gand binding data indicating differences among CCK receptors in various tissues, as well as to delineate the peripheral and central nervous system actions of CCK. The therapeutic utilities of potent antagonists, such as asperlicin, in CCK-related disorders of the gastrointestinal system remain to be determined.

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19. The animals were prepared for recording of gallbladder contractions in vivo as described by J. W. Fara and S. M. Erde [*Eur. J. Pharmacol.* **47**, 359 (1978)]. When administered intravenously, asperlicin was dissolved in 100 percent DMSO and administered in a volume of 0.1 ml per 100 g of body weight. Control studies demonstrated the reproducibility of cumulative dose-response curves to CCK-8 determined in the same animals before, 1, 3, and 4 hours after injection of vehicle.
20. For intraperitoneal administration, asperlicin was suspended in 0.5 percent methyl cellulose. In these studies the control CCK-8 ED_{50} was 5.5 (4.6 to 6.6) ng/kg injected intravenously (n = 5). The CCK-8 ED_{50} in the same animals 1 and 3 hours after asperlicin (30 mg/kg injected intraperitoneally) were 31.5 (23.3 to 42.6) and 33.5 (26.6 to 42.1) ng/kg (injected intravenously), respectively.
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Cloning of Shiga-Like Toxin Structural Genes from a Toxin Converting Phage of *Escherichia coli*

Abstract. *The genes controlling high-level production of Shiga-like toxin (SLT) in* Escherichia coli *were cloned from the SLT converting phage 933J. This phage was isolated from a strain of* E. coli *that caused a foodborne outbreak of hemorrhagic colitis. The genes that convert normal* E. coli *to organisms producing high levels of toxin were cloned into the plasmid pBR328 and expressed in* E. coli *HB101. DNA restriction mapping, subcloning, examination of the cloned gene products by minicell analysis, neutralization, and immunoprecipitation with antibodies to SLT were used to localize the toxin converting genes and identify them as structural genes for SLT. Southern hybridization studies established that the DNA fragment carrying the cloned toxin structural genes had homology with the DNA of* Shigella.

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Two Shiga-like toxin (SLT) converting phages, 933J and H19A/J, were isolated from *Escherichia coli* strains responsible for hemorrhagic colitis and infantile diarrhea, respectively (*1*). These two phages are members of a family of SLT converting phages that exist in *E. coli* strains in nature (*1–3*). The well-studied laboratory strain *E. coli* K12 makes small amounts of SLT (≤ 10^2 doses that have 50 percent cytotoxicity per milligram of protein in cell lysates) (*4, 5*) and is susceptible to infection by these phages. Lysogenization of *E. coli* K12 with phage 933J or phage H19A/J causes a 10,000-fold increase in the amount of SLT produced (*1*). These findings suggest that the toxin converting phages may either contain structural genes for SLT or regulatory elements that enhance the expression of structural genes for SLT in *E. coli* K12. We cloned the SLT converting genes from phage 933J, a phage isolated from *E. coli* 0157:H7 strain 933, examined the products of the cloned genes by minicell analysis, and demonstrated by neutralization and immunoprecipitation with antibodies that the products of the cloned genes expressed antigenic determinants of SLT. DNA-DNA hybridization studies showed that the cloned sequences encoding SLT were homologous to DNA sequences in Shiga toxin–producing strains of shigellae.

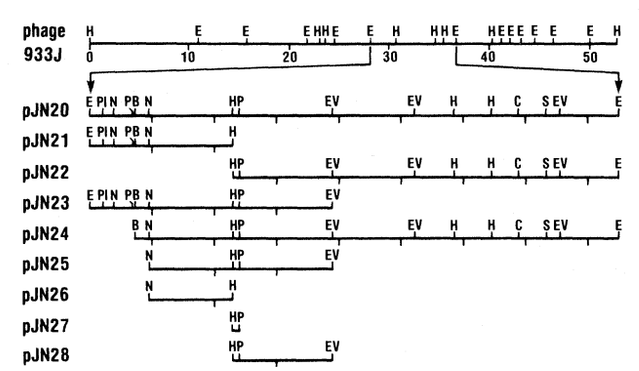

Fig. 1. Restriction endonuclease cleavage maps of phage 933J DNA and of DNA fragments subcloned into vector pBR328 to form plasmids pJN20 to pJN28. The circular map of phage 933J is displayed in a linear fashion and is arbitrarily broken between the two largest Hind III fragments. The molecular size of phage 933J is indicated in kilobases (kb) and increments of 1 kb are indicated below the maps for the cloned inserts. The toxinogenicity of *E. coli* strains lysogenic for phage 933J or containing each of the plasmids is indicated on the right. Toxin production by *E. coli* HB101 (pJN25) was seven times that of *E. coli* strain 933. Restriction enzymes are designated E, Eco RI; PI, Pst I; N, Nco I; P, Pvu II; B, Bam HI; H, Hind III; EV, Eco RV; C, Cla I; and S, Sma I.

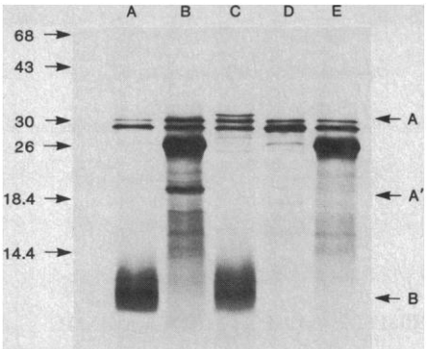

Fig. 2. Autoradiograph of the [^{35}S]methionine-labeled polypeptides synthesized in *E. coli* DS410 minicells containing the following hybrid plasmids: (lane A) pJN26, (lane B) pJN28, (lane C) pJN25, (lane D) pJN27, and (lane E) pBR328. Molecular masses of selected polypeptide standards (in kilodaltons) are indicated by the numbers and arrows on the left. Arrows on the right point to polypeptides not encoded by the pBR328 cloning vector.

Purification of phage 933J and its DNA has been described (*1*). Restriction endonuclease cleavage of the phage DNA by Eco RI or Hind III generated twelve and seven fragments, respectively (see Fig. 1). Eco RI–digested phage DNA was ligated into Eco RI–digested and alkaline phosphate–treated DNA of the vector pBR328, and the hybrid plasmids were transformed into *E. coli* HB101. Eleven of the twelve original fragments were present in hybrid plasmids that contained one or more of the Eco RI DNA fragments from 933J. Initial cloning experiments were conducted under P4 + EK1 containment, and all subsequent work with toxin-positive clones of *E. coli* HB101 was done under P3 + EK1 containment (*6*). *Escherichia coli* HB101 transformants containing cloned 933J phage DNA were incubated overnight in Luria broth. The culture supernatants were then diluted 1:250 and screened for cytotoxicity by testing 100-μl samples on HeLa cells. Negative controls for the HeLa cell assay included supernatants from *E. coli* HB101, and positive controls included supernatants from the parent strain, *E. coli* 933. The second largest (~ 8.5 kb) Eco RI fragment from phage 933J, which was present in all of the original transformants producing high levels of toxin, was cloned into vector pBR328 to construct the hybrid plasmid pJN20 (Fig. 1).

To identify more precisely the physical location of the SLT converting genes in pJN20, we constructed a series of subclones and examined their toxin phenotypes (Fig. 1). Only subclones that contained the segment of DNA flanking the leftmost Hind III restriction site gave positive results for toxin. The toxin was confirmed to be SLT by neutralizing it with hyperimmune rabbit antiserum to purified Shiga toxin. Selected subclones pJN25, pJN26, pJN27, and pJN28 were introduced into *E. coli* minicells, and the polypeptides they encoded were analyzed (Fig. 2) (*7*). The toxin-positive subclone pJN25 (lane C) encoded two polypeptides of approximately 31.5 kilodaltons (kD) and 7 kD that corresponded to the molecular mass of the A and B subunits of Shiga toxin (*8*) or SLT (*9*). Subclone pJN26 (lane A) contained the region of DNA extending leftward from the Hind III restriction site and encoded the 7-kD polypeptide corresponding to the B subunit of SLT, but not the 31.5-kD polypeptide corresponding to the A subunit. In contrast, subclone pJN28 (lane B) contained the region of DNA that extended rightward from the Hind III site and encoded a 20-kD polypeptide but not the 31.5- or 7-kD polypeptides. This 20-kD protein (A′) is probably a truncated polypeptide corresponding to the amino terminal portion of the 31.5-kD A subunit of SLT. This indicates that the orientation of transcription of the A subunit of the SLT gene is from right to left on the map in Fig. 1. The orientation of transcription of the B subunit has not yet been determined. Subclone pJN27 (lane D) contained a small insert (≤100 base pairs) that should correspond to an internal fragment of the structural gene for the A subunit and did not encode a unique polypeptide.

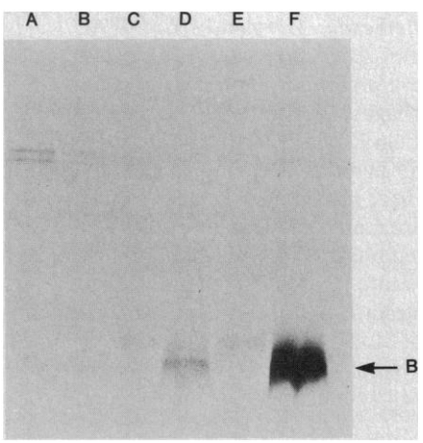

Fig. 3. Immunoprecipitation by monoclonal antibodies of [^{35}S]methionine-labeled polypeptides in minicell lysates. Immunoprecipitations with *Staphylococcus aureus* protein A were done essentially as described by Kessler (*12*) and analyzed by autoradiography on an SDS gel containing 15 percent polyacrylamide (*13*). Lanes B, D, and F contain polypeptides from minicells carrying pJN27, pJN25, and pJN26 that were immunoprecipitated by 16E6, a monoclonal antibody against the B subunit of SLT (*14*). Lanes D and F show that the 7-kD peptide of SLT encoded by pJN25 and pJN26 was immunoprecipitated, whereas no proteins were precipitated from the lysate of clone pJN27 in lane B. Lanes A, C, and E represent control experiments demonstrating that polypeptides from minicells carrying pJN27, pJN25, and pJN26 were not immunoprecipitated by 32D3, a monoclonal antibody against the B subunit of cholera toxin (*15*).

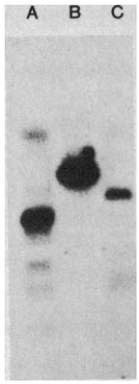

Fig. 4. Autoradiograph of ^{32}P-labeled pJN25 DNA hybridized to Southern blots of Eco RI–digested DNA from the following: (lane A) *Shigella dysenteriae*, (lane B) phage 933J, and (lane C) *S. flexneri*. The estimated molecular sizes of the restriction fragments producing the strongest reactions are (lane A) 4.5 kb, (lane B) 8.5 kb, and (lane C) 6.0 kb. Hybridizations were performed at 65°C in an aqueous system (*16, 17*).

Polypeptides of 28 kD and 30 kD corresponding to the products of the ampicillin resistance genes of the vector were present in all lanes (Fig. 2). The vector pBR328 (lane E) and subclone pJN28 (lane B) also code for the 26-kD chloramphenicol transacetylase polypeptide (*10*). We showed that the 7-kD polypeptide produced in minicells containing pJN25 or pJN26 could be immunoprecipitated with a monoclonal antibody designated 16E6, which was specific for the B subunit of SLT (Fig. 3). The 31.5-kD polypeptide produced in minicells containing pJN25 and released by treatment with sodium dodecyl sulfate (SDS) was not immunoprecipitated by polyclonal antiserum to SLT or Shiga toxin, presumably because the antibodies were directed against determinants of native toxin that were not expressed on the denatured A polypeptide. Monoclonal antibodies to the A subunit of SLT have not yet been described. No proteins were precipitated by monoclonal antibody 16E6 from extracts of minicells containing pJN27.

We also examined the relation between the insert in pJN25 and genes present in a total DNA extract from *Shigella*. ^{32}P-Labeled pJN25 DNA was hybridized in Southern blots with Eco RI–digested DNA from *S. dysenteriae* and *S. flexneri* and with Eco RI–digested DNA from phage 933J (Fig. 4). The probe was specific for a unique restriction fragment in the DNA from phage 933J and reacted strongly with only one DNA fragment from both *S. dysenteriae* 1 and *S. flexneri*. These results suggest that the SLT structural genes in pJN25 are homologous to the structural genes for Shiga toxin in *Shigella* and hybridize with them under high stringency condi-

tions. Smaller probes composed of internal fragments from the SLT structural genes are being characterized for use in additional hybridization experiments with *Shigella*, low-toxin producing *E. coli*, and other bacteria (*11*) that produce varying amounts of SLT. From the data presented we conclude that (i) the structural genes for SLT are present in the genome of phage 933J; (ii) the structural gene for the B subunit of SLT is encoded by the insert in pJN26, and a portion of the structural gene for the A subunit of SLT is encoded by the insert in pJN28; and (iii) the SLT genes from phage 933J appear to be homologous to structural genes for Shiga toxin in *Shigella*.

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Distinct Monoamine Oxidase A and B Populations in Primate Brain

Abstract. *Monoclonal antibodies specific for monoamine oxidase (MAO) A and MAO B, respectively, were used to localize these enzymes in primate brain. The reagents recognized different populations of neurons: those that recognized MAO A were located in cell groups containing catecholamines, including the substantia nigra, nucleus locus coeruleus, nucleus subcoeruleus, and the periventricular region of the hypothalamus, whereas those that recognized MAO B were observed in serotonin regions, including the nucleus raphe dorsalis and nucleus centralis superior. These data illustrate the physiological independence of MAO A and B and show that neurons may be specialized for their degradative as well as their synthetic functions.*

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Monoamine oxidase (E.C. 1.4.3.4) occurs in two forms, designated MAO A and MAO B, which differ in substrate preferences and inhibitor specificities (*1*), apparent molecular weights (*2*), and immunological properties (*3–5*). Two highly specific mouse monoclonal antibodies, one elicited in response to human placental MAO A and one to human platelet MAO B (*4–6*), were used to identify MAO A– and MAO B–containing structures in the primate central nervous system.

Three *Macaca fascicularis* monkey brains were stained to recognize MAO B and three others MAO A. Monkeys were prepared by serial cardiac perfusion with physiological saline, 4 percent buffered paraformaldehyde, and 20 percent buffered sucrose. Tissue sections (30 μm) were incubated overnight at room temperature with mouse monoclonal MAO A-3C9 or MAO B-1C2, followed by anti-mouse immunoglobulin G (diluted 1:50) for 30 minutes, then mouse peroxidase antiperoxidase (diluted 1:100) for 30 minutes, and finally diaminobenzidine-peroxide (0.25 percent/0.01 percent). Immunocytochemical controls included omission of one of the reagents in the peroxidase-antiperoxidase protocol, dilution of the antibody, incubation with ascites fluid containing nonreactive antibody, and inhibition of staining with antigen. Specific staining was not seen with the control stains or when specific antibody was adsorbed first with antigen.

Neurons positive for MAO A were observed in amine-containing regions systematically mapped in rat brain by Dahlström and Fuxe (*7*). Neurons containing MAO A were localized in the ventrolateral medulla (A1 cell group of Dahlström and Fuxe), in the dorsal medulla in the vicinity of the solitary and dorsal motor nuclei (A2), and in cells scattered tangentially between these two groups through the medullary tegmentum. In the caudal pons, cells were positively labeled in the dorsal and lateral walls of the fourth ventricle (A4) and in the ventrolateral pontine tegmentum (A5). The most densely populated sites were the nucleus locus coeruleus (A6) (Fig. 1A) and the subcoeruleus complex in the lateral pontine tegmentum (A7), including the Kölliker-Fuse nucleus and the medial and lateral parabrachial nuclei. Preliminary double-label staining studies indicate that MAO A is localized in cells of the locus coeruleus which in human brain contain the nonadrenergic synthetic enzyme, dopamine-β-hydrolyase. The MAO A–positive neurons were also observed in the lateral tegmentum at the junction of the pons and the midbrain (A8), in the substantia nigra (A9), especially in rostral portions of the nucleus, and sparsely in the midline throughout the midbrain (A10). Under the conditions used, the number of cells containing detectable stain for MAO A in the substantia nigra did not represent all dopaminergic cells normally observed in this region (*7, 8*).

Diencephalic cells labeled for MAO A were scattered dorsally around the ventral perimeter of the mammillothalamic tract in tuberal hypothalamic regions (A13), along the entire dorsal to ventral extent of the periventricular hypothalamic region (A11), and in the arcuate nucleus at the floor of the third ventricle (A12). A few stained neurons were located anteriorly in the periventricular region of the hypothalamus (A14). Some stained fibers were also observed, particularly near catecholamine cell groups, in ascending catecholamine pathways (Fig. 1C) and in some blood vessels, suggesting that this enzyme is probably distributed throughout the projection system in which it has been localized. Another large population of MAO A–positive neurons, which could not be ascribed to any previously identified catecholamine