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Infection by Verocytotoxin-Producing *Escherichia coli*†

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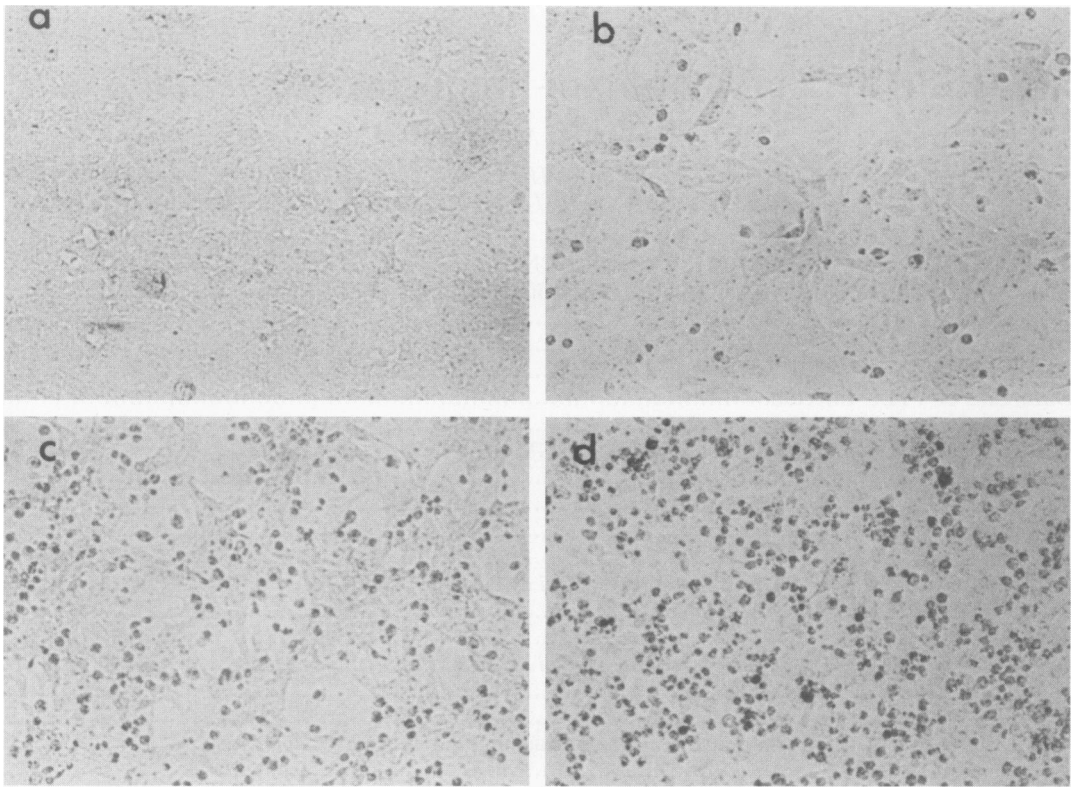

FIG. 1. Vero cell cytotoxicity on Vero cell monolayers exposed to a culture filtrate of a strain of VTEC for various periods. (a) Control, uninoculated monolayer; (b) 18-h exposure; (c) 36-h exposure; (d) 60-h exposure.

INTRODUCTION AND HISTORICAL BACKGROUND

The recognition of verocytotoxin (VT)-producing *Escherichia coli* (VTEC) as etiological agents of diarrhea represents one of the most important and exciting recent advances in the field of enteric infections. Not only do VTEC rival nontyphoidal salmonellae and *Campylobacter* spp. as the most frequent causes of diarrhea in some geographical settings (142), but a significant risk of two life-threatening complications, hemorrhagic colitis (158) and the hemolytic uremic syndrome (HUS) (80, 81), makes VTEC infection a public health problem of serious concern.

The pioneering work that led to the discovery of the *E. coli* VTs was done by Konowalchuk and colleagues during the late 1970s in Canada (92–94). While investigating the usefulness of Vero (African green monkey kidney) cells for detecting the heat-labile enterotoxin (LT) of *E. coli*, they observed that culture filtrates from some *E. coli* strains produced a profound irreversible cytopathic effect in Vero cells (Fig. 1) in contrast to the reversible cytotonic effect of LT. Culture filtrates from 10 of 136 *E. coli* strains from diverse sources produced a VT effect; 7 of the strains (serotypes O26, O128:B12, O111:B4, O18:B21, and O126:B16) were from infants with diarrhea, 1 isolate (serotype O138:K81) was from a weanling pig, and 2 VT^+ cultures (serotypes O68:H12 and O26:K60) originated from cheese. These observations led Konowalchuk and his colleagues (92–94) to speculate that VT may contribute to diarrheal disease. It should be noted that the "enterotoxic" activity of what later became known as VT had earlier been demonstrated in 1971 by Smith and Lingood (186).

Konowalchuk et al.'s findings were explored in two clinical studies in which the frequency of VTEC in stools of diarrhea patients was investigated. Wade et al. (W. G. Wade, B. T. Thom, and N. Evans, Letter, Lancet **ii**:1235, 1979) in England cultured VT^+ strains from stools of 3 of 56 children with diarrhea; each of these three patients (positive for VTEC serotype O26) had bloody diarrhea. Hardas et al. (65) in India found that 8 of 102 *E. coli* strains from patients with diarrhea were VT^+. These studies, however, failed to shed light on either the etiological significance of VTEC in diarrheal disease or the possible pathogenetic significance of VT.

The major breakthroughs occurred in 1983 with the publication of studies from the United States and Canada which linked VTEC infection to two conditions of previously unknown cause, hemorrhagic colitis (158) and HUS (81). A classic epidemiologic investigation from the Centers for Disease Control, Atlanta, Ga., (158; Morbid. Mortal. Weekly. Rep. **31**:580, 1982), linked two outbreaks of hemorrhagic colitis, a hitherto poorly understood bloody diarrheal condition, with what was then considered a "rare" *E. coli* serotype, O157:H7. Shortly thereafter, Johnson and colleagues (W. M. Johnson, H. Lior, and G. S. Bezanson, Letter, Lancet **i**:76, 1983) found that a strain of the same serotype that was implicated in an outbreak of hemorrhagic colitis in a Canadian nursing home was positive for VT. The American isolates of *E. coli* O157:H7 were subsequently confirmed by O'Brien and colleagues to be positive for a Shiga-like cytotoxin (A. D. O'Brien, T. A. Lively, M. E. Chen, S. W. Rothman, and S. B. Formal, Letter, Lancet **i**: 702, 1983). Earlier work by O'Brien's group (133) had led to the important observation that the cytotoxin (VT) from Konowalchuk's reference strain H30 was very closely related to Shiga toxin from *Shigella dysenteriae* type 1. Thus

developed the two different nomenclatures now used for the *E. coli* cytotoxins VT and Shiga-like toxin (SLT).

While the epidemiological studies on hemorrhagic colitis clearly established an association with *E. coli* O157:H7, the potential significance of VT in this condition remained uncertain. In 1983 and 1985, studies from Canada (80, 81) showed a close association between VTEC infection and the classical form of HUS, which is a leading cause of acute renal failure in childhood. HUS (42, 51, 54–56, 76) is defined by a triad of features: acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. In the classical form of the syndrome, which is by far the most common, these features develop a few days after an acute, usually bloody, diarrheal illness which shows marked similarities, clinicopathologically (156) and radiologically (5, 88, 159, 170), to hemorrhagic colitis. The association between VTEC and HUS (80, 81) was based not only on the isolation of VTEC from fecal cultures, but also on the demonstration of free VT in fecal filtrates and rising levels of VT-neutralizing antibodies in patients' sera. These findings, as well as the fact that VTEC isolates from these patients belonged to several different serotypes, in addition to O157:H7, emphasized the fact that VT production was probably of direct pathogenetic significance in HUS.

The pioneering studies described above laid the foundation and provided the stimulus for what is developing into a major area of multidisciplinary research interest in VTEC. Significant progress has been made in understanding the clinical and epidemiological features, natural history, laboratory diagnosis, and pathogenesis of VTEC infections in humans and other animals, as well as fundamental knowledge of the structure-function relationship and molecular biology of the VTs. This paper will review these developments within a broad perspective. Two other excellent review articles include one on the molecular biology of Shiga toxin and SLTs by O'Brien and Holmes (130) and another on the epidemiologic, clinical, and microbiologic features of hemorrhagic colitis by Riley (157). A summary of the first International Symposium and Workshop on VTEC Infections has been published by Edelman et al. (44).

CLINICAL AND PUBLIC HEALTH SIGNIFICANCE OF VTEC INFECTIONS

Evidence from studies of outbreaks (22, 68, 158, 186, 187, 194, 196; P. M. Griffin, S. M. Ostroff, R. V. Tauxe, et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-13; T. Itoh, et al., Annu. Rep. Tokyo Metrop. Res. Lab. Public Health **36**:16–22, 1985; V. Mai, A. Carter, L. Duncan, J. Carlson, and A. Borczyk, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-6; S. M. Ostroff, P. M. Griffin, R. V. Tauxe, J. G. Wells, K. D. Green, J. H. Lewis, P. A. Blake, and J. M. Kobayashi, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-8) and sporadic cases (80, 81, 142, 155, 174; N. Ish-Shalom, G. S. Arbus, M. A. Karmali, and M. Petric, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, HUS-11; P. I. Tarr, M. A. Neill, C. R. Clausen, D. L. Christie, P. Lehman, and R. O. Hickman. Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, HUS-2) of VTEC infection due in most cases to serotype O157:H7 indicates that the spectrum of illness includes asymptomatic infection, mild uncomplicated diarrhea, hemorrhagic colitis, HUS, and thrombotic thrombocytopenic purpura (TTP) (121; P. G. Ramsay, and M. A. Neill, Morbid. Mortal. Weekly Rep. **35**:549–551, 1986), a syndrome that is closely allied to HUS. Hemorrhagic colitis, HUS, and TTP have in the past been considered as isolated entities, although it is now clear that they span the spectrum of VTEC infection, and constitute the elements by which VTEC disease is recognized as a distinct clinicopathologic entity.

Hemorrhagic Colitis

Hemorrhagic colitis ("ischemic colitis") (157, 158) is a distinct clinical syndrome that presents typically with abdominal cramps and watery diarrhea followed by a hemorrhagic discharge resembling lower gastrointestinal bleeding. The disease is distinguished from inflammatory colitis by lack of significant fever and absence of an inflammatory exudate in the stools. In addition, in some patients a barium enema examination may reveal a characteristic radiological picture of filling defects referred to as a "thumbprinting" or "pseudotumor" appearance (30, 145, 157, 158). In a detailed review of the subject, Riley (157) has suggested that the disease was probably first recognized in 1971 (115) in five young adults with a condition referred to as "evanescent colitis." Subsequently, several sporadic cases of the syndrome, going by names such as ischemic colitis and "reversible segmental colitis," were described in the United States (87, 148, 159), Japan (168, 201), and Europe (30, 110, 200), although the etiology remained unclear.

In 1982, workers from the Centers for Disease Control investigated two outbreaks of hemorrhagic colitis in Michigan and Oregon (Morbid. Mortal. Weekly Rep. **31**:580, 1982). They identified 47 individuals with hemorrhagic colitis, using a case definition of severe abdominal pain, grossly bloody diarrhea, and the lack of evidence of infection by recognized enteric pathogens. Case control studies showed that the illness was associated with ingestion of hamburgers at outlets of a well-known fast-food restaurant chain. *E. coli* O157:H7 was recovered from the stools of about half the cases but from none of healthy controls. Apparently the same strain of *E. coli* was isolated from a beef patty from a suspected lot of meat in Michigan. This seminal study attracted widespread attention and set the stage for several additional studies, mostly in North America, to understand better the magnitude of the problem. In a large surveillance study of hemorrhagic colitis in the United States (155), 103 patients were identified as meeting the case definition of hemorrhagic colitis over a 20-month period. *E. coli* O157:H7 was identified in 28 (36%) of 76 cases in which stools were examined. In Calgary, Pai and colleagues (143) conducted a 6-month hospital-based prospective study of patients with bloody diarrhea and isolated *E. coli* O157:H7 from 19 (15%) of 125 patients; in a more recent report, the Calgary group (142) isolated this organism from 55 (40%) of 137 patients with bloody diarrhea presenting at the emergency departments of Calgary hospitals. Ratnam and March (152), in Newfoundland, recovered the organism from 7 (15%) of 47 patients with grossly bloody diarrhea. It soon began to emerge that *E. coli* O157:H7 was by no means a rare serotype as initially thought, but rather was a fairly common isolate from patients with hemorrhagic colitis and unspecified bloody diarrhea.

HUS

First described by Gasser et al. (54) in 1955 as a distinct clinical entity, HUS is defined by a triad of features: acute

renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. HUS has been reported in a variety of clinical and epidemiological settings, and several different agents, including drugs, chemicals, toxins, and microbes, have been postulated as potential causes (42, 51, 54–56, 76). The prevailing dogma for many years has been that HUS is probably a multifactorial disease, being the end result of a number of different inciting events and pathogenetic mechanisms.

By far the most common form of the syndrome is "idiopathic" or "classical" HUS which has its highest incidence in children. Classical HUS presents typically a few days after the onset of an acute diarrheal "prodromal" illness which is often bloody and shows remarkable similarities clinicopathologically (156) and radiologically (5, 88, 159, 170) to hemorrhagic colitis; some patients with hemorrhagic colitis have subclinical evidence of HUS (M. A. Neill, D. L. Christie, P. I. Tarr, and C. R. Clausen, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, HUS-6). In a variant of the classical syndrome, referred to as "atypical" HUS (86), the prodrome is nondiarrheal and may consist of upper or lower respiratory tract symptoms, fever, and vomiting. The other much less common types of HUS include a childhood form that is inherited (75) and adult forms that occur in association with pregnancy (29, 172), oral contraceptive use (13, 87), malignant hypertension (11, 105), and various chronic illnesses (42, 51).

HUS (42, 51, 54–56, 76, 208) is a leading, and in some centers the most common, cause of acute renal failure in childhood. The syndrome was at one time associated with a very high case fatality rate of about 50% (55, 56). However, improvement in the treatment of renal failure and the attendant biochemical disturbances, largely through the use of peritoneal dialysis, has substantially improved the outlook. Modern management techniques have reduced the case fatality rate to 10% or less, although up to 30% of survivors may develop long-term residual disability in the form of chronic renal failure, hypertension, or a neurological deficit (55, 56, 62, 106, 202, 206; Ish-Shalom et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987). Thus, even in the modern era HUS remains a disease with a significant mortality and morbidity.

Attempts have been made recently to estimate the incidence of HUS in North America. Rogers et al. (162) estimated the average incidence of HUS in Oregon during a 4-year period (January 1979 to December 1982) to be 0.97 and 2.65 cases per 100,000 children ≤18 and <5 years of age, respectively. In another study in Sacramento, Rogers and her colleagues (161) estimated the yearly incidence of HUS to be 0.41 case per 100,000 children ≤14 years during the study period of January 1979 and June 1982. The occurrence of an epidemic of HUS in Sacramento in November 1982 raised the yearly incidence to 11.2 cases per 100,000 children ≤14 years of age. Tarr and Hickman (195), in a study in King County, Wash., during a 10-year period (1971 to 1980 inclusive) estimated the yearly incidence of HUS to be 1.16 and 3.02 cases per 100,000 children <15 years of age and <3 years of age, respectively.

In South Africa (86), idiopathic HUS appears to be substantially more common in white than in black children. In England (202), the syndrome appears to be more common in rural than in urban areas. The reasons for such regional or sociocultural differences in prevalence remain to be established.

The occurrence of outbreaks of HUS (55, 56, 112, 188) lent support to a long-held view that the etiology of the syndrome had an infectious basis. Many microbes have been implicated as etiologic agents in HUS, including *Shigella* spp., particularly, *Shigella dysenteriae* type 1 (18, 23, 61, 95, 151); *Salmonella typhi* (3); *Campylobacter jejuni* (25, 36); *Yersinia pseudotuberculosis* (150); *Streptococcus pneumoniae* (P. J. Klein, M. Bulla, R. A. Newman, P. Muller, G. Uhlenbruck, H. E. Schaefer, G. Kruger, and R. Fisher, Letter, Lancet **ii:** 1024–1025, 1977; G. Lenz, U. Goes, D. Baron, H. Jonger, W. Heller, U. Sugg, and R. Lissner, Letter, Lancet **i:**292–293, 1984), rickettsia-like organisms referred to as microtatobiotes (113), and viruses such as coxsackievirus (59, 153), echovirus (139; R. P. B. Larke, J. K. Preiksaitis, and R. D. Devine, Abstr. Conj. Meet. Infect. Dis. Can. Public Health Assoc. 1979, VC-5), influenza virus (26), Epstein-Barr virus (178), and a Tacaribe group virus (Portillo) (55, 56). These reports fuelled the hypothesis that classical HUS was the result of many different inciting events, but little progress was made in consolidating this multiple-caused concept.

In 1980, my colleagues and I in Toronto became interested in the possible role of VTEC in HUS following the isolation of a VT^+ *E. coli* strain from the bowel of a fatal case. A search for VTEC was thus initiated in new cases of HUS by testing about 5 to 10 *E. coli* colonies from primary stool cultures for VT production. Initial results were negative, and this prompted us to examine fecal filtrates for the presence of VT activity. Astonishingly, fecal VT (FVT) was detected in a number of cases that were negative for VTEC by culture, although a VT^+ *E. coli* strain was subsequently recovered from the stools of some of the cases when a much larger number of *E. coli* colonies were tested. A preliminary report of our findings (81) was followed up by the results of a prospective controlled study (80) involving 40 patients with classical or idiopathic HUS from Ontario and Quebec. In the latter study, fecal VTEC belonging to at least six different O serogroups (O26, O111, O113, O121, O145, and O157) or FVT or both were detected in 24 (60%) of the cases, but in none of age-, sex-, and season-matched controls. Of 15 cases with microbiological evidence of VTEC infection (who provided acute and convalescent sera), 10 showed a fourfold or greater rise in VT-neutralizing antibody titer. However, six additional patients who were negative for VTEC and FVT also showed significant serological responses, giving a total of 30 (75%) who had evidence of VTEC infection by one or more criteria. The results suggested not only that there was a close relationship between HUS and VTEC infection, but also that VT was of direct pathogenetic significance because (i) VT production was a common factor in a serotypically heterogeneous group of VTEC strains, (ii) in vivo VT production (FVT activity) was demonstrated, and (iii) patients demonstrated significant serological responses to VT.

Our findings have now been confirmed in sporadic cases of HUS by other investigators (60, 126, 128, 196) as well as in outbreaks of VTEC-associated diarrhea and hemorrhagic colitis in which HUS occurred as a complication in one or more cases (Table 1). In one outbreak of *E. coli* O157:H7-associated diarrhea in a day-care center (187), 3 (8.3%) of 36 symptomatic children developed HUS; in another outbreak affecting a group of kindergarten children after a farm trip (43; Mai et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987), 3 (7%) of 42 symptomatic children developed the syndrome. In a family outbreak, all five (100%) affected children developed HUS (N. Ish-Shalom, G. S. Arbus, and M. A. Karmali, Pediatr. Res. **20:**228A, 1986). The most devastating outbreak of *E. coli* O157:H7 reported to date occurred in a Canadian nursing home (22) in which 12 (22%) of 55 affected residents

TABLE 1. Outbreaks of VTEC O157:H7 infection in Canada and the United States[a]

Date (reference)	Location	No. of cases	No. with HUS	No. of deaths	Setting	Source[b]
Feb.–Mar. 1982 (158)	Oregon[c]	26			Community	Hamburger
May–June 1982 (158)	Michigan[c]	21			Community	Hamburger
Nov. 1982 (68)	Ontario	31			Nursing home	Ground beef
May 1983 (68)	Labrador	19			Community	
Aug. 1983 (68)	Alberta	4	2		Family	Hamburger
Mar. 1984 (68)	Ontario	7			Nursing home	
Sept. 1984 (166)	Nebraska	34		4	Nursing home	Hamburger
Sept.–Oct. 1984 (186)	North Carolina	36	3		Day-care center	
Aug. 1985 (68)	Ontario[d]	5	5		Family	
Sept. 1985 (22)	Ontario[e]	73	12	17	Nursing home	Sandwich
Apr. 1986 (43)	Ontario	30	3		School trip	Raw milk
June 1986 (68)	Alberta	8	2		Nursing home	
June 1986 (68)	Ontario	2			Nursing home	
July 1986 (68)	British Columbia	20			Community	
Oct.–Nov. 1986 (196)[f]	Washington	37	3	2	Restaurant	Ground beef
Dec. 1986 (68)	Ontario	4			? Restaurant	
June 1987 (68)	Alberta	15		2	Nursing home	Ground beef
July 1987 (68)	Ontario	9			Nursing home	
July 1987 (68)	Ontario	6			Girls' camp	
Aug. 1987 (68)	Ontario	9		2	Nursing home	

[a] This table is based in design and content on an excellent summary of VTEC 0157:H7-associated outbreaks in Canada, 1982 to 1987, by Hockin and Lior (68) (reprinted by permission). The table has been suitably expanded to include U.S. data.

[b] Sources highly suspected or confirmed.

[c] Case definition included only hemorrhagic colitis.

[d] Ish-Shakom et al., Pediatr. Res. **20**:228A, 1986.

[e] The 73 affected cases included 55 (32.5%) of 169 elderly residents and 18 (13%) of 137 staff members. All cases of HUS and all fatalities occurred in the residents.

[f] Ostroff et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-8.

developed HUS which ended fatally in 11 cases. The latter outbreak emphasized that HUS, formerly thought to be a disease mostly of young children, should also be considered as a diagnosis in the elderly.

Unusual clinical manifestations have recently been reported in patients with *E. coli* O157:H7-associated HUS. Gransden et al. (W. R. Gransden, M. A. S. Damm, J. D. Anderson, J. E. Carter, and H. Lior, Letter, Lancet **ii**:150, 1985) reported one patient, a 2.5-year-old female, with hemorrhagic cystitis and a urine culture positive for *E. coli* O157:H7 and another patient, a 10-month-old boy, with an associated balanitis. Vickers et al. (D. Vickers, K. Morris, M. G. Coulthard, and E. J. Eastham, Letter, Lancet **i**:998, 1988) reported the presence of unusual anal signs in three young female children with *E. coli* O157:H7-associated HUS; the features consisted of reddening of the anal and perianal skin, gross anal dilatation, and, in two cases, bluish discoloration of the anal mucosa and venous swelling.

The association between VTEC infection and HUS has been recognized essentially with the classical form of the syndrome. The possible relationship of this infection to other forms of the syndrome is unclear, although Steele and colleagues (B. T. Steele, J. Goldie, I. Alexopoulou, and A. Shimizu, Letter, Lancet **i**:511, 1984) isolated a VTEC strain (serotype O6:H12) from the stools of a patient with postpartum HUS that was not preceded by a diarrheal illness; Stenger et al. (189) isolated a VTEC strain of serotype O111 from a young woman on oral contraceptives; oral contraceptive usage is thought to be a risk factor in the development of HUS (13, 87).

While HUS is now recognized as a complication in outbreaks of VTEC infection, controversy still exists as to the frequency of VTEC infection in sporadic cases of classical HUS. Recent findings from our laboratory (Ish-Shalom et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987) showed evidence of VTEC infection in 45 (88%) of 51 HUS cases. VTEC were isolated from only 23 (51%) of the 45 patients; the remainder were positive only for FVT. Interestingly, in five patients who were negative for VTEC but positive for FVT, VTEC were isolated from symptomatic family contacts. The O:H serotype distribution of the 28 VTEC isolates (23 from index cases and 5 from symptomatic family contacts) was 1 isolate each of serotypes O2:H5, O26:H11, O91:H21, O111:H8, O113:H21, O117:H4, O121:H19, and O145:H-, 4 isolates of serotype O111:H-, and 14 isolates of serotype O157:H7; two isolates were untypable. Thus, O157:H7 was clearly the most common serotype, accounting for 50% of VTEC isolates from our cases of HUS; the remaining 50% of the VTEC isolates were distributed among nine different OH serotypes.

In a prospective 1-year study of HUS in the northwest United States, Neill et al. (126) cultured *E. coli* O157:H7 from the stools of seven (58%) patients with classical HUS. These workers, however, did not examine their cases for FVT or for other VTEC serotypes.

In the United Kingdom (174), studies of patients with HUS were conducted by using multiple diagnostic approaches, including culture of VTEC, examination of FVT, and the use of colony hybridization with VT-specific deoxyribonucleic acid (DNA) probes. Evidence of VTEC was found in 22 (33%) of 66 cases. VTEC strains isolated from the feces of 19 patients consisted of 14 strains of serotype O157:H7 and 1 strain each of types O157:H-, O26:H11, O104:H2, O153:H25, and O163:H19.

The differences in frequency of VTEC infection in HUS cases in various laboratories must be interpreted with caution because the diagnosis of this infection is still in a developmental stage and optimal methods have yet to be defined and standardized. In addition, stools from HUS

patients may not be received in laboratories until several days after the onset of the initial diarrheal illness. For example, in a prospective study on HUS cases from our laboratory (80), the mean interval between the onset of the prodromal diarrheal illness and the diagnosis of HUS was 6.9 ± 3.3 days (range, 2 to 14 days). The first stool for examination from the 40 patients was received in a mean of 10.6 ± 5.9 (range, 3 to 28) days after the onset of the diarrheal illness. Factors which diminish the likelihood of diagnosing VTEC infection include a long interval between the onset of illness and examination of feces, delays in specimen transport, prolonged storage at either 4°C or freezer temperatures (−20 and −70°C), and repeated freezing and thawing of stools.

A fundamental principle of the clinical science of infectious diseases is that a unique relationship exists between a microbe and a specific disease such that the microbial etiology can often be predicted on the basis of the presenting clinical or clinicopathological features. Whether this principle applies as a general rule to VTEC infection and classical HUS remains to be established.

TTP

First described in 1924 (122), TTP closely resembles HUS in its clinicopathological features, but differs in that neurological signs and fever are more prominent in TTP and the peak age incidence is in the third decade. In a review of 271 cases, Amorosi and Ultmann (2) noted a rapidly progressive course, with 75% of patients dying within 90 days, and a pentad of clinical features: fever, thrombocytopenic purpura, microangiopathic hemolytic anemia, neurological manifestations which were often remittent, and renal dysfunction. Modern management techniques, in particular, plasmapheresis, have substantially improved the outlook (19). Most cases of TTP present without an antecedent illness, whereas a prodromal diarrheal illness is an essential feature of classical HUS.

Recently, however, two cases diagnosed as TTP (121; Ramsay and Neill, Morbid. Mortal. Weekly Rep. **35**:549–551, 1986) have been associated with *E. coli* O157:H7 infection. Both cases differed from the usual form of TTP in that the patients had an antecedent bloody diarrheal illness, and the disease therefore resembled classical HUS.

Outbreaks of VTEC Infection

Following the description of the initial outbreaks of *E. coli* O157:H7-associated hemorrhagic colitis in Oregon and Michigan (158), numerous additional outbreaks have been reported (Table 1), and these have provided new insights into the natural history and epidemiology of VTEC infection. It became clear that the clinical presentation of VTEC O157:H7 infection was not confined to hemorrhagic colitis, but spanned a spectrum ranging from asymptomatic infection, mild uncomplicated diarrhea, hemorrhagic colitis, HUS, and TTP to death. Second, many of the outbreaks were suspected or confirmed to be foodborne (Table 1), the foods being almost entirely of bovine origin. These observations helped to focus studies on possible sources and natural reservoirs of VTEC. Third, much has been learned about the incubation period, which in the case of VTEC infection is longer than the usual 12- to 36-h period associated with salmonellosis (6) or the typical 3- to 5-day incubation period of campylobacteriosis (6). The mean incubation periods in the original community outbreaks of VTEC O157:H7 infection in Michigan and Oregon were 3.8 and 3.9 days, respectively, but were significantly longer in subsequently reported outbreaks: 8 days in a nursing home outbreak in Nebraska (166), 5.7 days (range, 4 to 9 days) for residents in an Ontario nursing home outbreak (22), and 6.8 days (range, 1 to 14 days) in an outbreak that affected Ontario kindergarten children (43: Mai et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987). The reasons for the unusually long incubation period of VTEC infection have yet to be elucidated.

Of the 20 outbreaks summarized in Table 1, 9 (45%) were in nursing homes, 3 (15%) were among institutionalized children (day-care center, girls' camp, and kindergarten children on a farm trip), and the remainder were in the family and community setting. This pattern of outbreaks is similar to that associated with salmonellosis (6).

Outbreaks have also provided an appreciation of the frequency of complications (HUS or death or both) in VTEC infection (Table 1). Variability in the frequency of complications may be a function of several host and parasite factors including age, underlying condition (e.g., gastrectomy), previous antibiotic treatment (22), presence or absence of specific antitoxic immunity, and the inoculum size of the organism. Other potential host factors to be investigated include the presence or absence of specific receptors on target cells and blood group status.

Most outbreaks of VTEC infection have been associated with serotype O157:H7. While this may reflect a true predominance of this serotype, the paucity of reported outbreaks associated with other serotypes is also likely due to the fact that they are less easy to isolate than *E. coli* O157:H7. Outbreak of VTEC serotype O145:H- (Itoh et al., Annu. Rep. Tokyo Metrop. Res. Lab. Public Health **36**:16–22, 1985) and O111:H- (194) infection have been reported in Japan. Studies currently under way in our institution (unpublished data) of VTEC infection in family members of index cases of VTEC-associated HUS have revealed family outbreaks associated with several non-O157:H7 VTEC serotypes, including O111:H-, O117:H4, and O121:H19. A better appreciation of the epidemic potential of these VTEC serotypes will become apparent as methods for detecting them become more widely used.

Sporadic Cases of VTEC Infection

Many studies of sporadic VTEC infection have addressed either hemorrhagic colitis or HUS, but very few have investigated the role or frequency of this infection in a wide spectrum of diarrheal illness. In a major 2-year prospective study of VTEC infection among patients with diarrhea presenting at three hospitals in Calgary, Pai et al. (142) found VTEC to be the most commonly isolated enteric bacterial pathogen. Among 5,414 patients from whom stools were submitted, VTEC were recovered from 166 (3.1%) patients; *Salmonella* spp., from 145 (2.7%); *Campylobacter jejuni/coli* from 108 (2.0%); *Aeromonas* spp., from 71 (1.3%); *Shigella* spp., from 26 (0.5%); *Yersinia enterocolitica*, from 12 (0.22%); and enteropathogenic *E. coli* serotypes, from 8 (0.15%) cases. The vast majority of VTEC patients had bloody diarrhea, which probably was the major reason for seeking medical attention since the investigators identified many symptomatic, culture-positive family contacts who had nonbloody diarrhea. The relative age-specific incidence was highest in young children and the elderly, suggesting either that the infection is more severe at the extremes of age (thus leading to hospitalization) or that children and the

elderly are more susceptible to VTEC infection. Of the 166 patients with VTEC infection, 130 (78%) had serotype O157:H7, 29 had other VTEC serotypes, and 7 had both serotype O157:H7 and a second VTEC serotype. Bloody diarrhea was a feature in the vast majority of cases associated with VTEC serotype O157:H7 as well as non-O157:H7 serotypes. Serotypes (O157:H-, O111:H-, and O26:H11) other than O157:H7 have also been recovered from cases of hemorrhagic colitis in the United States (10).

Two other incidence studies have investigated the relative frequency of *E. coli* O157:H7 in fecal specimens submitted to diagnostic laboratories. Cahoon and Thompson (20) examined the relative frequencies of enteric bacterial pathogens in 7,252 stools submitted for investigation to two regional public health laboratories in Ontario from April to September 1986. *Campylobacter* spp. were isolated from 4.1%; *Salmonella* spp., from 4.0%; *E. coli* O157:H7, from 0.7%; *Y. enterocolitica*, from 0.5%; and *Shigella* spp., from 0.1% of samples. In a study of 2,552 stool samples from adults and children presenting at a large Chicago hospital, Harris and colleagues (66) isolated *E. coli* O157:H7 from only two patients.

VTEC have been isolated from infants with diarrhea in Mexico (A. Cravioto, V. Vazquez, A. Soria, and Z. M. Ort, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-3) and in Brazil (L. G. Giugliano and R. Giugliano, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-12). Giugliano and Giugliano in Brazil (Abstr. Int. Symp. Workshop on Verocytotoxin-producing *Escherichia coli* infections 1987) isolated VTEC significantly more frequently from diarrheic infants (<1 year of age) than from age-matched controls. However, in older children, the frequency of VTEC in controls was higher than in infected patients, a situation not dissimilar to that for campylobacteriosis in developing countries (9). The relative contribution of VTEC to the diarrhea problem in the third world requires considerably more study. Sack (167) has drawn attention to the fact that enteric infections, such as salmonellosis, that are transmitted largely through processed food are not major problems in developing countries. It remains to be established whether the frequency of VTEC in the third world agrees with this pattern.

CHARACTERISTICS AND MOLECULAR BIOLOGY OF VTs

Emerging evidence indicates that the *E. coli* VTs constitute a family of several related cytotoxins. At least two VTs, VT1 and VT2, are known to be associated with human disease (185, 192; M. A. Karmali, M. Petric, S. Louie, and R. Cheung, Letter, Lancet i:164, 1986; S. M. Scotland, H. R. Smith, and B. Rowe, Letter, Lancet ii:885–886, 1985). The prototype cytotoxin VT1 (SLT-I), was originally identified in strain H30 by Konowalchuk and colleagues (94) in 1977, although its enterotoxic activity in rabbit ileal loops had been recognized in 1971 by Smith and Lingood (186) in another *E. coli* strain, H19. VT was later shown to be closely related to Shiga toxin by O'Brien et al. in 1982 (133). First described at the turn of the century (33), Shiga toxin has been recognized as one of the most potent bacterial toxins that is known for eucaryotic cells (209). Much of our understanding about the *E. coli* VTs comes from information that has accumulated over the years on Shiga toxin (48, 130, 207); hence, it is instructive to summarize some of its pertinent features.

Shiga Toxin

Like several other bacterial toxins such as diphtheria, pertussis, cholera, *E. coli* LT, and *Pseudomonas* exotoxin A, Shiga toxin is a subunit toxin made up of an A (active) subunit and several B (binding) subunits (114). The general mode of action of such toxins involves binding to a specific receptor on the cell surface via the B subunits and then internalization of the A subunit, which interrupts cell function through interaction with specific components of the subcellular machinery.

Although past estimates of the molecular weight (MW) of Shiga holotoxin have ranged from 58,000 to 70,000 (14, 39, 131, 136, 137, 216), recent data based on subunit MW, subunit stoichiometry, and nucleotide sequence analysis of cloned Shiga toxin genes suggest that the true MW of Shiga toxin is probably about 70,000, with an A subunit of MW $\simeq$32,000 and five copies of a B subunit of MW $\simeq$7,600 (39, 176, 190). The genetic basis of Shiga toxin production is not known. Whereas it has been established that, in *E. coli*, VT1 and VT2 are encoded for by temperate bacteriophages, Strockbine et al. (190) were unable to correlate Shiga toxin production with the presence of toxin-converting phages in a strain of *S. dysenteriae* type 1. Timmis et al. (198) have localized the Shiga toxin gene on the *S. dysenteriae* type 1 chromosome.

Shiga toxin is cytopathic for a restricted number of cultured cell lines in vitro (45, 130, 209), is lethal to various laboratory animals (12, 24, 69, 130), and induces fluid accumulation in rabbit ileal loops (84). Cell lines susceptible to Shiga toxin include HeLa, Vero, Daudi (human B lymphoma), KB, human liver, and human foreskin fibroblast, the 50% cytotoxicity dose for a number of these cell lines being of the order of 1 to 2.5 pg (14, 39, 131, 136, 137); cell lines resistant to the cytopathic action of the toxin include WI-38, Henle 407 (human embryonic intestine), Chinese hamster ovary, L, BHK, and human melanoma cells, as well as several others of human neoplastic origin (45, 58, 130, 209).

In a systematic study of the effects of parenterally administered, partially purified Shiga toxin in laboratory animals, Cavanagh et al. (24), in 1956, observed that rabbits were more susceptible to the lethal action of the toxin than other animals that were studied. Monkeys, hamsters, mice, rats, and guinea pigs were 5, 40, 700, 5,000, and 10,000 times more resistant, respectively, to the lethal action of the toxin per unit weight than rabbits. O'Brien and LaVeck (131), using highly purified Shiga toxin, calculated the 50% lethal dose for mice (20-g body weight) to be 0.1 μg, equivalent to a 50% lethal dose of 5 g/kg of body weight. In addition to its lethal action in mice and rabbits, Shiga toxin also causes paralysis in these animals, which led to Shiga toxin being referred to as a "neurotoxin." Howard (69), Bridgewater et al. (12), and Cavanagh et al. (24) considered the term neurotoxin to be a misnomer because their work suggested that the paralytic effect was secondary to vascular damage (due to a proposed action of the toxin on endothelial cells) rather than a result of primary nerve toxicity as in the case of botulinum and tetanus toxins.

Shiga toxin has been known for over a decade to cause fluid accumulation in the rabbit ileal loop (84), supporting the hypothesis that a similar action in the human bowel might be responsible for the early water diarrheal phase of *Shigella* dysentery. However, no direct evidence to support this concept has been found in humans. The toxin does not appear to cause fluid secretion through the activation of

either cyclic adenosine 3′,5′-monophosphate or cyclic guanosine 3′,5′-monophosphate in a manner analogous to cholera toxin or the *E. coli* enterotoxins (130). O'Brien and Holmes (130) and Keenan et al. (82) have argued that the fluid accumulation might be the result of inhibition of water absorption by mature ileal absorptive cells due to their destruction by the direct action of Shiga toxin. Several workers have proposed that all biological activities associated with Shiga toxin (lethal, paralytic, enterotoxic, and cytotoxic) are due to the action of a single molecule (14, 39, 46, 132).

Evidence is accumulating that the action of Shiga toxin on eucaryotic cells is mediated via a specific receptor. Keusch and colleagues (85) have suggested that Shiga toxin binds in vitro to glycoproteins containing terminal β(1 → 4)-linked *N*-acetyl-D-glucosamine and have proposed that this may be the functional receptor for the toxin. However, Lindberg and colleagues (102, 103; J. E. Brown, K. A. Karlsson, A. Lindberg, N. Stromberg, and J. Thurin, Proc. 7th Int. Symp. Glycoconjugates 1983, p. 678), using a ^{125}I-labeled Shiga toxin-binding assay, were unable to confirm this but found that the toxin binds to glycolipids containing a terminal disaccharide Gal-α(1 → 4)-Gal, including galabiosyl ceramide and globotriosyl ceramide (Gb_3). They consider the latter to be the functional receptor for toxin-induced cytopathology in HeLa cells. Keusch's group (53, 72, 116) have now confirmed that Shiga toxin binds to Gb_3 isolated from adult rabbit jejunal mucosal cell membranes and have suggested that this glycolipid is the functional receptor for the toxin-induced fluid accumulation in ligated rabbit small bowel loops. However, they maintain that a glycoprotein (β(1 → 4)-linked *N*-acetyl-D-glucosamine) serves as the functional receptor for cytotoxicity in HeLa cells. They argue that there may be distinctive binding sites on different tissues for the toxin B subunit that result in diverse effects and that the B subunit has multiple binding domains that recognize these distinctive receptors.

Following receptor binding, the toxin is internalized by a specific receptor-mediated endocytic event (47, 85, 130, 138), although the mechanism for this is unclear. The active component of the holotoxin is the A subunit, which after it has been proteolytically nicked and reduced to the A1 fragment (136, 137), inhibits protein synthesis through the catalytic inactivation of the 60S ribosomal subunit (15–17, 154, 197). The precise mechanism involves inhibition of elongation factor 1-dependent aminoacyl-transfer ribonucleic acid binding to the eucaryotic ribosome (15, 135). This is achieved through ribonucleic acid *N*-glycosidase activity which cleaves the *N*-glycoside bond in the adenosine residue at position 4324 in 28S ribosomal ribonucleic acid of the 60S ribosomal subunit (K. Igarashi, T. Ogasawara, K. Ito, Y. Endo, K. Tsurugi, T. Yutsudo, N. Nakabayashi, and Y. Takeda, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, STF-1).

VTs

Konowalchuk and colleagues first described at least three distinct *E. coli* VTs (92–94), two of them being from human strains and the third from a porcine strain. Human isolates of VTEC are now known to produce either one or both of at least two antigenically distinct, bacteriophage-mediated VTs referred to as VT1 (SLT-I) and VT2 (SLT-II) (185, 192; Karmali et al., Lancet **i**:164, 1986; Scotland et al., Lancet **ii**: 885–886, 1985; H. R. Smith, N. P. Day, S. M. Scotland, R. J. Gross, and B. Rowe, Letter, Lancet **i**:1242–1243, 1984). Another VT from porcine *E. coli* strains associated with edema disease in pigs has recently been shown to be a variant of SLT-II and its production is not phage mediated (109; C. L. Gyles, C. Mackenzie, S. A. De Grandis, and J. L. Brunton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D169, p. 99); D. L. Weinstein, L. R. M. Marques, R. K. Holmes, and A. D. O'Brien, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, GEN-7). Bezanson and Johnson have reported that VT produced by an *E. coli* strain of serotype O113 was plasmid mediated (G. S. Bezanson and W. M. Johnson, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, GEN-8).

The phage-specified structural genes for both VT1 and VT2 have been cloned and characterized with respect to nucleotide and amino acid sequences (21, 35, 70, 73, 74, 97, 127, 134, 176, 192, 214, 215; M. P. Jackson, N. Strockbine, J. W. Newland, et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, GEN-2; S. M. Scotland, H. R. Smith, G. A. Willshaw, and B. Rowe, Letter, Lancet **ii**:216, 1983). The structural genes of the two toxins share 58% overall nucleotide and 56% projected amino acid sequence homologies (73, 74; Jackson et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987). Both toxins have a similar subunit structure (67, 73, 74, 130; Jackson et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987), bind to the same glycolipid receptor, Gb_3 (211; J. E. Brown, R. J. Neill, A. D. O'Brien, and A. A. Lindberg, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, STF-3), and inhibit protein synthesis by the same mechanism as Shiga toxin (71; Igarashi et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987). However, they fail to cross-neutralize and show differences in biological activities in tissue culture and animal models (67, 192).

VT1 (SLT-I)

First described by Konowalchuk et al. (92–94) and partially purified and characterized by them, the prototype *E. coli* cytotoxin VT1 (from strain H30) was subsequently studies in detail by O'Brien and LaVeck (131). These investigators found VT1 to have a subunit structure identical to that of Shiga toxin. Both holotoxins had identical isoelectric points, the same relative heat stabilities, and comparable biological activities, namely, cytotoxicity, lethal and paralytic action on mice, and enterotoxicity in rabbit ileal loops. Both showed complete cross-neutralization. However, the two toxins had apparently different MWs as determined by sucrose gradient analysis, gel filtration, and cross-linking experiments. The MWs of Shiga and H30 holotoxins were estimated to be 58,000 and 48,000, respectively. Furthermore, Shiga toxin was fivefold less active than H30 toxin in a rabbit ileal loop assay, but had a substantially greater lethal action on mice than the H30 toxin. As pointed out above, the true MW of Shiga toxin is probably ≈70,000, with an A subunit of MW ≈ 32,000 and five copies of a B subunit of MW ≈ 7,700. The ratio of B to A subunits in the H30 toxin is not known. The amino acid composition and sequence of the A and B subunits of the two toxins have been studied and have been shown to be similar (73, 74, 190; Jackson et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987), though not identical. It remains to be established whether there are indeed subtle differences in the biological activities of the two toxins and,

if so, whether they can be accounted for by differences either in minor variations in amino acid composition and sequence or in the B/A subunit ratio.

Lingwood et al. have shown that VT1, like Shiga toxin, binds specifically to the glycolipid Gb_3 and also that this substance is the major VT-binding glycolipid of Vero cells used to assay the toxin (104; C. A. Lingwood, M. Petric, J. Brunton, A. Cohen, and M. A. Karmali, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, STF-2). Cohen et al. (32) have correlated VT-induced cytopathology in the human Burkitt lymphoma cell line (Daudi) with the presence of VT-binding glycolipids (Gb_3 and galabiosyl ceramide) on its surface. A mutant selected from Daudi cells for VT resistance was found to be deficient in these glycolipids and failed to bind VT, suggesting that these substances are functional receptors for VT action in Daudi cells.

VT2 and SLT-II

Strockbine et al. (192) compared the biological activities of two phage-encoded toxins elaborated by *E. coli* O157:H7 strain 933. The two toxins, SLT-I (encoded for by phage 933J) and SLT-II (encoded for by phage 933W), were cytotoxic for the same cell lines (Vero and HeLa), caused paralysis and death in mice, and caused fluid accumulation in rabbit ileal loops. Antisera to the two toxins neutralized the activity of the homologous toxin in HeLa cells but showed no cross-neutralization.

Head et al. (67) have recently purified VT2 from strain E32511 (*E. coli* O157:H-) which has been characterized by Scotland et al. (Lancet **ii**:885–886, 1985) and Willshaw et al. (215) and is known to produce only a single toxin, VT2. VT2 from strain E32511 is presumed to be the same or similar to the 933W toxin (SLT-II), although this has yet to be confirmed. VT2 was purified by using a modification of a method used previously for purifying VT1 (147). The toxin was found to have a subunit structure similar to that of VT1, the main difference being a slightly larger size for the A subunit of VT2 than that elucidated for VT1. Jackson et al. (73, 74; Abstr. Int. Symp. Workship Verocytotoxin-Producing *Escherichia coli* Infections 1987), have predicted from genetic studies that the SLT-II A subunit is slightly larger than the SLT-I A subunit. Head et al., however, found that VT1 and VT2 differed in their biological activities (67). On a weight basis, VT2 was 1,000-fold less active on Vero cells than VT1, in keeping with the biological activity reported for SLT-II (130); furthermore, it had a 100-fold-higher 50% lethal dose for rabbits than VT1. The most striking difference (67), however, was that injected VT2 produced overt hemorrhagic cecitis in rabbits in contrast to VT1, which produces only mild nonbloody diarrhea. Barrett et al. (T. J. Barrett, M. E. Potter, and I. K. Wachsmuth, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, B87, p. 44) were unable to demonstrate such an effect with a preparation of SLT-II purified from a different strain. VT2 purified by Head et al. (67) and SLT-II purified by O'Brien's group have been shown to bind to the glycolipid Gb_3 (211; Brown et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987).

A subunit toxin corresponding to the predicted size of SLT-II (VT2) has also been purified by Yutsudo et al. (T. Yutsudo, N. Nakabayashi, T. Hirayama, and Y. Takeda, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, STF-11) and Downes et al. (40), from strains of *E. coli* O157:H7. The pIs of the latter two toxins were 4.1 and 5.2, respectively, in contrast to the VT2 isolated by Head et al. (67), which had a pI of 6.5. These differences could reflect slightly different toxins, especially since different parent strains were used. Kai et al. (A. Kai, T. Itoh, S. Yamada, Y. Kudoh, and M. Ohashi, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, STF-4) have isolated a non-VT1 toxin from a strain of *E. coli* serotype O145:H-. This toxin, which had a pI of 4.0, was not neutralized by anti-Shiga toxin, but reacted immunologically to anti-SLT-II from a strain of *E. coli* O157:H7.

Padhye et al. (140, 141) have purified a non-VT1 (SLT-I) cytotoxin from *E. coli* O157:H7 strain 932, isolated from a case of hemorrhagic colitis and reported by Marques et al. (108) to produce both SLT-I and SLT-II. The toxin was not neutralized by anti-Shiga toxin and is thus presumed to be SLT-II. It also differed from SLT-I in that it was shown not to have a subunit structure, which makes it different from the toxins (SLT-I or VT1) reported by Jackson et al. (73; Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987), Yutsudo et al. (Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987), Downes et al. (40), and Head et al. (67). Padhye's toxin, however, had potent biological activity in mice. When administered to mice intraperitoneally, it produced colonic hemorrhage which, the authors speculate, may be the counterpart of hemorrhagic colitis in humans.

Edema Disease Principle (VT Elaborated by *E. coli* of Porcine Origin)

Edema disease (31, 49, 177, 199), first described by Shanks (177) in 1932, is a usually fatal condition of weanling pigs characterized by anorexia, edema of the eyelids, and neurological abnormalities consisting of incoordination and paralysis. The characteristic finding seen at autopsy is edema affecting the eyelids and the stomach and also the bowel and other anatomic sites. The disease is associated with bowel colonization by specific *E. coli* serogroups, notably, O138, O139, and O141. It has long been postulated that the disease results from a toxemia (199), and the disease can be reproduced experimentally by injection of extracts of *E. coli* strains belonging to the associated serogroups (31, 49). The presence of a Vero cell cytotoxin in a porcine strain (strain E57, serogroup O138) was first reported by Konowalchuk et al. (94), an observation subsequently confirmed in other edema disease strains by Dobrescu (37).

VT from edema disease strains, also referred to as SLT-II variant (SLT-IIV) by Marques et al. (109), differs from VT1 and VT2 in that it is inactive on HeLa cells, but active on Y-1 adrenal cells (8). However, the cytotoxicity of VT from edema disease strains can be neutralized by antiserum to VT2 (109). The genes specifying VT from edema disease strains have been cloned and shown to hybridize with genes specifying VT2 (Gyles et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988) and Weinstein et al. (212; Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* infections 1987).

NOMENCLATURE AND DEFINITIONS

The terms verocytotoxins (VT) and Shiga-like toxins (SLT) are synonymous. *E. coli* strains that produce these toxins have been referred to as VT-producing *E. coli* (VTEC), SLT-producing *E. coli*, and enterohemorrhagic *E. coli* (EHEC). The term VTEC refers to all *E. coli* strains that

produce VT in culture supernatants (92–94). The term EHEC refers to strains that have the same clinical, epidemiological, and pathogenetic features associated with the prototype EHEC organism *E. coli* O157:H7 (98). Only two VTEC serotypes (O157:H7 and O26:H11) have been classified as EHEC by Levine (98).

Authors who use the different terminologies do so with their own reasonable justifications, and it is not the intention of this article to judge the merits of one or the other nomenclatural system, but rather to define the terms used here and to explain the context within which they are used. The original description of SLT-producing *E. coli* by O'Brien et al. (133) included three categories of strains: (i) trace-level, (ii) low-level, and (iii) high-level producers of the toxin. High-level SLT producers contained about 100- to 1,000-fold-greater amounts of toxin than the trace- and low-level producers. Furthermore, toxin was easily detectable in supernatants of high-level producer cultures, but not in culture supernatants of trace- and low-level producers. Hemorrhagic colitis and HUS have been associated only with high-level SLT producers (corresponding to VTEC), whereas the clinical relevance of trace- and low-level SLT-producing strains is uncertain. Thus, use of the term SLT-producing *E. coli* can be misleading unless qualified by the amount of toxin produced. Low-level SLT production has been observed in nonpathogenic strains such as *E. coli* K-12, as well as in environmental and human isolates of *Vibrio cholerae* and *Vibrio parahaemolyticus* (129), but there is no evidence that these groups of bacteria are associated with either HUS or hemorrhagic colitis. Shiga-like toxin quantitation, using the methods described by O'Brien et al. (133), is not practicable in clinical microbiology laboratories. On the other hand, for routine diagnostic purposes it is feasible to examine culture supernatants for cytotoxin, i.e., cultures of strains that are of known clinical relevance and that correspond to high-level, SLT-producing *E. coli* or VTEC. The term VTEC also has an ease of expression, being less cumbersome than the expression high-level SLT-producing *E. coli*.

CHARACTERISTICS OF VTEC

Distinction of VTEC from Other Pathogenic *E. coli*

The recognition of new potential virulence properties and disease syndromes associated with *E. coli* has led to a proliferation of new names and classifications for these organisms, often leading to confusion for users, especially clinicians and clinical microbiologists. Levine (98) has listed as many as 13 names that have been applied to diarrheagenic *E. coli*. The more commonly used names include enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli* (EPEC) serotypes, enteroadherent *E. coli*, "attaching and effacing" *E. coli*, VTEC, and EHEC. For a detailed background of the different classes of diarrheagenic *E. coli*, the reader is referred to reviews by Rowe (165), Levine (98), Levine and Edelman (99), and Robins-Browne (160).

Enterotoxigenic *E. coli*. Enterotoxigenic *E. coli* (98, 160, 165), the major cause of bacterial diarrhea in third world countries and of travellers' diarrhea, are distinguished by their ability to elaborate one or both of two plasmid-encoded cholera-like toxins, LT and heat-stable enterotoxins. The mechanism of pathogenesis involves attachment of the bacteria to small bowel enterocytes by means of specific fimbria, the best characterized of which are referred to as colonization factors I and II. The specific action of LT or heat-stable enterotoxin on the enterocyte at the subcellular level, through the stimulation of adenylate cyclase or guanylate cyclase, respectively, leads to fluid secretion, which results in watery diarrhea in the patient. Enterotoxigenic *E. coli* strains are generally confined to certain O:H serotypes.

Enteroinvasive *E. coli*. Enteroinvasive *E. coli* (98, 160, 165) cause a dysentery-like syndrome which resembles *Shigella* dysentery. Like shigellae, enteroinvasive *E. coli* have the capacity to invade epithelial cells in vitro and in vivo; plasmid-mediated factors have been shown to be involved in the invasive capacity of both groups of organisms.

EPEC serotypes, enteroadherent *E. coli*, and attaching and effacing *E. coli*. The term EPEC serotype (98, 160, 165) refers to specific serotypes of *E. coli* that were historically associated with outbreaks of infantile diarrhea, particularly during the 1940s, 1950s, and 1960s; the reported traditional EPEC O:H serotypes include O26:H11, O26:NM, O55:NM, O55:H6, O55:H7, O86:NM, O86:H34, O86:H2, O111:NM, O111:H2, O111:H12, O111:H21, O114:H2, O119:H6, O125ac:H21, O126:H27, O127:H9, O127:H21, O127:NM, O127:H6, O128ab:H2, O142:H6, and O158:H23 (*Manual of Investigations of Acute Enteric Infections*, World Health Organization publication no. CDD/83.3 Rev. 1., p. 27, 1983). As outbreaks of EPEC diarrhea declined in frequency during the 1970s, debate centered on the significance of isolating EPEC in sporadic cases of diarrhea, and research was directed towards identifying phenotypic markers of virulence and establishing the mechanisms of pathogenicity of epidemic EPEC strains. Cravioto et al. (34), in 1979, observed that many EPEC serotyped strains adhered to HEp-2 cells in vitro, a property not shared by nonpathogenic *E. coli* strains and other classes of pathogenic *E. coli*. Subsequent work (4) revealed that the HEp-2 cell adherence phenomenon was encoded for by plasmids of about 50 to 70 megadaltons (MDa) that were present in HEp-2 cell-adherent EPEC strains; these were referred to also as enteroadherent *E. coli* (EAEC) by Mathewson et al. (111). Later it was shown that EPEC strains exhibited two distinct patterns of adherence to HEp-2 cells (171), localized adherence and diffuse adherence. The localized adherence phenomenon was found to be plasmid mediated (100, 125); such plasmids were referred to as EPEC adherence or EAF plasmids (100, 124). A 1-kilobase segment of DNA from a reference EAF plasmid has now been cloned and has been shown to be useful as a DNA probe for detecting EPEC strains that harbor the EAF plasmid (100, 124). Levine and his colleagues (98, 100, 124) have classified EPEC strains into two classes on the basis of EAF DNA probe hybridization, class I (EAF^+) and class II (EAF^-).

Another major observation made on EPEC strains was that in vivo they show a specific type of attaching and effacing adherence to intestinal mucosal epithelial cells (120, 160). This effect is characterized by the destruction of microvilli and an intimate effacing adherence of the bacterium to the epithelial cell membrane which forms cups or pedestals at the base of the attached bacterial cell. The attaching and effacing lesion has been postulated to be responsible for the diarrhea associated with EPEC (160, 164), but the precise mechanism and genetic basis of the phenomenon remain to be established.

There is some controversy as to whether the plasmid-encoded, EAF-associated, localized HEp-2 cell adherence in vitro correlates with the attaching and effacing adherence seen in vivo (160). Knutton et al. (89, 90) examined the interaction of a recognized EPEC reference strain, its plasmid-cured derivative, and a recombinant K-12 strain con-

taining the HEp-2 adherence plasmid with both HEp-2 cells and cultured duodenal mucosa. Their findings provide compelling evidence that localized adherence to HEp-2 cells and attaching and effacing adherence are distinct phenomena, with the HEp-2 adhesin being of fimbrial origin and plasmid mediated, while the attaching and effacing adherence phenomenon is encoded for by genes located on the chromosome. They propose a two-stage model of EPEC adhesion, with a fimbrial-mediated initiation step and a subsequent phase of attaching and effacing adherence.

VTEC and EHEC. The term VTEC refers to strains that elaborate VT in their culture supernatants and can thus be distinguished from other classes of *E. coli* that do not do so. The term EHEC, coined by Levine (98), refers to strains that have the same clinical, epidemiological, and pathogenetic features associated with the prototype EHEC organism *E. coli* O157:H7. Only two VTEC serotypes (O157:H7 and O26:H11) have been classified as EHEC by Levine (98).

The criteria used by Tzipori et al. (205) for defining EHEC include association with hemorrhagic colitis, production of one or more verotoxins, possession of a large plasmid (50 to 70 MDa), and induction of distinct mucosal lesions, characterized by attaching and effacing adherence, in the large bowel of the gnotobiotic pig. They have extended the spectrum of EHEC strains to include VTEC serotypes O111:H-, O145:H-, O45:H2, and O4:H-. By this definition, all EHEC can be regarded as VTEC, but only those VTEC that show the same clinicopathological features as *E. coli* O157:H7 should be termed EHEC. Thus, EHEC constitute a defined theoretical subgroup of VTEC. It should be noted that Sherman et al. have demonstrated attaching and effacing adherence of several VTEC serotypes, including O157:H7, in the colon of rabbits (179, 180; P. Sherman, R. Soni, and M. Karmali, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-9).

Sherman et al. (180) found that VTEC O157:H7 strains exhibit localized and/or diffuse adherence to HEp-2 cells and the Henle 407 cell line of human intestinal origin. In contrast, Karch et al. (77) reported adherence of VTEC O157:H7 strains to Henle 407 cells but not to HEp-2 cells and provided evidence that adherence to the Henle cell line was determined by fimbriae encoded for by genes on a 60-mDa plasmid.

Levine et al. (101) found that *E. coli* O157:H7 strains, unlike class I EPEC strains, are negative for the EAF probe. They have now prepared a DNA probe from a 3.4-kilobase segment of a 60-mDa plasmid commonly present in *E. coli* of this serotype and reported that, among strains obtained from HUS patients, the probe hybridized with 99% of 107 VT^+ *E. coli* O157:H7 strains, 77% of 44 VT^+ *E. coli* O26:H11 strains, and 69% VT^+ *E. coli* belonging to several serotypes other than O157:H7 and O26:H11. Commensal *E. coli* and strains belonging to other classes of pathogenic *E. coli* gave a negative reaction.

Serotype Distribution of Human VTEC Isolates

The O:H serotypes of human VTEC isolates, reported by the Division of Enteric Pathogens, Central Public Health Laboratory, London, United Kingdom (174, 175, 183; B. Rowe, H. R. Smith, S. M. Scotland, and R. J. Gross, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-1), and the Enteric Reference Laboratory Centre for Disease Control, Ottawa, Ontario, Canada (H. Lior, personal communication), have included O1:NM, O2:H5, O2:H7, O4:NM, O4:H10, O5:NM, O5:H16, O6:H1, O18:NM, O18:H7, O25:NM, O26:NM, O26:H11, O26:H32, O38:H21, O39:H4, O45:H2, O50:H7, O55:H7, O55:H10, O82:H8, O84:H2, O91:NM, O91:H21, O103:H2, O111:NM, O111:H8, O111:H30, O111:H34, O113:H7, O113:H21, O114:H48, O115:H10, O117:H4, O118:H12, O118:H30, O121:NM, O121:H19, O125:NM, O125:H8, O126:NM, O126:H8, O128:NM, O128:H2, O128:H8, O128:H12, O128:H25, O145:NM, O145:H25, O146:H21, O153:H25, O157:NM, O157:H7, O163:H19, O165:NM, O165:H19, and O165:H25. Although some VTEC isolates belong to classical EPEC O:H serogroups, e.g., O26:NM, O26:H11, and O111:NM, only a minority of EPEC serotyped strains produce VT. Scotland and colleagues (173) found only 25 (10%) of 253 strains comprising 11 common EPEC serogroups to be VT^+. Twenty-three (42.5%) of 54 serogroup O26 and 2 of 34 serogroup O128 strains were VT^+, whereas VT was not detected in 228 strains belonging to O groups 18, 55, 111, 114, 119, 125, 126, 127, 128, and 142.

E. coli O157:H7 continues to be the VTEC serotype most commonly isolated from humans. The apparent predominance of serotype O157:H7 may be a reflection of the widespread use of sorbitol-MacConkey medium, which is designed to detect only this particular VTEC serotype. However, it has continued to be the most common VTEC serotype in studies that have included the detection of the other serotypes. Nevertheless, the number of different VTEC serotypes isolated from human sources continues to grow, and many have also been identified in animals (R. Clarke, S. McEwen, N. Harnett, H. Lior, and C. Gyles, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, P48, p. 282).

Types of VT Produced by Human VTEC Isolates

Several investigators have observed that human VTEC isolates elaborate one or both of the two cytotoxins VT1 and VT2. The relative distribution of VT1 and VT2 in individual isolates has been determined through the use of VT1- and VT2-specific DNA probes and specific neutralization tests.

In the most comprehensive study to date, Scotland et al. (175) examined 26 isolates of VTEC serogroup O157:H7 for the distribution of VT1 and VT2 by using both neutralization studies and VT1- and VT2-specific DNA probes. Two of the strains were nonmotile (H-) and 24 carried the H7 flagellar antigen. Fourteen strains (54%) elaborated both VT1 and VT2, 11 (42%) were positive for VT2 only, and 1 strain produced only VT1.

Workers at the Central Public Health Laboratory, Colindale, United Kingdom (174, 182, 183; Rowe et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987; S. M. Scotland, G. A. Willshaw, H. R. Smith, and B. Rowe, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, LFE-1), have examined several non-O157:H7 VTEC strains from humans and animals for distribution of VT1 and VT2, using specific DNA probes and neutralization assays. They found strains that produced only VT1, only VT2, or both toxins. Thus, available information indicates that VTEC isolates from patients with hemorrhagic colitis or HUS produce either one or both of the two toxins VT1 and VT2, suggesting that both toxins are involved in the pathogenesis of human disease.

Subtyping of VTEC Serotypes, Especially Serotype O157:H7

Because *E. coli* O157:H7 is the predominant human VTEC isolate, attempts have been made to subtype this serotype by

phage typing, biotyping, and plasmid profile analysis. Ahmed et al. (1) developed a phage-typing scheme which they used to separate 98 strains of *E. coli* O157:H7 into 14 different phage types. All strains possessed a 65-mDa plasmid, but many had additional smaller plasmids which enabled these investigators to subdivide the strains into at least four plasmid groups. Using a combination of phage typing and plasmid profile analysis, they were able to distinguish strains of *E. coli* O157:H7 from different outbreaks.

Studies by Scotland et al. (175) have shown that strains of *E. coli* O157:H7 are heterogeneous with respect to plasmid profile and colicin production. Krishnan et al. (96) examined the biochemical profiles of *E. coli* O157:H7 and were able to subdivide the strains into four biotypes on the basis of differential fermentation of dulcitol and rhamnose. There are thus a number of typing systems available for conducting studies on the sources and routes of spread of *E. coli* O157:H7. The enteric reference laboratory at the Laboratory Centre for Disease Control, Ottawa, Ontario, Canada has now separated *E. coli* O157:H7 strains into >46 phage types and 8 biotypes on the basis of differential fermentation of sucrose, dulcitol, and rhamnose (Lior, personal communication; R. Khakria, D. Duck, and H. Lior, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, LFE-13).

Based on the general observation that *E. coli* O157:H7 strains produce one or more Shiga-like toxins and lack the ability to rapidly ferment sorbitol, Whittam et al. (214) used multilocus enzyme electrophoresis to test the hypothesis that *E. coli* strains of this phenotype share a common ancestry. They examined 100 *E. coli* strains (97% VT^+), most of which were isolated from sporadic and outbreak cases of hemorrhagic colitis or HUS and cattle in North America. About two-thirds of the isolates belonged to serotype O157:H7 and the remainder comprised at least 12 non-O157:H7 serotypes from diverse sources. Genetic relatedness of the strains was determined for chromosomal genotypes on the basis of allelic variation at 17 enzyme-encoding loci detected by multilocus enzyme electrophoresis. Comparison of the observed combination of alleles among strains revealed 25 distinct multilocus genotypes, which were used to define naturally occurring cell lineages. It was found that O157:H7 strains fell into a well-defined group of clonal genotypes that was only distantly related to VTEC belonging to other serotypes, indicating that the O157:H7 organisms are of recent descent from an ancestral cell and belong to a pathogenic clone that occurs throughout North America.

EPIDEMIOLOGY OF VTEC INFECTIONS

Information currently available indicates that human VTEC infections occur most commonly in the summer and fall in Canada (80, 142; A. Borczyk, and H. Lior, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-9). VTEC are widely distributed in the intestines of animals (117, 118, 181; Borczyk and Lior, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* infections 1987; Clarke et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988; R. C. Clarke, S. A. McEwen, V. P. Gannon, V. E. O. Valli, H. Lior, and C. L. Gyles, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, LFE-15; M. L. Martin, L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, J. P. Davis, J. Arnoldi, and J. Tilleli, Letter, Lancet **ii**:1043, 1986; A. Mohammed, J. S. M. Peiris, and L. P. Perera, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-1; F. Orskov, I. Orskov, and J. A. Villar, Letter, Lancet **ii**:276, 1987; J. S. M. Peiris, A. Mohammad, and L. P. Perera, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, LFE-5; J. G. Wells, L. D. Shipman, K. D. Greene, F. P. Downes, M. L. Martin, R. V. Tauxe, and I. K. Wachsmuth, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, LFE-4), especially cattle, and most human infections probably occur as a result of consumption of undercooked foods of bovine origin (Table 1). Bovines have been investigated largely because of the epidemiological association of human disease with bovine products in the form of hamburger meat (Table 1) and unpasteurized milk (43). Large-scale systematic studies in other species of domestic animals have yet to be conducted. However, reports of the isolation of *E. coli* O157:H7 from retail pork, lamb, and poultry products (41) suggest that the animal reservoir of this organism may be wider than just bovines. While VTEC infection in most cases is probably foodborne, there is increasing evidence that VTEC infection can also be acquired through person-to-person transmission (22, 187).

Reservoirs of VTEC

VTEC isolates of serotype *E. coli* O157:H7 have been isolated from the feces of cattle by several investigators (Orskov et al., Lancet **ii**:276, 1987; A. A. Borczyk, M. A. Karmali, H. Lior, and L. M. C. Duncan, Letter, Lancet **i**:98, 1987). In a likely milkborne outbreak of this infection that affected many kindergarten children, the epidemic strain was of the same phage type as an isolate recovered from cattle at a farm where raw milk was served to the children (43; Borczyk et al., Lancet **i**:98, 1987; Mai et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987).

In a large prevalence study, Clarke et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1988) investigated 600 cattle for the presence of VTEC. They recovered these organisms from the feces of 21 (10.5%) of 200 beef cattle, 39 (19.5%) of 200 cull dairy cows, and 7 (3.5%) of 200 veal calves. The overall prevalence of VTEC in the 600 bovines sampled was 11.1%. Clarke and his colleagues concluded that bovines are an important reservoir of VTEC and that many clinically normal animals carry these strains into processing plants. The majority of veal calf isolates were multiply resistant to a variety of antimicrobial agents, an observation which Clarke et al. think reflects the practice of adding antimicrobial agents to veal calf milk replacer.

The 67 VTEC strains isolated by Clarke et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1988) represented 31 different serotypes, 9 of which have also been isolated from humans in Canada. Serotypes corresponding to human isolates comprised O153:H25 (6 isolates), O157:H7 (4 isolates), and O113:H21 (3 isolates), 2 isolates each of serotypes O103:H2, O126:H8, and O145:H-, and 1 isolate each of serotypes O26:H11, O91:H21, and O165:H-.

In studies in symptomatic animals, Sherwood et al. (181) isolated VTEC representing seven O serogroups (4, 8, 19, 26, 111, 149, and 168) from 9 (3%) of 306 diarrheic cows. Smith et al. (184) have reported the isolation of several VTEC serotypes from animals, including human O serotypes 26, 55, 111, and 153.

Mohammed et al. (117, 118; Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987),

in Sri Lanka, isolated VTEC from cattle and buffalo calves with diarrhea significantly more often than from controls, indicating that VTEC are causes of diarrhea in these animals. These bovine isolates consisted of 27 different O serogroups, 11 of which corresponded to human EPEC serotypes. In a separate prevalence study, the Sri Lankan investigators (117) recovered VTEC from 53 (16%) of 335 cattle, 3 (1.8%) of 164 pigs, and 3 (15%) of 20 goats.

In a major prevalence study in the United States (Wells et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* infections 1987), about 900 bovines from 16 farms and other agricultural facilities in four states were investigated. *E. coli* O157:H7 was isolated from 18 heifers or calves on nine farms, from 1 stockyard calf, and from 1 of 24 raw milk samples.

The above studies indicate that VTEC are prevalent in the intestines of domestic animals, particularly bovines, and that many animal isolates correspond to serotypes isolated from humans. The full picture will emerge only when a wide variety of animal species are investigated for VTEC in a systematic manner.

Sources for Human VTEC Infection

Foods of animal origin are probably the major sources of human VTEC infection (Table 1). Many outbreaks of *E. coli* O157:H7 have been linked to the consumption of inadequately cooked hamburger meat, and at least one has been linked to the consumption of unpasteurized milk.

In the two original outbreaks of *E. coli* O157:H7-associated hemorrhagic colitis in Michigan and Oregon (158, 213), organisms of the same serotype, plasmid profile, and restriction endonuclease profile were isolated from a portion of the implicated meat. In a suspected milkborne outbreak of *E. coli* O157:H7-associated illness affecting kindergarten children (43; Borczyk et al., Lancet i:98, 1987), the organism was isolated from cattle at a farm where raw milk was served.

In a survey of retail fresh meats and poultry obtained from grocery stores in Madison, Wis., and Calgary, Alberta, Canada, Doyle and Schoeni (41) isolated *E. coli* O157:H7 from 6 (3.7%) of 164 beef, 4 (1.5%) of 264 pork, 4 (1.5%) of 263 poultry, and 4 (2.0%) of 205 lamb samples. Investigators from the Agriculture Canada, Health of Animals Laboratory in Guelph, Ontario, Canada, investigated samples of ground beef from 50 randomly selected retail outlets in June 1987. Of 50 samples, 9 (18%) were positive for VTEC, and these comprised eight different serotypes, including one strain of O157:H7 (R. C. Clarke, personal communication). Konowalchuk et al. (94) recovered two VTEC isolates (serotypes O68:H12 and O26:H32) from cheese. At least two groups have isolated VTEC strains including serotype O157:H7 from milk and milk filters (Clarke et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987; Wells et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987).

Transmission of VTEC

Although ingestion of undercooked food products of animal origin seems the likely route of transmission in most cases of human infection, there is increasing evidence that VTEC infection can also be acquired via person-to-person transmission. In a day-care center outbreak (1987), no common food source was identified, and the sequential movement of illness from class to class was consistent with person-to-person transmission. In a large outbreak of *E. coli* O157:H7 infection in a Canadian nursing home (22) affecting 55 elderly residents and 18 staff members, the epidemic curve was biphasic. The first phase, which included the vast majority of the affected residents, was consistent with a point source infection presumed to be ingestion of a contaminated sandwich. The second phase, which involved many of the staff who cared for the sick patients, was indicative of person-to-person transmission. In an outbreak (43) among kindergarten children who probably acquired their infection after drinking raw milk at a farm, the occurrence of secondary cases among family members of some of the children suggested intrafamily spread of infection.

A nurse, working in a pediatric nephrology unit, developed HUS associated with *E. coli* O157:H7 infection (M. A. Karmali, G. S. Arbus, M. Petric, M. L. Patrick, M. Roscoe, J. Shaw, and H. Lior, Letter, Lancet i:526, 1988). Investigations revealed that she probably acquired the infection through contact with a child with HUS. The infecting strain in both the child and the nurse had an identical phage type and biotype pattern.

LABORATORY DIAGNOSIS OF VTEC INFECTIONS

Most VTEC studies have centered on the isolation of the single serotype, O157:H7, largely because the latter has a phenotypic property (sorbitol negative after 24 h) that facilitates detection in mixed flora on appropriate selective media. On the other hand, HUS and hemorrhagic colitis have now been associated with a wide array of different serotypes, even though O157:H7 continues to be the predominant one. The overall diagnostic strategy, therefore, must be directed towards detecting VTEC in general, rather than detecting a single serotype.

Detection of Free FVT and Isolation of VTEC

One of the main problems encountered in isolating VTEC (including serotype O157:H7) from primary media has been the relatively low numbers of VTEC in mixed flora. For example, during investigations on patients with hemorrhagic colitis, Wells et al. (213) screened five colonies from primary isolation media for the presence of serotype O157:H7, and they recovered this serotype from about three-quarters of the patients from whom stools were submitted within 4 days of onset of symptoms but from none of the stools that were sent 7 days or more after onset. A similar problem in isolating the organism from the stools of symptomatic patients has been noted by other investigators (187). In our studies of HUS patients (80, 81), we tested up to 20 individual colonies from primary stool culture media and found that the proportion of VT-positive colonies was often only 5 to 20%. Patients who had VTEC isolated from their stools were consistently positive for free FVT. However, we found that many patients who were negative for VTEC had positive results for FVT. These findings indicate that the most important procedure for diagnosing VTEC infection is the detection of FVT, an observation confirmed in other studies (43, 96, 143).

Although screening fecal filtrates for VT is a relatively straightforward procedure, isolating VTEC presents a considerable logistical problem because it involves examining a large number of *E. coli* colonies (at least 20) for VT production. Examination of a large number of colonies is particularly appropriate when investigating patients with HUS because their stools are often received 1 to 2 weeks after the

onset of the prodromal diarrheal illness. The problem of having to examine a large number of colonies can be overcome by using a sensitive screening procedure (79) that is able to detect low number of VTEC in mixed culture. This so-called VT/PECS (VT in polymyxin extracts of colony sweeps) procedure is based on the fact that high concentrations of VT present in the periplasmic space of VTEC can be released by treating pelleted cells with polymyxin B. When applied to sweeps of colonies taken from areas of semiconfluent growth on primary isolation media, the VT/PECS procedure can detect as few as about 1 VTEC colony in 100. The procedure is thus useful as a sensitive screening test for ruling out VTEC, but additional work has to be conducted to isolate individual VTEC colonies if the VT/PECS procedure is positive. The detection of FVT and VT/PECS can be made even more sensitive by adding 4 to 8 μg of cycloheximide per ml to the tissue culture medium in the Vero cell assay system used for detecting VT (146). Full details for performing the FVT and VT/PECS procedures and guidelines for interpreting results have been published (M. A. Karmali, Clin. Microbiol. Newsl. **9**:65–70, 1987).

VT production in *E. coli* may be assayed by inoculating a colony into an appropriate broth medium such as Penassay (80, 81) or Trypticase soy (143) medium and testing the culture filtrate for VT activity after overnight incubation at 37°C. The use of iron-depleted media such as iron-depleted Syncase medium appears to be essential for testing strains for low-level SLT production (i.e., cell-associated toxin that is not elaborated in culture filtrates) (133). However, iron restriction is not necessary for detection of VT production in culture filtrates, rather, it may retard growth and reduce VT production (28).

Sorbitol-MacConkey Agar for Detection of *E. coli* O157:H7

Many laboratories that do not have tissue culture facilities presently use a sorbitol-containing medium to detect the VTEC serotype O157:H7. This serotype does not ferment sorbitol at 24 h, whereas 95% of *E. coli* are sorbitol fermenters under these conditions (50, 96). A sorbitol-MacConkey agar (similar to standard MacConkey medium except that it contains 1% D-sorbitol instead of lactose) is a useful selective medium for this purpose. After inoculation with feces, this medium is incubated for 18 to 24 h at 37°C and examined for colorless (D-sorbitol-negative) colonies. Such colonies should then be tested by slide agglutination tests against O157 and H7 antisera, and, if positive, results should be confirmed by tube agglutination tests (107); the presence of the H7 antigen may also be tested by using a motility inhibition test (50). The extent to which slide or tube agglutinations or both can be performed will depend on the availability of appropriate antisera. If the culture is serotyped as O157:H7, it is highly likely to be VT positive, but it should be confirmed by submitting the culture to an appropriate reference laboratory. It should be noted that not all *E. coli* strains of O serogroup 157 have the H7 serotype (176). VT production is a feature of O157:H7 and O157:H-, both serotypes being sorbitol negative. On the other hand, human isolates belonging to other H types, e.g., O157:H45, O157:H39, O157:H19, and O157:H43 (may be LT producers), are typically sorbitol positive (175) and thus should not present a problem on the sorbitol-MacConkey medium.

Lior and Borczyk (H. Lior and A. Borczyk, Letter, Lancet **i**:333, 1987) have pointed out that a false-positive identification of *E. coli* O157:H7 may result with *Escherichia hermanii*, which resemble *E. coli* biochemically, are sorbitol negative, and give a positive agglutination reaction with O157 antiserum but are VT negative. They are distinguished from *E. coli* because, unlike the latter, they ferment cellobiose, grow in the presence of KCN, and produce a yellow pigment. Some VT-positive strains (e.g., some O18 isolates) may also be sorbitol negative, but would be missed because they do not agglutinate in O157 antiserum (Lior, personal communication).

Using sorbitol-containing selective media, Harris et al. (66) identified sorbitol-negative *E. coli* in 106 (4.1%) of 2,552 stool cultures, but only two isolates were serotype O157:H7. A higher yield was reported by March and Ratnam (107), who found 18 of about 1,000 stool cultures positive for this serotype; they found sorbitol-MacConkey medium to have a sensitivity of 100% and a specificity of 85% in the detection of *E. coli* O157:H7.

VT1- and VT2-Specific DNA Probes

Several groups have now cloned the genes specifying VT1 and VT2 (70, 97, 127, 193, 215, 216), and the cloned genes have been used in colony hybridization tests for detecting VT1- and VT2-producing *E. coli*. These probes have been used extensively in a diagnostic setting by workers (215, 216) at the Central Public Health Laboratory, Colindale, U.K. Using these probes, the Colindale group (174, 182) was able to detect VTEC that were present in numbers as few as 1 in 1,200 colonies. They found the DNA hybridization method to be at least as sensitive as the detection of FVT in fecal samples and suggested that, optimally, both procedures should be used in parallel. The probe method was significantly more sensitive than the sorbitol-MacConkey agar for detecting *E. coli* O157:H7. If these probes are made commercially available with nonradioactive labels, they are likely to become attractive in a variety of settings, including diagnostic and food microbiology and for epidemiological studies.

Enzyme-Linked Immunosorbent Assay Methods to Detect VT1 and VT2

A specific and sensitive immunospecific assay would offer considerable advantages over a tissue-culture-based assay for detection of FVT in the fecal filtrates of patients with suspected VTEC infection. Such an assay would be expected to have a shorter turnaround time than the use of DNA-DNA hybridization methods and would thus appeal to routine diagnostic laboratories as a screening test. FVT-positive samples would then need to be processed further, using an immunoassay, cell culture, or a DNA colony hybridization method to detect and isolate individual VTEC colonies.

A sandwich enzyme-linked immunosorbent assay method for detecting *Shigella* toxin in stool filtrates has been described by Donohue-Rolfe et al. (38), and a similar assay for detecting VT in bacterial isolates of *Shigella* spp. and *E. coli* has been reported by Kongmuang et al. (91) in Japan. Clearly, considerable developmental work is required in both of these assays before they become available as commercial kits for routine use. The discovery of the glycolipid Gb_3 as a specific receptor for VT1 (104) and VT2 (212) should result in attempts to develop receptor-binding enzyme-linked immunosorbent assays for detecting these toxins in the diagnostic setting.

Colony Blot Assay with VT Monoclonal Antibodies to Detect VTEC

Strockbine et al. (114) and Karch et al. (78) have reported a colony blot assay for detecting VTEC. Such an approach would be useful for identifying small numbers of VTEC in mixed culture, as it would be a useful screening procedure for VTEC in primary fecal cultures and cultures of foods and environmental samples. The method, analogous to the use of DNA probes, involves growing suspect colonies on a trimethoprim-sulfamethoxazole-containing medium; subinhibitory concentrations of trimethoprim-sulfamethoxazole have been shown to enhance toxin production, although the mechanism of this toxin-enhancing action is unclear (78). After 18 to 24 h of incubation, the colonies are blotted onto a nitrocellulose membrane, lysed with polymyxin B, and washed. Immunospecific staining with a VT-specific monoclonal antibody can then be used to detect VTEC colonies.

Other VT-specific method at a developmental stage include a rapid method to detect VTEC in bacterial smears, using an indirect fluorescent-antibody method with a VTI monoclonal antibody to detect VT1 directly in the bacteria (M. A. Fleming, M. A. Karmali, M. Petric, B. Rowe, S. Degrandis, M. Roscoe, and S. Mitra, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, LFE-12), and a method to detect VT in fecal filtrates and bacterial extracts by counterimmunoelectrophoresis (A. Maniar, T. Williams, and G. Hammond, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, B93, p. 45).

Serology

Patients with VTEC infection develop rising levels of VT-neutralizing antibodies, and this has been used to diagnose VTEC infection in patients without other evidence of infection (80; Karmali, Clin. Microbiol. Newsl. **9**:65–70, 1987).

Notenboom et al. (R. H. Notenboom, A. Borczyk, M. A. Karmali, and L. M. C. Duncan, Letter, Lancet **ii**:745, 1987) reported that patients with *E. coli* O157:H7 infection develop rising antibody titers to the somatic O157 antigen and suggest that O157 serology would be of diagnostic value in some settings and helpful in investigating epidemics. Extreme caution should be exercised in interpreting the results because the O157 antigen cross-reacts with *Brucella abortus* (193).

PATHOGENESIS OF VTEC INFECTION

Evidence That VTEC Are Human Pathogens

Several lines of evidence indicate that VTEC are causally related to human disease.

(i) VTEC belonging to several different serotypes, including O157:H7, have been linked consistently to sporadic cases of a well-defined syndrome, classical HUS. VTEC have been recovered from the feces of patients with HUS, but not from those of control children (80).

(ii) VTEC, in particular, serotype O157:H7, have been linked consistently with both sporadic cases and outbreaks of a well-defined syndrome, hemorrhagic colitis. The clinicopathological (156) and radiological features (5, 88, 158, 159, 170) of hemorrhagic colitis are remarkably similar to the bloody diarrheal phase of HUS, indicating that both syndromes are manifestations of the same underlying disease process. HUS, often affecting clusters of patients, has accompanied some outbreaks of hemorrhagic colitis (Table 1).

(iii) In both HUS and hemorrhagic colitis, VTEC organisms are most abundant in feces during the acute phase of the diarrheal illness and decline substantially in number during convalescence (80, 143).

(iv) VTEC-associated hemorrhagic colitis (or a similar illness) has been reported to occur naturally in calves (63, 123; D. H. Francis, B. H. Janke, and C. Y. Andraos, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-2; R. A. Moxley, and D. H. Francis, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections, AMV-3; M. Schoonderwoerd, R. C. Clarke, T. A. Van Dreumel, and S. A. Rawluk, Can. J. Vet. Res., in press. However, it has not yet been possible to reproduce the natural disease consistently by challenging experimental animals with VTEC, although the histopathological lesions in gnotobiotic pigs (52, 204, 205; Moxley and Francis, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987) and calves (27, 63, 123; Francis et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987; Schoonderwoerd et al., in press; G. A. Hall, N. Chanter, and A. P. Bland, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-12) challenged with VTEC in some studies have been suggested of hemorrhagic colitis.

Thus, substantial evidence links VTEC to human disease based on clinical, clinicomicrobiological, and epidemiological evidence. Implicit in the use of the term VTEC is the hypothesis that VT itself is a major determinant of the syndromes, HUS and hemorrhagic colitis, that are associated with VTEC infection. Evidence that VT is of direct pathogenetic significance in these syndromes includes the following.

(i) VT production is a common factor in a serotypically heterogeneous group of *E. coli* that are associated with the same distinct clinicopathological syndromes, HUS (80, 81) and hemorrhagic colitis (143, 158). This does not preclude the possibility of other factors being common to all HUS and hemorrhagic colitis strains, such as specific colonization factors.

(ii) In vivo VT production occurs in patients with both hemorrhagic colitis and HUS, and VT activity be detected in fecal filtrates (80, 81, 143).

(iii) The histopathology of HUS (42, 51, 62, 76, 156, 210) is characterized by widespread sterile systemic microangiopathic lesions consistent with systemic toxemia. These lesions, which occur in the microvasculature of the kidneys as well other organs and tissues such as the bowel, brain, and pancreas, are characterized by swelling of the endothelial cells, fibrin/platelet thrombi, and capillary occlusion.

(iv) The clinicopathological and radiological features of hemorrhagic colitis are remarkably similar to those of HUS, indicating that both conditions result from the same underlying vasculitic disease process.

(v) While the widespread histopathologic lesions of HUS or hemorrhagic colitis would be consistent with VT toxemia, VT has never been detected in the blood of patients with HUS or hemorrhagic colitis. There is thus no direct evidence that it is responsible for the vascular pathology underlying these conditions. On the other hand, the substantial indirect evidence for the involvement of systemic VT in this situation is as follows.

(a) Patients with VTEC infection develop rising levels of VT-neutralizing antibodies (80, 81).

(b) The injection of VT into rabbits leads to pathology in the bowel and central nervous system of these animals

characterized by microangiopathic lesions similar to those seen in humans with HUS or hemorrhagic colitis (156; S. E. Richardson, V. Jagadha, C. R. Smith, L. E. Becker, M. Petric, and M. A. Karmali, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-5). Injection of VT1 leads to the development of mild diarrhea and paralysis in rabbits (S. E. Richardson, V. Jagadha, C. R. Smith, L. E. Becker, M. Petric, and M. A. Karmali, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, B105, p. 43). Injection of VT2 (67) leads to a fulminant syndrome of bloody diarrhea resulting from an intense hemorrhagic cecitis which resembles, both clinically and pathologically, the syndrome of hemorrhagic colitis in humans. Injection of ^{125}I-labeled VT into rabbits (S. E. Richardson, M. Petric, and M. A. Karmali, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, B35, p. 35) is followed by rapid uptake of the toxin, within minutes, by tissues and organs which show the most intense histopathological changes. If a similarly rapid uptake occurs in humans, it would explain why circulating VT activity has not been detected in human cases.

(c) Edema disease in pigs is associated with strains of *E. coli* that elaborate a cytotoxin (37, 94)formerly known as the edema disease principle (31, 37, 49) and now known to be a variant of VT2 (109; Gyles et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). The pathology of edema disease is characterized by sterile vascular lesions in various tissues that resemble the microangiopathic lesions seen in HUS (31, 49, 177, 199). The proposal that edema disease results from a toxemia is supported by the fact that the disease can be reproduced experimentally by injection of crude toxin preparations (31, 49). Experimental evidence that a naturally occurring disease of animals that resembles HUS probably results from a VT toxemia supports the hypothesis that the pathological basis of HUS or hemorrhagic colitis is similar.

(d) VT has been demonstrated in the sera of gnotobiotic pigs (203) challenged orally with VTEC strains, although this was not correlated with the development of specific systemic lesions.

Initiation of Infection

Little is known about the inoculum size required to initiate infection. Presumably, after ingestion, the organisms have to overcome the gastric acid barrier. The importance of this was highlighted in a nursing home outbreak of *E. coli* O157:H7 infection (22) in which previous gastrectomy was correlated with an increased risk of acquiring the infection. An increased susceptibility to enteric infections has been recognized for other pathogens and suggests that gastric acid plays an important role in reducing the inoculum size of the infecting microorganism (57).

Colonization and Multiplication

No information is available from human cases to assess directly the nature, site, or mechanisms of intestinal colonization by VTEC. Nor are there any data on the manner in which VTEC interact with and penetrate the intestinal mucus barrier. On the other hand, a well-defined pattern of colonization has emerged from studies of natural and experimental VTEC infection in animals that is consistent with the attaching and effacing adherence characteristic of human EPEC infection (120, 160). To the extent that human EPEC strains exhibit the same pattern of attaching and effacing adherence in experimental animal models as occurs in humans, it could be inferred that the demonstration of attaching and effacing adherence by VTEC in animal models reflects a similar colonization process in humans. While the EPEC-associated attaching and effacing adherence has been demonstrated in the small bowel (160, 164), the VTEC-associated attaching and effacing adherence in animals has been observed primarily in the large bowel. The three animal models that demonstrate VTEC attaching and effacing adherence are the bovine, porcine, and lapine models.

VTEC of serogroups O111 and O5 have been associated with naturally occurring outbreaks of hemorrhagic colitis in calves (27, 63, 123; Francis et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987; Schoonderwoerd et al., in press). These serogroups have also been associated with HUS or hemorrhagic colitis in humans. Naturally occurring disease in calves is characterized by the typical attaching and effacing lesions in the bowel associated specifically with VTEC O111 and O5 strains; adherent bacteria are seen largely in the ileum and colon, but not in the jejunum or duodenum. Experimental inoculation of gnotobiotic or colostrum-deprived calves results in diarrhea, occasionally bloody, that is associated with the same attaching and effacing lesions seen in natural disease. The administration of VTEC serotype O157:H7, as well as other VTEC serotypes, to gnotobiotic or conventional pigs (52, 204, 205; Hall et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-12; C. L. Gyles and B. Wilcox, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-17), rabbits (144, 149, 179; J. J. Farmer, M. E. Potter, L. Riley, T. J. Barrett, P. A. Blake, et al., Letter, Lancet i:702–703, 1983; T. Itoh, A. Kai, Y. Kudoh, M. Ohashi, and K. Fukai, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-16; P. Sherman, R. Soni, and M. Karmali, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, AMV-9), and calves (27, 123; Francis et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987; Schoonderwoerd et al., in press) results in diarrhea that is also associated with attaching and effacing lesions. Such lesions occur characteristically in the large bowel of these animals.

Development of Diarrhea

Based largely on evidence from animal models, three possibly mechanisms have been postulated to account for the diarrhea associated with human infection.

(i) Diarrhea results from the local action of VT on the intestinal mucosa. This hypothesis has been driven by a number of experimental observations including that (a) Shiga toxin, VT1, and VT2 cause fluid accumulation in rabbit ileal loops (130, 186), (b) intragastric inoculation of young rabbits with VTEC O157:H7 leads to diarrhea (144, 149), and (c) mild, nonspecific, inflammatory changes may be seen in sections of rectosigmoid biopsies from adults with *E. coli* O157:H7-associated bloody diarrhea (83) which resemble changes in the colon of rabbits challenged with a crude VT preparation.

Keenan et al. (82) have conducted a detailed study of the histopathological changes associated with VT (SLT) and Shiga toxin in the rabbit ileal loop. They observed that both toxins appeared to act directly and selectively on the mature columnar epithelial cells of the intestinal villus, resulting in the premature expulsion of these cells from the lateral villus wall. The nonabsorptive crypt epithelium underwent a rapid

proliferation and maintained the epithelial integrity. Keenan et al. (82) and O'Brien and Holmes (130) propose that the mechanism of fluid accumulation in the ileal loop probably involves fluid and electrolyte malabsorption by the nonabsorptive crypt cells that release the sloughed-off mature absorptive columnar cells of the villus tip.

The histological features reported by Keenan et al. (82) are similar to those of Pai and colleagues (144), except that the latter observed that the epithelium of the small intestine was spared the changes despite a high concentration of VT in the small bowel, whereas Keenan et al. (82) observed the changes in the mucosa of small bowel loops. Possible explanations for these discrepancies have been discussed in detail by Riley (157). Mobassaleh et al. (116) have provided evidence that the fluid response to Shiga toxin in the rabbit small bowel loop is age dependent and correlates with the appearance of the specific toxin-binding glycolipid receptor Gb_3 in the microvillus membrane after 20 days of life. Pai et al. (144) observed a diarrheagenic response to VT in 3-day-old rabbits; the sparing of the small bowel in these young animals would be consistent with the lack of a specific receptor in the small bowel mucosa. On the other hand, the selective appearance of lesions in the colon of these young animals suggests either that the receptor Gb_3 appears earlier in the colonic mucosa than in the small bowel mucosa or the colonic lesions resulted from a non-Gb_3-mediated action of the toxin or other components in the crude preparation used by these workers.

The categorical demonstration of Gb_3 in the human intestinal mucosa would lend strong support to the hypothesis that the receptor-mediated toxin-induced fluid accumulation in rabbits is a valid model for VT-induced diarrhea in humans. However, a detailed study (7) of the structure of blood group glycosphingolipids in the small intestinal mucosa of four adults with different blood group types failed to reveal the presence of Gb_3.

(ii) Diarrhea is related to attaching and effacing adherence of VTEC to the intestinal cells. The development of diarrhea in animal models in association with characteristic destructive attaching and effacing lesions in the large bowel mucosa is strong suggestive evidence that this may be a mechanism by which VTEC cause diarrhea in humans. In a seminal study, Tzipori and colleagues (203) investigated the role of two putative virulence factors (a fimbrial antigen specified by a 60-MDa plasmid, and phage-mediated VT) in the diarrheagenic response of pigs to wild-type strains of *E. coli* O157:H7, isogenic mutants lacking one or the other of the two virulence actors, and recombinant *E. coli* K-12 strains that contained one or both factors. They found that neither VT (SLT) production nor the presence of the 60-MDa plasmid was required to cause diarrhea, whereas diarrhea was correlated with the attaching and effacing lesions which occurred independently of the plasmid or toxin-associated factors.

(iii) Diarrhea results from the systemic effects of the toxin on the intestinal vasculature. A strong case has been made that, irrespective of a possible local mucosal diarrheagenic action, VT is probably responsible for the systemic effects of VTEC-associated diseases, such as HUS, resulting from an action on the microvasculature of the bowel, kidneys, and other organs and tissues. It is possible that severe toxemia results in overt hemorrhagic colitis, whereas a mild toxemia produces bowel edema and mild diarrhea resulting from fluid malabsorption. This hypothesis is supported by the observation that the injection of VT1 into rabbits leads to cecitis and diarrhea (Richardson et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1987). However, other factors may also be relevant since, in contrast to the mild diarrheagenic action of VT1, injected VT2 is associated with overt hemorrhagic cecitis in the rabbit model (67).

Pathogenesis of Systemic Manifestations of VTEC Disease

Capillary endothelial cell damage is considered to be central to the pathogenesis of HUS (51, 210). Ultrastructural studies of capillaries in tissues from patients with HUS reveal a characteristic swelling of endothelial cells accompanied by widening of the subendothelial space. Similar appearances in rabbits challenged parenterally with VT1 (Richardson et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1987; Richardson et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987) support the hypothesis that endothelial cells are primary target sites for the toxin. Additional supporting evidence for this view is that endothelial cells are susceptible to the cytotoxic action of VT1 in vitro (J. Kavi, I. Chant, M. Maris, and P. E. Rose, Letter, Lancet **ii**:1035, 1987; J. Kavi, I. Chant, M. Taylor, and P. E. Rose, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, HUS-4; T. G. Obrig, P. J. Del Vecchio, J. E. Brown, M. A. Karmali, M. Petric, and C. Lingwood, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, HUS-5; T. G. Obrig, P. J. Del Vecchio, M. A. Karmali, M. Petric, T. P. Moran, and T. K. Judge, Letter, Lancet **ii**:687, 1987), and, moreover, contain Gb_3 (Lingwood et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987), which has also been found in large quantities in the human kidney (B. Boyd, C. Lingwood, Nephron, in press). Interestingly, the absence of renal microangiopathic lesions in rabbits challenged with VT1 has been correlated with the absence of Gb_3 in rabbit kidneys (C. A. Lingwood, personal communication).

The pathophysiological abnormalities in HUS include not only endothelial cell damage but also a reduction in the platelet count, an increase in plasma platelet aggregating activity (119), and the occurrence of an abnormal factor VIII molecule in acute-phase plasma (163). The factor VIII molecule, or complex, consists of a low-MW portion which has the factor VIII anticoagulant activity and a high-MW component referred to as factor VIII-related antigen (von Willebrand factor) (163; Kavi et al., Lancet **ii**:1035, 1987; Kavi et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987). Factor VIII-related antigen is increased and has an abnormal multimer pattern in acute-phase HUS plasma, but usually returns to normal during convalescence (163). It has been suggested that abnormal factor VIII-related antigen leads to platelet aggregation and thrombocytopenia (163). That factor VIII-related antigen is synthesized by endothelial cells suggests that the action of VT on these cells may be responsible for the factor VIII aberrations in patients with HUS. Demonstration by workers in the United Kingdom that VT promotes increased release of the abnormal antigen from cultured endothelial cells accords with this view (Kavi et al., Lancet **ii**:1035, 1987; Kavi et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987).

Another manifestation of HUS is a microangiopathic hemolytic anemia characterized by the presence of fragmented erythrocytes (11, 105). The damaged erythrocytes are removed from the circulation by the reticuloendothelial system. The mechanism of erythrocyte damage has yet to be

resolved, although it has been suggested that the cells become mechanically damaged as they traverse the fibrin strands in the occluded microvasculature (11). Erythrocytes in most individuals express the P antigen, which has structural similarities to Gb_3 (104). The possibility that VT binding to erythrocytes has a role in the development of the characteristic hemolytic anemia remains to be explored.

Shiga Dysentery and HUS

The only other microbe for which a convincing association exists with HUS is *S. dysenteriae* type 1 (Shiga'a bacillus) (23, 61, 95, 151). Shiga dysentery is the most severe form of bacillary dysentery, and outbreaks have been associated with morbidity and mortality, particularly at the extremes of age. Karmali et al. (M. A. Karmali, M. Petric, C. Lim, P. C. Fleming, and B. T. Steele, Letter, Lancet ii:1299–1300, 1985) have suggested that VT or Shiga toxin may be the common denominator responsible for the genesis of HUS associated with both VTEC and *S. dysenteriae* type 1. The question of interplay between the two types of bacteria in terms of eliciting cross-immunity to VT, and the part that this might play in epidemiology of both infections, is intriguing and should be addressed.

Susceptibility to VTEC Infection

Little is known about factors that determine susceptibility to VTEC infection and the risk factors for the development of systemic complications such as HUS. The peak age incidence (infants and young children) of classical HUS is a reflection of the age incidence of VTEC infection as a whole, suggesting that susceptibility to VTEC-associated HUS may, like other specific infectious diseases of childhood, be due to the lack of specific immunity, possibly to VT. The increasing recognition of VTEC-associated HUS in the elderly would be consistent with waning immunity in that age group.

Patients with VTEC-associated HUS develop rising levels of VT-neutralizing antibodies (80, 81). However, no definite information is available on the nature, class, and duration of these antibodies or their relationship to long-term immunity. Karmali et al (M. A. Karmali, M. Petric, and M. Roscoe, Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, LFE-11) found that 5 to 10% of 270 control children aged 1 month to 15 years had neutralizing antibody to VT1 but not to VT2. No age-related increase in seropositivity was noted. The frequency of VT1 seropositivity was 53% in 45 pregnant women and about 17% in 87 elderly patients. None of the senior citizens, and only one pregnant woman, had neutralizing antibody to VT2. The significance of these findings is unclear. It should be noted that, as in the case of human sera, neutralizing antibody to VT2 is rarely detected in animal sera (Peiris et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987).

The development of systemic complications such as HUS in susceptible individuals could be related to specific host factors such as the presence or absence of specific receptors (e.g., Gb_3) in target tissues. Boyd and Lingwood (in press) examined renal tissue from several patients in different age groups for the presence of Gb_3. All samples were positive for Gb_3, and no significant age-related differences were found in the distribution of the receptor in the tissues examined. On the other hand, the hypothesis that the development of complications is related to the inoculum size of the microorganism is supported by the occurrence of a very high incidence of HUS among elderly residents in a large nursing home outbreak (22) and the correlation of previous gastrectomy with more severe disease.

CONCLUDING REMARKS AND PROSPECTS FOR TREATMENT, PREVENTION, AND CONTROL OF VTEC INFECTION

There is now substantial evidence that VTEC are human pathogens and that they cause a wide spectrum of illness encompassing asymptomatic infection, mild-to-moderate diarrhea, hemorrhagic colitis, and HUS. There is also increasing evidence that VT is of direct pathogenetic significance, particularly in HUS and hemorrhagic colitis. Domestic animals, particularly bovines, appear to be the major reservoir of VTEC, and foods of animal origin are the major sources of human infection.

Programs for controlling human VTEC infection should address the reservoirs, although, as in the case of salmonellosis, eradication of VTEC from domestic animals will likely prove to be virtually impossible. Efforts should therefore be directed to abbatoirs and processing plants to institute practices that reduce cross-contamination between food products from different sources. Public education programs emphasizing the hazards of consuming unpasteurized milk and eating undercooked meat products should be beneficial. The more widespread use of food irradiation practices in the future could result in a substantial decrease in the transmission of VTEC and other foodborne pathogens to humans.

The treatment of individual patients with VTEC infection rests on the optimal management of the physiological complications of the infection such as fluid and electrolyte disturbances, anemia, renal failure, and hypertension. The role of antibiotics is questionable. In a nursing home outbreak of VTEC O157:H7 infection (22), the use of antibiotics prior to the onset of symptoms was considered to be a risk factor for acquiring the infection. The use of antibiotics in *Shigella* dysentery was considered a risk factor in the development of HUS (18). The mechanisms by which antibiotics increase the risk of infection or the risk of developing complications might involve the enhancement of toxin production by the bacteria or an alteration of the normal competing bowel flora leading to an overgrowth of VTEC. It has been reported (78, 191) that trimethoprim-sulfamethoxazole enhances toxin production by VTEC strains in vitro.

Calcium channel inhibitors, such as verapamil, have recently been shown to inhibit the cytotoxic action of Shiga toxin in vitro (169). Whether such agents would be of therapeutic value remains to be investigated.

The demonstration by Havens et al. (P. L. Havens, W. M. Dunne, Jr., and K. J. Sheth, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, B92, p. 45) that commercial immunoglobulin preparations contain VT1-neutralizing activity suggests that VT-specific antibody might be responsible for the reported beneficial effects of plasma infusion and immunoglobulin administration in HUS.

If future studies show that naturally acquired VTEC infection leads to long-term immunity, possibly antitoxic, then active immunization would be an option to prevent the serious complication of HUS associated not only with VTEC, but also with Shiga dysentery. Candidate vaccines are already available for such an eventuality in the form of immunogenic synthetic Shiga toxin B-subunit peptides (64). Rabbit antisera to these synthetic peptides are able to neutralize the biological activity of Shiga toxin in cell cul-

ture, and active immunization protects mice against the lethal effects of Shiga toxin.

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