicity of *E. coli* strains considered pathogenic because of their serotype; recently developed sensitive in vitro techniques were employed.

METHODS

Thirty-eight strains of *E. coli* isolated from children with diarrhea in 1974 and 1975, which agglutinated in

pools (A or B)* of antisera used to identify enteropathogenic *E. coli* (Difco), were obtained from Drs. H. D. Riley, Jr., University of Oklahoma Medical Center, and Peter Wright, Vanderbilt Medical Center. No attempt was made to identify specific serotypes. Three strains isolated from victims of nursery epidemics of diarrhea in 1970 and 1971 in Houston, Texas (M3458, serotype 0111), Augusta, Ga. (AU316, serotype 0119) and Birmingham, Ala. (30192, serotype 0128), and from a Washington, D.C., epidemic in adults in 1967 (SPU237, serotype 0111) were obtained from the Center for Disease Control. Strains from Drs. Riley and Wright were received on nutrient agar slants, usually within two months of isolation and stored in the dark until tested within five months of their arrival. Isolates from the CDC were handled in the identical fashion although they were isolated prior to 1971.

Assay for heat-labile enterotoxin. Isolates were grown in Syncase media (glucose substituted for sucrose) in 13 mm × 100 mm tubes at 37°C for 48 hours. Cultures were tested for enterotoxin production in duplicate using the Y1 adrenal cell mini-culture assay as described by Sack and Sack.⁷ Cultures of well-studied heat-labile toxigenic *E. coli* (334A, B7A and B2C)† and an isolate known to be negative in the rabbit ileal loop assay were included as positive and negative controls.

Assay for heat-stable enterotoxin. The infant mouse assay was performed by procedures described by Dean and associates.⁸ Briefly, enterotoxigenic material was prepared by inoculating 10 ml of Syncase media in 250 Erlenmeyer flasks with culture grown overnight in broth. Flasks were shaken at 37°C at 200 rpm for 24 hours. One tenth of a milliliter of the culture, with one drop of 0.5% Evan's blue per ml, was inoculated through the body wall into milk-filled stomachs of four, one- to four-day-old, Swiss albino suckling mice. After inoculation, mice were held at 25°C for four hours and then sacrificed by anesthesia with chloroform. The abdomen was opened, and the intestines from the pylorus to the rectum were removed. The ratio of gut weight to remaining body weight was calculated. No mice were included if dye was not present within the intestinal tract. Each strain was tested three to five times (four mice/assay) and the degree of distension expressed as the mean of the individual assays. A mean ratio of greater than 0.085 was considered positive for the presence of heat-stable toxin. Strains (334A, B7A, and B2C) known to produce heat-labile and

heat-stable toxin were tested unheated and after incubation at 100°C for 20 minutes.

Test for invasiveness. Approximately 10⁶ organisms were placed into the conjunctival sac of adult albino guinea pigs according to the methods of Mackel and associates.¹⁰ An *E. coli*, serotype 0124, isolated from an epidemic of gastroenteritis in adults in 1971 in Washington, D.C., and previously shown to be invasive by the Sereny test, was included as a positive control.¹¹ Eyes were examined daily for seven days for keratoconjunctivitis.

RESULTS

None of the *E. coli* which agglutinated in pools of antisera used to identify enteropathogenic strains produced either heat-labile toxin as determined by the adrenal cell assay or heat-stable toxin as determined by the suckling mouse assay. The cultures of three well-studied strains known to produce heat-labile and heat-stable toxin produced mean gut to remaining body weight ratios of greater than 0.120 (unheated or heated) and also caused typical rounding of the adrenal cell tissue cultures. None of the strains tested produced keratoconjunctivitis, whereas the positive control *E. coli* 0124 produced obvious corneal opacification with purulent discharge after 48 hours.

DISCUSSION

None of the enteropathogenic *E. coli* tested produced heat-labile enterotoxin as determined by the adrenal cell assay. Since this assay has excellent correlation with the adult rabbit ileal loop method and has been shown to be at least 40 times more sensitive, it is unlikely that heat-labile toxin-producing strains would not have been detected.^{8, 12}

Investigators differ on criteria for deciding whether an *E. coli* strain produces heat-stable toxin. Dean and associates⁸ considered a gut-to-remaining-body-weight ratio of less than 0.070 negative, 0.070 to 0.090 questionably positive, and greater than 0.090 positive for heat-stable toxin. Others have considered ratios above 0.071 or 0.085 positive.^{13, 14} In our experience, isolates must be tested on multiple occasions to produce reliable results, since the range in ratios produced by a single isolate was broad. For example, one of the isolates that agglutinated in enteropathogenic *E. coli* antisera produced ratios of 0.064, 0.073, 0.089, 0.073, and 0.068 and 0.070, 0.081, and 0.081, respectively. The means of these ratios 0.073 ± 0.010 and 0.077 ± 0.006 , however, were not significantly different from each other by the Student's *t* test ($p > 0.10$). Well-studied strains which produced heat-stable toxin also had similar variation but ratios were never below 0.085. For example, B7A produced a mean ratio of 0.149 ± 0.017

*Bacto *E. coli* OB antisera polyvalent A and B contain serotypes 026:B6, 055:B5, 0111:B4, 0127:B8, 086:B7, 0119:B14, 0124:B17, 0125:B15, 0126:B16, 0128:B12.

†*Esch. coli* 334A, B7A and B2C were kindly supplied by R. B. Hornick.

(range 0.111-0.170) on one occasion and 0.148 ± 0.002 (range 0.148-0.151) on another ($p > 0.10$). This assay variation is similar to observation of Whipp and associates¹⁴ on the limits of the suckling mouse enterotoxin assay. Those authors chose a minimum mean ratio of 0.085 as positive after three determinations. This value was chosen with the knowledge that 5% of their samples containing the same quantity of enterotoxin would be considered as a negative response. This value, however, was selected to assure against a false positive interpretation; 3.5% of negative control samples fell in the range of 0.075 to 0.085. Using this criterion (mean-gut-remaining-body-weight ratio of greater than 0.085), none of the enteropathogenic strains we tested produced heat-stable toxin.

None of the enteropathogenic serotypes produced keratoconjunctivitis when inoculated into guinea pig eyes, indicating that none of these strains had the ability to produce intestinal ulceration.¹⁵

None of the *E. coli* tested which agglutinated in pools of antisera used to identify enteropathogenic strains and which would be considered pathogens by most bacteriology laboratories were capable of causing diarrhea according to results using these three techniques. Either these strains cause diarrhea by unexplained mechanisms not detected by these assays, or, more likely, these strains may share the same surface antigens as other isolates responsible for epidemics of diarrheal disease but do not possess plasmids which make them pathogenic. It is possible that the strains tested lost their plasmids on storage before being studied, however most were not passed on multiple occasion and were stored in the dark. Furthermore, positive controls *E. coli* 334A, B7A, B2C and serotype 0124, which had been isolated in 1970 and 1971 and had been handled in identical fashion, did not lose their enterotoxigenic or invasive capacity.

The value of routine serotyping of *E. coli* isolated from children with diarrhea needs reevaluation since most "enteropathogenic" isolates are not pathogenic as determined by available technics, and toxigenic and invasive strains are often not of recognized "enteropathogenic" serotypes.^{11, 16}

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