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Escherichia coli diarrhoea

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INTRODUCTION

Ever since Escherich (1885) first isolated the organism now known as *Escherichia coli* from the stools of infants, medical microbiologists have been faced with the problem of distinguishing between those strains capable of causing diarrhoea and those that are harmless gut commensals. Epidemiological investigations were greatly facilitated by the description by Kauffmann (1947) of a serotyping scheme for *E. coli*, and Taylor (1961) later reported that 17 O serogroups of *E. coli* had been implicated as possible causes of epidemic infantile enteritis. These infantile enteropathogenic *E. coli* (EPEC), having been discovered by epidemiological studies using serotyping, belonged by definition to a restricted range of serogroups. More recently it was shown that other *E. coli* strains may produce enterotoxins, and these enterotoxigenic *E. coli* (ETEC) usually belong to particular serogroups which are different from those associated with EPEC. *E. coli* strains belonging to a third range of serogroups may cause an illness resembling shigella dysentery, and these may be regarded as entero-invasive *E. coli* (EIEC). *E. coli* strains that cause diarrhoea may therefore be considered as three groups, as follows.

(1) Enterotoxigenic *E. coli* (ETEC)
Common serogroups: O6, O8, O15, O25, O27, O63, O78, O115, O148, O153, O159, O167.

(2) Entero-invasive *E. coli* (EIEC)
Common serogroups: O28ac, O112ac, O124, O136, O143, O144, O152, O164.

(3) Enteropathogenic *E. coli* (EPEC)
Common serogroups: O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142.

While ETEC and EIEC are defined on the basis of known pathogenic mechanisms, EPEC are defined by serotype. It is not surprising therefore that new knowledge suggests that the EPEC group may include strains with a variety of potentially pathogenic characters. Furthermore, these characters may be shared by other strains that do not belong to recognized EPEC serotypes. Additional groups may need to be established to include such strains, but for the time being in this review they will all be considered in the section on EPEC.

ENTEROTOXIGENIC *E. COLI* (ETEC)

In areas of good hygiene and nutrition ETEC are uncommon, although they occasionally cause outbreaks of diarrhoea among infants in hospital nurseries and among individuals of all ages in the community. In addition, ETEC are one of the

most common causes of diarrhoea among those travelling from such areas to regions with poor hygiene, especially in the tropics. In the developing countries diarrhoeal disease, together with underlying malnutrition, is a major cause of death in children under 5 years of age. ETEC are responsible for a high proportion of the acute diarrhoeas of childhood in these areas and therefore contribute significantly to the high level of mortality. ETEC typically cause a watery diarrhoea, and in the most severe cases the illness is indistinguishable from that due to *Vibrio cholerae* O1. This cholera-like syndrome has been described in Western countries but is more commonly reported in areas where cholera is endemic. However, even within a developed country populations may exist that have a relatively poor standard of hygiene and, particularly if the climate is warm, the incidence of ETEC infections may be similar to that found in the tropics. For example, in a study of Apache Indian children in Arizona, Sack *et al.* (1975) found ETEC in 16% of those hospitalized with acute diarrhoea.

Epidemic infantile enteritis in developed countries

Epidemic infantile enteritis due to ETEC was first reported by Gross *et al.* (1976). Twenty-five babies in the nursery of a Glasgow hospital were infected. Five babies required intravenous therapy, but none died. The causative organism belonged to a previously undescribed serogroup, *E. coli* O159, and produced ST but not LT. In the same year Ryder *et al.* (1976) reported an outbreak which affected 55 of 205 infants in the special-care nursery of a large hospital in the United States. The epidemic strain belonged to serogroup O78 and again produced ST but not LT. Rowe *et al.* (1978) described a further outbreak in Britain that affected 10 of 18 babies in the special-care unit of a Gloucester hospital and was caused by a strain of *E. coli* O6 that produced ST and LT. The source and route of transmission of infection in these outbreaks was uncertain. In the Gloucester outbreak the evidence suggested that the index case was a premature baby who developed diarrhoea 4 days after birth, and further cases resulted from cross-infection. The outbreak in the United States continued for 9 months, and investigations of the hospital environment showed heavy contamination with *E. coli* O78. The epidemic strain was also isolated from milk feeds.

Diarrhoea in the community in developed countries

Several surveys have shown that ETEC are not a common cause of sporadic, endemic diarrhoea in developed countries with good standards of hygiene. A study of Boston children suggested that ETEC were unimportant (Echeverria, Blacklow & Smith, 1975) and studies in Canada reached the same conclusion (Gurwith & Williams, 1977). In Europe a Swedish study and a small survey in Britain found that ETEC were uncommon as a cause of indigenous sporadic diarrhoea in those countries (Back, Blomberg & Wadstrom, 1977; Gross, Scotland & Rowe, 1979) and ETEC were not found in a Swiss study of 119 adults with diarrhoea (Loosli *et al.* 1985). However, contamination of food or water may occasionally lead to outbreaks of infection. In the summer of 1975 more than 2000 staff and visitors at a national park in the United States developed diarrhoea caused by a strain of *E. coli* O6 which produced ST and LT (Rosenberg *et al.* 1977). The source of the infection was found to be drinking water which had been contaminated with

sewage. In Japan seven outbreaks of adult enteritis were studied in Tokyo between 1969 and 1974 (Kudoh *et al.* 1977). Six were caused by *E. coli* O159 and one by *E. coli* O11. In all the outbreaks the *E. coli* produced ST but not LT. Two outbreaks were caused by contaminated water supplies and two others were thought to be due to food-borne infection.

Riordan *et al.* (1985) described an outbreak of diarrhoea with abdominal pain that occurred among staff members from a school in Manchester, England following a social function. Food history analysis showed a correlation between the disease and the consumption of curried turkey mayonnaise, but no food sample was available for bacteriological examination. Faecal specimens from 13 cases were examined. *E. coli* O6 H16 (ST^+LT^+) was found in 9 and *E. coli* O27 H20 (ST^+) in 11. Eight specimens contained both strains, and only one specimen was negative for ETEC.

Traveller's diarrhoea

Traveller's diarrhoea is a world-wide illness usually of brief duration, often beginning with the rapid onset of loose stools and sometimes accompanied by other symptoms including nausea, vomiting and abdominal cramps. It occurs most frequently among those travelling from areas of good hygiene and temperate climate to areas with lower standards, particularly in the tropics.

Rowe, Taylor & Bettelheim (1970) first demonstrated a relationship between *E. coli* and traveller's diarrhoea in a study of British troops in South Arabia. In this study serotyping showed that about half the diarrhoea among new arrivals might be due to *E. coli* O148, a previously undescribed serogroup. It was subsequently discovered that this strain produced ST (Rowe, Gross & Scotland, 1976). Enterotoxigenic *E. coli* O148 also caused diarrhoea among United States soldiers in Vietnam (DuPont *et al.* 1971), and ETEC of various serotypes were the most common pathogens found among U.S. military personnel in the Philippines (Echeverria *et al.* 1979*a*). In contrast, a study of U.S. troops in South Korea, an area with a relatively temperate climate, showed that ETEC infections were uncommon even though 55% of the soldiers developed diarrhoea (Echeverria *et al.* 1979*b*).

There have been several studies of traveller's diarrhoea in Mexico, where 'Turista' has an attack rate of 29–48%. In one study ETEC was the most common cause and accounted for 45% of the diarrhoea cases (Merson *et al.* 1976), while in another survey 72% of those with diarrhoea were excreting ETEC (Gorbach *et al.* 1975). Travellers from Europe to tropical or even Mediterranean countries may develop diarrhoea due to ETEC. In a study in Sweden (Back *et al.* 1977) ETEC were found in 11% of those developing diarrhoea while abroad or shortly after returning from abroad, and in a similar study in Great Britain the figure was also 11% (Gross, Scotland & Rowe, 1979).

The sea cruise is a form of travel which has often been plagued by outbreaks of diarrhoea. Salmonella or shigella are often the cause, but some outbreaks have been associated with ETEC. Hobbs *et al.* (1976) reported that a strain of *E. coli* O27 that produced ST was isolated from 55%, 61% and 20% of travellers with diarrhoea on three successive cruises by the same ship. On this ship the infections were probably food-borne. In another ship it was reported that an *E. coli* O25 strain that produced ST was isolated from 83% of diarrhoea cases in outbreaks occurring

during two successive cruises (Report, 1976). Studies on board the ship revealed many deficiencies in the handling of food and drinks. When these procedures were improved no further outbreak of diarrhoea occurred.

Diarrhoea in tropical countries

There is no doubt that ETEC are an important cause of diarrhoea in all age groups in areas of poor hygiene, particularly in the tropics. In a study of 354 Ethiopian children and infants with diarrhoea ETEC were isolated from 14% of the patients (Wadstrom *et al.* 1976). In black South African infants with acute summer gastroenteritis ETEC were found in 9 of 34 infants (Schoub *et al.* 1977). In Mexico, Donta *et al.* (1977) found ETEC in 16% of 50 children admitted to hospital with diarrhoea while Evans, Evans & DuPont (1977) isolated ETEC from 40% of 71 children in a retrospective study. In the Philippines Echeverria *et al.* (1978) found ETEC in 11% of 82 children hospitalized with diarrhoea, and Echeverria *et al.* (1985) found ETEC in 10% of 177 Thailand villagers with diarrhoea.

Clinical cholera, characterized by profuse 'rice-water' stools, is traditionally associated with *V. cholerae* O1, but there is often a significant proportion of patients from whom no aetiological agent can be isolated. Sack *et al.* (1971) used the rabbit ileal loop test to demonstrate LT production by ETEC isolated from jejunal aspirates of four adult patients with cholera-like diarrhoea in Calcutta. A subsequent survey in Bangladesh showed than LT-producing ETEC could be isolated from 19·2% of all patients with diarrhoea, from 55% of hospital in-patients with diarrhoea and from 70% of those severely ill with non-vibrio cholera (Nalin *et al.* 1975). In a further study Sack *et al.* (1977) isolated ETEC from 23 of 65 patients (35%) with acute cholera-like disease in Dacca, Bangladesh. Diarrhoea caused by ETEC affected individuals of all ages and could not be distinguished from cholera on clinical grounds.

Nature and mode of action of E. coli *enterotoxins*

Heat-labile enterotoxin (LT)

LT consists of two polypeptide subunits. Subunit A has a molecular weight of about 25000 and has the ability to stimulate adenylate cyclase activity. Subunit B has a molecular weight of about 11500 and forms aggregates of five monomers which are able to adsorb to the Y1 adrenal cells used in LT tests. Immunological and biological neutralization tests show that there is a close similarity between *E. coli* LT and the *V. cholerae* enterotoxin (CT). Furthermore, there is significant homology between the amino acid sequences of the B subunits of LT and CT. In both toxins the B subunit is responsible for binding to the Gm1 ganglioside of the epithelial cells of the small intestine while subunit A stimulates adenylate cyclase activity, thereby increasing the concentration of cyclic AMP in the cells. The increased level of cAMP appears to act in two ways to cause fluid and electrolyte loss into the gut lumen. In the villus cells cAMP inhibits the absorption of Na^+ and hence of Cl^- and water. In the crypt cells cAMP exerts a direct secretory effect by increasing Na^+ secretion and consequently causing loss of Cl^- and water (Field, 1979).

Heat-stable enterotoxin (ST)

More than one form of ST exists: ST_A is methanol-soluble and active in the infant mouse test and in neonatal piglets while ST_B is methanol-insoluble, inactive in infant mice and active in ligated intestinal loops of older piglets and rabbits. *E. coli* strains may produce ST_A, ST_B or both (Burgess *et al.* 1978). ST_A is found in strains of human and animal origin while ST_B strains are usually of porcine origin. Studies using genetic probes have shown that ST_A can be further divided into two types, ST_{A1} and ST_{A2}. ST_{A1} has been found in strains from human and animal sources while ST_{A2} has been found only in human strains (Moseley *et al.* 1982). The nomenclature of these toxins can be confusing. ST_A is also known as ST_I or ST_a, ST_B as ST_{II} or ST_b, ST_{A1} as ST_{Ia} or ST_P, and ST_{A2} as ST_{Ib} or ST_h.

ST_A does not affect the concentration of cAMP but instead stimulates the activity of guanylate cyclase, causing an increase in cGMP levels. This occurs only in intestinal epithelial cells and not in a variety of other tissues and cell lines, suggesting that a unique toxin receptor is present in intestinal cells. The mechanism by which increased cGMP leads to a net secretion of water and electrolytes is not well understood, but the action of ST appears to be mainly anti-absorptive and lacks the secretory activity of LT and cholera toxin (Newsome, Burgess & Mullan, 1978; Guerrant *et al.* 1980). The fluid secretion induced by ST_B appears to take place with no alteration of cGMP levels, and the mechanism of action of ST_B remains unknown (Kennedy *et al.* 1984).

Adhesive factors

It was first shown in studies of piglet enteritis that the ability to produce enterotoxin alone is not sufficient to enable an *E. coli* strain to cause diarrhoea. The bacteria must also be able to colonize the mucosal surface of the epithelial cells of the small intestine. This colonization depends on adhesion that is often mediated by filamentous protein fimbriae which bind to specific receptors in the cell membrane. These adhesive or colonization factors are antigenic, and their presence can be determined by means of agglutination, immunodiffusion or ELISA tests using specific antisera. Their presence can also be detected by haemagglutination, by experimental colonization of animal intestines or by tissue or organ culture methods, as well as by electron microscopy. In contrast to the type 1 pili of *E. coli*, adhesive fimbriae associated with diarrhoeal disease cause a mannose-resistant haemagglutination (MRHA). The genetic determinants controlling the production of *E. coli* adhesive factors are carried on plasmids which may be transferable, and plasmids may simultaneously carry genes for both an adhesive factor and enterotoxin production.

The first adhesive factor found to be important in *E. coli* diarrhoea was the K88 antigen. Its role in piglet enteritis was confirmed in experiments that showed that loss of the K88 plasmid from a strain of *E. coli* O 141 resulted in the loss of its ability to cause diarrhoea when given orally to piglets. This ability was restored by introducing a K 88 plasmid from another strain of *E. coli* (Smith & Linggood, 1971). A further antigen designated as 987 P is also important in infections of pigs (Isaacson, Nagy & Moon, 1977). The K 99 antigen has been shown to be important

in diarrhoea of sheep and cattle (Ørskov *et al.* 1975), and an antigen known as F41 has been found in strains from calf diarrhoea (de Graaf & Roorda, 1982).

The human colonization factor antigen I (CFA/I) was discovered in an enterotoxigenic strain of *E. coli* O78 isolated from a patient with cholera-like diarrhoea (Evans *et al.* 1975). CFA/I appears as a fimbrial surface structure, and its loss correlates with the loss of a plasmid coding for both CFA/I and ST production. Strains of *E. coli* that possess CFA/I cause MRHA of human group A erythrocytes, but this is of little value as a laboratory test for CFA/I since non-enterotoxigenic strains from extra-intestinal sources frequently cause group A MRHA but do not possess CFA/I (Cravioto *et al.* 1979*a*). The presence of CFA/I appears to be restricted mainly to a few serogroups of ETEC including O25, O63, O78 and O153. It has been confirmed by oral challenge experiments in human volunteers that the original CFA/I strain causes diarrhoea while its laboratory derivative without CFA/I does not (Satterwhite *et al.* 1978). A second human colonization factor (CFA/II) has been found and differs from CFA/I antigenically and in its ability to cause MRHA of bovine but not human red blood cells. CFA/II is commonly found in ETEC belonging to serogroups O6 and O8 (Evans & Evans, 1978). CFA/II production is also plasmid-determined, and plasmids encoding the synthesis of ST, LT and CFA/II have been described (Penaranda *et al.* 1980). Studies using antisera prepared against strains of *E. coli* O6 H16 of different biotypes have shown that three different antigenic components of CFA/II can be detected (Cravioto, Scotland & Rowe, 1982; Smyth, 1982). These three components have been designated coli surface-associated antigens; CS1, CS2 and CS3. Strains of *E. coli* O6 H16 may produce CS1+CS3 or CS2+CS3, while strains of other serogroups usually produce CS3 alone. Plasmid transfer studies show that production of CS1+CS3 and CS2+CS3 is controlled by a single plasmid which also codes for ST and LT. The combination of CS antigens that is expressed depends on the properties of the bacterial host strain into which the plasmid has been transferred (Mullaney *et al.* 1983). Levine *et al.* (1984) showed by immune-electron microscopy that CS1 and CS2, like CFA/I, consist of rigid fimbriae of 6–7 nm diameter. In contrast, CS3 resembles K88 and F41 in being a flexible, wiry structure of 2 nm diameter. A third factor (CFA/III) was described in a single enterotoxigenic strain of *E. coli* O128. CFA/III resembles CFA/I in causing MRHA of human and bovine erythrocytes but was reported to be antigenically distinct from CFA/I and CFA/II (Darfeuille *et al.* 1983). Work in the authors' laboratory suggests that this strain actually possesses CFA/I (Scotland, unpublished results). Honda, Arita & Miwatani (1984) demonstrated the presence of a hydrophobic fimbrial antigen in a strain of ETEC and also called it CFA/III. These fimbriae did not cause MRHA and differed antigenically from CFA/I and CFA/II. Thomas *et al.* (1982) have described another antigenically distinct putative colonization factor provisionally known as E8775 which has been found in ETEC of other serogroups including O25, O115 and O167 (Table 1). E8775 also has three components, CS4, CS5 and CS6 (Thomas *et al.* 1985) and resembles CFA/II in that strains may produce CS4+CS6, CS5+CS6, or CS6 alone. CS4 and CS5 are fimbrial while the structure of CS6 is still uncertain. Thomas & Rowe (1982) have examined a large number of ETEC strains for the presence of CFA/I, CFA/II and E8775 and found that only 34% of strains possessed one of these adhesive factors. Thomas (1985) reported that

Table 1. *Colonization factors and O serogroups*

	O serogroup
CFA/I	4, 15, 25, 63, 78, 90, 110, 114, 126, 128, 153
CFA/II	6, 8, 9, 78, 80, 85, 115, 139
E8775	25, 27, 92, 115, 148, 153, 167, 169

Table 2. *Summary of a survey for CFA/I, CFA/II and the E8775 factor on ETEC strains isolated in Bangladesh, Thailand and travellers returning to Japan*

Enterotoxin production	Number of strains	Positive for adhesive factor		
		CFA/I	CFA/II	E8775
ST/LT	293	51 (17·4%)	81 (27·6%)	28 (9·6%)
LT	98	0 (0%)	2 (2%)	0 (0%)
ST	347	46 (13·3%)	15 (4·3%)	26 (7·5%)
Total	738	97 (13·1%)	98 (13·3%)	54 (7·3%)

strains that produced LT alone rarely possessed any of these three factors (Table 2). It therefore seems likely that further colonization factors are yet to be discovered. Deneke, Thorne & Gorbach (1981) investigated adhesion of human ETEC strains to human buccal epithelial cells and found three distinct fimbrial antigens. However, these antigens were not compared with CFA/I and CFA/II. Thomas (1985) has shown that two of the three antigens are identical to CFA/I and CFA/II respectively.

Laboratory techniques

Detection of LT

Tissue culture methods using Y1 mouse adrenal cells (Donta, Moon & Whipp, 1974) and Chinese hamster ovary (CHO) cells (Guerrant *et al.* 1974) are the standard tests for the detection of LT, although other cell lines, including Vero monkey kidney cells, are also sensitive. In these tests the tissue culture cells are exposed to bacterial culture supernatants, and when LT or cholera toxin is present the intracellular concentration of cAMP increases, leading to a morphological response which can be seen microscopically. CHO cells elongate while Y1 cells become rounded. The response is distinct from the cytotoxic effect of supernatants of some *E. coli* O26, O126 and O157 cultures on Vero cells (see EPEC, below).

Antisera to LT can be prepared in animals, and immunological techniques have been developed for the detection of LT. A passive immune haemolysis method has been described in which sheep erythrocytes sensitized with LT are lysed by exposure to antitoxin and complement. A further development of this method enables tests to be performed in media solidified with agar (Bramucci & Holmes, 1978). In this way large numbers of individual colonies of *E. coli* or *V. cholerae* can be tested for LT production and mutants can be selected for genetic studies. An enzyme-linked immunosorbent assay (ELISA) has also been described, and uses cholera antitoxin prepared in guinea-pigs and a goat anti-guinea-pig serum conjugated with alkaline phosphatase (Yolken *et al.* 1977). The discovery that the Gm1 ganglioside binds LT and cholera toxin led to the development of a

ganglioside ELISA test for the detection of these toxins. Test samples are added to microtitre wells coated with Gm1 ganglioside. Bound toxin is then detected by the addition of guinea-pig cholera antitoxin followed by enzyme-labelled goat anti-guinea-pig serum (Sack *et al.* 1980).

A solid phase radio-immunoassay (RIA) for *E. coli* LT has also been developed. In this method an LT antitoxin prepared in goats is bound to polystyrene tubes and LT is assayed by competitive inhibition of the binding of radio-iodinated LT (Ceska, Grossmuller & Effenburger, 1978). Of particular interest is a precipitin test (the Biken test) performed directly on cultures growing on special agar media. The Biken test promises to be a valuable, simple technique suitable for use in field laboratories (Honda *et al.* 1981). Staphylococcal co-agglutination (Honda *et al.* 1983; Ronnberg & Wadstrom, 1983) and latex particle agglutination tests (Finkelstein & Yang, 1983; Ito, Kuwahara & Yokota, 1983) have also been described.

Detection of ST

Surveys suggest that almost half of all enterotoxigenic *E. coli* isolates produce ST alone, while only a small number produce LT alone. Tests for ST production are therefore essential. Unfortunately the usual tests are cumbersome, time-consuming and extravagant in their use of experimental animals.

Injection of enterotoxin preparations into ligated ileal loops of rabbits, pigs, calves and dogs causes accumulation of fluid. In these tests the action of ST can be distinguished from that of LT by its relative stability to heat and by its rapidity of action. Nevertheless, these tests have not been widely used in investigations of human disease. The most widely used method is the infant mouse test described by Dean *et al.* (1972). In this test culture supernatants are injected through the abdominal wall directly into the milk-filled stomach of infant mice. After 4 h the mice are killed and the intestines examined for dilation due to accumulated fluid. The intestines are then removed and the ratio of gut weight to remaining body weight determined as an objective measure of fluid accumulation. The test works well for ST_A but not for ST_B.

The non-immunogenic nature of ST prevented the development of more convenient immunological test methods for many years, but antiserum to purified ST has now been prepared by coupling the toxin to a bovine serum albumin carrier (Frantz & Robertson, 1981; Kauffman, 1981) or by glutaraldehyde polymerization (van Wijnendaele, Dobrescu & Boon, 1982). Frantz & Robertson (1981) developed a radioimmunoassay for ST and Klipstein *et al.* (1984) have developed an ST ELISA test. Further immunological tests may become available in the future.

Genetic probes for the detection of ST and LT

Radiolabelled DNA fragments from enterotoxin genes may be used as specific probes to detect homologous DNA sequences in *E. coli* strains. In this way the genes coding for ST or LT may be detected in ETEC strains or in stool or water samples containing ETEC. The method is complicated by the existence of more than one kind of ST (see above). Nevertheless, surveys have been successfully carried out using ST probes and LT probes (Moseley *et al.*, 1980, 1982; Echeverria *et al.* 1982).

ENTERO-INVASIVE *E. COLI* (EIEC)

Epidemiology

Compared with the extensive literature on ETEC there have been few studies of EIEC, and our understanding of their epidemiology is limited. The first description of EIEC diarrhoea was in American soldiers in the Mediterranean region between 1943 and 1945 (Ewing & Gravatti, 1947). Hobbs, Thomas & Taylor (1949) reported an outbreak of gastroenteritis among school children in Britain and the epidemic organism was identified as a 'paracolon bacillus'. This strain and that of Ewing & Gravatti were later identified as *E. coli* O 124. Strains of this serogroup have since caused outbreaks in hospitals in Britain (Rowe, Gross & Allen, 1974), water-borne outbreaks in the community in Hungary (Lanyi *et al.* 1959) and a large outbreak in the United States in 1971 caused by contaminated French cheese (Marier *et al.* 1973). Outbreaks due to entero-invasive *E. coli* O 164 have also been reported. This organism was first isolated in 1946 from the faeces of a British prison inmate who developed dysentery, and outbreaks and sporadic cases were subsequently reported in Australia (Riley, 1968), Britain (Rowe, Gross & Woodroof, 1977) and Israel (Shmilovitz, Kretzer & Levy, 1974).

In most studies adults and children are reported to have been affected. Patients usually develop the symptoms of bacillary dysentery including fever and diarrhoea, with blood and mucus in the stools. The stools are frequent but of low volume in contrast to the watery stools seen in ETEC diarrhoea.

Pathogenesis

The ability of EIEC to cause dysentery-like diarrhoea has been confirmed in human volunteers by feeding experiments using strains of *E. coli* O 124, O 136 and O 143 (DuPont *et al.* 1971). Sigmoidoscopy of infected volunteers showed changes similar to those seen in mild shigella dysentery, and sigmoidoscopy of patients in the cheese-borne outbreak in the United States showed ulceration of the colon. Laboratory investigations using rabbits and guinea-pigs have shown the importance of epithelial cell invasion and confirm that the pathogenesis of EIEC diarrhoea resembles that of shigella dysentery: epithelial invasion and intracellular multiplication in the large bowel leads to inflammation and ulceration of the mucosa. Genetic studies show that in shigella and EIEC the pathogenic mechanism depends on both chromosomal and plasmid-borne genes (Hale *et al.* 1983).

Laboratory investigation

The identification of EIEC presents the laboratory with a number of problems. They are frequently less reactive in biochemical tests than other *E. coli*; they may ferment lactose late or not at all, and may be anaerogenic and non-motile. Furthermore, most of the serogroups to which EIEC commonly belong are antigenically related to various serogroups of shigella. For these reasons laboratories may mistakenly identify these strains as shigella. Such a misidentification is of little consequence clinically since EIEC diarrhoea is indistinguishable from shigella dysentery. Nevertheless, accurate identification is important for epidemiological purposes.

The correlation between serogroup and pathogenicity in the EIEC is not perfect,

and serogrouping is therefore of limited value in distinguishing EIEC from other *E. coli* strains. It has been reported that most EIEC strains fail to decarboxylate lysine and differ in this respect from most other *E. coli* strains (Silva, Toledo & Trabulsi, 1980). It is therefore possible that serogrouping and biochemical testing combined might be valuable in the recognition of EIEC; more studies are needed.

Laboratory tests are available for the detection of 'invasiveness' in EIEC and shigella, but these are not widely used in clinical laboratories. In the Sereny test a dense suspension of live organisms is instilled into the guinea-pig eye, and both EIEC and virulent strains of shigella cause an ulcerative keratoconjunctivitis, usually within three days (Sereny, 1957). Tissue culture tests have also been described using either HeLa (DuPont *et al.* 1971) or HEp-2 cells (Day, Scotland & Rowe, 1981). An outer membrane protein detectable by ELISA has been found in virulent EIEC and shigella, and an OMP-ELISA method has been proposed as a test for invasiveness (Pal *et al.* 1985).

INFANTILE ENTEROPATHOGENIC *E. COLI* (EPEC)

Epidemiology

Outbreaks of infantile enteritis were reported in hospitals and nurseries in Britain during the 1940s, but at first neither the aetiological agent nor the manner of spread of infection was recognized. Nevertheless, it was discovered that outbreaks could be controlled by the sterilization of feeding bottles, the pasteurization or sterilization of milk feeds shortly before use and the application of strict measures to control the transmission of the disease from patient to patient. Studies using serotyping eventually showed that such epidemics could be caused by strains of *E. coli* that belonged to particular O serogroups, and members of these serogroups later became known as enteropathogenic *E. coli* (EPEC). During the 1950s further outbreaks of infantile enteritis due to EPEC were reported among infants in hospitals and nurseries in Europe and North America, and during the 1960s and early 1970s a number of serious epidemics occurred in Britain (Love *et al.* 1972) and Ireland (Hone *et al.* 1973). Since 1971 few outbreaks of EPEC enteritis have been reported in Britain or the United States, but no satisfactory explanation has been put forward for this.

Although outbreaks of EPEC enteritis now appear to be uncommon in temperate areas with good standards of hygiene, it has been shown that EPEC are still a common cause of enteritis in tropical countries (Maiya *et al.* 1977) and among communities with poor standards of hygiene (Gurwith & Williams, 1977). The epidemiology of EPEC enteritis in tropical countries differs from that in Europe and North America in that although outbreaks in institutions are often reported in the tropics, sporadic cases and outbreaks occur more frequently in the community. Breast feeding has an important effect, and the peak incidence of enteritis in some countries occurs in the few months after the beginning of the weaning period. Weaning often occurs later in developing countries, and the age distribution of EPEC enteritis consequently differs from that in Europe and North America.

The importance of EPEC as a cause of enteritis in adults is difficult to assess, since most laboratories look for EPEC only in patients under 3 years of age. However, a few outbreaks have been reported. A water-borne outbreak due to *E.*

coli O111 affected adults attending a conference centre in the United States (Schroeder *et al.* 1968) and two food-borne outbreaks have been reported in Britain (Report, 1974; Vernon, 1969).

Pathogenesis

The ability of EPEC to cause diarrhoea has been confirmed by human volunteer studies (Levine *et al.* 1978), but the mechanism by which they elicit a fluid response in the human gut is unclear. They usually do not produce ST or LT, nor do they possess genes coding for the production of these enterotoxins (Gross, Scotland & Rowe, 1976; Robins-Browne *et al.* 1982). They also lack the entero-invasive capacity associated with EIEC and shigella. However, recent studies have demonstrated certain factors that may be of importance in the pathogenesis of EPEC diarrhoea and suggest that the EPEC group may need to be further subdivided.

Some strains of *E. coli* belonging to EPEC serogroups O114 and O128 do produce ST and/or LT (Scotland *et al.* 1981). These strains possess particular flagella or H antigens which are different from those found in members of the same O groups from outbreaks of infantile enteritis. These enterotoxigenic strains should therefore be considered as members of the ETEC group. Several studies have shown that EPEC strains cause accumulation of fluid in the rabbit ligated ileal loop test and, in the later study of Klipstein *et al.* (1978), extracts of EPEC strains from outbreaks of infantile enteritis were shown to cause a net efflux of water in the perfused rat gut. These studies suggest that EPEC may produce an enterotoxin that is distinct from ST and LT and is not detected by standard methods for the detection of ETEC. Konowalchuk, Speirs & Stavric (1977) showed that culture filtrates of certain *E. coli* strains had a cytotoxic effect on monolayers of Vero cells which contrasted with the cytotonic effect of LT, and this cytotoxin is now known as VT. VT differs from LT and ST and has no effect on the Y1 or CHO cell lines commonly used to detect LT, nor does it cause fluid accumulation in the infant mouse test used to detect ST. Partially purified VT does however induce some fluid accumulation in the rabbit ileal loop test. Any relationship between VT and the toxic principle demonstrated by Klipstein *et al.* (1978) has yet to be determined. In their initial study Konowalchuk, Speirs & Stavric (1977) showed VT production by 7 of 35 EPEC strains isolated from infants with diarrhoea. Three of the seven strains belonged to O group 26, and subsequently a particular association of VT production with strains of this O group has been reported. In the study of Scotland, Day & Rowe (1980), 25 strains were VT^+ amongst 253 EPEC strains of 11 O serogroups isolated from infants with diarrhoea in the United Kingdom. VT^+ strains included 20 strains belonging to serotype O26 H11, 2 of serotype O26 H- and 2 of O128 H2. O'Brien *et al.* (1982) demonstrated the production of VT by EPEC strains of other serogroups when grown in an iron-depleted medium. The significance of these findings has yet to be established.

Interest in VT has recently increased as a result of reports that certain strains of *E. coli*, especially those of serotype O157 H7, associated with outbreaks of haemorrhagic colitis in the United States and Canada produce VT (Riley *et al.* 1983; Johnson, Lior & Bezanson, 1983). Some workers have referred to these strains as enterohaemorrhagic *E. coli* (EHEC). VT^+ strains have also been associated with

cases of haemolytic-uraemic syndrome (HUS) in Canada (Karmali *et al.* 1983, 1985) and Britain (authors' unpublished results). Further studies show that VT closely resembles 'Shiga toxin', a cytotoxin produced by strains of *Shigella dysenteriae* 1 (O'Brien *et al.* 1983), and it is well documented that infections with *Sh.dysenteriae* 1 may lead to HUS (Koster *et al.* 1978). The role of Shiga toxin in the pathogenesis of bacillary dysentery remains unclear since entero-invasive shigella strains lacking the ability to produce Shiga toxin can still cause bacillary dysentery (Levine *et al.* 1973).

Post-mortem studies and intubation techniques have shown that the ability to colonize the epithelial mucosa of the small intestine is important in the pathogenesis of EPEC diarrhoea. However, in contrast to ETEC, the mechanism of this colonization is not known. Cravioto *et al.* (1979*b*) described an *in vitro* method that tested for the ability of *E. coli* to adhere to HEp-2 cells grown in tissue culture. Adhesion to HEp-2 cells was found in a significantly higher proportion of strains belonging to EPEC serogroups and isolated from outbreaks of infantile enteritis than in strains from healthy humans. The ability to adhere to HEp-2 cells was uncommon in ETEC strains even when they possessed the fimbrial colonization factors CFA/I and CFA/II. Scotland, Richmond & Rowe (1983) have shown that adhesion to HEp-2 cells is mediated by non-fimbrial adhesins, and Baldini *et al.* (1983) showed that HEp-2 adhesion correlated with the presence of a 50–70 mD plasmid. *E. coli* strains that adhere to HEp-2 cells may do so in a diffuse manner or they may form localized clusters. Nataro *et al.* (1985) have recently demonstrated by means of DNA probes that localized and diffuse adhesion by EPEC are due to at least two genetically distinct adhesins each encoded by plasmids. Several recent clinical studies have produced *in vivo* evidence to suggest that adherence to the intestinal mucosa in EPEC diarrhoea leads to local destruction of microvilli and the formation of 'pedestals' of the epithelial cell membranes at the site of EPEC adhesion (Edelman & Levine, 1983). A report in the United States described the isolation of *E. coli* O 125 from the duodenal aspirate of a 7-day-old infant with protracted diarrhoea (Ulshen & Rollo, 1980). The *E. coli* strain was found to be non-enterotoxigenic and non-invasive, but biopsy showed the organism to adhere closely to the epithelium of the small bowel. The authors proposed that this represented a new mechanisn of diarrhoea. A similar report described a strain of *E. coli* O 111 isolated from the small bowel of two infants with chronic diarrhoea (Clausen & Christie, 1982). Again the strain was non-enterotoxigenic and non-invasive. Small-bowel biopsy revealed focal adherence of the bacteria to the epithelium accompanied by inflammation of the tissue, and *in vitro* tests showed that the organism adhered to HEp-2 tissue culture cells in densely packed aggregates. The authors suggested that the inflammatory changes might result solely from the close attachment of the organisms to the intestinal epithelium. In a study of 15 infants with severe diarrhoea Rothbaum *et al.* (1982) showed that *E. coli* O 119 was present in small-intestine fluid cultures and in stools. In all the patients jejunal biopsy showed adhesion of the bacteria to mucosal cells. The organisms did not produce ST, LT or VT. A similar phenomenon has been observed in strains of *E. coli* that attach to the intestinal epithelial cells and cause diarrhoea in rabbits (Cantey & Blake, 1977). These so-called RDEC strains resemble human EPEC strains in that they do not produce ST or LT and are non-invasive. RDEC

diarrhoea in the rabbit may therefore be an animal model for diarrhoea caused by EPEC in humans.

The HEp-2 cell adhesion test has also been used to show an association between adhesive strains and traveller's diarrhoea in adults (Mathewson *et al.* 1985). These strains rarely belonged to EPEC serotypes.

It is not yet clear how frequently the production of VT and the ability to adhere to enterocytes or to HEp-2 cells in tissue culture may be found together. Cravioto *et al.* (1979*b*) studied a strain of *E. coli* O26 designated H19 and showed that it adhered to HEp-2 cells, and McNeish *et al.* (1975) showed that the same strain adhered to human foetal small intestine. Scotland *et al.* (1980) examined the same strain and showed that it also produced VT. However, in the study of Rothbaum *et al.* (1982) the adhesive *E. coli* O119 strains did not produce VT.

It therefore seems that much diversity exists among the strains previously designated as EPEC. A few strains are enterotoxigenic and best regarded as ETEC. Some strains produce VT and might tentatively be regarded as Vero toxin-producing *E. coli* (VTEC). Some strains are strongly adhesive and might be considered as entero-adherent *E. coli* (EAEC). The relationship between VTEC, EAEC and EPEC, as determined by serotype, is yet to be fully determined. Further toxins may be as yet undiscovered in VT^- EPEC strains.

Laboratory investigations

Faecal specimens from children less than 3 years old with diarrhoea are usually investigated for the presence of EPEC. Specimens are streaked on to non-selective media such as MacConkey or eosin-methylene blue agar, and several colonies are examined by slide agglutination using polyvalent antisera for the EPEC serogroups. Colonies giving positive reactions are then tested using monovalent antisera. The serogroup is confirmed by tube agglutinations using heated antigen suspensions. Strains serogrouped in this way should also be confirmed as *E. coli* by means of biochemical tests, since there is widespread sharing of antigens among the members of the Enterobacteriaceae. When outbreaks of infantile enteritis occur and EPEC are not found the assistance of a reference laboratory should be sought. Full serotyping using over 160 O antisera and over 50 H antisera may lead to the recognition of new EPEC serogroups.

While serotyping is always worthwhile when outbreaks of diarrhoea occur, the value of routine O group determination in sporadic cases is open to question since many strains of *E. coli* belonging to EPEC O groups are probably not capable of causing diarrhoea. Improvements in our understanding of the pathogenesis of EPEC diarrhoea may lead to the development of more conclusive laboratory tests in the near future.

CONCLUSIONS

Although *E. coli* was first isolated in 1885 most of the work done during the next 50 years was aimed at distinguishing *E. coli* and related organisms (termed 'coliforms') from the known pathogens such as salmonella and shigella. Goldschmidt (1933) first used serotyping to show an association between a particular *E. coli* strain and infantile enteritis, and epidemiological studies and outbreak investiga-

tions using similar methods continued for almost four decades. Studies of the pathogenic mechanisms of *E. coli* diarrhoea received an enormous stimulus in the early 1970s from the development of the suckling mouse test and tissue culture tests for enterotoxins. During the later part of the decade the idea was established that *E. coli* that cause diarrhoea might usefully be considered as belonging to three groups – EPEC, ETEC and EIEC. During the first half of the 1980s our understanding of the genetics of virulence of ETEC and EIEC has developed greatly, and studies of verotoxin and entero-adhesion have begun to explain at least some of the mysteries of EPEC. However, at the same time further complexity has been added by the discovery of VT^+ entero-haemorrhagic strains and entero-adherent strains that do not belong to accepted EPEC serogroups. Only recently has our understanding of ETEC pathogenesis reached the stage where the development of suitable vaccines may be possible, particularly oral vaccines directed against the fimbrial adhesins. By the end of the present decade such vaccines could be in widespread use for the prevention of traveller's diarrhoea. Unfortunately, the use of such vaccines among the population most at risk from ETEC diarrhoea, that is infants in the developing countries, may be more difficult. The distribution of an oral vaccine in the tropics, especially if it is a live vaccine, and its administration to an infant population present considerable problems.

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