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Purification of an *Escherichia coli* Serogroup O157:H7 Verotoxin and Its Detection in North American Hemorrhagic Colitis Isolates

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Verotoxin (VT), which is immunologically unrelated to VT1 (Shiga-like toxin I), was purified from the culture filtrate of *Escherichia coli* hemorrhagic colitis serogroup O157:H7 strain 3657 by copper ion chelate affinity chromatography followed by anion-exchange chromatography. The isoelectric point by sucrose density gradient isoelectric focusing was 5.0, the molecular weight by gel filtration on Superose 12 was about 60,000, and the 50% cytopathic dose for Vero cells was about 1 pg. This toxin was found by immunological methods to be the predominant VT in *E. coli* O157 isolates associated with illness in North America, with 38 of 42 strains tested producing this toxin, 20 in combination with VT1. VT from strain 3657 is immunologically identical to the described Shiga-like toxin II (VT2) of *E. coli* strains (from the United States) K-12(pEB1) and C600(933W) but only partially related to VT of strain E32511 (from the United Kingdom), the first to be named VT2.

In 1982, three outbreaks of hemorrhagic colitis (HC) caused by *Escherichia coli* serogroup O157:H7 occurred in North America, at fast-food restaurants in Oregon and Michigan and a nursing home in Ontario, Canada (23, 25, 28). Prior to these outbreaks, this serotype was rarely encountered (28; W. M. Johnson, H. Lior, and G. S. Bezanson, Letter, Lancet **i**:76, 1983), but since 1982, its isolation from sporadic cases and outbreaks has increased steadily in both countries (4, 22). Food-borne transmission is suspected in most cases, and dairy cattle are considered to be a reservoir (A. A. Borczyk, M. A. Karmali, H. Lior, and L. M. C. Duncan, Letter, Lancet **i**:98, 1987; 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). Persons in all age groups are at risk, but the young and the elderly are more prone to serious complications, such as hemolytic uremic syndrome, and death. The responsible agent of illness is thought to be one or more cytotoxins which are produced in high titers by strains of *E. coli* O157:H7. Konowalchuk et al. (9) in 1977 reported that culture filtrates from several strains of enteropathogenic *E. coli* induced a distinctive cytotoxic effect on Vero cells. Three different cytotoxins (verotoxins [VTs]) were distinguished based on molecular weight, isoelectric point, and immunology; VT of enteropathogenic *E. coli* O26:H11 strains H30 and H19 was found in 8 of the 10 positive *E. coli* strains (8). In 1982 and 1983, O'Brien and co-workers (14, 15) reported that the cytotoxin produced by strain H30 was similar to toxin produced by *Shigella dysenteriae* 1 in structure and biological activity and that its effect on HeLa cells was neutralized by antiserum against Shiga toxin. Scotland et al. (S. M. Scotland, H. R. Smith, G. A. Willshaw, and B. Rowe, Letter, Lancet **ii**:216, 1983) subsequently reported that the toxin was bacteriophage mediated. Johnson et al. (Lancet **i**:76, 1983) were the first to report that VT was produced by isolates of *E. coli* O157:H7. Scotland et al. (S. M. Scotland, H. R. Smith, and B. Rowe, Letter, Lancet **ii**:885–886, 1985) reported that six *E. coli* O157:H7 or O157:H− strains isolated from patients with HC or hemolytic uremic syndrome in the United Kingdom produced a VT not neutralized by antiserum to Shiga toxin and proposed the names VT1 (for that in *E. coli* O26:H11 strain H19 and *S. dysenteriae* 1) and VT2 (for that produced by *E. coli* O157: H− strain E32511). Strockbine et al. (27) in 1986 reported that *E. coli* O157:H7 strain 933 contained two distinct phages each coding for a different cytotoxin and proposed the names Shiga-like toxin (SLT) I (for that in *S. dysenteriae* 1) and SLTII (for the immunologically distinct cytotoxin). (Antiserum against the latter toxin neutralized cytotoxic activity of *E. coli* E32511.)

Although *E. coli* serogroup O157:H7 has been the causative agent in HC outbreaks, other serotypes have been implicated in sporadic cases of HC and hemolytic uremic syndrome (1, 6). Karmali et al. (6) isolated six different serotypes, including O157, from children with hemolytic uremic syndrome; all isolates were strong VT producers. A supply of antibody against VT1 (SLTI) is now available with the release of the anti-VT1 hybridoma 13C4 (26) to laboratories working with VT. A second cytotoxin is known to be produced in many strains of O157 in high titer (27; Scotland et al., Lancet **ii**:885–886, 1985). To detect this second toxin in North American strains, purification and antiserum production were performed with *E. coli* O157:H7 strain 3657 as the source, which appeared by immunological methods to produce VT unrelated to VT1.

MATERIALS AND METHODS

Toxin production. *E. coli* serogroup O157 strains associated with illness in North America were from collections maintained at our facility and those obtained from G. K. Morris, N. A. Strockbine, and C. A. Bopp, Centers for Disease Control, Atlanta, Ga.; H. Lior, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada; C. H. Pai, University of Calgary, Calgary, Alberta, Canada; S. M. Scotland, Central Public Health Laboratory, London,

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United Kingdom, via M. A. Karmali, Hospital for Sick Children, Toronto, Ontario, Canada; and M. P. Doyle, University of Wisconsin–Madison, Madison. Strains were grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) for 24 h at 35°C and centrifuged (20,000 × *g* for 30 min), and the supernatants were filtered through 0.22-μm-pore-size membranes (Syrfil-MF; Nuclepore Canada, Inc., Toronto, Ontario, Canada). *E. coli* O157:H7 strain 3657 was isolated from a patient with HC and selected for VT purification since it produced a VT in high titer which was not neutralized by anti-VT1 monoclonal antibody 13C4 (26). For production and purification of the VT, bacteria were grown for 48 h at 35°C with agitation (140 rpm) in 1-liter flasks containing 250 ml of tryptic soy broth. Batches of 1 liter were handled separately. The culture filtrate (crude toxin) was freeze-dried for storage until purified.

Copper ion chelate affinity chromatography. For binding of VT to gel-immobilized copper ion, we chose the batchwise technique because it is more rapid than the column technique. Furthermore, high-capacity gels might clog in a column procedure. Chelating Sepharose 6B (Pharmacia, Uppsala, Sweden) was converted to the copper chelate form by equilibrating 25 ml of the settled gel in a sinter with aqueous $CuSO_4 \cdot 5H_2O$ (5 mg/ml). Buffers used were either 0.05 M sodium dibasic phosphate containing 0.5 M sodium chloride (buffer A) or 0.05 M sodium phosphate (pH 6.5) containing 0.5 M sodium chloride (buffer B). After being washed with 10 volumes of distilled water followed by 10 volumes of buffer A, the charged gel was transferred to a solution of protein in a flask and the mixture was shaken gently for 3 h at 10°C or, for the sake of convenience, overnight. The pH of the medium was sufficiently high (7.5 to 8.0) to ensure that mass action would allow the copper to compete with protons bound to ligand atoms. Following extensive washing with the phosphate-saline buffer, the gel was packed into a 1.0-cm-diameter glass column to a height of about 33 cm. Uncharged gel was layered on top to a height of several centimeters to remove trace amounts of copper from the eluate during development by upward flow of buffer through the column. After a preliminary wash with 50 ml of buffer B, the adsorbed proteins eluted with a linear gradient of from 0 to 0.05 M imidazole in buffer B (total volume, 500 ml). Fractions (3 ml each) were collected every 30 min at 10°C. Between experiments, the column was freed of copper by being washed with 0.05 M EDTA (pH 7.0) containing 1 M sodium chloride and then extensively with distilled water before the gel was reloaded with copper. The optical density of the fractions was monitored at 278 nm. Protein concentration was determined spectrophotometrically by the formula of Layne (11).

Gel filtration and anion-exchange chromatography. Gel filtration and anion-exchange chromatography were done in prepacked glass columns of Superose 12 and Mono Q HR 10/10, respectively, with the automated fast protein, polypeptide, and polynucleotide liquid chromatography (FPLC) system according to the recommendations of the manufacturer (Pharmacia). For anion-exchange chromatography of partially purified VT, the sample was first desalted in a small disposable column of Sephadex G-25M (Pharmacia) which was previously equilibrated with 20 mM bis-Tris hydrochloride (pH 6.46) (start buffer). The conditions of ion-exchange chromatography were as follows: buffer 1 (start buffer); buffer 2, 1 M sodium chloride in start buffer; concentration change of chloride ion, 17.5 mM/ml of gradient volume at a flow rate of 1 ml/min.

PAGE and electrofocusing. Polyacrylamide gel electrophoresis (PAGE) and electrofocusing were performed on slab gels by the PhastSystem (Pharmacia) following the instructions and standards of the manufacturer. PhastGel IEF 3-9 was used for isoelectric point determination in a pH range of 3 to 9. For sodium dodecyl sulfate (SDS)-PAGE, SDS buffer strips with PhastGel gradient 10-15 were used for a molecular weight range of 10,000 to 250,000. For denaturation of toxin, samples were mixed 1:1 with a disruption buffer of 10 mM Tris hydrochloride (pH 8.0), 1.0 mM EDTA, 5% β-mercaptoethanol, and 2.5% SDS and heated at 100°C for 5 min.

Vero cell culture and cytotoxin assay. Vero cells, purchased originally from the American Type Culture Collection, Rockville, Md., were cultured in medium 199 (GIBCO Canada, Burlington, Ontario) supplemented with 4% fetal bovine serum (GIBCO). For assay of toxin, cells were seeded at 1.5×10^5/ml of culture medium in either 48-well Costar dishes (3548; Johns Scientific, Toronto, Ontario, Canada) at 0.5 ml per well or 96-well Falcon dishes (3072; Becton Dickinson Canada Inc., Mississauga, Ontario, Canada) at 0.1 ml per well. Samples for assay were added to freshly seeded cells at 0.05 ml per well in the 48-well dish or 0.01 ml per well in the 96-well dish. (Comparative experiments indicated that the two systems yielded similar results.) Cultures were incubated at 37°C for 3 days and observed microscopically for cytopathic effect. One 50% cytotoxic dose (CD_{50} unit) was defined as the amount of VT activity that caused cytopathic effect in 50% of a Vero monolayer.

Assay of lethal activity. Twofold dilutions of 0.1-ml samples of purified VT were injected intraperitoneally into BALB/c mice (weighing about 20 g). Animals were observed for 7 days, and the numbers that died between days 2 and 7 were recorded. The 50% lethal dose was determined by the method of Reed and Muench (20) with groups of three to five mice.

Antisera and neutralization tests. Three adult albino rabbits were each immunized with purified VT as follows. A 16.7-μg portion in 0.5 ml mixed 1:1 with Freund complete adjuvant (Difco) was administered subcutaneously at multiple sites on day 0 and with Freund incomplete adjuvant at days 7 and 21, followed by up to seven weekly intravenous inoculations without adjuvant. A serum dilution of 1:300 neutralized 30 CD_{50} units of VT from strain 3657.

The hybridoma cell line 13C4 which secretes monoclonal antibody (MAb 13C4) to SLTI (VT1) of *E. coli* H30 (26) was kindly provided by A. D. O'Brien, Bethesda, Md. Mouse ascites fluid was prepared as a source of antibody. A dilution of 1:300 neutralized 30 CD_{50} units of VT1 (culture filtrate of *E. coli* H30 [9]). Strains C600(933W) (27) and K-12(pEB1), which produce SLTII (VT2), and reference rabbit antiserum against toxin from the latter strain were kindly provided by N. A. Strockbine and I. K. Wachsmuth, Centers for Disease Control, Atlanta, Ga. Antiserum diluted 1:800 neutralized 30 CD_{50} units of VT from strain K-12(pEB1).

Neutralization tests were done by mixing equal volumes of VT CD_{50} units (as indicated) with rabbit antiserum against 3657 VT (1:100) or reference rabbit antiserum against VT2 (1:300) with or without MAb 13C4 (1:100) and incubating the mixture for 2 h at 37°C before adding Vero cells.

RESULTS

Freeze-dried crude VT (1.3×10^9 CD_{50} units) from 1 liter of *E. coli* 3657 culture medium was reconstituted in 80 ml of distilled water, and 40-ml portions were applied to gel-

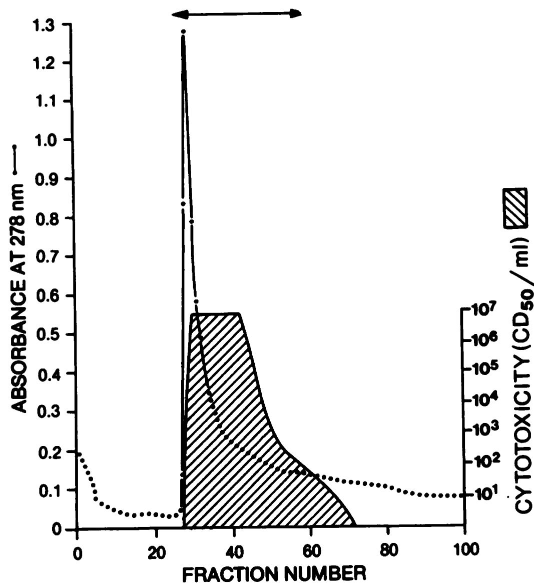

FIG. 1. Copper ion chelate affinity chromatography of *E. coli* 3657 culture filtrate. The bar indicates the fractions collected for further purification.

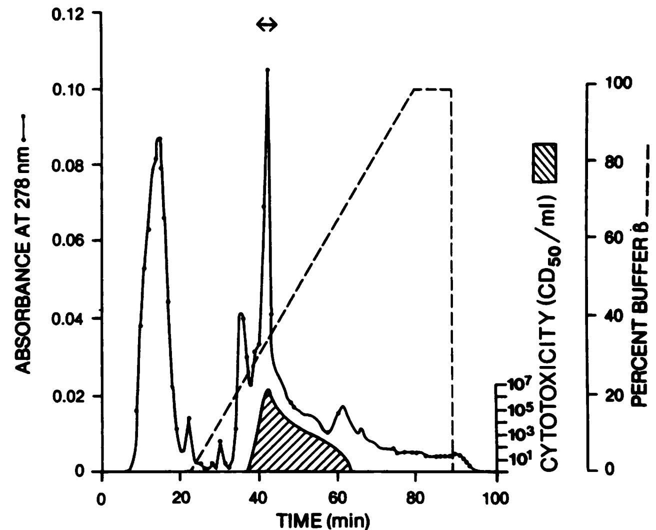

FIG. 2. Anion-exchange chromatography on Mono Q of active fractions obtained from the copper ion chelate column (Fig. 1). The bar indicates the zone collected for further study.

immobilized copper ion as described in Materials and Methods. After buffer B was used to wash the gel following application of sample, bound material was eluted by use of an imidazole gradient at constant high ionic strength (Fig. 1). Elution curves from gel-immobilized copper ion had sharp leading edges and long trailing edges, indicating that some high-affinity interactions were not fully disrupted. VT activity was as great in fractions 31 to 40 along the trailing edge as in the peak (fractions 28 to 31); activity gradually declined to insignificance at fraction 50. The recovery of cytotoxic activity at this purification step averaged 43% (38 to 47%). The toxic material eluted with imidazole was transferred to small disposable columns of Sephadex G-25M to remove salts and imidazole. The high-molecular-weight material was then further purified by anion-exchange chromatography. The recovery of toxic activity at this step (Fig. 2) averaged 31% (23 to 40%), with the overall recovery being 13%. The purity of the final preparation was assessed by isoelectric focusing in polyacrylamide gel (130 ng of protein applied), which revealed a single band by silver staining corresponding to a pI value close to pH 5.0.

When toxin from the first step in purification was analyzed by sucrose density gradient isoelectric focusing (21), the zone of pH 5.0 contained the peak of toxin activity. Gel filtration on Superose 12 revealed the toxin to have a molecular weight of about 60,000. With SDS-PAGE, neither Coomassie blue nor silver staining (as recommended for the PhastSystem by the manufacturer) was sufficient to detect resolved bands. By using the former stain followed by a modification of the latter, copper ion chelate chromatography fractions pooled from the relatively pure trailing edge (Fig. 1, fractions 31 to 40, 485 ng applied) showed weak staining at molecular weights of 33,000 and 22,000, but the bulk of material travelled with the buffer front and had a molecular weight of ≤10,000 (Fig. 3, lane A). Only the latter material was detectable after anion-exchange chromatography (130 ng applied) (Fig. 3, lane B). Specific activity on Vero cells after anion-exchange chromatography was 1.9 × 10^9 CD_{50} units/mg of protein (0.54 pg for 1.5 × 10^4 cells). The 50% lethal dose for mice was 14 ng. Subcutaneous and intravenous injections of 16.5 μg were nonlethal and antigenic for rabbits.

Rabbit anti-VT of strain 3657 (1:100) or reference rabbit anti-VT2 of strain K-12(pEB1) (1:300) neutralized ≤256 CD_{50} units of VT from culture filtrates of strain 3657 and reference VT2 strains K-12(pEB1) and C600(933W) from the United States. Neutralization titers were not enhanced by the addition of anti-VT1 (MAb 13C4) (1:100), which neutralized ≤128 CD_{50} units of VT1 from the culture filtrate of strain H30.

Culture filtrates of 42 strains of *E. coli* serogroup O157 (39 were O157:H7 and 3 were O157:H−) associated with illness in North America were tested for VT activity; all had CD_{50} titers of 1,000 to 20,000 (Table 1). When 128 CD_{50} units were tested for neutralization with mouse ascites fluid against VT1 and rabbit antiserum against VT of strain 3657 or VT2 of strain K-12(pEB1), 38 of the 42 isolates were found to

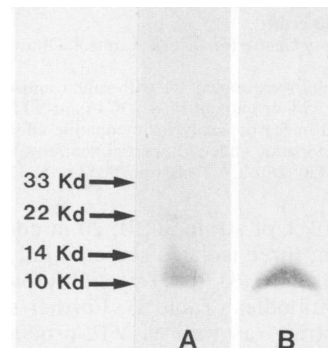

FIG. 3. SDS-PAGE molecular weight determination after Coomassie blue followed by silver staining of copper ion chelate chromatography-purified VT of *E. coli* 3657 fractions 31 to 40 (Fig. 1), 485 ng of protein applied (lane A) and anion-exchange chromatography fraction 42 (Fig. 2), 130 ng of protein applied (lane B). A line was drawn freehand through several standard points, and the molecular weight was estimated by interpolation. Kd, Kilodaltons.

TABLE 1. Type of VT found in culture filtrates of North American strains of *E. coli* serogroup O157

Serotype and strain	Origin[a]	Source[b]	CD_{50} titer in Vero cells (10^3)	Neutralization with antibodies against[c]:			VT type[d]
				VT1	3657 VT or K-12(pEB1) VT2	Both VT1 and VT2	
O157:H7							
ACH5	Alberta, 1982 or 1983	Pai	1	+	−	+	1
3124-85	Massachusetts, 1985 (1)	CDC	2	+	−	+	1
O59	Alberta, 1983	LCDC	20	−	+	+	2
670	Newfoundland, 1983	LCDC	20	−	+	+	2
756	Quebec, 1983	LCDC	10	−	+	+	2
794	United States, 1983	CDC	20	−	+	+	2
1032	Alberta, 1983	LCDC	10	−	+	+	2
1340	British Columbia, 1983	HPB	20	−	+	+	2
1948	United States, 1982	CDC	10	−	+	+	2
2027	Ontario	LCDC	10	−	+	+	2
2035	Ontario	LCDC	10	−	+	+	2
5683	United States, 1986	LCDC	20	−	+	+	2
5684	United States, 1986	LCDC	20	−	+	+	2
5685	United States, 1986	LCDC	20	−	+	+	2
A39	Ontario, 1986	HPB	20	−	+	+	2
A43	Ontario, 1986	HPB	20	−	+	+	2
H3	Ontario, 1986	HPB	20	−	+	+	2
H16	Ontario, 1986	HPB	20	−	+	+	2
O60	Oregon, 1983	LCDC	20	−	P	+	1 + 2
O62	Michigan, 1983	LCDC	20	−	P	+	1 + 2
932	(16, 17)	Doyle	2	−	−	+	1 + 2
1083	United States, 1983	CDC	20	−	P	+	1 + 2
1091	United States, 1983	CDC	4	−	P	+	1 + 2
1118	Alberta, 1983	LCDC	1	−	P	+	1 + 2
1122	Alberta, 1983	LCDC	20	−	P	+	1 + 2
1215	United States, 1983	CDC	10	−	P	+	1 + 2
1668	United States, 1982	CDC	4	−	P	+	1 + 2
1669	United States, 1982	CDC	20	−	−	+	1 + 2
1883	United States, 1982	CDC	1	−	−	+	1 + 2
2011	Ontario, 1983	LCDC	1	−	−	+	1 + 2
2018	Ontario	LCDC	10	−	P	+	1 + 2
2030	Ontario, 1982	LCDC	4	−	−	+	1 + 2
2163	Ontario, 1982	LCDC	4	−	P	+	1 + 2
19386	Not recorded	HPB	20	−	P	+	1 + 2
161-84	Ohio, 1982 (1)	CDC	4	−	−	+	1 + 2
1011-84	Maine, 1984 (1)	CDC	4	−	−	+	1 + 2
FRQ5	British Columbia, 1987	HPB	10	−	P	+	1 + 2
FRQ5-2	British Columbia, 1987	HPB	10	−	P	+	1 + 2
3174-87	Texas, 1987 (1)	CDC	2	−	P	P	?
O157:H−							
3123-85	North Dakota, 1985 (1)	CDC	1	+	−	+	1
3199-85	Massachusetts, 1985 (1)	CDC	1	−	+	+	2
3344-85	Maryland, 1985 (1)	CDC	4	−	+	+	2

[a] All isolates were from human feces except O62, FRQ5, and FRQ5-2 from meat and A39 and A43 from cattle feces. Numbers in parentheses indicate references where the strain is described.
[b] LCDC, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada; CDC, Centers for Disease Control, Atlanta, Ga.; HPB, Health Protection Branch, our facility.
[c] 128 CD_{50} VT units were mixed 1:1 with rabbit antiserum against VT of strain 3657 (1:100) or reference rabbit antiserum against SLTII (VT2) of strain K-12(pEB1) (1:300), with or without MAb 13C4 (anti-VT1) (1:100), and incubated for 2 h at 37°C before the addition of Vero cells. Cultures were incubated for 3 days and observed microscopically for cytopathic effect. −, No neutralization (all cells with cytopathic effect, as controls without antisera); +, complete neutralization (no cytopathic effect); P, partial neutralization (some but not all cells with cytopathic effect).
[d] 1, VT of strain H30 (26); 2, VT of strain 3657 or K-12(pEB1).

secrete VT2 (or VT of strain 3657), 20 in combination with VT1. In addition, three isolates secreted only VT1, and VT activity of one isolate (3174-87) was only partially neutralized by these antibodies (Table 1). Further studies showed that the latter strain, as well as VT2-producing prototype strain E32511 from the United Kingdom, were notably different from the others in this study in that only 2 CD_{50} units of toxin were completely neutralized by anti-VT of strain 3657 with or without anti-VT1 (Table 2). A fourfold concentration of antiserum neutralized 8 CD_{50} units of the two strains but 1,024 CD_{50} units of strains 3657, K-12(pEB1), and C600(933W).

DISCUSSION

A number of workers have described *E. coli* cytotoxins that are either immunologically related or unrelated to Shiga toxin, but all share similar biological properties such as lethality to mice in low doses (nanograms) and cytotoxicity to Vero and HeLa cells in the low picogram range. No apparent discrepancies exist to date with the characterization within the former group of toxins found in serotypes O26:H11 and O157:H7 (7, 13, 14, 19; A. D. O'Brien, T. A. Lively, T. W. Chang, and S. L. Gorbach, Letter, Lancet **ii**:573, 1983). Isoelectric points from 6.7 to 7.2 and additional

TABLE 2. Neutralization of VT secreted by *E. coli* strains with antiserum against VT of strain 3657

Strain	Dilution of anti-VT of strain 3657	Neutralization of cytopathic effect on Vero cells after incubation of antiserum with the following CD_{50} units[a]:										
		1	2	4	8	16	32	64	128	256	512	1,024
3174-87 or E32511	1:100	+	+	P	P	P	P	P	–	–	–	–
	1:25	+	+	+	+	P	P	P	P	P	–	–
3657 or K-12(pEB1) or C600(933W)	1:100	+	+	+	+	+	+	+	+	+	P	P
	1:25	+	+	+	+	+	+	+	+	+	+	+

[a] CD_{50} units were mixed 1:1 with rabbit antiserum against VT of strain 3657 and incubated for 2 h at 37°C before the addition of Vero cells. Cultures were incubated for 3 days and observed microscopically for cytopathic effects. Code is described in Table 1, footnote *c*.

properties indicate that the toxin is similar to Shiga toxin (14). These toxins are now known as VT1 (SLTI) (27; Scotland et al., Lancet **ii**:885–886, 1985). With the cytotoxins that are immunologically unrelated to Shiga toxin, differences in characteristics were seen within serotype O157. For strain E32511, the isoelectric point, molecular weight, and specific activity on cultured cells (3) differed markedly from those of other reported VTs or SLTs (distinct from VT1) (Table 3). For this strain, as well as strains J-2 (29), K-12(pEB1) (2), and 3657, the toxin consisted of subunits. With strain E32511, the major (or more darkly stained) band on SDS-PAGE was the A subunit with a molecular weight of 35,000; with strains J-2, K-12(pEB1), and 3657, the major band was the B subunit of about 10,000 molecular weight. Padhye et al. (16, 17), however, reported that a VT of strain 932 lacked subunits.

The toxins from strains K-12(pEB1), 3657, and C600(933W) (the first to be called SLTII [27]) appeared to be similar immunologically. Antisera against the first two VTs neutralized toxin activity of strain 932 if anti-VT1 was included, but they were 100-fold less effective in neutralizing VT activity of strain E32511, with or without anti-VT1. The results suggest an immunological variant or the presence of an additional toxin (other than VT1 and VT2). The latter possibility seems unlikely since complete neutralization was achieved either with a comparatively low CD_{50} level or at a higher CD_{50} level with a greater concentration of antiserum. The presence of an additional immunologically unrelated toxin should not be influenced by varying concentrations of antiserum to VT2. It appears more likely that the VT of strain E32511 is related to VT of strains 3657 and K-12(pEB1) but is less efficiently neutralized by their antisera.

The VT of strain 3657 [or VT2 of strain K-12(pEB1)] appears to be the predominant cytotoxin in North American strains of *E. coli* serogroup O157. Of the isolates examined, 91% produced this toxin, while 55% produced VT1. Of the isolates, 50% appeared to secrete only one of these toxins, but relatively low levels of a second toxin may go undetected, i.e., our assay method would not detect the minor toxin with CD_{50} ratios greater than 1 to 128. It is interesting, and in our plans, to compare these results with those obtained with DNA probes for the toxins. One of the isolates examined, like strain E32511, was only partially related immunologically to the above toxins. All isolates produced VT titers of at least 1,000 and therefore may be considered moderate to high producers (12).

In a number of human diarrheal outbreaks caused by *E. coli* serogroup O157:H7, improperly cooked ground beef and raw milk have been implicated (10, 18, 23, 24, 28; Borczyk et al., Lancet **i**:98, 1987; Martin et al., Lancet **ii**:1043, 1986); healthy dairy cattle are a suspected reservoir for this serotype. Two isolates of O157:H7 used in our study were from feces of healthy cattle that were collected at a dairy farm in Ontario, following an outbreak of gastroenteritis caused by this serotype among visiting kindergarten children who consumed unpasteurized milk (Borczyk et al., Lancet **i**:98, 1987). Three other isolates used in our study originated from meat (beef) implicated in illness (23, 28). In addition to *E. coli* O157, several other serotypes that have been isolated from calves or implicated in human illness have also been shown to produce VT1 or VT immunologically unrelated to VT1 (5, 6, 9, 12; M. A. Karmali, M. Petric, S. Louie, and R. Cheung, Lancet **i**:164–165, 1986). Antisera to purified toxins will be useful for detecting VT in food and clinical specimens

TABLE 3. Characteristics of VTs (SLTs) unrelated immunologically to VT1

Strain	Isoelectric point	Mol wt by:		Sp act/mg of protein (cell type)	Reference
		SDS-PAGE	Other methods		
E32511	6.5	35,000 <14,000	42,000[a]	1.4×10^6 (Vero)	3
K-12(pEB1)	5.2	32,000 (A) 25,000 (A1) 10,200 (B)		9.1×10^8 (HeLa)	2
932	5.2	64,000	54,000[b]	4.0×10^7 (Vero)[d]	16, 17
J-2	4.1	35,000 (A) 10,700 (B)		1.3×10^9 (Vero)[d]	29
3657	5.0	33,000 22,000 ≤10,000	60,000[c]	1.9×10^9 (Vero)	This report

[a] Nonreducing, nondenaturing PAGE.
[b] Sephacryl S-200 Superfine.
[c] Gel filtration on Superose 12.
[d] Calculated from CD_{50} values reported by authors.

and also for determining relationships among the various *E. coli* cytotoxins.

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