INFECTION AND IMMUNITY, July 1978, p. 171-178
0019-9567/78/0021-0171$02.00/0

Vol. 21, No. 1
Printed in U.S.A.

Enterotoxigenicity of Enteropathogenic Serotypes of *Escherichia coli* Isolated from Infants with Epidemic Diarrhea

FREDERICK A. KLIPSTEIN,[1]* BERNARD ROWE,[2] RICHARD F. ENGERT,[1] HELEN B. SHORT,[3] AND ROGER J. GROSS[2]

Departments of Medicine[1] and Microbiology,[3] University of Rochester School of Medicine and Dentistry, Rochester, New York, 14642, and the Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, Colindale, London, England[2]

Received for publication 31 January 1978

Enteropathogenic serotypes of *Escherichia coli* which have been incriminated by epidemiological evidence as responsible for epidemics of acute diarrhea in infants are often found to be nontoxigenic when tested by conventional systems such as Y1-adrenal, Chinese hamster ovary, and suckling mouse assays. Twelve such strains, representing four different enteropathogenic serotypes, were examined for their capacity to elaborate toxic materials which alter water transport. Ultrafiltration fractions prepared to contain either a high-molecular-weight, heat-labile or a low-molecular-weight, heat-stable form of toxin from each strain were perfused through rat jejuna in graded concentrations ranging from 100 μg to 0.1 ng/ml. Ten of the twelve enteropathogenic strains produced one or both toxin forms that induced water secretion at concentrations of 1 to 10 ng/ml. Values in this range are considered indicative of clinically significant enterotoxigenicity in this assay system, and toxins from well-documented toxigenic strains examined in this study were active at these same concentrations. Similar preparations from ten control strains from healthy persons were either inactive or evoked water secretion only at concentrations of 10 to 100 μg/ml. These observations suggest that enteropathogenic serotypes of *E. coli* isolated from epidemics of infantile diarrhea produce diarrhea by elaborating potent heat-labile and heat-stable toxin forms which alter water transport but which are inactive in conventional assay systems. The manner in which these toxins differ either quantitatively or qualitatively from those which stimulate the conventional test systems is unknown.

The relationship between intestinal contamination by serotypes of *Escherichia coli* designated as enteropathogenic and nursery epidemics of acute diarrhea has been firmly established by epidemiological as well as other forms of evidence accrued during the past quarter century (36). More recently, two separate pathogenic mechanisms have been elucidated by means of which *E. coli* can produce diarrheal disease: (i) mucosal invasion and (ii) intraluminal production of enterotoxins (7). Enterotoxigenic strains which produce a high-molecular-weight, heat-labile (LT) and/or a low-molecular-weight, heat-stable (ST) form of toxin are capable of causing diarrhea (32, 41, 44). The enterotoxigenicity of these strains is evaluated under usual circumstances by in vitro tissue culture systems such as the Y1-adrenal cell and Chinese hamster ovary assays for LT and the suckling mouse assay for ST (34). The application of these tests to strains of enteropathogenic serotypes isolated from infants in nursery epidemics (20, 45) or from sporadic cases collected over a period of time (10, 17, 18, 22) or to those obtained during surveys of diarrheal subjects among specific groups (5, 43, 47) has shown that these strains only rarely elaborate enterotoxins. Conversely, many strains not included among the enteropathogenic serotypes which have been isolated from persons with acute diarrhea have been shown to be toxigenic by these assay techniques (44).

These observations have generated debate as to the value of serotyping strains of *E. coli* isolated either from victims of epidemic outbreaks or individuals with acute diarrhea (11, 40, 45, 46). Furthermore, the mechanism by which apparently nontoxigenic strains of enteropathogenic serotypes produce diarrhea remains obscure. The same also holds true for those strains of non-enteropathogenic serotypes isolated from persons with acute diarrhea that has

no other apparent cause, which have also been found to be inactive in the conventional tests for invasive and enterotoxigenic properties (4, 8, 35, 47, 48). It has been suggested that such strains may produce enterotoxins which differ qualitatively from those detected by the usual assay systems (39) or, alternatively, that other as yet unidentified mechanisms may exist by means of which *E. coli* induces diarrhea in humans, such as has recently been described in the case of rabbits (3).

In vivo perfusion of either the viable organisms or crude enterotoxins of toxigenic strains of *E. coli* evokes water secretion in the small intestine of experimental animals (21, 38). We have recently reported that when this procedure is used to determine the effect of semipurified ultrafiltration fractions of *E. coli* LT and ST on water transport it provides an assay system with a millionfold range of sensitivity for both of these toxin forms (29, 31). By using this technique, we have been able to show that strains of several species of coliform bacteria that are isolated from persons with acute diarrhea consistently elaborate highly potent toxins, whereas those cultured from healthy individuals or other sources are either inactive or produce only weakly active toxins (29). In the present study, we applied this assay technique to strains of four different enteropathogenic serotypes, each of which was isolated from victims of a separate epidemic of diarrhea, and compared the results with those obtained for ten control strains of non-enteropathogenic serotypes which were isolated from healthy individuals. The strains of enteropathogenic serotypes had been identified as the etiological agents responsible for nursery epidemics occurring in Great Britain; all were previously tested by conventional assay techniques and found to be nontoxigenic (20).

MATERIALS AND METHODS

Strains examined. Clinical, epidemiological, and bacteriological investigations conducted during the four outbreaks described above have been reported in detail (20, 27, 33, 49). The strains isolated during these epidemics were studied by using full serotyping techniques and, in each instance, the particular serotype was clearly implicated as the epidemic strain. The nursery epidemics that occurred at Glasgow (O142:H6), Manchester (O114:H2), and Teeside (O128:H2) are described clinically as severe in view of the attendant high mortality rate; such was not the case in the epidemic, described as mild, which occurred among infants at Taunton (O127:H6). All of the strains of enteropathogenic serotypes have been previously shown to be inactive for toxin production when tested by the Y1-adrenal, Chinese hamster ovary, and suckling mouse assays (20). Representative strains of three serotypes were also assayed by the lysis inhibition and passive immune hemolysis assay systems, which are based on the reaction of polymyxin-released *E. coli* LT to antiserum to LT or cholera toxin, as measured by complement-mediated hemolysis (or inhibition) of enterotoxin-sensitized sheep erythrocytes (13, 14). Polymyxin extracts from either the growth media that were used to prepare toxin fractions in the present study (Trypticase soy broth) or the Casamino Acids-based medium (CYE) used by Evans and Evans (13, 14) yielded a positive response in both of these assay systems in the case of a positive toxigenic control strain of *E. coli* (H-10407) and a negative response in both assays for enteropathogenic serotype O142:H6 strain E 2772/170, serotype O114:H2 strain E 380/69, and serotype O128:H2 strain E 63/68.

Positive toxigenic controls consisted of two *E. coli* strains, 334 (O15:H11) and H-10407 (O78:H11), both of which were isolated from persons with acute diarrhea and are well recognized as enterotoxin producers as determined by conventional assay techniques (10, 13, 17). The ten nontoxigenic strains used as controls were cultured from healthy individuals; all of these strains were negative in the Y1-adrenal, Chinese hamster ovary, and suckling mouse assay systems.

Assay technique. The procedures used for the culture and preparation of ultrafiltration retentates containing enterotoxin fractions and for the assay of this material by in vivo marker perfusion in rat jejuna have been described in detail previously (29–31). Briefly, preparations referred to as LT consisted of the retentate after passage through a PM-30 ultrafiltration membrane (Amicon Corp., Lexington, Mass.) of whole cell lysates derived by sonic oscillation (Branson Sonic Power Co., Plainview, N.Y.) of harvested confluent surface growth on Trypticase soy agar. The fractions referred to as ST were obtained from the UM-05 retentate after previous passage through a UM-10 membrane of the acetone precipitate of an aerobic culture in Trypticase soy broth.

Serial 10-fold dilutions of these toxin preparations in isosmolar-balanced electrolyte solution (30) were perfused at a constant rate with a model 1201 Harvard peristaltic pump (Harvard Apparatus Co., Millis, Mass.) through the jejuna of anesthetized, tracheotomized Sprague-Dawley rats (150 to 200 g each). Osmolalities were determined by measuring the freezing point depression with an Advanced DigiMatic osmometer (Advanced Instruments, Newton Heights, Mass.). All solutions containing toxin fractions were isosmotic with rat plasma at 317 mosmol/kg (30).

The net transport of water, expressed in microliters per centimeter per 30 min, was calculated from changes in the concentration of polyethylene glycol 4000 by the usual marker technique formula (30). Absorption, or net lumen-to-blood transport, is signified by a plus sign, whereas a minus sign indicates secretion. For reasons related to the different time of onset of action of LT versus that of ST toxins, which we have discussed previously (29, 31), the values reported are for that 30-min perfusion period during which there was maximal secretion or minimal absorption. A positive enterotoxin effect is defined as the presence of water secretion, and the minimal effective concentration (MEC) is that concentration of toxin (in dry weight per milliliter) which induces secretion.

Perfusion of 10 rats with the electrolyte solution alone yielded absorption with a range of +12 to +66 and a mean ± standard error of the mean of +38 ± 5 μl per cm per 30 min.

RESULTS

Nontoxigenic control strains. The ultrafiltration preparations of two of the ten strains did not evoke fluid secretion even at the highest concentration perfused and were regarded as inactive (Table 1). One or both of the ultrafiltrate fractions of the eight other strains contained material which induced water secretion but only at the highest concentrations used (either 100 or 10 μg per ml). In four of these strains, there was activity only in the case of the high-molecular-weight fractions referred to as LT; in two, only the low-molecular-weight fractions referred to as ST were active; and in two strains, both fractions induced water secretion (Fig. 1).

Toxigenic control strains. Ultrafiltrate preparations of both strains contained both LT and ST toxins which induced water secretion at an MEC of 10 ng or less per ml (Table 2). We regard values in this range as indicative of clinically significant toxigenicity based on the fact that those strains of *E. coli* isolated from persons with acute diarrhea which we have studied to date have consistently yielded one or both toxin fractions with an MEC in this range, whereas such has never been the case for strains obtained from healthy individuals (27).

TABLE 1. *Effect of ultrafiltrates of control strains on water transport in rat jejuna*

Serotype	Strain	Toxin form	Water transport at toxin concn in perfusate (per ml) of:[a]		
			100 μg	10 μg	1 μg
O22:H1	E3285/1	LT	−17	−4	+28
		ST	−8	+20	
O81:H27	E2708/0	LT	+4	+17	
		ST	+19		
O99:H4	E2939/3	LT	+17		
		ST	+6	+34	
O71:H48	E2698/1	LT	+3	+23	
		ST	−16	+23	
O40:H3	E2722/1	LT	−9	+7	+17
		ST	+21		
O113:H21	E2698/2	LT	−23	−8	+20
		ST	+9	+31	
O9:H4	E2744/4	LT	−22	−13	+25
		ST	+6	+20	
O?:H14	10405	LT	−12	+23	
		ST	+42		
O?:H14	C1-3	LT	+24	+26	
		ST	−27	+17	
O18:H7	C7-2	LT	−39	+26	
		ST	−10	+20	

[a] Values indicate microliters per centimeter per 30 min. +, Net absorption; −, secretion.

Enteropathogenic serotypes. All 12 strains examined elaborated both LT and ST toxin fractions that yielded water secretion (Table 2). Ten strains produced one or both toxin fractions for which the MEC was 10 ng/ml or less (Fig. 1). All nine of the strains isolated from the three epidemics that were characterized clinically as severe produced at least one or, in seven instances, both toxin forms with this degree of activity. In contrast, of the three strains tested that were isolated from persons in the Taunton epidemic which was characterized as mild, two did not produce either toxin with this degree of potency.

Characterization of the toxin material. Exposure to 100°C for 30 min abolished the secretory activity of each of the preparations considered to have LT from the toxigenic and enteropathogenic strains as well as the weakly active material produced by some of the nontoxigenic control strains (Table 3). In all but one instance, the heated material yielded values for absorption that were within the same range as that for the balanced electrolyte solution alone.

To compare further the active toxin fractions derived from the various strains, cell-free filtrates of the whole-cell lysates of a positive toxigenic control strain (334), an enteropathogenic strain (Teeside E/63), and a nontoxigenic control strain (E 3285/1) were passed sequentially through a series of ultrafiltration membranes, and the activity of the retentates of the different membranes, which contained fractions of various molecular weights, was assessed (Table 4). All of the ultrafiltration fractions from the control nontoxigenic strain were inactive. Both the toxigenic control strain and the enteropathogenic strain yielded two potent toxin fractions: (i) a heat-labile toxin in the PM-30 retentate (molecular weight between 30,000 and 100,000) and (ii) a heat-stable toxin in the UM-05 retentate (molecular weight between 500 and 30,000). Both of these strains also produced weakly active, heat-stable material with a high molecular weight which was 100,000 or more in the case of strain E/63 and greater than 300,000 in the case of strain 334.

DISCUSSION

Although epidemiological evidence has clearly incriminated the strains of enteropathogenic se-

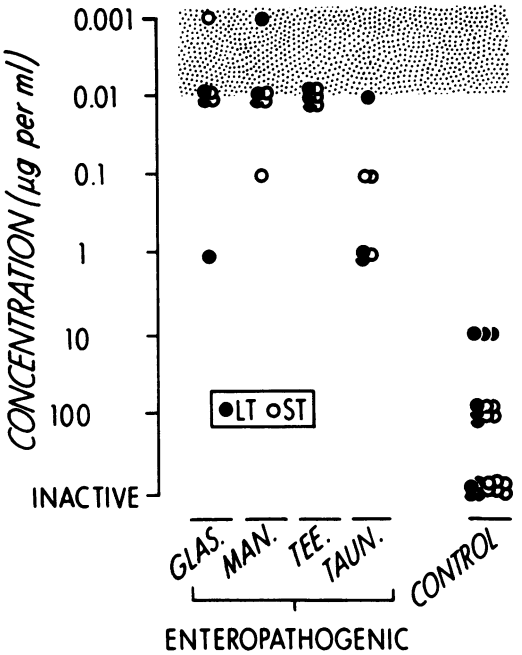

FIG. 1. *MECs for toxins produced by enteropathogenic strains (identified by the site of the epidemic: Glas., Glasgow; Man., Manchester; Tee., Teesdale; Taun., Taunton) and nontoxigenic control strains isolated from healthy individuals. The stippled area indicates the range of activity of toxins produced by documented enterotoxigenic strains. Values in this range are considered indicative of clinically significant enterotoxigenicity in this assay system.*

rotypes evaluated in this study as the causal agents of acute epidemics of diarrhea, none of these strains has been found to be enterotoxigenic when subjected to assay by conventional laboratory techniques, including the Y1-adrenal cell, Chinese hamster ovary, and suckling mouse assays. The results of the present study indicate that some of these enteropathogenic strains share with those strains whose toxigenicity is documented by the conventional assays the capacity to elaborate one or several forms of enterotoxin which induce water secretion (the basic pathophysiological abnormality by which enterotoxigenic bacteria produce diarrhea). Ten of the 12 enteropathogenic strains examined elaborated a high-molecular-weight, heat-labile and/or a low-molecular-weight, heat-stable form of toxin which induced water secretion at an MEC of 10 ng or less per ml. We regard potency of this degree as indicative of clinically significant enterotoxigenicity in the rat perfusion assay employed. This is based on our findings both in the present and previous studies (29) that strains of *E. coli* which have been isolated from persons with acute diarrhea and shown to be toxigenic by conventional assay systems consistently produce toxins which are active at these concentrations, whereas similarly produced materials elaborated by strains obtained from healthy individuals are either inactive or evoke secretion only at concentrations which are anywhere from a thousand- to a millionfold greater.

The in vivo marker perfusion assay system employed in the present study appears to be capable of detecting potent materials responsible for causing water secretion that are elaborated by strains of *E. coli* isolated from persons with acute diarrhea, which can be characterized either as toxigenic or nontoxigenic on the basis of response in conventional assay systems. We have referred to the substances produced by the enteropathogenic strains as toxins since the materials elaborated by both types of strain share the physiological property upon which enterotoxins are defined—the ability of a cell-free growth filtrate to induce net water secretion into the intestinal lumen. We also refer to the toxins elaborated by the enteropathogenic strains as LT and ST since these fractions were prepared by techniques which have been used by other laboratories to make these specific toxins (2, 24), and they share the same apparent molecular weight and heat-lability characteristics as the LT and ST toxin forms produced by toxigenic strains (6, 26). Nevertheless, it is quite obvious that some quantitative or qualitative difference exists between the toxins elaborated by those strains which are active in conventional assays and those which are not. Our preliminary observations with several strains by using the passive immune hemolysis technique also suggest that there may be an immunological dissimilarity between the toxins produced by toxigenic and enteropathogenic strains. The nature of this difference is unknown. Separation of the toxin fractions on the basis of molecular weight by sequential passage through graded polymeric membranes in the present study failed to show any difference between toxins produced by toxigenic and enteropathogenic strains.

The nature of the weakly active material elaborated by some strains of *E. coli* isolated from healthy individuals and the relationship of this material to the much more potent fractions derived by the same preparatory techniques from well-documented enterotoxigenic strains and from some of the enteropathogenic strains is uncertain. Separation on the basis of molecular weight by sequential ultrafiltration of the cell-free whole cell lysate of a nontoxigenic control strain failed to reveal any evidence of activity in any of the fractions so obtained. This observation raises the question as to whether the active materials produced by these strains, which we

TABLE 2. *Effect of ultrafiltrates of enteropathogenic and enterotoxigenic strains on water transport*

Outbreak serotype	Strain	Toxin form	Water transport at toxin concn in perfusate (per ml) of:[a]					
			1 μg	100 ng	10 ng	1 ng	100 pg	10 pg
Glasgow O142:H6	E2772/70	LT			−19	+9		
		ST			−34	−7	+26	
	E2831/70	LT		−23	−7	+21		
		ST			−10	+6	+14	
	E851/71	LT	−13	+3	+8	+13		
		ST		−22	−5	+7	+23	
Manchester O114:H2	E380/69	LT			−21	+19		
		ST			−28	+21		
	E554/69	LT		−10	−17	+11		
		ST			−17	+18		
	E508/69	LT			−50	−14	+26	
		ST		−16	+15			
Teeside O128:H2	E63/68	LT			−39	+6	+18	
		ST			−35	+6	+12	
	E74/68	LT			−17	+35		
		ST			−13	+12		
	E79/68	LT			−17	+14		
		ST			−25	+8	+7	
Taunton O127:H6	E2347/69	LT	−21	+8	+9			
		ST	−8	+4	+19			
	E2348/69	LT	−12	+3	+22			
		ST	−27	−8	+17			
	E2349/69	LT			−24	+13		
		ST		−17	+3	+18		
Toxigenic O15:H11	334	LT			−42	−18	+6	
		ST			−37	−35	−23	+11
Toxigenic O78:H11	H-10407	LT			−24	+2	+18	
		ST			−15	+11		

[a] Values indicate microliters per centimeter per 30 min. +, Net absorption; −, secretion. No water transport was recorded at a toxin concentration of 100 or 10 μg/ml.

have referred to in the past as enterotoxins (27), are identical to those present in the much more potent fractions produced by strains isolated from persons with acute diarrhea.

Variations in the potency of toxins elaborated by toxigenic strains of *E. coli* have been detected when these have been assayed by means of stimulation of adenyl cyclase in intestinal tissue (28) and of cyclic AMP in Chinese hamster ovary cells (9) or by distention of rabbit ligated intestinal loops (9, 12). These previous studies have suggested that a correlation exists between the degree of enterotoxigenic potency and the severity of diarrhea. Although the small number of strains examined in the present study precludes any definite conclusion, it is of interest that all of the enteropathogenic strains isolated from persons in epidemics that were characterized clinically as severe produced highly potent toxins, whereas such was the case in only one of three strains tested that had been isolated from individuals in an epidemic characterized as mild.

The precise significance and role of enteropathogenic or other specific serotypes of *E. coli* in the pathogenesis of acute diarrhea remain to be defined. The property of enterotoxigenicity is clearly not restricted to enteropathogenic serotypes; both enteropathogenic and non-enteropathogenic serotypes have been shown to be toxigenic as determined by both conventional assay systems and by the in vivo perfusion assay employed in the present study. The common denominator of the toxigenic strains, as defined either by conventional or in vivo perfusion assays, appears to be that all are isolated from persons with acute diarrhea. The classic antigenic determinants of enteropathogenic serotypes are chromosomally mediated, whereas both enterotoxin production and the presence of surface antigens which promote adhesion to the mucosal surface are plasmid mediated (16, 23). The association of those factors which confer

virulence with transmissible plasmids implies that they are independent of serotype; nevertheless, certain evidence suggests that in some instances serotype is significant. First, specific serotypes which are found to be toxigenic by conventional assay techniques have been shown to be responsible for outbreaks of epidemics of diarrhea in nurseries and in confined adult groups (1, 19, 25, 41). Second, enteropathogenic serotypes which have been clearly incriminated by epidemiological evidence as the causative factor responsible for nursery epidemics, but which were negative on routine testing for enterotoxigenicity, have been shown in the present study to produce toxins or related toxic substances that induce water secretion.

Finally, it has recently been recognized that a relatively small number of serotypes are commonly included among toxigenic strains isolated from persons with acute diarrhea in scattered parts of the world (15, 41, 42). In reviewing toxigenic strains isolated from different locations that were collected at the World Health Organization Collaborative Centre, Ørskov and his colleagues noted that certain serotypes which have not been classified as enteropathogenic in the past are found present with an unusually high frequency and have questioned whether these are special serotypes which have been selected to carry the plasmids necessary to invoke diarrhea (37). Evans and his associates have carried the matter one step further by demonstrating an apparent relationship among serotype, stability of enterotoxin production, particularly that of LT, and isolation from individuals with acute diarrhea as opposed to healthy controls (15). This observation has led them to suggest that these serotypes may serve as a reservoir of toxin-inducing plasmids in nature, which in turn transmit the plasmids to strains of *E. coli* of other serotypes.

TABLE 3. *Effect of heat on the secretory activity of the LT toxin preparations*

Serotype	Strain	Water transport[a]	
		Before heating[b]	After heating[b]
Toxigenic[c]			
O15:H11	334	−42	+17
O78:H11	H-10407	−24	+23
Enteropathogenic[c]			
O142:H6	E2772/70	−19	+33
O114:H2	E508/69	−50	+15
O128:H2	E79/68	−17	+16
O127:H6	E2349/69	−24	+13
Nontoxigenic controls[c]			
O22:H1	E3285/1	−17	+8
O40:H3	E2722/1	−9	+20
O113:H21	E2698/2	−23	+14
O9:H4	E2744/4	−22	+17
O?:H14	10405	−12	+40

[a] Values indicate microliters per centimeter per 30 min. +, Net absorption; −, secretion.
[b] Exposure to 100°C for 30 min.
[c] Toxigenic control and enteropathogenic strains were perfused at a concentration of 10 ng/ml; nontoxigenic control strains were perfused at 100 μg/ml.

TABLE 4. *Effect of whole-cell lysate fractions separated by sequential ultrafiltration on water transport*

UF membrane[a]	Strain	Water transport at concn in perfusate (per ml) of:[b]						
		100 μg	10 μg	1 μg	100 ng	10 ng	1 ng	100 pg
XM-300	334	−16 (−19)	−13	+6	+13			
>300,000	Teeside E/63	+7	+28					
	Control E3285/1	+33						
XM-100A	334	+10	+16					
>100,000	Teeside E/63	−17 (−12)	+3	+13				
	Control E3285/1	+36						
PM-30	334					−45 (+7)	−5	+13
>30,000	Teeside E/63					−13 (+5)	+7	+29
	Control E3285/1	+29						
UM-05	334					−16 (−14)	−3	+11
>500	Teeside E/63					−21 (−23)	+6	+21
	Control E3285/1	+36						

[a] UF, Ultrafiltration. Values indicate molecular weight of material retained by given membrane.
[b] Values indicate microliters per centimeter per 30 min. Values in parentheses are for the same preparation after exposure to 100°C for 30 min. +, Net absorption; −, secretion.

ACKNOWLEDGMENTS

This study was supported by grants from the Research Corporation, New York City, N.Y., and the Hillsdale Fund, Greensboro, N.C., and by contracts DAMD 17-77-C-7032 from the U. S. Army Medical Research and Development Command and NR 204-060 from the Office of Naval Research.

LITERATURE CITED

1. **Boyer, K. M., N. J. Petersen, I. Farzaneh, C. P. Pattison, M. C. Hart, and J. E. Maynard.** 1975. An outbreak of gastroenteritis due to *E. coli* O142 in a neonatal nursery. J. Pediatr. **86**:919–927.
2. **Bywater, R. J.** 1972. Dialysis and ultrafiltration of a heat-stable enterotoxin from *Escherichia coli*. J. Med. Microbiol. **5**:337–343.
3. **Cantey, J. R., and R. K. Blake.** 1977. Diarrhea due to *Escherichia coli* in the rabbit: a novel mechanism. J. Infect. Dis. **135**:454–462.
4. **Dean, A. G., Y. C. Ching, R. G. Williams, and L. B. Harden.** 1972. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J. Infect. Dis. **125**:407–411.
5. **Donta, S. T., R. B. Wallace, S. C. Whipp, and J. Olarte.** 1977. Enterotoxigenic *Escherichia coli* and diarrheal disease in Mexican children. J. Infect. Dis. **135**:482–485.
6. **Dorner, F., H. Jaksche, and W. Stockl.** 1976. *Escherichia coli* enterotoxin: purification, partial characterization and immunological observations. J. Infect. Dis. **133**(Suppl.):142–156.
7. **DuPont, H. L., S. B. Formal, R. B. Hornick, M. J. Snyder, J. P. Libonati, D. G. Sheahan, E. H. Labrec, and J. P. Kalas.** 1971. Pathogenesis of *Escherichia coli* diarrhea. N. Engl. J. Med. **285**:1–9.
8. **Echeverria, P., N. R. Blacklow, and D. H. Smith.** 1975. Role of heat-labile toxigenic *Escherichia coli* and reovirus-like agent in diarrhoea in Boston children. Lancet **ii**:1113–1116.
9. **Echeverria, P., C. J. Louria, A. L. Smith, and D. Smith.** 1977. Variation in enterotoxigenicity of *Escherichia coli*. J. Infect. Dis. **135**:195–200.
10. **Echeverria, P. D., and D. A. Smith.** 1976. Enterotoxigenicity and invasive capacity of "enteropathogenic" serotypes of *Escherichia coli*. J. Pediatr. **89**:8–10.
11. **Editorial.** 1975. *E. coli* enteritis. Lancet **ii**:1131–1132.
12. **Etkin, S., and S. L. Gorbach.** 1971. Studies on enterotoxin from *Escherichia coli* associated with acute diarrhea in man. J. Lab. Clin. Med. **78**:81–87.
13. **Evans, D. J., Jr., and D. G. Evans.** 1977. Direct serological assay for the heat-labile enterotoxin of *Escherichia coli*, using passive immune hemolysis. Infect. Immun. **16**:604–609.
14. **Evans, D. J., Jr., and D. G. Evans.** 1977. Inhibition of immune hemolysis: serological assay for the heat-labile enterotoxin of *Escherichia coli*. J. Clin. Microbiol. **5**:100–105.
15. **Evans, D. J., Jr., D. G. Evans, H. L. DuPont, F. Øskov, and I. Ørskov.** 1977. Patterns of loss of enterotoxigenicity by *Escherichia coli* isolated from adults with diarrhea: suggestive evidence for an interrelationship with serotype. Infect. Immun. **17**:105–111.
16. **Evans, D. G., R. P. Silver, D. J. Evans, Jr., D. G. Chase, and S. L. Gorbach.** 1975. Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. Infect. Immun. **12**:656–667.
17. **Goldschmidt, M. C., and H. L. DuPont.** 1976. Enteropathogenic *Escherichia coli*: lack of correlation of serotype with pathogenicity. J. Infect. Dis. **133**:153–156.
18. **Gorbach, S. L., and C. M. Khurana.** 1972. Toxigenic *Escherichia coli*. A cause of infantile diarrhea in Chicago. N. Engl. J. Med. **287**:791–795.
19. **Gross, R. J., B. Rowe, A. Henderson, M. E. Byatt, and J. C. Maclaurin.** 1976. A new *Escherichia coli* O-group, O159 associated with outbreaks of enteritis in infants. Scand. J. Infect. Dis. **8**:195–198.
20. **Gross, R. J., S. M. Scotland, and B. Rowe.** 1976. Enterotoxin testing of *Escherichia coli* causing epidemic infantile enteritis in the U.K. Lancet **i**:629–631.
21. **Guerrant, R. L., U. Ganguly, A. G. T. Casper, E. J. Moore, N. F. Pierce, and C. C. J. Carpenter.** 1973. Effect of *Escherichia coli* on fluid transport across canine small bowel. J. Clin. Invest. **52**:1707–1714.
22. **Gurwith, M. J., D. A. Wiseman, and P. Chow.** 1977. Clinical and laboratory assessment of the pathogenicity of serotyped enteropathogenic *Escherichia coli*. J. Infect. Dis. **135**:736–742.
23. **Gyles, C., M. So., and S. Falkow.** 1974. The enterotoxin plasmids of *Escherichia coli*. J. Infect. Dis. **130**:40–49.
24. **Gyles, C. L., and D. A. Barnum.** 1969. A heat-labile enterotoxin from strains of *Escherichia coli* enteropathogenic for pigs. J. Infect. Dis. **120**:419–429.
25. **Hobbs, B. C., B. Rowe, M. Kendall, P. C. B. Turnbull, and A. C. Ghosh.** 1976. *Escherichia coli* O 27 in adult diarrhoea. J. Hyg. **77**:393–400.
26. **Jacks, T. M., and B. J. Wu.** 1974. Biochemical properties of *Escherichia coli* low-molecular-weight, heat-stable enterotoxin. Infect. Immun. **9**:342–347.
27. **Jacobs, S. I., A. Holtzel, B. Wolman, J. H. Keen, V. Miller, J. Taylor, and R. J. Gross.** 1970. Outbreak of infantile gastroenteritis caused by *Escherichia coli* O114. Arch. Dis. Child. **45**:656–663.
28. **Kantor, H. S., P. Tao, and S. L. Gorbach.** 1974. Stimulation of intestinal adenyl cyclase by *Escherichia coli* enterotoxin: comparison of strains from an infant and an adult with diarrhea. J. Infect. Dis. **129**:1–9.
29. **Klipstein, F. A., R. F. Engert, and H. B. Short.** 1977. Relative enterotoxigenicity of coliform bacteria. J. Infect. Dis. **136**:205–215.
30. **Klipstein, F. A., I. R. Horowitz, R. F. Engert, and E. A. Schenk.** 1975. Effect of *Klebsiella pneumoniae* enterotoxin on intestinal transport in the rat. J. Clin. Invest. **56**:799–807.
31. **Klipstein, F. A., C.-S. Lee, and R. F. Engert.** 1976. Assay of *Escherichia coli* enterotoxins by in vivo perfusion in the rat jejunum. Infect. Immun. **14**:1004–1010.
32. **Levine, M. M., E. S. Caplan, D. Waterman, R. A. Cash, R. B. Hornick, and M. J. Snyder.** 1977. Diarrhea caused by *Escherichia coli* that produce only heat-stable enterotoxin. Infect. Immun. **17**:78–82.
33. **Love, W. D., R. J. Gross, A. M. Gordon, and B. Rowe.** 1972. Infantile gastroenteritis due to *Escherichia coli* O142. Lancet **ii**:355–357.
34. **Morris, G. K., M. H. Merson, D. A. Sack, J. G. Wells, W. T. Martin, W. E. Dewitt, J. C. Feeley, R. B. Sack, and D. M. Bessudo.** 1976. Laboratory investigation of diarrhea in travelers to Mexico: evaluation of methods for detecting enterotoxigenic *Escherichia coli* J. Clin. Microbiol. **3**:486–495.
35. **Mutanda, L. N.** 1976. Enteropathogenic *E. coli*. Lancet **i**:1412.
36. **Neter, E.** 1976. *Escherichia coli* as a pathogen. J. Pediatr. **89**:166–168.
37. **Ørskov, F., I. Ørskov, D. J. Evans, Jr., R. B. Sack, D. A. Sack, and T. Wadstrom.** 1976. Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhea in adults and children. Med. Microbiol. Immunol. **162**:73–80.
38. **Pierce, N. F., and C. K. Wallace.** 1972. Stimulation of jejunal secretion by a crude *Escherichia coli* enterotoxin. Gastroenterology **63**:439–448.

39. **Rowe, B., R. J. Gross, and S. M. Scotland.** 1976. Rheovirus and *E. coli* in infantile enteritis. J. Clin. Pathol. **29**:761.
40. **Rowe, B., R. J. Gross, and S. M. Scotland.** 1975. Serotyping of enteropathogenic *Escherichia coli*. Lancet **ii**:925-926.
41. **Ryder, R. W., I. K. Wachsmuth, A. E. Buxton, D. G. Evans, H. L. Dupont, E. Mason, and F. F. Barrett.** 1976. Infantile diarrhea produced by heat-stable enterotoxigenic *Escherichia coli*. N. Engl. J. Med. **295**:849-853.
42. **Sack, D. A., D. C. Kaminsky, R. B. Sack, I. A. Wamola, R. Ørskov, I. Ørskov, R. B. C. Slack, R. R. Arthur, and A. Z. Kapikian.** 1977. Enterotoxigenic *Escherichia coli* diarrhea of travelers: a prospective study of American Peace Corps volunteers. Johns Hopkins Med. J. **141**:63-70.
43. **Sack, D. A., J. C. McLaughlin, R. B. Sack, R. Ørskov, and I. Ørskov.** 1977. Enterotoxigenic *Escherichia coli* isolated from patients at a hospital in Dacca. J. Infect. Dis. **135**:275-280.
44. **Sack, R. B.** 1975. Human diarrheal disease caused by enterotoxigenic *Escherichia coli*. Annu. Rev. Microbiol. **29**:333-353.
45. **Sack, R. B.** 1976. Serotyping of *E. coli*. Lancet **i**:1132.
46. **Sack, R. B.** 1976. Enterotoxigenic *Escherichia coli*—an emerging pathogen. N. Engl. J. Med. **295**:893-894.
47. **Sack, R. B., N. Hirschhorn, I. Brownlee, R. A. Cash, W. E. Woodward, and D. A. Sack.** 1975. Enterotoxigenic *Escherichia coli*-associated diarrheal disease in Apache children. N. Engl. J. Med. **292**:1041-1045.
48. **Shore, E. G., A. G. Dean, K. J. Holik, and B. R. Davis.** 1974. Enterotoxin-producing *Escherichia coli* and diarrheal disease in adult travelers: a prospective study. J. Infect. Dis. **129**:577-582.
49. **Taylor, J.** 1970. Infectious infantile enteritis, yesterday and today. Proc. R. Soc. Med. **63**:1297-1306.