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Host Damage from Bacterial Toxins [and Discussion]

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Host damage from bacterial toxins

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Bacterial infection often involves toxin-mediated damage to the host. This can occur at mucosal epithelial surfaces, in subepithelial tissues (involving connective tissue, blood vessels and host defence cells), or at organ or tissue sites distant from the focus of infection. This paper deals with host damage at each of these levels and examples have been selected of toxins that have a well defined role in pathogenesis and for which evidence is less clear cut. Current views of mechanisms of host damage are presented along with summaries of mode of action at the molecular level where this is known. Certain unifying features of mechanisms of toxin action on host cells are emphasised.

Modern genetic methods and gene cloning techniques should help in the assessment of the role of individual toxic factors where pathogenesis is multifactorial, and preliminary examples of this approach are mentioned.

The search for new toxins continues and this is illustrated with reference to the toxins involved in the staphylococcal scalded skin syndrome and staphylococcal toxic shock syndrome.

This overview is intended to convey an impression of the rapid development that has taken place in knowledge of the role of toxins in pathogenesis.

Introduction

In infections caused by bacterial pathogens the complex series of interactions that compose pathogenesis often involve damage to host tissues. This may occur at the level of epithelial surfaces, subepithelial connective tissues and specific target sites in internal organs, or tissues of the body (figure 1). The soluble bacterial extracellular factors responsible for such damage are referred to as bacterial exotoxins.

There is sometimes disagreement as to which factors should be given the status of a toxin. This is hardly surprising in view of the rather imprecise definition: 'exotoxins are extracellular proteins that cause harmful effects when injected into experimental animals in small doses'.

In some diseases the role of toxin(s) in pathogenesis is well established, e.g. diphtheria, tetanus, botulism, cholera, *Escherichia coli* gastroenteritis, clostridial and staphylococcal food poisoning, and staphylococcal scalded skin syndrome. In others where several extracellular toxins and enzymes are produced it is less easy to pinpoint the contribution of individual toxic factors; indeed synergism between toxins and auxiliary factors probably occurs (figure 2).

In assessing the pathogenic mechanisms of Gram-negative bacteria, it is necessary to consider the possible role of cell-associated endotoxin (lipopolysaccharide) in which the unique lipid A region of the molecule is capable of triggering a variety of pathophysiological changes. Where Gram-negative organisms penetrate host defences to enter the bloodstream, endotoxin becomes an important virulence factor. Endotoxic shock is a serious consequence of Gram-negative septicaemia.

Toxins represent a diverse group of bacterial products and often exert several different pathophysiological effects. For this reason it is difficult to generalize. However, in the context

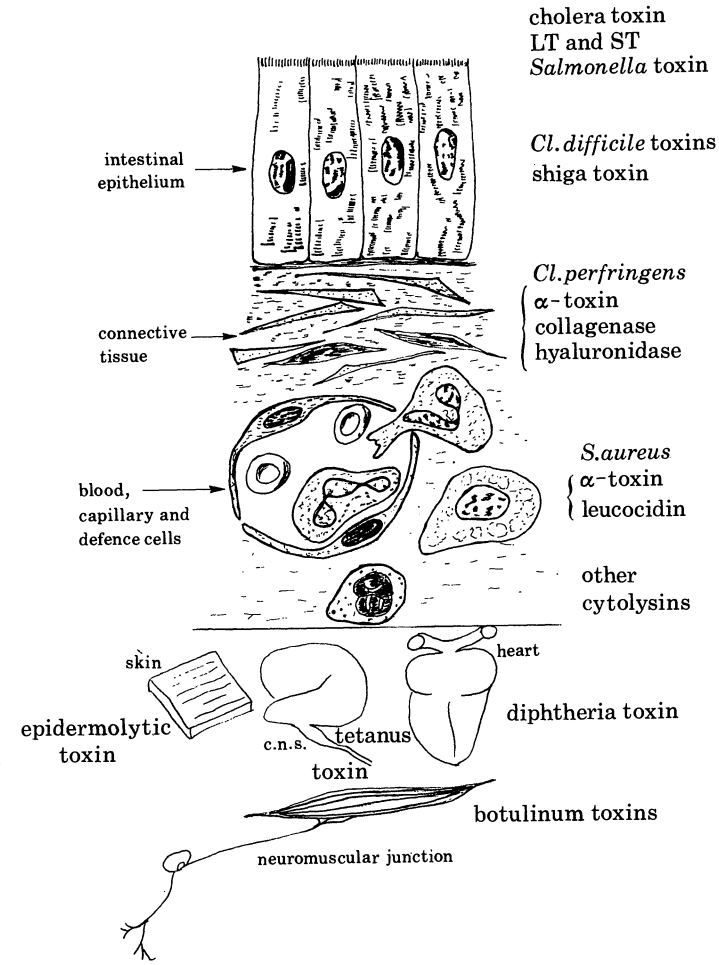

FIGURE 1. A diagrammatic representation showing examples of bacterial exotoxins that damage the host at different levels, i.e. at epithelial surfaces, subepithelial sites, and tissues or organs distant from the focus of infection.

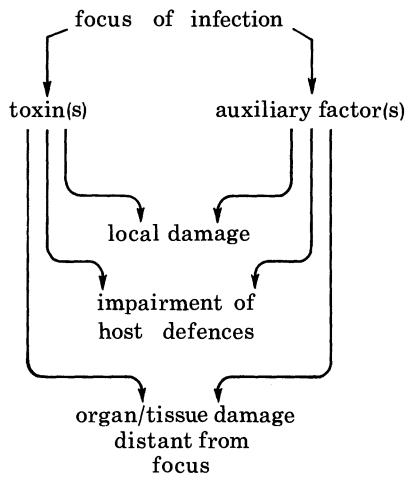

FIGURE 2. Possible interactions between bacterial exotoxins and auxiliary factors (e.g. proteases, lipases, nucleases) in bacterial pathogenesis.

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of this meeting it is helpful to consider the main types of host-damaging effects where these are well established. Three levels of tissue damage have been selected: local damage of epithelial surfaces, local damage in a subepithelial focus of infection, and damage to tissues and organs distant from the focus of infection.

The examples given include toxins whose role in pathogenesis is well known and toxins where evidence is less clear cut. Mechanisms of tissue damage are summarized and new approaches of study are indicated.

Toxin action on mucosal epithelial surfaces

The damaging effects of certain bacterial pathogens are limited to epithelial surfaces. This holds particularly for organisms that cause diarrhoeal disease. In recent years there has been an explosion of interest in the role of the toxins, known as enterotoxins, that act on the intestinal mucosa (table 1).

In some instances detailed information is available on the role of enterotoxins in pathogenesis and on the mode of toxin action. Cholera enterotoxin and the LT and ST enterotoxins of *E. coli* are examples of well characterized enterotoxins (for reviews see van Heyningen (1982), Johnson (1982) and Greenberg & Guerrant (1981)).

Cholera toxin

After colonization of epithelial cells of the small intestine, cholera vibrios release an enterotoxin that binds specifically to a GM1 ganglioside receptor on epithelial cells. The symptomatic damaging effect of cholera, namely the loss of large amounts of fluid (up to 20 l per day) results not from a cytotoxic action of the enterotoxin on epithelial cells but from a passive efflux of water from the mucosal surface after alterations in ion fluxes across the membrane of intestinal cells. The pioneering physiological studies of Field (1971) revealed that the direction of chloride ion transport was reversed with active secretion of chloride ions, whereas absorption of sodium ions was reduced. The molecular events responsible for altered ion transport are well understood. The A1 fragment of subunit A of cholera toxin hydrolyses NAD and transfers the ADP-ribose moiety of NAD to a regulatory protein (GTP-binding protein) in the adenylate cyclase complex. Activation of cyclase activity results in increased levels of cyclic AMP, which in turn effects abnormal alteration of ion fluxes (see review by Johnson 1982).

With precise knowledge of the mode of action at the molecular level it is possible to consider strategies for combating the action of cholera toxin. In experimental systems, antibody blocks toxin action, the GM1 ganglioside can be blockaded to prevent receptor-mediated binding of toxin, and antisecretory drugs such as chlorpromazine reverse the effects of altered ion flux (Holmgren 1981). These findings will, it is hoped, lead to the development of new preventative measures against cholera and other diarrhoeal diseases in man and animals.

E. coli enterotoxins

Enterotoxigenic strains of *E. coli* are important aetiological agents of diarrhoeal disease in man, pigs and calves. Two well characterized enterotoxins, heat-labile toxin (LT), and heat-stable toxin (ST) are produced by such strains under the control of plasmid-encoded genes. Strains may produce either ST or LT alone, or both together. Epidemiological studies have

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TABLE 1. SUMMARY OF EXAMPLES OF EXOTOXINS THAT ACT AT DIFFERENT LEVELS

toxin	mode of action	role in pathogenesis
	(*a*) *Toxins that act at epithelial surfaces*	
cholera toxin	Bipartite structure. 5B (binding) subunits linked to A (active) subunits. Toxin binds to GM_1 ganglioside receptor. A_1 fragment of subunit A penetrates membrane and causes ADP ribosylation of GTP-binding protein in adenylate cyclase complex	Release of cholera toxin after colonization of small intestine by *V. cholerae* leads to massive fluid and electrolyte loss. Impaired physiological function not accompanied by cell death
Escherichia coli enterotoxins	Two enterotoxins implicated. Heat-labile enterotoxin (LT) closely resembles cholera toxin in structure and mode of action. Heat-stable enterotoxin (ST); small molec. mass peptide acts by stimulation of guanylate cyclase	Organisms colonize small intestine by means of fimbrial adhesins (K88, K99, 987, CFAI, CFAII). Release of enterotoxin induces fluid loss. Impaired physiological function not accompanied by cell death
Salmonella enterotoxin	Purified enterotoxin resembles cholera toxin	Organisms colonize small intestine and penetrate columnar epithelial cells. Role of enterotoxin not yet clear
Shigella toxin	Enterotoxic, cytotoxic (inhibition of protein synthesis) and lethal activities associated with a single component	Role of toxin still unclear. Enterotoxic activity could account for water diarrhoea. Cytotoxic activity with inhibition of protein synthesis may occur in colonic epithelial cells invaded by shigellae
Clostridium perfringens enterotoxin	Enterotoxin action is accompanied by cytotoxic damage, net secretion of fluid and electrolytes and impairment of other cell functions	Sporing organisms in small intestine release enterotoxin, which is responsible for symptoms of diarrhoea
Clostridium difficile enterotoxin	Two toxins involved. Toxin A is lethal, enterotoxic, and necrotizing. Toxin B is a potent cytotoxin that does not induce fluid accumulation	Outgrowth of *Cl. difficile* after administration of antibiotics leads to pseudomembranous colitis. Combined action of toxins A and B probably involved in diarrhoea and necrosis
	(*b*) *Toxins that act locally on connective tissue, blood vessels and host defence cells*	
Clostridium perfringens α-toxin	Cytotoxic and lethal properties of α-toxin due to enymatic degradation of phospholipid components of host cell membranes	α-Toxin together with collagenase, hyaluronidase and other toxic factors, e.g. θ-toxin, combine to cause spreading necrosis and oedema typical of gas gangrene. Toxin also acts on host defence cells at sub-toxic doses
Staphylococcus aureus α-toxin	Cytotoxic and lethal properties of α-toxin due to insertion into hydrophobic region of cell membranes and subsequent disruption of normal permeability properties	α-Toxin acting together with other virulence factors (coagulase, leucocidin, other cytolytic toxins) causes local necrosis and promotes multiplication and spread of infection. Sub-toxic doses inhibit host defence cells

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toxin	mode of action	role in pathogenesis
Streptococcus pyogenes streptolysins O and S	Streptolysin O is an example of a group of toxins that bind to cholesterol in cell membranes. Streptolysin S is a small peptide that is cytolytic in a carrier-bound form	Streptolysins S and O probably act together with other factors to cause local tissue damage, and both attack host defence cells even at sub-toxic doses. Streptolysin O is also cardiotoxic
Escherichia coli α-haemolysin	Mechanism of cytolytic action poorly understood	A number of studies (epidemiology, animal models, plasmid analysis and transfer) indicate that the haemolysin is a virulence factor. Affects functions of human leucocytes and may be tissue-damaging
Pasteurella haemolytica cytolytic toxin	Mechanism unknown	Soluble cytolytic toxin specific for bovine leucocytes including alveolar macrophages. May be involved in impairment of lung defences in pneumonic pasteurellosis of cattle
	(*c*) *Toxins that act at a distance from focus of infection*	
diphtheria toxin (*Corynebacterium diphtheriae*)	A bipartite toxin that binds through B (binding) region to specific receptor. Proteolytic nicking and reduction of –S–S– bonds releases A (active) fragment, which inhibits protein synthesis by ADP-ribosylation of EF2	Symptoms of disease due to release into circulation of toxin produced at infection site. Anti-toxin immunity protective
tetanus toxin (*Clostridium tetani*)	Single polypeptide chain consists of heavy (H) and light (L) chains. Possibly bipartite. Toxin binds specifically to certain gangliosides. Inhibits release of inhibitory neurotransmitters (glycine and γ-aminobutyric acid) at inhibitory neural endings. Possibly inhibits exocytosis	Toxin produced at infection site reaches target by retrograde axonal transport. Removal of inhibitory control leads to tetanic muscular spasm. Anti-toxin immunity protective
botulinum toxins (*Clostridium botulinum*)	Single polypeptide chain activated by proteolytic nicking and reduction. Interferes with Ca^{2+} ion flux and impairs release of acetylcholine at neuromuscular junctions	Consumption of preformed toxin or toxin found in gut (infant botulism) reaches target site and causes flaccid paralysis
epidermolytic toxin (*Stephylococcus aureus*)	Toxin acts specifically at *stratum granulosum* in outer epidermis. Affected cells lose normal adhesive mechanism. Separation leads to extensive intraepidermal split	Toxin produced at focus of infection causes either localized blisters or extensive epidermal splitting (scalded skin syndrome)
Clostridium perfringens type D, ε-toxin	ε-Toxin is activated by proteolytic enzymes. Binds to luminal surface of endothelial lining of blood vessels in several tissues	Toxin produced in gut of affected animals (e.g. lambs) affects permeability of small intestine. Uptake into circulation leads to damage of vascular endothelium. Endothelial damage in brain is severe and could account for neurological symptoms

shown the importance of *E. coli* as a cause of diarrhoeal disease in man. For instance in Bangladesh, enterotoxigenic *E. coli* were found to be the commonest enteric pathogens in human adults and the second commonest, after rotaviruses, in hospitalized children (Black *et al.* 1979). Adhesive fimbrial antigens, K88, K99, CFAI and CFAII, are responsible for attachment of the organisms to the small intestinal mucosa. Like *Vibrio cholerae*, enterotoxigenic *E. coli* exert pathological effects on the intestinal epithelium through toxin-mediated interference with ion transport. The LT toxin closely resembles cholera toxin in both subunit structure and mode of action. Only 20 of the 103 amino acid residues of LTB subunits differ from cholera toxin B subunits and LT cross-reacts immunologically with cholera toxin (Dallas & Falkow 1980; Clements & Finkelstein 1978). Although GM1 ganglioside has been identified as a receptor for LT, recent work (Holmgren *et al.* 1982) has shown the existence of a glycoprotein receptor in rabbit intestine that binds LT but not cholera toxin. The distribution of this glycoprotein in other species remains to be determined. Like cholera toxin, LT acts by ADP ribosylation (Moss *et al.* 1979; Gill & Richardson 1980) and activation of adenylate cyclase.

E. coli ST differs in many respects from cholera toxin and LT, though its pathological effect (fluid loss) is similar. ST toxins are small molecular mass (1980–5100 Da) heat-stable peptides that act on the small intestine by reducing absorption of sodium and chloride ions. Differences have been reported in amino acid content, methanol solubility and animal-species specificity for ST toxins prepared from different strains (see review by Greenberg & Guerrant 1981). Unlike cholera toxin and LT, the effects of STs on ion and fluid transport are immediate and the toxins are inactive in most non-intestinal assay systems. ST_A (methanol soluble) causes fluid accumulation in neonatal mice and newborn pigs, whereas ST_B (methanol insoluble) induces fluid accumulation in rabbit ileal loops and in older pigs. A specific receptor for ST has not yet been identified, though it seems likely that interaction with a receptor serves as the initial signal for subsequent events. By contrast with cholera toxin and LT, ST activates guanylate cyclase rather than adenylate cyclase and the cyclic GMP analogue 8-bromo cyclic GMP has a similar effect to ST. Various agents prevent ST-induced activation of guanylate cyclase (table 2), and these might be of value in combating the effect of this toxin.

Salmonella toxin

The pathogenesis of salmonellosis is more complex than that of cholera and *E. coli* gastroenteritis. Whereas cholera vibrios and *E. coli* colonize the surface of epithelial cells, salmonellae often invade the intestinal epithelium and penetrate to the lamina propria. Nevertheless the symptoms of *Salmonella* gastroenteritis include diarrhoea, which can be severe (Axon & Poole 1973). Several groups have attempted to demonstrate the existence of a *Salmonella* enterotoxin. Living cultures of salmonellae cause an accumulation of fluid in ligated rabbit intestinal loops (see review of Peterson 1980). However, it has proved more difficult to demonstrate an accumulation of fluid with culture filtrates and purified 'toxin' preparations. Indeed, Gianella (1979) provided evidence that the inflammatory reaction resulting from invasion by *Salmonella* organisms is responsible for secretion of fluid. However, a partly purified enterotoxin that causes accumulation of fluid in rabbit intestinal loops was reported by Sedlock *et al.* (1978*a*, *b*). Also, a factor, termed permeability factor (PF), which causes a delayed type of increased skin permeability, was isolated by Peterson & Sandefur (1979); it competed for cholera toxin binding sites in rabbit small intestine. This factor showed several similarities to cholera toxin and LT, and concentrated preparations of purified PF caused accumulation of

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TABLE 2. POSSIBLE STRATEGIES FOR INTERFERING WITH *E. COLI* ST

(From Greenberg & Guerrant (1981).)

ingestion of ST-producing *E. coli*	← hygiene
↓	
adherence and colonization of upper small bowel	← receptor blocking, or antibody prevention of fimbrial binding
↓	
? binding of ST	
↓	
activation of prostaglandin	← quinacrine
↓	
release of free radicals	← indomethacin, free radical scavangers, e.g. chlorpromazine
↓	
particulate guanylate cyclase activation	
↓	
synthesis of cyclic GMP	
↓	
? activation of protein kinase	← phenothiazines (chlorpromazine)
↓	
impaired Cl^- absorptive flux	
↓	
net isotonic fluid secretion (non-inflammatory diarrhoea)	

fluid in rabbit intestinal loops (unpublished result quoted by Peterson (1980)). Characterization of these enterotoxic factors is still incomplete, and the role of such factors in *Salmonella* gastroenteritis is not yet clear.

Shigella toxin

Diseases caused by *Shigella* can have two different clinical presentations: (*a*) watery diarrhoea resulting from fluid loss through the small intestine, and (*b*) acute inflammatory bacterial colitis with frequent passage of bloody mucus-containing stools, i.e. dysentery. Considerable controversy surrounds the role of toxins in these conditions.

Interest in the possible role of 'enterotoxin' arose as a result of the findings of Keusch *et al.* (1972*a*, *b*), who showed that *Shigella* produced a toxin active in ligated ileal loops from rabbit. Since then it has become clear that several toxic activities are associated with shigellae: enterotoxicity, cytotoxicity, neurotoxicity and lethal activity (see review by Keusch *et al.* 1981). Progress was hampered by low yields of purified material and difficulty in determining whether all the toxicities were manifestations of a single moiety. Anti-shiga toxin affinity chromatography was employed by one group (O'Brien *et al.* 1980) to obtain purified toxin possessing cytotoxicity for HeLa cells, enterotoxicity and lethal activity. The isolation of milligram quantities of highly purified shiga toxin having a molecular mass of 70 kDa (Brown *et al.* 1982) that possessed all three toxic activities should facilitate further study of its mode of action.

Keusch *et al.* (1981) propose that enterotoxin activity is involved in the diarrhoeal syndrome and that in dysentery, cytotoxic activity inhibits protein synthesis in colonic epithelial cells invaded by *Shigella* organisms; killing of epithelial cells could of course lead to inflammatory colitis.

Clostridium perfringens enterotoxin

The production of enterotoxins is not restricted to Gram-negative organisms. For instance the symptoms of *Cl. perfringens* food poisoning result from the action of an enterotoxin released during sporulation of the organism of the gut; the toxin appears to be a spore coat protein

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(Frieben and Duncan 1975). In rabbits, net secretion of fluid and electrolyte in the small intestine is accompanied by histological damage due to cytotoxic action on the ileum and jejunum (see review by McDonel 1980). The toxin also caused erythema in guinea-pig skin after intradermal injection. Inhibition of plating efficiency of Vero cells in tissue culture is a sensitive *in vitro* test. Fluid loss in the intestine results from reversal of transport from absorption to secretion of fluid, sodium ions and chloride ions; desquamation of cells occurs at the villus tips. Most evidence points to the toxin's being responsible for damage to cell membranes, and in ultrastructural studies the first indication of this is formation of blebs in the membrane (McDonel *et al.* 1978). In addition to alterations in ion and water transport, such membrane damage leads to inhibition of other cell functions such as glucose uptake, energy production and macromolecule synthesis.

Clostridium difficile toxins

Pseudomembranous colitis (p.m.c.) results from the outgrowth of *Cl. difficile* in the intestine in some patients receiving orally administered antibiotics. A model for this disease in hamsters, termed antibiotic associated caecitis, has been used to study the role of toxins in the pathogenesis of p.m.c. Most patients have diarrhoea and show the development of a pseudomembrane in the colon. Histopathogical features range from focal epithelial necrosis to complete necrosis of the lamina propria surmounted by a thick firmly attached pseudomembrane consisting of fibrin, mucus, polymorphonuclear cells and bacteria derived from the colon (see review by Chang *et al.* 1981). A potent cytotoxin was found in the stools of patients with p.m.c. (Larson & Price 1977); indeed the discovery of this toxin played a major part in the subsequent identification of *Cl. difficile* as the causative organism. More recently, Taylor *et al.* (1981) described two antigenically distinct toxins, which have been partly characterized (Libby *et al.* 1982).

Toxin A has the properties of an enterotoxin: it is also weakly cytotoxic and when injected into the hamster caecum induces multifocal necrosis of the epithelium. Toxin B is a potent cytotoxin toxin (1000 times more cytotoxic than toxin A) that does not cause fluid accumulation but in the hamster caecum induces epithelial cell necrosis with inflammation and haemorrhage.

The combined action of toxins A and B probably account for the symptoms of p.m.c.

In summary, extracellular toxic factors play a very important role in pathogenesis at the level of the intestinal epithelium. Such toxins are basically of two types: either they involve net fluid and ion loss by interfering with intracellular regulation of ion fluxes or they act principally by cytotoxic damage of epithelial cells. In some disease syndromes synergism almost certainly occurs between different toxins produced by the causative organism. The examples given are of toxin action on intestinal epithelium. Less is known of the involvement of toxins in damage to other epithelia. However, there is considerable evidence that toxic factors of *Bordetella pertussis* are envolved in localized damage to epithelial cells in the respiratory tract.

Toxin-mediated local damage to connective tissue, blood vessels and defence cells

After penetrating mucosal epithelia, some bacterial pathogens exert powerful damaging effects through the action of their toxins on connective tissue, blood vessels and host defense cells (figure 1). These damaging effects may result in a localized lesion, or they may lead to further invasion and spread of the organism. The outcome depends not only on the toxinogenicity of the species and strain of bacteria but also on the susceptibility of the individual

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host. The multifactorial nature of pathogenesis is clearly evident in such infections. To establish itself in the tissues of the host such a pathogen must be capable of resisting a barrage of host defences and in particular must be capable of resisting the onslaught of phagocytic cells delivered to the focus of infection by means of the inflammatory response (for review see Arbuthnott 1981). The analysis of the contribution of individual virulence factors to pathogenicity in such complex situations is at an early stage because of the difficulty in genetically manipulating the organisms concerned. Clostridia, staphylococci and streptococci are examples of pathogens that can cause infections in which toxins play a role in local tissue damage after invasion.

Clostridial toxins in necrosis and toxaemia

Cl. perfringens strains produce a large number of toxic or potentially toxic extracellular factors (for review see McDonel 1980), and strains are typed on the basis of their patterns of major lethal and necrotizing toxins (α, β, ε, ι). In addition these organisms produce a large number of auxiliary toxic factors, which probably augment the damaging effects of the principal toxins.

Type A strains of *Cl. perfringens* are the commonest cause of gas gangrene, and this disease is an example of a situation in which tissue damage probably involves several toxic factors. Pathological changes include necrosis and oedema involving damage to connective tissue, blood vessels and muscle tissue; systemic effects can follow rapidly, with the development of a fatal toxaemia. Owing to the large number of toxic factors produced by these organisms and the difficulty of reconciling results with different experimental animal models it is not possible to pinpoint the role of individual toxins precisely. However, it is accepted by most workers that α-toxin is a dominant factor in the development of necrosis and toxaemia. This is supported by results obtained in animals immunized with purified α-toxin. For instance, Kameyama *et al.* (1975) demonstrated that guinea-pigs immunized with purified α-toxin did not develop gas gangrene when challenged simultaneously with live organisms, α-toxin and κ-toxin (collagenase).

The α-toxin has been well characterized (see review by McDonel 1980). It is a cytolytic membrane-damaging toxin that is haemolytic, cytotoxic, dermonecrotic and lethal. These toxic effects on cells result from enzymic degradation of phospholipid components present in mammalian cell membranes; the toxin is a phospholipase C (see review by Mollby 1978). Auxiliary factors implicated with α-toxin in gas gangrene are θ-toxin (thiol-activated cytolysin, κ-toxin (collagenase) and ν-toxin (hyaluronidase). Further analysis of the pathogenic role of individual virulence factors produced by *Cl. perifringens* will require the application of modern genetic techniques.

The role of staphylococcal toxins in tissue damage

Like *Cl. perfringens*, *Staphylococcus aureus* produces a large number of toxins and auxiliary toxic factors (see review by Freer & Arbuthnott 1983), including a group of membrane-damaging cytolysins (α-, β-, γ- and δ-toxin and leucocidin), each of which is a candidate for involvement in localized tissue damage or in more subtle perturbation of host defences (Arbuthnott 1981). Much work points to the importance of α-toxin in this respect.

The development of a neonatal mouse model (MacKay & Arbuthnott 1980) allowed the comparison of strains with different toxin patterns; α-toxinogenic strains produced spreading necrotic lesions, whereas a poorly toxinogenic strain gave localized abscesses. Later, Kinsman *et al.* (1980), working with three different mouse models, showed that α-toxin-deficient mutants of *S. aureus* had reduced virulence. These observations confirmed the conclusions of Adlam

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et al. (1977) that α-toxin played a determining role in development of the spreading lesion in a gangrenous form of staphylococcal mastitis in rabbits.

Whereas mutant strains and purified antigens have proved valuable in investigating the role of α-toxin in pathogenesis, the availability of cloning techniques will facilitate a more detailed analysis of staphylococcal toxins and virulence factors. The α-toxin gene has been cloned in *E. coli* and *Bacillus subtilis* (Kehoe *et al.* 1983). This opens the possibility of reintroducing the α-toxin gene into *S. aureus* strains with a known genetic background and, perhaps more importantly, will allow the introduction of specific α-toxin mutations back into virulent strains of *S. aureus* either by using plasmid vectors or by homologous recombination with residual wild-type α-toxin determinant.

Genetic analysis of the role of E. coli haemolysin

The ease of genetic analysis in *E. coli* has greatly facilitated the study of the pathogenic role of *E. coli* haemolysin (Welch *et al.* 1981; Waalwijk *et al.* 1982). There is now considerable evidence in favour of the haemolysin's being a virulence factor (see Cavalieri & Snyder (1982) for recent references). Haemolytic strains of *E. coli* are frequently associated with extra-intestinal infections. Haemolytic strains are more virulent for mice and rats than non-haemolytic strains. A plasmid encoding for haemolysin is associated with virulence in nephropathogenic strains, and the elimination of the haemolysin plasmid results in a loss of nephropathogenicity. The DNA sequence encoding for haemolysin, when incorporated in a non-haemolytic faecal strain resulted in increased virulence for rats (Welch *et al.* 1981).

Whether this cytolytic toxin contributes to pathogenesis directly by virtue of its membrane-damaging activity (e.g. on leucocyte function (Cavalieri & Snyder 1982)) or indirectly by some other mechanism (e.g. by provision of increased levels of iron after the release of haemoglobin by lysis of erythrocytes) is not known. There is no doubt, however, that this is a useful model system for applying modern advances in molecular biology to the analysis of virulence.

Toxin action on host defence cells

There are several points at which bacterial toxins can attack host defence cells: (i) inhibition of the chemotactic response of phagocytic cells, (ii) killing or impairing the action of phagocytic cells that arrive at the focus of infection, and (iii) alteration of the responses of lymphocytes.

Several membrane-damaging cytolytic toxins have been shown to kill phagocytic cells or to inhibit phagocyte function (for review see Arbuthnott 1981). This aspect of cytolytic toxins produced by *S. aureus*, *Streptococcus pyogenes* and *Cl. perfringens* has been well studied; however, it is interesting to note more recent suggestions for the involvement of cytolytic toxins.

Pasteurella haemolytica is associated with pneumonic pasteurellosis, which causes major economic loss of feedstock cattle. The organism produces a cytolytic toxin that specifically kills bovine leucocytes (alveolar macrophages, cultured monocytes and neutrophils) (Shewen & Wilkie 1982). This toxic factor may contribute to pathogenesis by impairing lung defences and the subsequent immune response or by initiating an inflammatory response after leucocyte lysis; alternatively both types of reaction might contribute to disease.

Interestingly, production of cytolytic toxin has also been demonstrated in *Legionella pneumophila* (Friedman *et al.* 1982). The small molecular mass (1300 Da) heat-stable toxic factor interferes with certain neutrophil functions and could be involved in the intracellular survival of the pathogen.

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Interference with leucocyte function by sub-toxic levels of cytolytic toxins

In the context of interference with host defences, it is interesting to note that in many instances cytolytic toxins at sub-toxic concentrations can interfere with leucocyte functions such as phagocytosis, chemotaxis and random movement (Wilkinson 1975, 1977). Such studies have revealed the need for caution in interpretation because individual cytolytic toxins have different effects on the leucocytes of different species (see summary in table 3). One of the most complete studies of chemotactic responses and its susceptibility to interference by bacteria was that of Russell *et al.* (1976) who examined whole bacterial cells, culture supernatants and fractions prepared by isoelectric focusing of nine strains of *S. auerus*. This revealed a complex pattern of both stimulatory and inhibitory activities.

TABLE 3. INHIBITION OF CHEMOTAXIS BY SUBLETHAL BACTERIAL CYTOLYTIC TOXINS

(This information represents a synopsis of work published up to 1981: +ve denotes inhibition; −ve denotes no effect; n.d. denotes no published information. H, human; S, sheep; R, rabbit.)

		effect on	
	toxin	polymorphonuclear cells	monocytes/ macrophages
Cl. perfringens	streptolysin O	+ve (H > S > R)	n.d.
	α-toxin	−ve (H)	+ve (H)
		+ve (R)	n.d.
	θ-toxin	+ve (H)	−ve (H)
S. aureus	α-toxin	+ve (H)	+ve (H)
	β-toxin	−ve (H)	+ve (H)
		+ve (R)	n.d.
	leucocidin	+ve (H)	+ve (H)

TOXIN-MEDIATED DAMAGE AT SITES DISTANT FROM THE FOCUS OF INFECTION

So far, attention has focused on localized action of toxins on epithelial surfaces or in tissues close to a focus of infection. In some diseases toxin absorbed across an epithelial surface or diffusing out of a focus of infection penetrates, through the bloodstream or by another route, to affect single or multiple targets at distant sites. Examples include not only the classical toxinoses (diphtheria, tetanus and botulism) but clostridial enterotoxaemia of domestic animals, streptococcal scarlet fever, and staphylococcal scalded skin syndrome.

Diphtheria toxin

This powerful lethal toxin is synthesized by *Corynebacterium diptheriae* in the diphtheritic pseudomembrane in the throat, and passes into the bloodstream where it exerts a toxic action on various tissues and organs. The pathological symptoms result from an inhibition of protein synthesis through the action of diphtheria toxin on susceptible cells.

The molecular mechanisms involved have been very thoroughly studied and have been reviewed extensively (see review by Uchida 1982). Afer binding of the toxin to a glycoprotein receptor through its B fragment (molecular mass 38 kDa), proteolytic cleavage and thiol reduction lead to the entry of fragment A (molecular mass 24 kDa) of diphtheria toxin, which catalyses, the hydrolysis of NAD^+ and ADP-ribosylation of elongation factor 2 (EF2); death of the cell results from the inhibition of protein synthesis.

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The mechanism of entry of fragment A into the target cell is still far from clear. This has proved to be an extremely challenging problem, partly because the number of molecules of toxin that bind to a cell does not necessarily reflect the number of molecules that penetrate the cell. Indeed, Yamaizumi *et al.* (1978) showed that a single molecule of fragment A can kill a cell. The exotoxin A of *Pseudomonas aeruginosa* also inhibits protein synthesis by ADP-ribosylation of EF2. Diphtheria toxin and *Pseudomonas* exotoxin A are related to the extent that the immunodeterminant of *Pseudomonas* toxin A cross-reacts with a normally inaccessible determinant of fragment A of diphtheria toxin. However, no hybridization was detected between the DNA encoding for the two toxins (Sadoff *et al.* 1982). It is therefore probable that diphtheria toxin and exotoxin A evolved independently. Adaptation to a similar substrate could explain the limited degree of antigenic similarity.

From the point of view of public health the success of toxoid immunization has played a major part in reducing the incidence of diphtheria in the population. Recently antitoxic immunity to diphtheria toxin was achieved with a synthetic peptide (representing the sequence between residues 188 and 201) covalently linked to a protein carrier. As a further development of this approach the same workers (Audibert *et al.* 1982) have obtained immunity in mice and guinea-pigs with a synthetic octadecapeptide linked, with adjuvant, to a synthetic carrier. This constitutes a totally synthetic diptheria vaccine with built-in adjuvanticity and represents a major advance in the field of synthetic vaccines.

Tetanus toxin

Released by sporing organisms at the site of infection, tetanus toxin is taken up at neuromuscular junctions and passes to its site of action in the central nervous system by a process known as retrograde axonal transport (see review by van Heyningen 1980). This can be demonstrated by immunofluorescence and radioautography (Price *et al.* 1975; Carroll *et al.* 1978). Uptake of the toxin by the axon could depend on the high specificity of tetanus toxin for the gangliosides GT_1 and GD_{1b} present in the membrane at the motor nerve ending (Stockel *et al.* 1977); entry of toxin may be by endocytosis with subsequent transport of toxin with endocytic vessels. The specificity of tetanus toxin for binding to gangliosides GT_1 and GD_{1b} first reported by van Heyningen (1963) has been thoroughly studied. Dimpfel *et al.* (1975) confirmed that toxin binds to gangliosides in the membranes of neuronal cells.

The pathology of tetanus, namely convulsive spasm of the voluntary musculature, results from continual excitation of the motor neurons of the central reflex apparatus in the spinal cord. After reaching the synapse, tetanus toxin exerts this action by preventing the release of the inhibitory neurotransmitters, glycine and γ-aminobutyric acid, that under normal conditions would regulate stimulation of the motor neuron (see review by Bizzini 1979).

The molecular mode of action of tetanus toxin is still not fully understood. However, the toxin molecule consists of an H chain (molecular mass 100 kDa), which contains the ganglioside-binding site (van Heyningen 1976) and an L chain (molecular mass 50 kDa). The H and L fragments are formed by proteolytic nicking of the native polypeptide chain and can be separated after reduction of disulphide bonds. This type of structure suggests an analogy with known bipartite toxin (van Heyningen 1982). If this model is correct, then binding of the H fragment probably results in penetration by the L region of the membrane of the inhibitory motor neuron at the inhibitory synapse. Inhibition of release of transmitter may result from the inhibition of exocytosis of synaptic vesicles.

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Botulinum toxin

Cl. botulinum produces a number of serologically distinct, highly potent neurotoxins. Absorption of preformed bolutinum toxin from the intestine after consumption of food contaminated with *Cl. botulinum* induces the symptoms (nausea, dizziness, neurological disorders) of botulism. The recently recognized form of the disease known as infant botulism differs from this in that *Cl. botulinum* toxin is elaborated by organisms growing in the intestine of infants (Arnon *et al.* 1977). Botulinum toxins all have similar structures (see review by Sugiyama 1980). They are secreted as single polypeptide chains having a molecular mass around 150 kDa. Like tetanus toxin, proteolysis results in nicking with the formation of fragments of molecular masses 100 kDa (H chain) and 50 kDa (L chain) respectively.

Botulinum toxins are the most potent known bacterial toxins, with specific toxicities of 10^7 to 10^8 l.d.$_{50}$ per milligram when tested by intraperitoneal challenge in mice. The action of toxin on the peripheral nervous system results in flaccid paralysis by contrast with the muscular spasms of tetanus. The most likely explanation of the mode of action of botulinum toxin is that it acts on peripheral nerve endings to prevent the release of acetylcholine (see Sugiyama 1980). A striking feature of nerve muscle junctions poisoned with botulinum toxin is the improved release of acetylcholine on addition of agents such as 4-amino pyridine that increase the intraneuronal calcium ion concentration, indicating a link between calcium ion flux and the action of the toxin. There is increasing evidence from work with synaptosomes (see Sugiyama 1980) that the toxin binds preferentially to the presynaptic membrane through the H chain (molecular mass 100 kDa). The precise molecular events involved in the action of botulinum toxin are not known. However, it appears that binding of the toxin is followed by a series of events that result in blocking the calcium-dependent fusion of synaptic vesicles with the presynaptic membrane (Pumplin & del Castillo 1975) and the subsequent release of acetylcholine.

Staphylococcal epidermolytic toxin

Epidermolytic toxins (A and B) act on the outer layers of the epidermis, causing the cells in this region to lose their normal mechanisms of cell–cell contact. Separation of the cells occurs with the formation of an intraepidermal cleavage plane (see review by Freer & Arbuthnott 1983). This action explains the role of epidermolytic toxins in staphylococcal blistering diseases of the skin. These range from localized blistering conditions such as pemphigus neonatorum and bullous impetigo (due to localized production of toxin) to the scalded skin syndrome, in which extensive epidermal splitting over large areas of the skin results from the spread of toxin in the bloodstream.

The discovery of epidermolytic toxin in the early 1970s (Melish *et al.* 1970; Arbuthnott *et al.* 1971; Kapral & Miller 1971) triggered an explosion of interest in the bacteriological, dermatological and paediatric aspects of these diseases (see review by Freer & Arbuthnott 1983). Purification of the toxins (A and B), studies of genetic control, and biological aspects such as species specificity have advanced rapidly. However, the molecular events that trigger separation of cells in the epidermis remain a mystery at present.

Staphylococcal toxic shock syndrome: codicil and conundrum

Toxic shock syndrome is characterized by the sudden development of high fever, vomiting, diarrhoea, hypotension and the onset of erythrematous rash (Todd *et al.* 1978; United States

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C.D.C. report 1980). It is associated with the use of vaginal tampons and with the carriage of phage group I strains of *S. aureus*. Because bacteraemia is rare the multisystem effects are consistent with the elaboration of one or more staphylococcal toxic factors. A 'toxic' protein (molecular mass approx. 20 kDa) variously termed staphylococcal enterotoxin F (Bergdoll *et al.* 1981), pyrogenic exotoxin C (Schlievert *et al.* 1981) and toxic factor (de Azavedo *et al.* 1983) is produced by most toxic shock syndrome strains. Although the role of this factor in the pathogenesis of toxic shock syndrome remains to be firmly established, its ability to induce pyrogenicity and a lethal shock-like syndrome in the rabbit (Schlievert *et al.* 1981; Schlievert 1982; de Azavedo *et al.* 1983) might lead to the development of a model system for further study. The findings of de Azavedo *et al.*, showing a paradoxical age-related susceptibility to toxic factor in rabbits, point to an indirect rather than a direct toxic action. The ability of toxic factor to potentiate the lethal effect of endotoxin is consistent with such an indirect mechanism.

The extent to which this toxic factor, acting alone or in combination with minute amounts of endogenous endotoxin, accounts for the symptoms of toxic shock syndrome remains to be further investigated.

Concluding remarks

Toxins can exert their effects at many levels in the infected host. There are some unifying features of their mechanism of interaction with susceptible cells (e.g. the delivery of active moieties from bipartite toxins, and membrane damage by cytolytic toxins). Some toxins from different pathogens have similar pathophysiological effects. Progress in the study of modes of action has been considerable, and knowledge so gained will help in developing new approaches to prevention and therapy. However, in many cases there are vital gaps, either in the identification of the molecular events involved in toxin damage or in pinpointing the role of individual toxins in pathogenesis. These problems will continue to be the targets of active research interest.

References

Adlam, C., Ward, P. D., McCartney, A. C., Arbuthnott, J. P. & Thorley, C. 1977 Effect of immunization with highly purified alpha- and beta-toxins on staphylococcal mastitis in rabbits. *Infect. Immun.* **17**, 250–256.

Arbuthnott, J. P. 1981 Membrane-damaging toxins in relation to interference with host defence mechanisms. In *Microbial perturbation of host defences* (ed. F. O'Grady & H. Smith), pp. 97–115. London: Academic Press.

Arbuthnott, J. P., Kent, J., Lyell, A. & Gemmell, C. G. 1971 Toxic epidermal necrolysis produced by an extracellular product of *S. aureus*. *Br. J. Derm.* **85**, 145–149.

Arnon, S. S., Midura, T. F., Clay, S. A., Wood, R. M. & Chin, J. 1977 Infant botulism. Epidemiological, clinical and laboratory aspects. *J. Am. med. Ass.* **237**, 1946–1951.

Audibert, F., Jolivet, M., Chedid, I., Arnon, R. & Sela, M. 1982 Successful immunization with a totally synthetic diphtheria vaccine. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5042–5046.

Axon, A. T. R. & Poole, D. 1973 Salmonellosis presenting with cholera-like diarrhoea. *Lancet* i, 745–746.

de Azavedo, J., Hartigan, P. J., de Saxe, M. & Arbuthnott, J. P. 1983 The rabbit as an animal model for the toxic shock syndrome. (Submitted.)

Bergdoll, M. S., Cross, B. A., Reisser, R. F., Robbins, R. N. & Davis, J. P. 1981 A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome *Staphylococcus aureus* isolates. *Lancet* ii, 1017–1021.

Bizzini, B. 1979 Tetanus toxin. *Microbiol. Rev.* **43**, 224–240.

Black, R. E., Merson, M. H., Rowe, B., Taylor, P. R., Mizanur Rahman, A. S. M., Huq, A., Aleem, A. R. M. A., Sack, D. A. & Curlin, G. T. 1979 Epidemiology of enterotoxigenic *Escherichia coli* in rural Bangladesh. In *Symposium on cholera* (ed. K. Takeya & Y. Zinnaka), pp. 292–301. Tokyo: Fuji Printing Company.

Brown, J. E., Griffin, D. E., Rothman, S. W. & Doctor, B. P. 1982 Purification and biological characterization of shiga toxin from *Shigella dysenteriae* 1. *Infect. Immun.* **36**, 996–1005.

Carroll, P. T., Price, D. L., Griffin, J. W. & Morris, J. 1978 Tetanus toxin immunocytochemical evidence for retrograde transport. *Neurosci. Lett.* **8**, 335–339.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Cavalieri, S. J. & Snyder, I. S. 1982 Effect of *Escherichia coli* alpha hemolysin on human peripheral leukocyte function *in vitro*. *Infect. Immun.* **37**, 966–974.
Chang, T.-W., Bartlett, J. G. & Taylor, N. S. 1981 *Clostridium difficile* toxin. *Pharmac. Ther.* **13**, 441–452.
Clements, J. D. & Finkelstein, R. A. 1978 Demonstration of shared and unique immunologic determinants in enterotoxins from *Vibrio cholerae* and *Escherichia coli*. *Infect. Immun.* **22**, 709–713.
Dallas, W. S. & Falkow, S. 1980 Amino acid sequence homology between cholera toxin and *Escherichia coli* heat-labile toxin. *Nature, Lond.* **288**, 499–501.
Dimpfel, W., Neale, J. H. & Habermann, E. 1975 ^{125}I-labelled tetanus toxin as a neuronal marker in tissue cells derived from embryonic CNS. *Nauyn-Schmiedebergs Arch. Pharmac.* **290**, 329–333.
Field, M. 1971 Intestinal secretion effect of cyclic AMP and its role in cholera. *New Engl. J. Med.* **284**, 1137–1144.
Freer, J. H. & Arbuthnott, J. P. 1983 Toxins of *Staphylococcus aureus*. *Pharmac. Ther.* (In the press.)
Frieben, W. R. & Duncan, C. L. 1975 Heterogeneity of enterotoxin-like protein extracted from spores of *Clostridium perfringens* type A. *Eur. J. Biochem.* **55**, 455–463.
Friedman, R. L., Lochner, J. E., Bigley, R. H. & Iglewski, B. H. 1982 The effects of *Legionellea pneumophila* toxin on oxidative processes and bacterial killing of human polymorphonuclear leukocytes. *J. infect. Dis.* **146**, 328–334.
Gianella, R. A. 1979 Importance of the intestinal inflammatory reaction in *Salmonella*-mediated intestinal secretion. *Infect. Immun.* **23**, 140–145.
Gill, D. M. & Richardson, S. H. 1980 Adenosine diphosphate-ribosylation of adenylate cyclase catalysed by heat-labile enterotoxin of *Escherichia coli*: comparison with cholera toxin. *J. infect. Dis.*, **141**, 64–70.
Greenberg, R. N. & Guerrant, R. L. 1981 *E. coli* heat-stable enterotoxin. *Pharmac. Ther.* **13**, 507–531.
van Heyningen, W. E. 1963 The fixation of tetanus toxin, strychnine, serotonin, and other substances by ganglioside. *J. gen. Microbiol.* **31**, 375–387.
van Heyningen, S. 1976 Binding of ganglioside by the chains of tetanus toxin. *FEBS Lett.* **68**, 5–7.
van Heyningen, S. 1980 Tetanus toxin. *Pharmac. Ther.* **11**, 141–157.
van Heyningen, S. 1982 Similarities in the action of different toxins. In *Molecular action of toxins and viruses* (ed. P. Cohen & S. van Heyningen), pp. 169–190. Amsterdam, New York and London: Elsevier Biomedical Press.
Holmgren, J. 1981 Actions of cholera toxin and the prevention and treatment of cholera. *Nature, Lond.* **292**, 413–417.
Holmgren, J., Fredman, P., Lindblad, M., Svennerholm, A. M. & Svennerholm, L. 1982 Rabbit intestinal glycoprotein receptor for *Escherichia coli* heat-labile enterotoxin lacking affinity for cholera toxin. *Infect. Immun.* **38**, 424–433.
Johnson, G. L. 1982 Cholera toxin action and the regulation of hormone-sensitive adenylate cyclase. In *Molecular action of toxins and viruses* (ed. P. Cohen & S. van Heyningen), pp. 33–49. Amsterdam, New York and London: Elsevier Biomedical Press.
Kameyama, S., Sato, H. & Murata, R. 1975 The role of α-toxin of *Clostridium perfringens* in experimental gas gangrene in guinea pigs. *Japan J. med. Sci. Biol.* **25**, 200.
Kapral, F. A. & Miller, M. M. 1971 Product of *S. aureus* responsible for the scalded skin syndrome. *Infect. Immun.* **4**, 541–545.
Kehoe, M., Dougan, G., Foster, T. J. & Duncan, J. 1983 Cloning, expression and mapping of the *Staphylococcus aureus* α-haemolysin determinant in *Escherichia coli* K12. (Submitted.)
Keusch, G. T., Donohue-Rolfe, A. & Jacewicz, M. 1981 Shigella toxin(s): description and role in diarrhoea and dysentery. *Pharmac. Ther.* **15**, 403–446.
Keusch, G. T., Grady, G. F., Mata, L. J. & McIver, J. 1972*a* The pathogenesis of *Shigella* diarrhoea I enterotoxin production by *Shigella dysenteriae* 1. *J. clin. Invest.* **51**, 1212–1218.
Keusch, G. T., Grady, G. F., Takeuchi, A. & Sprinz, H. 1972*b* The pathogenesis of *Shigella* diarrhoea. II. Enterotoxin induced acute enteritis in the rabbit ileum. *J. infect. Dis.* **126**, 92–95.
Kinsman, O., Jonsson, P., Haraldson, I., Lindberg, M., Arbuthnott, J. P. & Wadstrom, T. 1980 Decreased virulence of alpha-haemolysin negative and coagulase negative mutants of *S. aureus* in experimental infections in mice. In *Staphylococci and Staphylococcal infections* (ed. J. Jeljaszewicz) (*Zbl. Bakt. Suppl.* no. 10), pp. 651–659. Stuttgart and New York: Gustav Fischer Verlag.
Larson, H. E. & Price, A. B. 1977 Pseudomembranous colitis: presence of clostridial toxin. *Lancet* ii, 1312–1314.
Libby, J. M., Jortner, B. S. & Wilkins, T. D. 1982 Effects of the two toxins of *Clostridium difficile* in antibiotic-associated cecitis in hamsters. *Infect. Immun.* **36**, 822–829.
McDonel, J. L. 1980 *Clostridium perfringens* toxins (type A, B, C, D, E). *Pharmac. Ther.* **10**, 617–655.
McDonel, J. L., Chang, L. W., Pounds, J. G. & Duncan, C. L. 1978 The effects of *Clostridium perfringens* enterotoxin on rat and rabbit ileum: an electron microscopic study. *Lab. Invest.* **39**, 210–218.
McKay, S. E. & Arbuthnott, J. P. 1979 Age-related susceptibility of mice to staphylococcal infection. *J. med. Microbiol.* **12**, 99–105.
Melish, M. E., Glasgow, L. A. & Turner, M. D. 1970 Staphylococcal scalded skin syndrome: experimental model and isolation of a new exofoliative toxin. *Paediat. Res.* **4**, 378.
Mollby, R. 1978 Bacterial phospholiphases. In *Bacterial toxins and cell membranes* (ed. J. Jeljaszewicz & T. Wadstrom), pp. 367–424. London: Academic Press.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Moss, J., Garrison, S., Oppenheimer, N. J. & Richardson, S. H. 1979 NAD-dependent ADP-ribosylation of arginine and proteins by *Escherichia coli* heat-labile enterotoxin. *J. biol. Chem.* **254**, 6270–6272.
O'Brien, A. D., La Veck, G. D., Griffin, D. E. & Thompson, M. R. 1980 Characterisation of *Shigella dysenteriae* 1 (shiga) toxin purified by anti-shiga toxin affinity chromatography. *Infect. Immun.* **30**, 170–179.
Peterson, J. W. 1980 Salmonella toxin. *Pharmac. Ther.* **11**, 719–724.
Peterson, J. W. & Sandefur, P. D. 1979 Evidence of a role for permeability factors in the pathogenesis of salmonellosis. *Am. J. clin. Nutr.* **32**, 197–209.
Price, D. L., Griffin, J., Young, A., Peck, K. & Stocks, A. 1975 Tetanus toxin: direct evidence for retrograde intraaxonal transport. *Science, Wash.* **188**, 945–947.
Pumplin, D. W. & del Castillo, J. 1975 Release of packets of acetylcholine from synaptic vesicles elicited by brown widow spider venom in frog motor nerve endings poisoned by botulinum toxin. *Life Sci.* **17**, 137–142.
Russell, R. J., Wilkinson, P. C., McInroy, R. J., McKay, S., McCartney, A. C. & Arbuthnott, J. P. 1976 Effects of staphylococcal products on locomotion and chemotaxis of human blood neutrophils and monocytes. *J. med. Microbiol.* **8**, 433–449.
Sadoff, J. C., Buck, G. A., Iglewski, B. H., Bjorn, M. J. & Groman, N. B. 1982 Immunological cross-reactivity in the absence of DNA homology between *Pseudomonas* toxin A and diphtheria toxin. *Infect. Immun.* **37**, 250–254.
Schlievert, P. M. 1982 Enhancement of host susceptibility to lethal endotoxin shock by staphylococcal pyrogenic exotoxin type C. *Infect. Immun.* **36**, 123–128.
Schlievert, P. M., Shands, K. N., Dan, B. D., Schmid, G. P. & Nishimura, R. D. 1981 Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic shock syndrome. *J. infect. Dis.* **143**, 509–516.
Sedlock, D. M. & Deibel, R. H. 1978*a* Detection of *Salmonella* enterotoxin using rabbit ileal loops. *Can. J. Microbiol.* **24**, 268–273.
Sedlock, D. M., Koupal, L. R. & Deibel, R. H. 1978*b* Production and partial purification of *Salmonella* enterotoxin. *J. Infect. Immun.* **20**, 375–380.
Shewen, P. E. & Wilkie, B. N. 1982 Cytotoxin of *Pasteurella haemolytica* acting on bovine leukocytes. *Infect. Immun.* **35**, 91–94.
Stockel, K., Schwab, M. & Thonen, H. 1977 Role of ganglioside in the uptake and retrograde axonal transport of cholera and tetanus toxin as compared to nerve growth factor and wheat germ agglutinin. *Brain Res.* **132**, 273–285.
Sugiyama, H. 1980 *Clostridium botulinum* neurotoxin. *Microbiol. Rev.* **44**, 419–448.
Taylor, N. S., Thorne, G. M. & Bartlett, J. G. 1981 Comparison of two toxins produced by *Clostridium difficile*. *Infect. Immun.* **34**, 1036–1043.
Todd, J., Fishaut, M., Kapral, F. & Welch, T. 1978 Toxic shock syndrome associated with phage-group I straphylococci. *Lancet* ii, 1116–1118.
Uchida, T. 1982 Diphtheria toxin: biological activity. In *Molecular action of toxins and viruses* (ed. P. Cohen & S. van Heyningen), pp. 1–31. Amsterdam, New York and London: Elsevier Biomedical Press.
United States C.D.C. report 1980 Toxic shock syndrome. In *United States C.D.C. Morbid. Mortal. Rep.* no. 29, pp. 229–230.
Waalwijk, C., van den Bosch, J. F., MacLaren, D. M. & de Graff, J. 1982 Hemolysin plasmid coding for the virulence of a nephropathogenic *Escherichia coli* strain. *Infect. Immun.* **35**, 32–37.
Welch, R. H., Dellinger, E. P., Minshew, B. & Falkow, S. 1981 Haemolysin contributes to virulence of extra-intestinal *E. coli* infections. *Nature, Lond.* **294**, 665–667.
Wilkinson, P. C. 1975 Inhibition of leukocyte locomotion and chemotaxis by specific bacterial toxins. *Nature, Lond.* **255**, 485–487.
Wilkinson, P. C. 1977 Action of sphingomyelinase C and other lipid specific agents as inhibitors of Fc binding and locomotion in human leucocytes. *Immunology* **33**, 407–412.
Yamaizumi, M., Mekada, E., Uchida, T. & Okada, Y. 1978 One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. *Cell* **15**, 245–250.

Discussion

J. M. Rutter (*Institute for Research on Animal Diseases, Compton, U.K.*). Professor Arbuthnott emphasized in his paper the multifactorial nature of the action of many bacterial toxins *in vivo*. With regard to the development of toxoid vaccines, does he consider that purified component vaccines will be developed, or that because of the complex interactions of these toxins *in vivo* we shall still rely on relatively crude but often outstandingly successful products such as the clostridial vaccines used in veterinary medicine?

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J. P. Arbuthnott. Purified component vaccines are successful in prevention of the classical toxinoses (e.g. tetanus and diphtheria). Indeed in diphtheria toxin the antigenic region of the toxin molecule that confers protective immunity has been identified and a synthetic peptide of 16 amino acids has been prepared which, when coupled to adjuvant, raised specific immunity in experimental animals.

Where pathogenesis is multifactorial and several toxins are involved, the task is more difficult. We are still at an early stage in identifying the determinants of virulence. For some time to come we shall have to rely on relatively crude mixtures of antigens. However, successful identification of virulence factors will provide a better strategy for vaccine development. It is to be hoped that existing vaccines will be improved and new vaccines, where none exist, will be developed.

A. A. Glynn (*Central Public Health Laboratory, London, U.K.*). From the galaxy of toxins so well described by Professor Arbuthnott, I should just like to pick out staphylococcal α-toxin. Its importance in the pathogenesis of staphylococcal lesions has been stressed for many years, but has not always been satisfactorily demonstrated. Today Dr Adlam mentioned [discussion not submitted for publication] how antibody to α-toxin was beneficial in staphylococcal mastitis of sheep. This is not necessarily proof of α-toxin's pathogenic role. In the subcutaneous staphylococcal abscess model developed by Noble in mice, Charles Easmon and I (*Immunology* **29**, 67–76 (1975)) could prevent dermonecrosis not only by anti-α-toxin but by antibodies to non-toxigenic non-pathogenic strains of staphylococci or by non-specific local inflammation. Thus, although we did not exclude neutralizing effects of antibody, the inflammation produced by a local Arthus reaction seemed to be the major protective factor. As Professor Smith said earlier, proving the role of putative virulence factors is difficult.

J. P. Arbuthnott. Professor Glynn has raised a good point, which I did not have time to cover. Staphylococcal pathogenesis is truly multifactorial and difficult to analyse. Much depends on the experimental animal model used and the strain of *S. aureus* selected. Much evidence points to the importance of α-toxin as a cause of local tissue damage. Clearly, however, as pointed out by Profesor Glynn, other factors are also involved.

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