CRC HANDBOOK OF MICROBIOLOGY

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Volume VIII
Toxins and Enzymes

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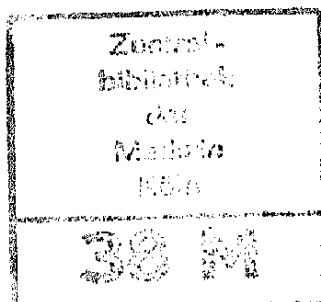Waksman Institute of Microbiology
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BACTERIAL TOXINS: DESCRIPTION

D. Michael Gill

INTRODUCTION

Bacterial exotoxins are soluble agents that, in small amounts, damage living cells or organisms. They are usually secreted and can be recovered from the culture medium or can be found at some distance from bacterial colonies on agar. The Greek origin of the word (*toxicon pharmakon*) referred to the poison used on arrow tips. Although "toxin" is used for animal and plant poisons of any chemical type, when applied to bacteria the word is used only for proteins (except for endotoxins, see elsewhere in this series). There is no accepted lower limit to the toxicity that qualifies a protein as a toxin, but as can be seen from the accompanying Table of Lethal Amounts the majority are dangerous at 100 μg/kg or less.

Classification Based on Mechanisms of Action

The degree of association with bacterial cells is no longer considered to be a useful basis for classifying the protein toxins. Classifications based on the physiological effect (e.g., enterotoxin, cardiotoxin, neurotoxin) may be useful but do not reflect common fundamental properties. With recent advances in understanding the mechanisms of damage, however, a more informative grouping can be made with respect to the type of interaction the toxin makes with target cells (or organisms). In principal a toxin might (1) pass across plasma (or vesicular) membranes and affect cytosolic metabolism, (2) bind to a transmembrane component in such a way that the toxin remains outside but a signal is generated internally, (3) alter the properties of the plasma membranes or internal membranes of cells without passing across, or (4) alter some extracellular material. It simplifies the description of individual toxins to discuss some general properties of the four groups here.

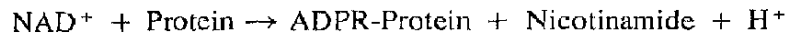### A-B Toxins Which Cross Cell Membranes

The plasma membrane is an effective barrier to the passage of proteins, but a few toxins (and bacteriocins) possess the unusual ability to pass efficiently from the extracellular space to the cytosol — the inverse of secretion. Such toxins possess separable functional components that are usually designated A and B. The B (*binding*) components bind to the exterior cell surface, and in some cases also intercalate into the phospholipid bilayer of the plasma membrane or its endocytosed derivative, to form sites at which the A components cross from the outside to the inside. The A (enzymically *active*) components are enzymes that catalyze various changes within the cytosol. Full details of the passage mechanism are usually lacking, but it is thought that the A components pass as unwound polypeptide chains that spontaneously regain their enzymically active three-dimensional configurations inside. This ability to easily regain their functional configuration means that most A components are only temporarily inactivated by denaturing conditions. (Reference 7 is a general reference on entry mechanisms.)

The various A-B toxins exhibit a variety of organizations of the A and B components.[9] Some are domains of a single polypeptide chain. Others are separate polypeptides connected by disulfide bonds or only noncovalently as subunits of multimers. A few seem to be distinct proteins that only come together on the surfaces of target cells. When proteolysis or disulfide bond reduction is necessary to separate the components in vitro, such events seem also to occur at the cell surface before, or while, the A component makes its passage.

The first three toxins listed below all inhibit protein synthesis. They modify components

of the ribosomal machinery that are different from those in eubacteria. The next five all elevate target cell cyclic AMP by means that do not operate in the parent bacteria. The selective advantage of raising cyclic AMP may be, or may have been during their evolution, that cyclic AMP diminishes phagocyte activities such as chemotaxis, ingestion, and superoxide production.[10,11] Those with asterisks all catalyse ADP-ribosylations of the type

$$NAD^+ + Protein \rightarrow ADPR\text{-}Protein + Nicotinamide + H^+$$

Examples include diphtheria toxin*, *Pseudomonas aeruginosa* exotoxin A*, Shigella toxin; cholera toxin* and other heat-labile enterotoxins*, Bordetella pertussis pertussigen* and adenylate cyclase and *Bacillus anthracis* "edema factor".

Toxins That Signal Across Cell Membranes

There is no well-established example. Candidates are the heat-stable enterotoxins (STa) of *E. coli* and other enterobacteriaceae which seem to bind to the outsides of intestinal cells and to signal an increase of guanylate cyclase activity inside the cells.

Toxins That Disrupt Cellular Membranes

Cytolysins are common. Those that lyse erythrocytes are often termed hemolysins, but this term may reflect the usual mode of detection (on blood agar plates) rather than the true role of the toxin. In most cases leukocytes are more sensitive than erythrocytes and the primary role of the toxin in pathogenesis may be to combat immune cells of the host. Among phagocytes, polymorphonuclear leukocytes are usually more sensitive than mononuclear cells.

Most cytolysins increase membrane permeability and (if lysis is important) induce lysis osmotically. The membrane first becomes permeable to ions which equilibrate, but the intracellular excess of macromolecules which cannot equilibrate generates a net influx of water that first swells, then bursts, the cell. In some cases, endocytosis of the toxin followed by disruption of lysosomal membranes may be more important than disruption of the plasma membrane. At sublytic doses of toxins, the increased flux of ions may cause damage indirectly. In particular the entry of calcium ions has profound pharmacologic consequences, including the inappropriate production of inflammatory mediators. The loss of potassium blocks protein synthesis. Too great a flux of sodium and potassium depletes ATP.

Enzymes — Many of the osmotically active cytolysins are phospholipases; that is, enzymes which remove the hydrophilic head groups from phospholipids. The most important substrates are phosphatidylcholine (lecithin) and sphingomyelin, for these tend to be the most abundant lipids of the outer leaflet of the plasma membranes. (For a review see References 12.) Examples include *Clostridium perfringens* alpha toxin and Staphylococcal beta toxin.

Nonenzymic "pore-forming" cytolysins — Other proteins that lyse osmotically are too small to be enzymes or, if large, have not been shown to act enzymically. In general these agents bind to the surface or the thickness of cell membranes and cause changes in structural organization that increase permeabilities. Examples include Staphylococcal alpha toxin, Streptolysin S and Streptolysin O.

Detergent-like cytolysins — A few agents cause direct solution of the plasma membrane. Unlike osmotic lysis, detergent lysis cannot be prevented by placing high-molecular-weight molecules in the medium. Staphylococcal delta toxin is an example of a detergent-like cytolysin.

Extracellular Enzymes

Enzymes that digest extracellular material are rarely particularly toxic in themselves but more often assist the process of infection and contribute only indirectly to tissue damage.

Hyaluronidase, and proteases such as Streptokinase and *Clostridium perfringens* kappa toxin (a collagenase) may assist bacterial spread. The elastase of *Pseudomonas aeruginosa* damages the lung. Other proteases deplete complement components or cleave immunoglobulins and may reduce bacterial clearance. Hydrolases such as amylase, NADase, or DNase, are probably only digestive in function.

Aeromonas hydrophila

Enterotoxin ("cytotonic enterotoxin") — Causes intestinal fluid accumulation. It also alters the shapes of certain tissue culture cells without cell death, but it is not related to cholera toxin or LT. Its gene has been cloned.[13] It is distinct from any lysin, and is synthesized by all strains.

Aerolysin (beta lysin, cytotoxic lysin) — Causes complete hemolysis in blood agar and kills Vero cells. Rat erythrocytes are the most sensitive. The mechanism of lysis and cytotoxicity is not clear. It is not a phospholipase. The cellular receptor may be a glycoprotein. Gangliosides inhibit lysis. Purification.[14-16] Molecular weight is ~50,000. Its gene is adjacent to a regulatory gene. Produced also by *A. sobria*.

Other lysin(s) — Most *Aeromonas* isolates produce a zone of incomplete hemolysis as well as the zone of complete hemolysis due to aerolysin. The responsible agent has been called alpha lysin[17] and hemolysin B. Culture filtrates contain a phospholipase,[15] in a complex with an acyltransferase.[18] Their relationships to alpha lysin are not clear.

Bacillus anthracis

Toxin — Three easily separated proteins, probably constituting an A-B system in which the protective antigen constitutes a common B component. Toxin is centrally important in anthrax. Physiologically the primary effects of the mixture are an increased vascular permeability and a neurotoxicity that results in central respiratory failure. It is generally cytotoxic and edema-producing. All three components are encoded on a large plasmid, loss of which generates the "Pasteur strain". (For a review, see Reference 19.) The protein called *protective antigen* (Factor II) binds to target cells. It is harmless alone and can be used as a vaccine. It is thought to act as a "B component" that assists either of the other components across a cell membrane. It may be prebound to target cells and later used to transport the other factors. Possibly because they share sites upon the protective antigen, lethal factor and edema factor antagonize the effects of each other. Molecular weight is 85,000. The *edema factor* (Factor I) is a potent adenylate cyclase that absolutely requires calcium-calmodulin for its activity[20] and is effective only inside target cells (cf. *Bordetella pertussis* cyclase). It raises internal cyclic AMP levels profoundly in many types of cell (but not all); thereby, it blocks phagocyte function. It requires the protective antigen for the effect on intact cells and tissues. Molecular weight is 89,000. The *"lethal factor"* (Factor III) also requires the protective antigen, optimally in a fivefold excess, for its effect.[21] It is not in general cytopathic, but it kills certain cells (e.g., mouse macrophages) and it causes such rapid pulmonary edema in rats, mice, etc. as to cause death in 40 min.[22] Molecular weight is 83,000.

Bacillus cereus

For a general review, see Reference 23.

Enterotoxin (diarrheagenic toxin, Mouse lethal factor I) — Responsible for diarrheal-type food poisoning. It causes intestinal fluid secretion and necrosis and is dermonecrotic and lethal. It has a molecular weight of ~50,000. (NB an emetic agent responsible for vomiting-type food poisoning, mol wt <5,000, is probably not a protein).

Cereolysin — A thiol activated lysin (see "Streptolysin O"); mol wt 60,000.

Lysin — An unrelated hemolysin consists of two proteins mol wt 29,000 and 34,000. Molecular cloning: see Reference 24.

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Phospholipase C — Responsible for the diagnostic lecithinase reaction but may not be important in pathogenesis. Not hemolytic.

Bacillus thuringiensis

Crystal toxins (delta endotoxins) — A set of related cytotoxins. Protoxins of mol wt 130,000 are produced during sporulation as single large insoluble bipyramidal crystals that are released upon the dissolution of the spore mother cells. The protoxins are solubilized and activated by partial proteolysis to insecticidal fragments of mol wt ~60,000. The toxins or the cells are used commercially as insecticides to kill the larvae of lepidoptera, coleoptera, and diptera. Upon consuming the crystals these larvae suffer rapid destruction of their gut linings. Large differences in the sensitivities of different insects to the different toxins seem to be due mainly to differences in the mode of proteolytic processing by insect gut enzymes and partly to different susceptibilities of target cells. The gene is sometimes chromosomal but is usually carried on a plasmid, loss of which leaves a microbe indistinguishable from *B. cereus*. In *B. thuringiensis* var. *israeliensis* a soluble protein mol wt 27,000 to 28,000 has been identified that is broadly cytolytic but is not insecticidal. It is debated whether this is a fragment of the crystal toxin or the product of a separate gene. For a general review, see Reference 25.

Thuringiolysin — A thiol-activated cytolysin. (See "Streptolysin O".)

Bacillus spp.

Other thiol-activated lysins (see "Streptolysin O") are produced by *B. alvei* (alveolysin) and *B. laterosporus*.

Bordetella pertussis

The three toxins described, plus a dermonecrotic toxin and a tracheal cytotoxin are simultaneously lost or gained by a phase change.[26]

Adenylate cyclase (heat-labile factor) — A soluble adenylate cyclase that is most active in the presence of calcium-calmodulin that it finds inside target cells. Unlike the *Bacillus anthracis* cyclase (qv) there is a calmodulin-independent form. The cyclase itself mol wt ~70,000 (possibly an oligomer) may constitute the A component of an A-B toxin: a form that penetrates many types of cells has a molecular size of ~190,000.[27] Penetration is continuous and the cytosolic cyclase is fairly soon inactivated. Cyclic AMP levels rise profoundly and impair the activities of phagocytes,[10] natural killer cells, platelets, and other cells. Lymphocytes die. There is no penetration of human or rat erythrocytes or reticulocytes. It is also produced by *Bordetella parapertussis* and *Bordetella bronchiseptica*.

Pertussigen (pertussis toxin, islet activating protein, histamine sensitizing factor, lymphocytosis promoting factor) — An A-B toxin with six subunits. The B (binding) oligomer consists of two heterodimers (S2 + S4, S3 + S4). These interface with the enzymically active A subunit (also known as S1) through a single C subunit (also known as connecting subunit or S5; see Figure 1). A = 28,000; S2 = 23,000; S3 = 22,000; S4 = 11,500; S5 = 9,300, total = 105,700. The A subunit when activated by thiols and provided with ATP catalyses the ADP-ribosylation of the α subunit of Ni,[28] a membrane-bound GTP-binding protein that regulates a variety of hormonal effects. These include inhibitory effects on adenylate cyclase of hormones such as alpha adrenergic agents, cholinergic muscarinic agents, and opiates.[29] ADP-ribosylation uncouples such inhibitory hormones from adenylate cyclase.[29] However, several physiologic responses mediated by Ni may not depend on rises in cyclic AMP but may for example be mediated by the mobilization of calcium[30] and can even be the reverse of those anticipated from a cyclic AMP increase. Physiologically, nanomolar concentrations of pertussigen enhance secretagog-stimulated insulin secretion, increase the lethality of histamine, and inhibit macrophage chemotaxis and

Pertussigen   A   S2 / S4 / S3 / S4   C

Botulinal neurotoxins   S—S   Light   Trypsin   Heavy

Tetanus toxin   Trypsin   Light   Heavy   S—S   B   Papain   A

|— H45 —|

Diphtheria toxin   S—S   A   B   Trypsin

LT/Cholera toxin   S—S A_1 A_2 5B

Pseudomonas aeruginosa toxin A.   b   a   Chymotrypsin, etc.

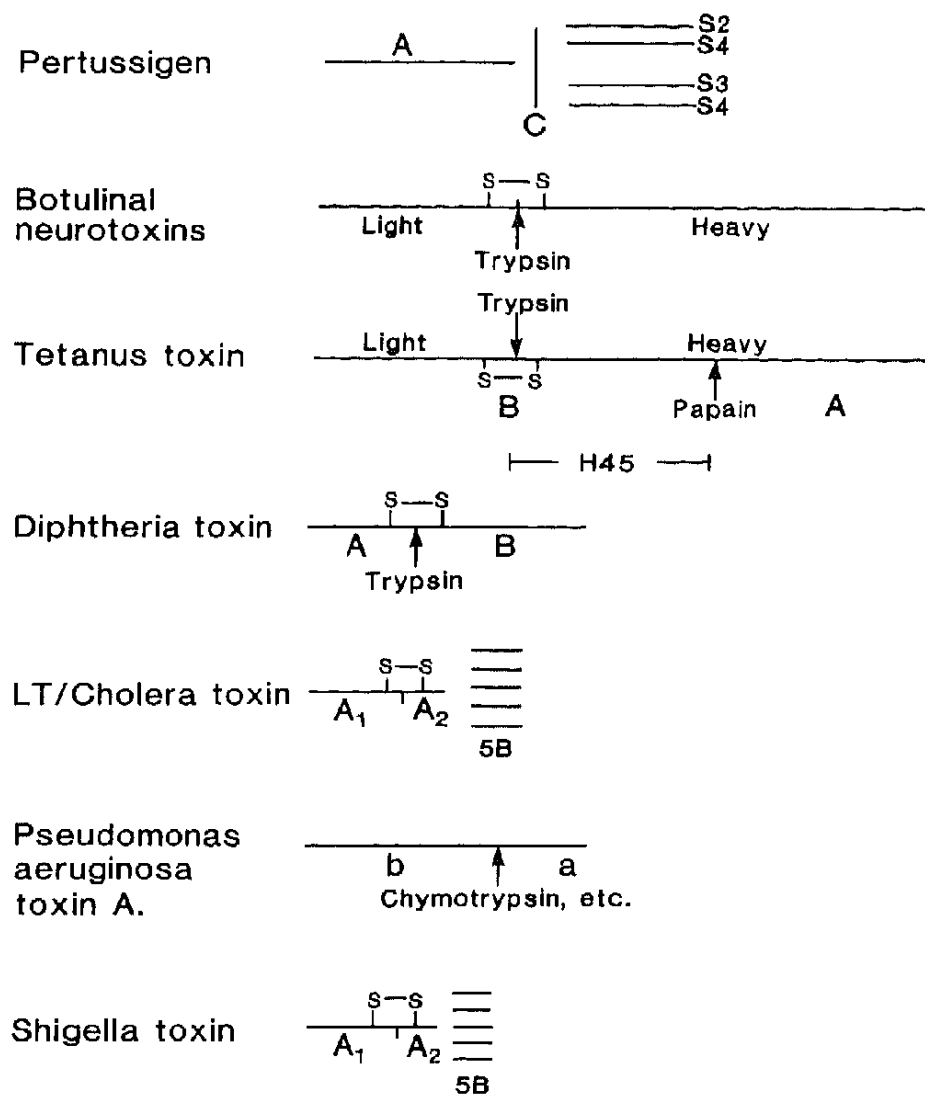Shigella toxin   S—S A_1 A_2 5B

FIGURE 1.   Molecular arrangement of certain A-B toxins.

spreading. Micromolar amounts induce lymphocytosis and (probably thereby) act as an adjuvant. This seems to be attributable to the mitogenic effect of the multivalent B assembly alone.[31]

Hemolysin — Less important for virulence.

Campylobacter jejuni

Enterotoxin, of the cholera toxin-LT family, responsible for diarrhea.[32]

Clostridium botulinum

Neurotoxins (Botulinum toxins, botulinal toxins) — Seven serologically distinct but related neurotoxins (A_1, B, C_1 [formerly C], D, E, F, G). *C. botulinum* is classified by the serotype of the toxin it produces. Most human disease is caused by types A, B, E, and F. All are originally single chains of mol wt 140,000 to 160,000 that are nicked extracellularly beneath the disulfide bridge to generate heavy (~100,000) and light (~50,000) chains linked by a disulfide bridge (cf., tetanus toxin; see Figure 1). Type E bacteria lack a suitable protease and so their toxin is usually recovered intact: tryptic nicking of intact E toxin

increases its toxicity. They are possibly A-B toxins. Isolated heavy chains compete for binding with whole toxin and also form channels in lipid bilayers. The toxins are taken up at the presynaptic side of neuromuscular junctions and block the exocytosis of acetylcholine-containing granules. This results in a flaccid paralysis. The toxin is considerably less effective at inhibiting the exocytosis of noncholinergic transmitters. The biochemical basis of the antisecretory action is unknown, but it is believed that a stage subsequent to the entry of calcium is affected. The botulinal neurotoxins are all extremely potent but the serotypes have differential toxicities among species. Oral toxicities are increased when the toxins are complexed with other botulinal proteins. Types C_1 and D, and probably others are encoded by lysogenic bacteriophages. For reviews see References 33 through 35.

C_2 **toxin** — Consists of a heavy chain (component I) (mol wt 105,000) and a light chain (component II) (mol wt 55,000) which are not covalently linked. Toxicity is increased by tryptic action on the heavy chain. Despite its structural resemblance to the botulinal neurotoxins it is not neurotoxic. Rather it kills by increasing vascular permeability.[36] It can also cause intestinal secretion.[37] It is possibly an A-B toxin: the heavy chain is thought to bind to tissue receptors. The light chain catalyzes ADP-ribosylations.[128,129]

Clostridium difficile [38,39]

Enterotoxin (Toxin A) — Elicits hemorrhagic diarrhea. It is the major cause of the damage and inflammation in the antibiotic-induced pseudomembranous colitis caused by *C. difficile*.[40] It probably acts at cell membranes.[41]

Cytotoxin (Toxin B) — Much more cytotoxic than toxin A.[38] At sublethal doses it causes some cells to lose microfilaments and hence their shape. CHO cells clump and round with long thin processes. They cannot divide further. The biochemical basis of the action of the cytotoxin is unclear, but it seems to act within the cytosol and to get there via lysosomes. Both toxins are probably also made by *Clostridium sordelii* since whole *sordelii* antitoxin is protective.

Clostridium perfringens

The four major lethal toxins (alpha, beta, epsilon and iota) are used to group the species into five toxigenic types. Type A produces mainly α toxin, B: $\beta + \epsilon$, C: $\beta + \gamma$, D: ϵ, E: ι. For a review see Reference 42.

Alpha toxin — A phospholipase C that digests lecithin as well as sphingomyelin, phosphatidylethanolamine, and phosphatidylserine. It requires that calcium be bound to the substrate. It is cytolytic, necrotic, and lethal. Erythrocyte sensitivities: cattle, mice > rabbits, sheep, men, pigeons > horses, goats. It is the main lethal agent of type A strains that cause gas gangrene and is probably responsible for necrosis in infections. It gives "hot-cold" hemolysis of certain erythrocytes (cf., Staphylococcal beta lysin). Commercial preparations are contaminated with theta toxin. Purification.[43] Molecular weight is ~3400. For a review see Reference 44.

Beta toxin — Necrotizing and lethal. Thought to be involved in necrotic enteritis of man ("pig bel") and animals, particularly on diets low in protein and rich in antitrypsin so that the usual tryptic inactivation of the toxin does not occur. Intravenous toxin causes vascular changes.[45] Partial purification.[46] Probably encoded by a plasmid.[47] Molecular weight is 28,000.

Delta toxin — Cytolysin. Particularly active on sheep erythrocytes. Basic protein, mol wt ~40,000 with amphiphilic properties. The receptor is suggested to be ganglioside G_{M2}. Produced by young cultures of some strains of types B and C. Its role in disease is unclear. It causes gut fluid accumulation.

Epsilon toxin — A cytotoxin secreted by *Clostridium perfrigens* strains types B and D as a nontoxic or almost nontoxic protoxin. This is activated by tryptic removal of the amino

terminal 14 amino acids.[49] Responsible for enterotoxemia of herbivores in which it is produced and activated in the intestine, itself increases intestinal permeability, escapes to the general circulation and causes various lesions by increasing vascular permeability. It also damages some macrophages Molecular weight is 25,000 to 33,000.

Iota toxin — Released as a protoxin that has to be proteolytically activated. Necrotic and lethal. Not purified.

Theta toxin (Perfringolysin O) — A thiol-activated cytolysin (see Streptolysin O). Mechanism of lysis discussed in References 50 and 51. Purification.[52] Molecular weight is 53,000.

Enterotoxin — Diarrheagenic agent of food-poisoning strains of types A, C, and D. Membrane-damaging agent. Cytotoxic, lethal to mice. The cellular receptor is a protein. It increases sodium chloride secretion in the gut; an early effect on gut epithelium is an inhibition of amino acid transport. Produced in the gut by ingested cells when they sporulate (stage II or III sporulating cells) and seems to be a precursor of a spore coat protein.[1] Molecular weight is 34,262 with one free SH group.[53] Tryptic removal of the amino terminal 25 residues increases the activity threefold.

Clostridium tetani

Tetanus toxin (tetanospasmin) — A potent neurotoxin that inhibits transmitter release from nerve terminals. Many types of synapses are affected, including neuromuscular junctions, but the clinical effect is dominated by a central blockade of GABA and glycine release at inhibitory synapses which results in a local or generalized spastic paralysis. Circulating toxin enters the nervous system by endocytosis at peripheral junctions and is transported via smooth vesicles up axons to the central nervous system (CNS). While many vesicles fuse with lysosomes and their contents are destroyed, some of the toxin-containing vesicles fuse with postsynaptic membranes and presumably discharge the toxin (or a modified form of it) into synaptic clefts where it is able to bind and paralyse presynaptic terminals. The biochemical basis of the paralysis is not known, but the extraordinary potency suggests that the toxin affects the presynaptic terminals catalytically and possibly intracellularly. It seems to affect the process of exocytosis in the presynaptic neurone at a stage after the entry of calcium. It is possibly an A-B toxin. Molecular weight is ~146,000 (see Figure 1). It is often already nicked when purified or can be experimentally nicked by trypsin. Reduction by a thiol then yields a light (or α) chain, mol wt 51,500, and a heavy (or β) chain, mol wt 95,000. Digestion at a second site by papain yields fragments B (96,500) and C (50,000). (Note that the nomenclature of the papain fragments does not conform to the A-B system.) Fragment B in high concentration can cause a flaccid paralysis. The light chain is suspected of carrying the postulated enzymic function. The central region (''H45'': 54) is hydrophobic, makes conducting channels in artificial membranes, and is thought to be involved in membrane-interaction in an acidic compartment.[54] Heavy chain and fragment C bind gangliosides G_{D1b} and G_{T1} and so are thought to be involved in cell receptor binding. However, it is not certain that gangliosides constitute functional receptors, for their binding is not sufficiently tight to inactivate soluble toxin, and treatment with neuraminidase does not protect cells against the toxin. Clinical disease can be entirely prevented by immunization with tetanus toxoid (formaldehyde-modified toxin) which is often administered with diphtheria toxoid (qv). The toxin may be encoded by a plasmid.[55] For reviews, see References 56 and 57.

Tetanolysin — A thiol-activated cytolysin (see ''Streptolysin O'').

Clostridium spp.

Thiol-activated lysins (see Streptolysin O) have been reported for *C. bifermentans*, *C. botulinum*, *C. histolyticum* (ϵ-toxin), *C. novyi* (γ toxin), and *C. septicum.*)

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Corynebacterium diphtheriae

Diphtheria toxin[58,59] — The archetypal A-B toxin. A single polypeptide chain of 535 residues with an amino terminal A domain (21,150) and a carboxy-terminal B domain (37,200) connected by the peptide backbone and a disulfide bond (see Figure 1). Both bonds must be broken for activation. Thiol-reduced Fragment A catalyses the ADP-ribosylation of elongation factor 2 (see "Introduction"). ADP-ribosyl elongation factor 2 is unable to support protein synthesis; hence, cellular death ensues. The amino acid residue that is ADP-ribosylated is a modified histidine, "diphthamide"[60] apparently unique to elongation factor 2. The entry of fragment A requires at least three functions of the B domain: the binding of a carboxy terminal region to specific protein receptors, binding of a region near the center of the toxin molecule to lipid head groups, and the insertion of a hydrophobic section into the lipid bilayer. The latter event occurs following endocytosis and acidification of the endosome. B forms voltage-sensitive channels through which fragment A presumably passes. The passage requires a pH gradient across the vesicle membrane and the presence of permeant anions such as chloride. Approximately 1 molecule of toxin is sufficient to kill a cell. Mutant cells that are resistant to diphtheria toxin are either defective in the mechanism whereby fragment A enters the cell or have altered elongation factor 2. The toxin gene is carried by various temperate corynebacteriophage. Double lysogens are common. Its expression is reduced by iron. The DNA sequence is known.[61,62] Diphtheria is a simple toxinosis: the toxin is responsible for most aspects of the syndrome. Hence, immunization by diphtheria toxoid is remarkably successful and has dramatically cut the incidence of diphtheria. Toxoid is inactivated toxin generally formed by exposing the toxin to formaldehyde or a similar agent. Diphtheria toxoid is usually administered in conjunction with tetanus toxoid. The toxin affects most vertebrates, but rats and mice lack the toxin receptor and are resistant. It was one of the first toxins discovered (Roux and Yersin 1888). Produced also by pathogenic *C. ulcerans.*

Corynebacterium equi

Cytolysin — A phospholipase C which also hydrolyses ceramide phosphate, the product of the reaction of spingomyelinase D, and thereby enhances the hemolysis by *Corynebacterium ovis.*[63]

Corynebacterium ovis (C. pseudotuberculosis), C. ulcerans and C. hemolyticum

Ovis toxin (sphingomyelinase D). Hydrolyses sphingomyelin to ceramide phosphate plus choline.[64] Weakly hemolytic, more lytic in acid conditions,[65] and hence hemolysis is enhanced by a reduced oxygen supply. In combination with Corynebacterium equi lysin (qv) or Staphylococcal delta toxin, hemolysis is much enhanced. It inhibits the hemolysis by *Clostridium perfrigens* alpha toxin and by Staphylococcal beta toxin by modifying their substrates.

The toxin is important in establishing infections. It has been reported to increase vascular permeability.[66] It is presumably responsible for the hemoglobinurea seen, but its major effect is probably on leukocytes. Experimentally it is dermonecrotic and lethal. It consists of a single polypeptide chain, mol wt 31,000.[67]

Corynebacterium pyogenes

Cytolysin. Lethal. Not known to affect abscess formation but it is adsorbed from abscesses and causes generalized symptoms.[68] Poorly characterized.

Escherichia coli

(Similar toxins are found widely among the enterobacteriaceae.)

Heat-labile enterotoxins (LT) — A-B toxins identical in action to cholera toxin (qv).

Several slightly different types have been identified among human and porcine *E. coli*. They form a family with cholera toxin and LTs of other enteric organisms. The genes are generally carried on plasmids with slightly overlapping cistrons for subunits A and B. Several of the genes have been sequenced (see, for example, Reference 69). An "LT-like" enterotoxin that elevates cyclic AMP but is not neutralized by anti-LT also has been reported.[70]

Heat-stable enterotoxins (STa, also called ST$_I$; subtypes ST$_{Ia}$ ST$_{Ib}$) — A set of small methanol-soluble peptides, initially about 72 amino acid residues long, of which the functional region consists of the carboxyl-terminal 18 residues. This region includes three disulfide bridges required for activity. The stable toxins cause intestinal secretion in suckling mice and piglets. They activate particulate guanylate cyclase (only) of intestinal epithelial cells, or brush border vesicles, or membranes, with little or no lag and thereby cause a prolonged cholera-like secretion. The intestine appears to have specific receptors distinct from guanylate cyclase.[71] The guanylate cyclase of other tissues does not respond. The activation can be mimicked by hemin and certain divalent metal ions. Encoded on plasmids. The ST$_{Ia}$ gene is a transposon.[72] DNA sequences of ST$_{Ia}$: 72, of ST$_{Ib}$: 73. For a review, see Reference 74.

Heat-stable enterotoxin STb (also called STII) — Methanol insoluble. Active in suckling piglets and weaned pigs, but not in suckling mice or in adult ileal loops. Effective with no apparent lag. The effect is largely reversed by washing. The secretion does not involve cyclic GMP or cyclic AMP.[75] Anion secretion is not inhibited by furosemide (that of LT and STa is) and probably involves bicarbonate rather than chloride. The gene has been sequenced and shows no relation to STa. The protein has 71 amino acid residues.

Hemolysin (alpha hemolysin) — Made late in log phase. Important in systemic infection but not in enteric infections. It is active as a complex with a phospholipid and is inactivated by phospholipases. Complex genetic organization: the *hlyA* gene is adjacent to *hlyBa*, *hlyBb*, and *hlyC* genes, the products of which are required for the secretion of the hemolysin. There are usually one or two chromosomal copies of the gene cluster that are located in large "islands" that also encode other virulence factors. It is sometimes carried as a transposon on a plasmid (76). Molecular weight is 107,000.

Cytotoxin (Shiga-like toxin, Vero cell toxin) — Widely distributed but is often synthesized in very small amounts. Substantial amounts are made by human and animal strains that cause hemorrhagic colitis.[77] It has the same effects on cells in culture as does Shigella toxin and it is neutralized by antiserum against Shigella toxin, but it is not genetically identical. Produced under iron stress. Sometimes encoded by phage.[78]

Fusobacterium necrophorum
   Leucocidin — Assists the establishment of *Corynebacterium pyogenes* in the combined infection "foot rot".

   Klebsiella — STa (see "*Escherichia coli*").

Legionella pneumophila
   Cytolysin[79]

Listeria Ivanorii
   Listeriolysin A, Listeriolysin B

Listeria monocytogenes
   Listeriolysin — A thiol-activated lysin (see "Streptolysin O"). There is also a phospholypase.[80]

Plesiomonas shigelloides
   Enterotoxin — Encoded by a plasmid.

Pseudomonas aeruginosa

For a review see Reference 81.

Exotoxin A — Lethal A-B toxin consisting of a single polypeptide chain mol wt 66,583 with four disulfide bonds. Activated either by partial proteolysis of reduced toxin, e.g., by elastase or by chymotrypsin plus NAD which release C-terminal fragment a: mol wt ~26,000[82] or by urea denaturation of the reduced toxin without proteolysis.[83] The activated toxin, or fragment a, catalyses the same ADP-ribosylation of elongation factor 2 as does diphtheria toxin,[84] but the toxin is unrelated to the latter and uses a different cellular receptor. Exotoxin A seems important in infections but other toxins and enzymes contribute. Single copy chromosomal gene. Sequence: 85. The level of expression is governed by a positive regulator gene. Transcription is reduced by high iron unless there are multiple copies of the regulator gene. Protein purification: 86. Found in 80 to 90% of isolates.

Cytotoxin (Leucocidin) — Membrane active on most cells. Synergized by calcium and possibly acts by altering phospholipid metabolism.[87] Molecular weight is ~25,000.

Other toxins — Includes a lecithinase, a possible enterotoxin and several proteases of low toxicity that necrotize skin and eyes. Among activities of elastase that seem important are degradation of elastin, IgG, and serum proteinase inhibitors. An ADP-ribosyl transferase called exoenzyme S may be toxic. A toxin Z mentioned in the older literature turned out to be exotoxin A.

Shigella dysenteriae

Shigella toxin (Shiga toxin, Shigella dysenteriae type 1 toxin, Vero cell toxin (also called "Shigella neurotoxin" but this is a misnomer for the clinical neurotoxicity is secondary to vascular damage) — An A-B toxin. The A subunit (mol wt 31,244) can be proteolytically nicked to an A_1 peptide (~27,000) and A_2 (~5000) (Figure 1). A_1 inhibits protein synthesis by inactivating an unidentified component of 60S ribosomes.[88] There are five identical B subunits of mol wt 3782. Thus, as for cholera toxin, the delivery system seems to consist of $A_2.5B$. The target cell receptor may be a glycoprotein terminating in N acetyl glucosamine or a protein or lipid containing digalactose. The toxin is cytotoxic for several cell lines including Vero cells, a variety of epithelial cells, and some intestinal epithelial cell lines. This cytotoxicity may contribute to the colonic epithelial damage in shigellosis, but the exact role in pathogenesis is controversial. The susceptibilities of different HeLa cell clones vary by over 10^6-fold. The same toxin causes fluid secretion from the small intestine (without cell death) and may be responsible for the diarrhea in shigellosis. The mechanism of this secretion induction is not clear. A suggested involvement of cyclic AMP is disputed. A similar or identical toxin is produced by other species of *Shigella*, by some strains of *E. coli* (qv) and rarely by *Salmonella*, and other Enterobacteriaceae. Encoded on the chromosome. Production is increased by iron stress. For a review see Reference 89.

Staphylococcal aureus[90,91]

General — A master regulator gene *agr* on the chromosome regulates the expression of several of these toxins.

Alpha lysin (alpha toxin) — Hemolytic, cytotoxic to leukocytes, paralytic to the innervation of smooth muscle, and lethal. Acute lethality may be neurologic in origin and need not be accompanied by hemolysis. All of the actions may involve the disorganization of cell membranes by the formation of ring-like hexameric "pores" 2 to 3 nm in diameter.[92] Rabbit erythrocytes are particularly sensitive and their lysis by low doses (50 to 100 ng/mℓ) entails binding to a specific proteinaceous receptor which may provide a model for more relevant effects on the nervous system. Effects on myelin also involve specific binding, possibly to a similar receptor as that on rabbit erythrocytes. Much higher doses (30 to 100 μg/mℓ) lyse a variety of cells and protein-free liposomes "nonspecifically". Alpha toxin

appears important in the formation of abscesses. It causes aggregation of platelets and stimulates the constriction of small vessels thus promoting ischemia and necrosis. It is toxic to macrophages, and at sublytic doses reduces phagocytosis and the respiratory burst.[93] There are similar effects on neutrophils.[94] Molecular weight is 33,000. For gene sequence see Reference 95. For a review see Reference 96. Before about 1975 many functional studies were performed with impure alpha toxin contaminated with delta toxin.

Beta lysin (β toxin) — A cytolytic sphingomyelinase. It is toxic to most tissue culture cells and to macrophages, neutrophils, and platelets. It causes the leakage of lysosomal enzymes. It is hemolytic, particularly to sheep erythrocytes. Hemolysis exhibits the hot-cold phenomenon: after exposing erythrocytes to toxin in the warm, hemolysis is only complete during a further period in the cold, or upon removing membrane-bound magnesium ions. The hemolytic zone around colonies then has a sharp margin (that of alpha toxin has a diffuse margin). Hemolysis is also enhanced by the so-called "CAMP factor", a protein secreted by group B Streptococci. Present in most strains. There is some variety in sequence.

Gamma lysin (γ toxin) — Cytolytic, probably leukotoxic. Hemolysis requires Na^+ and Ca^{++}. Molecular weight is 32,000. Molecular cloning: 97. Reviewed in Reference 91.

Delta lysin (δ toxin) — Causes detergent-like disorganization of membranes. It lyses erythrocytes of all species, and all lipid vesicles. It increases vascular permeability, and is inactivated by various lipids. A small amphiphilic protein with a molecular weight of 2977. For sequence see Reference 98. Stable to boiling. Soluble in lipid solvents. Poor immunogen.

Enterotoxins (SEA, SEB, etc.) — Cause vomiting when injected to monkeys. Responsible for all of the symptoms of Staphylococcal food poisoning including emesis and diarrhea. Mitogenic. Seven related serological types (A, B, C_1, C_2, C_3, D, E) which compose two groups: A, D, E and B, C_1, C_2, C_3. Molecular weight is from 26,000 to 29,000. A, B and C_1 have been sequenced. Enterotoxin B contains a disulfide loop enclosing a nicking site. Trypsin and pepsin resistant. The gene for enterotoxin A is associated with a variable genetic element [99] that can be plasmid borne or chromosomal. The protein once called enterotoxin F is now called toxic shock syndrome toxin 1 (TSST-1). Some of the enterotoxins, particularly B, are also associated with cases of toxic shock. For a review see Reference 100.

Pyrogenic exotoxins A and B — Both are proteins of mol wt 12,000 that exhibit properties of agents which induce interleukin-1 production (fever, mutagenesis, etc.).[101,102] The substance formerly known as pyrogenic exotoxin C is now called TSST-1.

Toxic Shock Syndrome Toxin(s) — One (TSST-1), or perhaps several, proteins with pleiotropic effects. Many effects are mediated by the induction of interleukin-1 production by mononuclear phagocytes.[103] Thus the toxin indirectly causes fever, B and T cell mitogenesis, headache, arthralgia, and neutropenia. May also cause the rash of toxic shock syndrome (cf., Streptococcal erythrogenic toxins). Molecular weight is 29000. For genetic organization, see Reference 104.

Exfoliatin (epidermolytic toxin) — Toxin that causes generalized sloughing of skin — the "scalded skin syndrome" — in babies and, experimentally, in young mice.[105] The epidermis splits at the level of the stratum granulosum. Unknown mechanism. Exfoliatin A is chromosomal, mol wt 30,000, and its gene has been cloned.[106] Exfoliatin B is controlled by a plasmid found in phage group II staphylococci. Mol wt: 29,500.

Leukocidin — Unlike the α, β, γ, and δ lysins, leukocidin is specific for granulocytes, macrophages and mast cells of man and rabbit. No effect on erythrocytes, lymphocytes, or cell lines in culture. Two fractions: F (mol wt 32,000) and S (mol wt 31,000) act synergistically to increase cation permeability.[107] Details are lacking. The role of leukocidin in disease is unclear.

Streptococcus pneumoniae

Pneumolysin — A thiol-activated cytolysin (see "Streptolysin O"). Molecular weight is ~53,000. Purification.[108] Only weak antigenic cross-reactivity with Streptolysin O.[108]

Streptococcus pyogenes

Streptolysin O (SLO) — The best characterized of a group of thiol-activated (oxygen-labile) lysins that each interact with membranes containing cholesterol and are inactivated by free cholesterol.[109] They are also inactivated by oxidation of free SH groups, and can be reactivated by reducing agents such as thiols. They are cytolytic, cardiotoxic, and lethal.[110] They are thought to generate channels in membranes,[111] and the damage may be exacerbated by the deposition of complement on the membrane of the target cell.[112] The SLO gene has been cloned.[113] Although various thiol-activated lysins share common antigenic sites, the extent of DNA homology is limited. Molecular weight is 52,600.

Streptolysin S (SLS)[114,115] — Oxygen-stable cytolysin. Antiphagocytic. Nonspecific for white cells, but T cells are especially sensitive. The principal cause of the aerobic "beta hemolysis" of many pyogenic streptococci. Small protein of approximately 15 amino acid residues with a tendency to aggregate. Not antigenic. Needs to form a complex with some carrier molecule for stability (e.g., RNA, BSA, triton, lipoteichoic acid).

Erythrogenic toxins (scarlet fever toxins, pyrogenic exotoxins) — Specific to scarlet fever strains. Pyrogenic, T cell mitogenic, transiently immunosuppressive; these effects may be mediated by interleukin-1 released from mononuclear phagocytes. They damage small vessels to give the rash of scarlet fever. Three antigenic types: A, B, and C. Genes carried by bacteriophage are expressed in lysogenic and vegetative states. The Dick test, the injection of a small quantity of toxin i.d., tests for immunity to these toxins.

Vibrio cholerae[116-118]

Cholera toxin (choleragen) — A-B toxin of six subunits: A5B (Figure 1). Subunit A consists of fragment A_1 (mol wt 21,817) and fragment A_2 (mol wt 5398) joined initially by a peptide backbone (easily nicked) and a disulfide bond. A_2 is linked noncovalently to a ring of five identical B subunits (mol wt 11,677 each), each of which binds ganglioside GM_1 on cell surfaces. The 5B assembly is known as choleragenoid. Thus the complex $A_2 5B$ constitutes the delivery system. The nicking of A accelerates the effect on cells. Thiol-reduced A_1 is an enzyme that catalyses the intracellular ADP-ribosylation of the α subunit of Ns, the GTP-binding regulatory subunit of adenylate cyclase. The Ns modification results in hormone-independent activation of adenylate cyclase. In intestinal epithelium the resulting high intracellular cyclic AMP leads to electrolyte secretion and hence diarrhea. Cholera is thus a simple toxinosis and can be effectively combated solely by reversing the fluid loss caused by the toxin. Intestinal toxin is lethal only as a result of the fluid loss. The toxin generally contacts the intestinal lining only, but experimentally it raises cyclic AMP in most cell types, with a variety of physiological consequences, mostly nonlethal. Efficient ADP-ribosylation requires (1) reduction of A_1, (2) concurrent or prior binding of GTP to a membrane site "S" distinct from Ns,[118] (3) a membrane-environment for Ns, and (4) the absence of complete activation of cyclase by GTPγS. At much lower efficiencies the toxin also ADP-ribosylates many other proteins. The gene is chromosomal. Operon organization: promoter-A_1-A_2-B with a 2-bp overlap between A and B. A and B have separate SD sequences. That for B is more efficient and allows B:A translation ratios of at least five. The gene appears to be on a transposon that is often duplicated at separated points on the chromosome (Classical strains) or in tandem (some El tor strains) and can be further amplified by animal passage.[119] Closely related to the heat-labile enterotoxins (LTs) of *E. coli* and various other organisms including non-O1 *Vibrio cholerae*, and *V. mimicus*.

Hemolysin — In El tor strains. Single gene copy.

Shiga-like toxin — See *"Escherichia coli"*.

Vibrio fluvialis

Enterotoxin — Also causes shape changes in cultured cells.[120]

Vibrio parahemolyticus

"Enterotoxin" of unknown mechanism. Also causes CHO cells to elongate.

Cytolysin (thermostable direct hemolysin, Kanagawa lysin) — Cardiotoxic, cytotoxic. Responsible for hemolysis on a special medium (Wagatsuma medium); the "Kanagawa phenomenon". Heat stable when crude. Receptor may be a trisialoganglioside.[121] Probably unimportant in food poisoning by this organism. Molecular weight is 45,000. Other lysins are also present.[122]

Shiga-like toxin — See *"Escherichia coli."*

Vibrio vulnificus

Cytolysin, mol wt ~56,000.[123]

Yersinia enterocolitica

Heat-stable enterotoxin — Clinical isolates produce material resembling STa of *E. coli.*[124,125]

Yersinia pestis

"Murine toxin" rapidly kills mice and rats especially at low temperatures. It is suggested to act as an antagonist of epinephrine but the biochemical mechanism is not known.[126] Not well characterized, possibly a membrane protein. Probably unimportant in human plague. For a review see Reference 127.

REFERENCES

General Reviews and Collections

1. **Ajl, S., Kadis, S., and Montie, T. C., Eds.,** *Microbial Toxins,* Vol. I, IIA, and III, ("Bacterial Protein Toxins"), Academic Press, New York, 1970.
2. **Alouf, J. E., Fehrenbach, F. J., Freer, J. H., and Jeljaszewicz, J., Eds.,** *Bacterial Protein Toxins,* Academic Press, London, 1984, (A conference report).
3. **Falmagne, P., Alouf, J. E., Fehrenbach, F. J., Jeljaszewicz, J., and Thelestram, M., Eds.,** *Bacterial Protein Toxins,* Supplement 15 of Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene, 1986.
4. **Bernheimer, A. W., Ed.,** *Mechanisms in Bacterial Toxinology,* Wiley Medical, New York, 1976.
5. **Jeljaszewicz, J. and Wadström, T., Eds.,** *Bacterial Toxins and Cell Membranes,* Academic Press, New York, 1978.
6. *Microbial Toxins and Diarrhoeal Disease,* CIBA Found. Symposium, Vol. 112, Pitman, London, 1985.
7. **Middlebrook, J. L. and Kohn, L. D.,** *Receptor-Mediated Binding and Internalization of Toxins and Hormones,* Academic Press, New York, 1981.
8. **Stephen, T. and Pietrowski, R. A.,** *Bacterial Toxins,* Thomas Nelson & Sons, "Aspects of Microbiology" series, 1981.

Original Articles

9. **Gill, D. M.,** in Jeljaszewica, J. and Wadström, T., Eds., *Bacterial Toxins and Cell Membranes,* Academic Press, New York, 1978, 291—332.
10. **Confer, D. L. and Eaton, J. W.,** *Science,* 217, 948, 1982.
11. **Lowrie, D. B., Aber, V. R., and Jackett, P. S.,** *J. Gen. Microbiol.,* 110, 431, 1979.
12. **Möllby, R.,** in Jeljaszewicz, J. and Wadström, T., Eds., *Bacterial Toxins and Cell Membranes,* Academic Press, New York, 1978, 367—424.
13. **Chakraborty, T., Montenegro, M. A., Sanyal, S. L., Helmuth, R., Bulling, E., and Timmis, K. N.,** *Infect. Immun.,* 40, 435, 1984.
14. **Buckley, J. T., Halasa, L. N., Lund, K. D., and MacIntyre, S.,** *Can. J. Biochem.,* 430, 1981.
15. **Bernheimer, A. W. and Avigad, L. S.,** *Infect. Immun.,* 9, 1016, 1974.
16. **Asao, T., Kinoshita, Y., Kozaki, S., Uemura, T., and Sakaguchi, G.,** *Infect. Immunol.,* 46, 122, 1984.
17. **Ljungh, A., Wretlind, B., and Mollby, R.,** *Acta Pathol., Microbiol. Scand. Sect. B,* 89, 1981.
18. **McIntyre, S. and Buckley, J. T.,** *J. Bacteriol.,* 135, 402, 1978.

16   *CRC Handbook of Microbiology*

19. Stephen, J., *Pharmacol. Ther.*, 12, 501, 1981.
20. Leppla, S. H., *Proc. Natl. Acad. Sci.*, 79, 3102, 1982.
21. Ezzell, J. W., Ivins, B. E., and Leppla, S. H., *Infect. Immun.*, 45, 761, 1984.
22. Beall, F. A. and Dalldorf, F. G., *J. Infect. Dis.*, 116, 377, 1966.
23. Turnbull, P. C. B., Pharmacol. Ther. 13, 453, 1981.
24. Kreft, J., Berger, H., Hartlein, M., Muller, B., Weidinger, G., and Goebel, W., *J. Bacteriol.*, 155, 681, 1983.
25. Dean, D. H., *Biotechnol. Genet. Eng. Rev.*, 2, 341, 1984.
26. Weiss, A. A. and Falkow, S., *Infect. Immun.*, 43, 263, 1984.
27. Hanski, E. and Farfel, Z., *J. Biol. Chem.*, 260, 5526, 1985.
28. Katada, T. and Ui, M., Proc. Natl. Acad. Sci., 79, 3129, 1982.
29. Ui, M., Katada, T., Murayama, T., and Kurose, H., *Adv. Exp. Med.*, 175, 1, 1984.
30. Okajima, F., Katada, T., and Ui, M., *J. Biol. Chem.*, 260, 6761, 1985.
31. Tamura, M., Nogimori, K., Yajima, M., Ase, K., and Ui, M., *J. Biol. Chem.*, 258, 6756, 1983.
32. Klipstein, F. A. and Engert, R. F., *Infect. Immun.*, 48, 629, 1985.
33. Sakaguchi, G., *Pharmacol. Ther.*, 19, 165, 1983.
34. Sellin, L. C., *Trends Pharm. Sci.*, 6, 80, 1985.
35. Sugiyama, H., *Microbiol. Rev.*, 44, 419, 1980.
36. Ohishi, I., Musakazu, I., and Sakaguchi, G., *Infect. Immun.*, 31, 890, 1981.
37. Ohishi, I., *Infect. Immun.*, 40, 691, 1983.
38. Taylor, N. S., Thorne, G. M., and Bartlett, J. G., *Infect. Immun.*, 34, 1036, 1981.
39. Sullivan, N. M., Pellett, S., and Wilkins, T., *Infect. Immunol.*, 35, 1032, 1981.
40. Thelestam, M. and Bronnegard, M., *Scand. J. Infect. Dis. Suppl.*, 22, 16, 1980.
41. Rothman, S. W., Broom, J. E., Diecidue, A., and Foret, D. A., *Infect. Immun.*, 46, 324, 1985.
42. McDonel, J. L., *Pharmacol. Ther.*, 10, 617, 1980.
43. Möllby, R. and Wadström, T., *Biochim. Biophys. Acta*, 321, 569, 1973.
44. Möllby, R., in Jeljaszewicz, J. and Wadström, T., Eds., *Bacterial Toxins and Cell Membranes*, Academic Press, New York, 1978, 385—391.
45. Sakurai, J., Fujii, Y., Matsuura, M., and Endo, K., *Microbiol. Immunol.*, 25, 423, 1981.
46. Jolivet-Reynaud, C., Popoff, M. R., Vinit, M.-A., Ravisse, P., Moreau, H., and Alouf, J. E., in Reference 3, pp. 145—151.
47. Duncan, C. L., Rokos, E. A., Christenson, C. M., and Rood, J. I., *Microbiology*, 1978, 246, 1978.
48. Jolivet-Reynaud, C. and Alouf, J. E., *J. Biol. Chem.*, 258, 1871, 1983.
49. Worthington, R. W. and Mulders, M. S., *Infect. Immun.*, 18, 549, 1977.
50. Mitsui, K., Sekiya, T., Nozawa, Y., and Hase, J., *Biochim. Biophys. Acta*, 554, 68, 1979.
51. Thelestam, M. and Möllby, R., *Infect. Immun.*, 29, 803, 1980.
52. Smyth, C. J., *J. Gen. Microbiol.*, 87, 219, 1975.
53. Richardson, M. and Granum, P. E., *FEBS Lett.*, 182, 479, 1985.
54. Roa, M. and Boquet, P., *J. Biol. Chem.*, 260, 6827, 1985.
55. Finn, C. W., Silver, R. P., Habig, W. H., and Hardegree, M. C., *Science*, 224, 881, 1984.
56. Mellanby, J. and Green, J., *Neuroscience*, 6, 281, 1981.
57. Dowell, V. R., *Rev. Infect. Dis.*, 6, S202, 1984.
58. Pappenheimer, A. M., *Annu. Rev. Biochem.*, 46, 69, 1977.
59. Collier, R. J., Hayaishi, O., and Ueda, K., Eds., *ADP-ribosylation Reactions*, Academic Press, New York, 1982, 575—592.
60. Van Ness, B. G., Howard, J. B., and Bodley, J. W., *J. Biol. Chem.*, 255, 10710, 1980.
61. Ratti, G., Rappuoli, R., and Giannini, G., *Nucleic Acids Res.*, 11, 6589, 1983.
62. Kaczorek, M., Delpeyroux, F., Chenciner, N., Streek, R. E., Murphy, J. R., Boquet, P., and Tiollas, P., *Science*, 221, 855, 1983.
63. Bernheimer, A. W., Linder, R., and Avigad, L. S., *Infect. Immun.*, 29, 123, 1980.
64. Souček, A., Michaelec, C., and Součková, A., *Biochim. Biophys. Acta*, 144, 180, 1967.
65. Burrell, D. H., *Res. Vet. Sci.*, 26, 333, 1979.
66. Jolly, R. D., *J. Comp. Pathol.*, 75, 417, 1965.
67. Linder, R., and Bernheimer, A. W., *Biochim. Biophys. Acta*, 530, 236, 1978.
68. Lovell, R., and Cotchin, E., *J. Comp. Pathol.*, 57, 20, 1947.
69. Dallas, W. S., *Infect. Immun.*, 40, 647, 1983.
70. Green, B. A., Neill, R. J., Ruyechan, W. T., and Holmes, R. K., *Infect. Immun.*, 41, 383, 1983.
71. Gariepy, J. and Schoolnik, G. K., *Proc. Natl. Acad. Sci.*, 83, 483, 1986.
72. So, M. and McCarthy, B. J., *Proc. Natl. Acad. Sci.*, 77, 4011, 1980.
73. Moseley, S. L., Hardy, J. W., Huq, M. I., Echeverria, P., and Falkow, S., *Infect. Immun.*, 39, 1167, 1983.

74. Rao, M. C., in *Microbial Toxins and Diarrhoeal Disease*, CIBA Found. Symp., Vol. 112, Pitman, London, 1985, 74—93.
75. Kennedy, D. J., Greenberg, R. W., Dunn, J. A., Abernathy, R., Ryerse, J. S., and Guerrant, R. L., *Infect. Immun.*, 46, 639, 1984.
76. Goebel, W. and Hedgpeth, J., *J. Bacteriol.*, 151, 1290, 1982.
77. O'Brien, A. D., Lively, T. A., Chen, M. E., Rothman, S. W., and Formal, S. B., *Lancet*, 1, 702, 1983.
78. O'Brien, A. D., Newland, J. W., Miller, S. F., Williams Smith, H., and Formal, S. B., *Science*, 226, 694, 1984.
79. Baine, W. B., Rasheed, J. K., Maskel, D. C., Bopp, C. A., Wells, J. G., and Kaufmann, A. F., *J. Gen. Microbiol.*, 9, 453, 1979.
80. Vicente, M. F., Baquero, F., and Perez-Diaz, J. C., *FEMS Microbiol. Lett.*, 30, 77, 1985.
81. Woods, D. E. and Iglewski, B. H., *Rev. Infect. Dis.*, 5, S715, 1983.
82. Lory, S. and Collier, R. J., *Infect. Immun.*, 28, 494, 1980.
83. Leppla, S. H., Martin, O. C., and Muehl, L. A., *Biochem. Biophys. Res. Commun.*, 81, 532, 1978.
84. Iglewski, B. H. and Kabat, D., *Proc. Natl. Acad. Sci.*, 72, 2284, 1975.
85. Gray, G. L., Smith, D. M., Baldridge, S., Harkins, R. N., Vasil, M. L., Chen, E. Y., and Heyneker, H. L., *Proc. Natl. Acad. Sci.*, 81, 2645, 1984.
86. Leppla, S. H., *Infect. Immun.*, 14, 1077, 1976.
87. Hirayama, T. and Kato, I., *Infect. Immun.*, 43, 21, 1984.
88. Reisbig, R., Olsnes, S., and Eiklid, K., *J. Biol. Chem.*, 256, 8739, 1981.
89. Keusch, G. T., Donohue-Rolfe, A., and Jacewicz, M., in *Microbial Toxins and Diarrhoeal Disease*, CIBA Found. Symp., Vol. 112, Pitman, London, 1985, 193; Keusch, G. T., Donohue-Rolfe, A., and Jacewicz, M., *Pharm. Ther.*, 15, 403, 1981.
90. Freer, J. H. and Arbuthnott, J. P., *Pharmacol. Ther.*, 19, 355, 1982.
91. Jeljaszewicz, J., Szmigielski, S., and Hryniewicz, W., in Jeljaszewicz, J. and Wadström, T., Eds., *Bacterial Toxins and Cell Membranes*, Academic Press, New York, 1978, 185—227.
92. Fussle, R., Bhakdi, S., Sziegoleit, A., Tranum-Jensen, J., Kranz, T., and Wellensiek, H. J., *J. Cell Biol.*, 91, 83, 1981.
93. McGee, M. D., Kreger, A., Leake, E. S., and Harshman, S., *Infect. Immun.*, 39, 439, 1983.
94. Gemmel, C. G., Peterson, P. K., Schmeling, D. J., and Quie, P. G., *Infect. Immun.*, 38, 975, 1982.
95. Gray, G. S. and Kehoe, M., *Infect. Immun.*, 46, 615, 1981.
96. Harshman, S., *Mol. Cell. Biochem.*, 23, 143, 1979.
97. Mulvey, M. and Arbuthnott, J. P., in Reference 3, pp. 287—288.
98. Fitton, J. E., Dell, A., and Shaw, W. W., *FEBS Lett.*, 115, 209, 1980.
99. Betley, M. J., Lofdahl, S., Kreiswirth, B. N., Bergdoll, M. S., and Novick, R. P., *Proc. Natl. Acad. Sci.*, 81, 5179, 1984.
100. Bergdoll, M. S., in *Staphylococci and Staphylococcal Infection*, Vol. 2, Easman, C. F. S. and Adlam, C., Eds., Academic Press, London, 1983, 559—598.
101. Schlievert, P. M., Schoettle, D. J., and Watson, D. W., *Infect. Immun.*, 23, 609, 1979.
102. Schlievert, P. M., *Biochemistry*, 19, 6204, 1980.
103. Ikejima, T., Dinarello, C. H., Gill, D. M., and Wolff, S. M., *J. Clin. Invest.*, 73, 1312, 1984.
104. Kreiswirth, B. N., O'Reilly, M., and Novick, R. P., *Surv. Syn. P.*, 3, 73, 1984.
105. Elias, P. M., Fritsch, P., and Epstein, E. H., *Arch. Dermatol.*, 113, 207, 1977.
106. Toole, P. W. and Foster, T. J., in Reference 3, pp. 293—294.
107. Woodin, A. M., in *Microbial Toxins, Vol. 3*, Montie, T. C., Kadis, S., and Ajl, S. J., Eds., Academic Press, New York, 327—350.
108. Kanclerski, K. and Möllby, R., in Reference 3, pp. 179—180.
109. Bernheimer, A. W., Ed., *Mechanisms in Bacterial Toxinology*, Wiley Medical, New York, 1976, 85—97.
110. Smyth, C. S. and Duncan, J. L., in Jeljaszewicz, J. and Wadström, T., Eds., *Bacterial Toxins and Cell Membranes*, Academic Press, New York, 1978, 130—184.
111. Bhakdi, S., Tranum-Jensen, J., and Sziegoleit, A., *Infect. Immun.*, 47, 52, 1985.
112. Bhakdi, S. and Tranum-Jensen, J., *Infect. Immun.*, 48, 713, 1985.
113. Kehoe, M. and Timmis, K. N., *Infect. Immun.*, 43, 804, 1984.
114. Bernheimer, A. W., *J. Bact.* 93, 2024, 1967.
115. Loridan, C. and Alouf, J. E., in Reference 3, pp. 63—64.
116. Finkelstien, R. A. and Dorner, F., *Pharmacol. Ther.*, 27, 37, 1985.
117. Ouchterlony, O. and Holmgren, J., Eds., *Cholera and Related Diarrheas*, 43rd Nobel Symp., S. Karger, Basel, 1978.
118. Gill, D. M. and Woolkalis, M., in *Microbial Toxins and Diarrhoeal Disease*, CIBA Found. Symp., Vol. 112, Pitman, London, 1985, 57—73.

18   *CRC Handbook of Microbiology*

119. Mekalanos, J. J., Swartz, D. J., Pearson, G. D. N., Harford, N., Groyne, F., and deWilde, M., *Nature*, 306, 551, 1983.

120. Lockwood, D. E., Kreger, A. S., and Richardson, S. H., *Infect. Immun.*, 35, 702, 1982.

121. Takeda, Y., Takeda, T., Honda, T., and Miwatani, T., *Infect. Immun.*, 14, 1976.

122. Yanagase, Y., Inoue, K., Ozaki, M., Ochi, T., Amano, T., and Chazono, M., *Biken J.*, 13, 77, 1970.

123. Gray, L. D. and Kreger, A. S., *Infect. Immun.*, 48, 62, 1985.

124. Boyce, J. M., Evans, D. J., Evans, D. G., and DuPont, H. L., *Infect. Immun.*, 25, 532, 1979.

125. Rao, M. C., Guandalini, S., Laird, W. J., and Field, M., *Infect. Immun.*, 26, 875, 1979.

126. Brown, S. D. and Montie, T. C., *Infect. Immun.*, 18, 85, 1977.

127. Montie, T. C. and Ajl, S. J., in *Microbial Toxins*, Vol. 3, Montie, T. C., Kadis, S., and Ajl, Eds., Academic Press, New York, 1970, 1—38; Kadis, S. and Ajl, S. J., in *Microbial Toxins*, Vol. 3, Montie, T. C., Kadis, S., and Ajl, S. J., Eds., Academic Press, New York, 1970, 39—67.

128. Ohishi, I. and Tsuyama, S., *Biochem. Biophys. Res. Commun.*, 136, 802, 1986.

129. Aktories, K., Bärmann, M., Ohishi, I., Tsuyama, S., Jakobs, K. H., and Habermann, E., *Nature*, 322, 390, 1986.