OCCASIONAL REVIEW

COMPARATIVE STUDY OF THE NATURE AND BIOLOGICAL ACTIVITIES OF BACTERIAL ENTEROTOXINS

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Introduction

Within the last 20 years there has been a great increase in the number of aetiological agents associated with gastrointestinal infection and intoxication. Concomitant with this has been the realisation that many of the microorganisms responsible elaborate enterotoxins that contribute to the pathogenesis of the infection. Most of these enterotoxins give rise to a hypersecretion of fluid from the intestinal mucosa by a mechanism that may or may not involve cytolytic damage to the epithelium lining the intestinal tract (Keusch and Donta, 1975). Enterotoxins that are not cytotoxic are now designated cytotonic, mainly because the efflux of water is passive and follows the active secretion of chloride ions into the intestinal lumen (Field et al., 1969). In general, these toxins are thought to exert their effects by perturbations of the cyclic nucleotide system at the level of the cyclase. There are many similarities between the different enterotoxins in terms of their activation of this system. In this review I shall attempt to bring together the various features of the interactions of enterotoxins and intestinal cells to achieve an integrated approach to the different modes of action. Table I summarises the different types of enterotoxin which will be used to illustrate the different ways in which they act.

Within the cytotonic group of enterotoxins, two types of toxin that differ in their molecular size and mode of action within the intestinal cell can be distinguished (table II). One group comprises enterotoxins with a mol. wt of 70 000–90 000, made up of various active subunits and capable of activating adenylate cyclase. The enterotoxins in the other group have a much lower mol. wt (5000–10 000) and are capable of activating guanylate cyclase instead of adenylate cyclase. Very much less is known about the biological activities of the enterotoxins elaborated by gram-positive bacteria.

A more detailed picture of the structural features of the individual toxins has emerged in recent years with the discovery that several subunits with different sizes and biological functions make up the native enterotoxin molecules (table III). As will be shown later when the individual enterotoxins are discussed, there appear to be three types of toxin present among the gram-negative intestinal pathogens. There are heat-labile cytotonic enterotoxins with a subunit structure, heat-stable cytotonic enterotoxins with no subunit structure and cytotoxic enterotoxins with a subunit structure and active on subcellular functions. The enterotoxins of gram-positive organisms appear to include substances with similar properties.

Much of our knowledge about the way in which bacterial enterotoxins act has been

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TABLE I
Classification of bacterial enterotoxins

<table>
<thead>
<tr>
<th>Source of enterotoxin</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotonic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>A. hydrophila</td>
<td></td>
</tr>
<tr>
<td>C. difficile</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>toxin A</td>
<td>V. cholerae</td>
<td>Salmonella sp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y. enterocolitica</td>
</tr>
<tr>
<td>Cytotoxic</td>
<td>B. cereus</td>
<td>Sh. dysenteriae</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>E. coli</td>
<td></td>
</tr>
</tbody>
</table>

TABLE II
Cytotoxic toxins classified according to molecular size and biological activity

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Molecular size</th>
<th>Biochemical system activated</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. cholerae</td>
<td>84 000</td>
<td>Adenylate cyclase</td>
</tr>
<tr>
<td>E. coli LT</td>
<td>85 000</td>
<td></td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>90 000</td>
<td></td>
</tr>
<tr>
<td>Sh. dysenteriae</td>
<td>70 000</td>
<td></td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>78 000</td>
<td></td>
</tr>
<tr>
<td>E. coli ST</td>
<td>2500</td>
<td>Guanylate cyclase</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>9700</td>
<td></td>
</tr>
</tbody>
</table>

TABLE III
Sub-unit structure of certain bacterial enterotoxins

<table>
<thead>
<tr>
<th>Producer bacterium</th>
<th>Toxin</th>
<th>Number of subunits</th>
<th>Molecular size</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. cholerae</td>
<td>Choleragen</td>
<td>1 × A</td>
<td>28 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 × B</td>
<td>11 500</td>
</tr>
<tr>
<td>E. coli</td>
<td>LT</td>
<td>1 × A</td>
<td>25 500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 × B</td>
<td>11 500</td>
</tr>
<tr>
<td>Sh. dysenteriae</td>
<td>Dysentery (Shiga)</td>
<td>1 × A</td>
<td>30 000</td>
</tr>
<tr>
<td></td>
<td>toxin</td>
<td>4 or 5 × B</td>
<td>7000–11 000</td>
</tr>
</tbody>
</table>

TABLE IV
Models used for enterotoxin assays in vitro and in vivo

<table>
<thead>
<tr>
<th>In-vitro tests</th>
<th>In-vivo tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte ghosts</td>
<td>Suckling mice</td>
</tr>
<tr>
<td>Adrenal cells</td>
<td>Infant rabbit</td>
</tr>
<tr>
<td>Isolated fat cells</td>
<td>Rabbit ileal loop</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Skin permeability</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td></td>
</tr>
</tbody>
</table>
derived from the use of laboratory models—either whole animals or isolated tissue cells. A list of the commonly used models is shown in table IV. For example, Y1 adrenal cells derived from mouse adrenal cortex grow as a confluent monolayer which, in the presence of enterotoxin, undergoes morphological and metabolic changes that include rounding up and detachment from the plate surface and secretion of increased amounts of δ-4,3 ketosteroids. Similar changes can be induced by cyclic adenosine monophosphate (cAMP) and adrenocorticotrophin (ACTH), and increased intracellular concentrations of cAMP are detectable in the intoxicated cells (Donta, King and Sloper, 1973; Donta, 1974).

The different cell types have been used (Richards and Douglas, 1978) to study the binding and physiological effects of choleragen and the heat-labile enterotoxin (LT) of *Escherichia coli*. Close similarities in membrane receptors have been shown and the sensitivities of the cells to these toxins have been compared on the basis of the numbers of cell receptors and activation sites on each cell type; e.g., toad erythrocytes, cultured melanocytes and fibroblasts have 20 000–50 000 binding sites/cell but only 1000–3000 activation sites.

With the increasing interest in enteropathogenic microorganisms, their virulence factors and their role in the disease process, it will be impossible in this review to cover all aspects of infection and intoxication by these organisms. I shall concentrate on the comparative aspects of the potent enterotoxins produced, in terms of their biological activity, their active sites, receptors and substrates, leading to a discussion of their mode of action at a molecular level where known and so attempt to draw together the various strands of information available for the different toxins to produce an integrated picture of their similarities and differences.

**Vibrio cholerae**

Of the various enterotoxins to be described in this review, that produced by *Vibrio cholerae* has been well defined both in terms of its structure and mode of action. It consists (fig. 1) of five B subunits (mol. wt 11 500 each) that are responsible for the binding of the toxin to specific receptors on the brush border epithelium, and the A subunit (mol. wt 28 000). The receptors are composed of GM₁ ganglioside, of which the lipid moiety is embedded in the lipid matrix of the cell membrane with the hydrophobic oligosaccharide moiety sticking out from the outer surface of the cell membrane (fig. 2). The B subunits stick to this oligosaccharide, one per receptor molecule. After the toxin has bound to the membrane, translocation of the single A subunit through the membrane takes place. The A subunit comprises a single polypeptide that can split through a disulphide bridge into two fragments—A₁ (mol. wt 20 500) and A₂ (mol. wt 7500).

The active toxin moiety is accessible to antitoxin for only 30–50 s after binding. Thereafter, its presence remains undetected until at least 10 min have elapsed during which activation of adenylate cyclase occurs. Little is known of what happens during this time except that some evidence has been presented showing that some protein synthesis occurs (Moss et al., 1976).

Two hypotheses have been proposed: (a) the active moiety may become enclosed within a vesicle and later released within the cytosol; or (b) it may pass directly through the plasma membrane. The B subunits may facilitate these processes by forming a
CHOLERA TOXIN

CHOLERAGENOID (pI 7.75) 56,000 (toxoid)

CHOLERAGEN (pI 6.6) 84,000

Fig. 1.—Subunit structure of cholera toxin.

channel through which the A subunit can pass in an unfolded form (Gill, 1976) with fraction A₂ leading the way (fig. 3). Gill et al. (1981) have proposed that the B subunits remain on the surface of the cell membrane and play no further part in the entry process. Saturation of the binding sites by B subunits renders the cell immune to cholera toxin. However, if the cells are broken open they become sensitive to intoxication by fragment A₁.

Once in the cytosol, the A₁ fragment catalyses a transfer of ADP-ribose from NAD to membrane-bound proteins, principally a part of the regulatory GTP-binding component of adenylate cyclase; this target has a subunit structure of mol. wt 42,000 (Cassel and Pfeuffer, 1978). Such an event “locks” adenylate cyclase in an active form by inhibiting an inherent feed-back regulatory mechanism by which stimulatory GTP is hydrolysed to inhibitory GDP and inorganic phosphate; cAMP then accumulates intracellularly (fig. 4). Hormones increase the cyclase activity by increasing the rate of GTP binding. Cholera toxin, on the other hand, inhibits the GTPase “turn-off” reaction and thus increases the activity of adenylate cyclase (Cassel and Selinger, 1977).

Ceramide—Glucose—Galactose—N-Acetyl galactosamine—Galactose

N-Acetyl neuraminic acid

Fig. 2.—Structure of ganglioside GM₁, the cell receptor for cholera toxin.
BACTERIAL ENTEROTOXINS

Fig. 3.—Binding to (I) and translocation through (II) the mammalian cell membrane of cholera toxin. A₁, A₂, and B represent subunits of cholera toxin; □ = ganglioside, GM₁; ○ = phospholipid molecule; □ = protein molecule.

Fig. 4.—Cholera toxin and the adenylate cyclase system. A₁ = subunit of toxin, C = component responsible for conversion of ATP to cAMP, R = component responsible for GTP-dependent regulation of enzyme activity, H = hormone receptor.
The accumulation of cAMP stimulates active secretion of chloride and bicarbonate by crypt cells and inhibits the normal absorption of chloride coupled to sodium ions by villus cells. Because cholera toxin increases cAMP in both cell types, the diarrhoea probably results from both mechanisms acting simultaneously (Field, 1981). In addition, it has been postulated that cholera toxin potentiates the release of serotonin from the enterochromaffin cells of the small intestine resulting in further fluid secretion by a nervous reflex (Cassuto et al., 1979).

Activation of membrane-bound adenylate cyclase is dose-dependent (Bennett and Cuatrecasas, 1975) and results in the conversion of ATP to cAMP. This activation is distinct from that caused by epinephrine, prostaglandins, glucagon (Berkenstein and Delaney, 1976), catecholamines or ACTH. Cholera toxin exerts its maximum effect after 1–3 h and cAMP levels remain high for 12 h whereas *E. coli* LT acts best in 15–90 min and activation is short-lived (Finkelstein et al., 1976). Several enterotoxins can also potentiate hormonal stimulation of adenylate cyclase. The variety of physiological changes induced in different types of cell is summarised in table V.

**Table V**

Interaction of cholera toxin with isolated cell functions

<table>
<thead>
<tr>
<th>Cell system</th>
<th>Effect of toxin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse adrenal cells</td>
<td>Morphological change; steroidogenesis increased</td>
<td>Dona et al. (1973)</td>
</tr>
<tr>
<td>Chinese hamster ovary cells</td>
<td>Morphological change; cAMP increased</td>
<td>Guerrant et al. (1974)</td>
</tr>
<tr>
<td>Enterochromaffin cells</td>
<td>Granule release; cAMP increased</td>
<td>Fujita et al. (1974)</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>Lysosomal enzyme release reduced</td>
<td>Bourne et al. (1973)</td>
</tr>
</tbody>
</table>

**Shigella dysenteriae**

The molecular structure and mechanism of action of *Shigella dysenteriae* toxin (Shiga toxin) has been largely elucidated in the last 4 years. Like cholera toxin and *E. coli* LT, it consists of two kinds of polypeptide chain (Olsnes and Eiklid, 1980; Olsnes, Reisbig and Eiklid, 1981). One of the chains, the A chain or its active A1 fragment, enters into the enterocyte cytoplasm and inhibits protein biosynthesis by inactivating catalytically, the 60S ribosomal sub-units (Brown, Rothman and Doctor, 1980; Reisbig, Olsnes and Eiklid, 1981). Only one A chain is present in each molecule of toxin. The remainder of the toxin consists of six or seven B chains that are probably concerned in the binding of the toxin to sites at the cell surface (Eiklid and Olsnes, 1980). So far, only certain primate-derived epithelial cell lines bind the toxin. A possible mechanism has been proposed (Keusch, Donahue-Rolfe and Jacewicz, 1981) whereby the toxin penetrates the intestinal cell and releases its active subunit A so that protein synthesis can be impaired (figs 5A and 5B).

There is conflicting evidence about whether the toxin directly stimulates adenylate cyclase activity leading to increased levels of cAMP and fluid accumulation in the gut.
lumen (Donowitz, Keusch and Binder, 1975; Charney et al., 1976). Injection of the toxin cause haemorrhages in the intestinal mucosa, probably as a result of its cytotoxic activity, and it seems more likely, therefore, that any changes in fluid balance, adenylate cyclase activity and cAMP levels might be a secondary phenomenon (table VI). As well as inducing fluid accumulation in rabbit ileal loops, the toxin is also lethal to monkeys, rabbits and mice (Keusch et al., 1972) and the intoxicated animals show evidence of neurological disorder, viz., hind leg paralysis.

In an elegant study, Eiklid and Olsnes (1983) have shown that the cytotoxic, enterotoxic and neurotoxic activities of the purified toxin are engendered by the same
molecule. They showed that the relative concentrations of the three activities remained the same during purification of the toxin and were equally destroyed by heating to 80°C and precipitation by neutralising antibody. In summary, therefore, this enterotoxin ought to be regarded as a cytotoxic enterotoxin that is potentially lethal, and is different in its mechanism of action from either cholera toxin or E. coli LT.

**Salmonella species**

Until the mid 1960's, there was little evidence to suggest that the pathogenesis of human salmonellosis involved anything more than endotoxin (Kohler and Bohl, 1966; Thomlinson and Buxton, 1963). Koupal and Deibel (1975) identified an envelope-associated enterotoxin that caused fluid secretion in newborn mice and this finding started a different train of events that resulted in the discovery of two skin permeability factors in rabbits (Sandefur and Peterson, 1976). The salmonella toxin(s) closely resembled cholera toxin and E. coli LT in its biological activities in vitro. However, the salmonella toxin failed to induce fluid and electrolyte secretion in rabbit intestinal loop tests (Molina and Peterson, 1980). More recently, Baloda et al. (1983) examined various strains of *Salmonella enteritidis* and *S. typhimurium* and described the presence therein of a heat-labile enterotoxin that gave a positive intestinal loop assay, a positive rabbit skin permeability test and morphological changes in Y1 adrenal cells and CHO tissue cells. Their strains had been isolated in India whereas those of Peterson and his colleagues were derived from American sources. Thus there may be geographical differences amongst *Salmonella* strains in their enterotoxigenicity. It appears that the two toxins differ in their sensitivity to proteolytic digestion (Peterson, 1980).

Because the salmonella enterotoxin can bind to GMI ganglioside, it is likely that it may act at the cellular and sub-cellular level in the same way as cholera toxin. However, no subunit structure has yet been described in this enterotoxin.

**Escherichia coli**

The association between travellers' diarrhoea and toxigenic *E. coli* is well recognised and is usually the result of the action of heat stable (ST) or heat-labile (LT) enterotoxin or both on the small intestine (Sack, 1975). ST from *E. coli* strains of human origin is a simple protein with a mol. wt of c. 2500 (Madsen and Knoop, 1980).
Specific binding of ST to the rat intestinal cells and, in particular, brush border membranes has been demonstrated (Giannella, Luttrell and Drake, 1980) and a proteinaceous receptor has been implicated in the binding process. Thereafter, the toxin stimulates guanylate cyclase, so increasing the intracellular concentration of cGMP (Newsome, Burgess and Mullan, 1978).

Usually the biological activity of the toxin is measured in vivo and there is evidence to suggest that calcium and prostaglandins play a part in the secretion process (Guerrant et al., 1980) and a mechanism of action of ST has been proposed by Greenberg and Guerrant (1981) which is outlined in table VII. Hypotheses of the roles for free radicals and the activation of prostaglandin synthesis have developed from the findings that (a) butylated hydroxy anisole (BHA), a free radical scavenger, can inhibit the action of ST, and (b) indomethacin, by inhibiting cyclo-oxygenase, which is involved in the arachidonic acid cascade, can impair ST action. In summary, therefore, ST causes fluid secretion within the intestinal lumen by activation of guanylate cyclase and impairment of chloride absorption. Experiments with a variety of pharmacologically active inhibitors indicate that ST may act through the prostaglandin synthesis pathway, may involve free-radical activation of guanylate cyclase and may be regulated by intracellular calmodulin.

On the basis of significant differences in heat stability and amino-acid composition, the production of two distinct STs by E. coli has been recognised (Kapitany et al., 1979). Likewise, differences between Yersinia enterocolitica ST (see later) and E. coli ST may reflect a family of ST molecules that are similar in their biological activity and mechanism of action but somewhat different in structure.

In contrast to the relatively small amount of information available about E. coli ST, LT has received considerable attention because of its close similarity with cholera toxin. By cloning of the LT structural gene and translation of the chimeric plasmid produced in E. coli minicells, the structure of LT has been shown (Dallas, Gill and Falkow, 1979) to consist of a protein of mol. wt 25 500 that has adenylate-cyclase-stimulating activity as well as NADase activity and several copies of a protein of mol. wt 11 500 that is bound to Y 1 adrenal cells; both proteins react with anticholera toxin sera (Dallas and Falkow, 1979). LT cross-reacts immunologically mainly with antisera directed against the B subunit of cholera toxin; this indicates some homology between the binding domains of each toxin (Klipstein and Engert, 1977). Like cholera toxin,
LT needs NAD$^+$ to activate adenylate cyclase and can also potentiate the effect of hormones on this enzyme.

As well as the immunological similarities between the B subunits of *E. coli* LT and cholera toxin, LT appears to bind specifically to ganglioside GM$_1$, but it has been suggested that the binding is less avid because the amounts of GM$_1$ needed to neutralise its biological activity are somewhat larger than for cholera toxin (Zenser and Metzger, 1974). Another difference between the binding of the two toxins is that the biological activity of *E. coli* LT is not blocked to the same extent by cholera toxoid (choleragenoid) as is the activity of cholera toxin (Holmgren, 1973). Otherwise it appears that *E. coli* LT and cholera toxin bear a close resemblance to each other.

A significant immunological cross-reaction between cholera toxin and *E. coli* LT has been recognised (Holmgren, 1980) to the extent that both the A and B sub-units of the two toxins are antigenically similar. Using an enzyme-linked immunosorbent assay (ELISA), Holmgren and Svennerholm (1979) observed that monospecific anti-cholera A and anti-cholera B antibodies both reacted with *E. coli* LT bound to GM$_1$ ganglioside adsorbed onto plastic. It is likely that *E. coli* LT, as well as sharing determinants with cholera toxin, also contains unique antigenic determinants.

The production of enterotoxins by non-invasive enterotoxigenic strains of *E. coli* is responsible for intestinal fluid secretion and, hence, diarrhoea. In contrast, other *E. coli* strains that are serologically related to the shigellae (Formal et al., 1978) are able to penetrate and multiply within colonic epithelial cells (Dupont et al., 1971). Strains of a third group recognised by serotype as enteropathogenic *E. coli* (EPEC), have been incriminated as agents of epidemic and endemic diarrhoeal disease in infants (table VIII) but they are not enteroinvasive nor do they produce LT or ST (Gurwith, Wiseman and Chow, 1977). Many EPEC and some other *E. coli* intestinal isolates that produce the classic enterotoxins also make a cell-associated cytotoxin that can be neutralised by antitoxin prepared against the purified toxin of *Sh. dysenteriae* (O’Brien et al., 1982). Moreover, like shigella extracts, cell lysates of certain EPEC strains are enterotoxic for rabbit ileal loops and lethal and paralytic for mice. This cytotoxin is probably a recognisable virulence determinant in EPEC strains and this suggestion is supported by the destruction of intestinal epithelial cell microvilli seen in small bowel biopsies from infants with EPEC diarrhoea (Clausen and Christie, 1982). The Shiga-like toxin has been isolated and purified (O’Brien and La Veck, 1983) and shown to be composed of one A subunit (mol. wt 31 500±1000) and several copies of a B subunit (mol. wt 4000), making a native toxin molecule of mol. wt 48 000.

The A subunit can be nicked by trypsin or by endogenous proteases to form an A$_1$ component (mol. wt 27 000) and an A$_2$ component (mol. wt 4500). There are, therefore, many similarities between this toxin and that produced by *Sh. dysenteriae*

### Table VIII

*E. coli* serotypes and diarrhoeal disease

<table>
<thead>
<tr>
<th>Pathogenic group</th>
<th>O-serotypes that have the given pathogenic mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteropathogenic</td>
<td>26, 55, 86, 111, 114, 119, 125, 126, 127, 128, 142</td>
</tr>
<tr>
<td>Enterotoxigenic</td>
<td>6, 8, 15, 25, 27, 78, 148, 149</td>
</tr>
<tr>
<td>Enteroinvasive</td>
<td>28ac, 112ac, 124, 136, 143, 144, 152, 164</td>
</tr>
</tbody>
</table>
and antibody to the latter toxin can neutralise the former. So far, no studies have been published on the mechanism of action of the E. coli cytoxin but it is likely that there is a family of Shiga-like toxins as there is a family of cholera-like toxins.

**Aeromonas hydrophila**

Another member of the Vibrionaceae, *Aeromonas hydrophila* produces both a heat-stable and a heat-labile enterotoxin. Strains of this species that have caused opportunistic infection in compromised patients and diarrhoea in normal people produce only the heat-labile enterotoxin (Wadström, Ljungh and Wretlind, 1976; Annapurna and Sanyal, 1977) whereas strains isolated from fish produce both enterotoxins (Boulanger, Lallier and Cousineau, 1977). Little is known about the heat-stable enterotoxin, but several interesting facts about the heat-labile enterotoxin are known (Ljungh, Eneroth and Wadström, 1982a and b; Ljungh and Kronevi, 1982). The biological features resemble those of cholera enterotoxin and *E. coli* LT but there are several significant differences (table IX). Some of these differences may be attributable to the nature of the toxin itself. It has a much lower mol. wt, only 15 000, has no apparent subunit structure and is very sensitive to proteolytic digestion which inactivates it. The native form of this toxin may have a higher mol. wt and be broken down immediately after intracellular biosynthesis. Such a hypothesis would account for the inability of gangliosides or antibody to cholera toxin or *E. coli* LT to neutralise its biological effects. It should also be noted that the enterotoxin induces cytotoxic changes in adrenal Y1 cells and elongation of Chinese hamster ovary cells similar to those produced by cholera toxin and *E. coli* LT (Ljungh, Wretlind and Möllby, 1981). However, the sensitivity of the adrenal cells to aeromonas toxin was much lower than their sensitivity to cholera toxin, but β-glucosidase or β-galactosidase treatment of the cells increased their sensitivity to the aeromonas toxin.

Similar differences in the degree of induction of intracellular cAMP, but not of cGMP, by the toxin in either intestinal or adrenal cells were noted by Ljungh, Eneroth and Wadström (1982b). Aeromonas enterotoxin was further shown to induce steroidogenesis in adrenal Y1 cells but was less effective on the basis of equivalent weights than cholera toxin, although the lag phase was significantly shorter with aeromonas enterotoxin than with either cholera toxin or *E. coli* LT.

<table>
<thead>
<tr>
<th>Test system</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethality</td>
<td>negative</td>
</tr>
<tr>
<td>Fluid accumulation (rabbit ileal loop)</td>
<td>positive</td>
</tr>
<tr>
<td>Adrenal Y1 cell elongation</td>
<td>positive</td>
</tr>
<tr>
<td>Rabbit skin permeability</td>
<td>positive</td>
</tr>
<tr>
<td>Potentiation of cAMP synthesis</td>
<td>positive</td>
</tr>
<tr>
<td>Steroidogenesis in adrenal cells</td>
<td>positive</td>
</tr>
<tr>
<td>Neutralisation by gangliosides</td>
<td>negative</td>
</tr>
<tr>
<td>Neutralisation of effects by antiserum to cholera toxin or <em>E. coli</em> LT</td>
<td>negative</td>
</tr>
</tbody>
</table>
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Yersinia enterocolitica

This organism has been recognised recently as an important cause of gastroenteritis in children (Kohl, Jacobson and Nahmias, 1976) and part of its pathogenicity has been attributed to its ability to elaborate a low mol. wt (9700) heat-stable enterotoxin (Okamoto et al., 1982). This toxin has strong similarities with E. coli ST in its activity in the suckling mouse assay (ED₉₀ₐₜ = 25 ng) through causing fluid accumulation within the intestine by activation of cGMP (Robins-Browne et al., 1979), and in its failure to induce morphological changes in Chinese hamster ovary cells or Y1 adrenal cells.

Compared with the enterotoxins elaborated by gram-negative bacteria, the enterotoxins of the gram-positive bacteria (table X) have only recently been subjected to the same kind of analysis, with Clostridium perfringens enterotoxin foremost.

TABLE X
Enterotoxins of gram-positive bacteria

<table>
<thead>
<tr>
<th>Producer species</th>
<th>Mol. wt</th>
<th>Isoelectric point (pI)</th>
<th>Number of electrophoretic forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. aureus</td>
<td>28 000-35 000</td>
<td>7-0-8-6</td>
<td>Several</td>
</tr>
<tr>
<td>B. cereus</td>
<td>55 000-60 000</td>
<td>4-85</td>
<td>One</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>35 000</td>
<td>4-3</td>
<td>Two</td>
</tr>
<tr>
<td>C. difficile</td>
<td>450 000</td>
<td>5-5</td>
<td>One</td>
</tr>
</tbody>
</table>

Clostridium perfringens

This enterotoxin, although cytotoxic, probably falls into a separate group from the other cytotoxic enterotoxins because it inhibits glucose uptake, energy production and macromolecular synthesis within the intestinal epithelium (McDonel and Duncan, 1976, 1977; McDonel, 1979).

The toxin has been estimated to have a mol. wt of 35 000 and binds to intestinal cells with a suggested binding site density of 1.96 × 10⁶ sites/cell (McDonel, 1979). However, this binding is not reversible, which suggests that some form of trapping mechanism occurs within the cell. This would account for the fact that the toxin produces functional “holes” in Vero (African green monkey) cells according to the methods described by Thelestam and Möllby (1976). The “holes” allowed the release of nearly 100% of the ¹⁴C-aminobutyric acid (mol. wt 103) and 50-60% of ³H-uridine (mol. wt 224) from the cells. Markers with higher molecular sizes were not released, which suggests that the functional holes were small but, nevertheless, large enough or frequent enough to cause changes in cell morphology, viability and macromolecular synthesis. Within the intestinal mucosa, levels of cAMP remain unaltered throughout enterotoxin treatment. Using everted ileal sacs from rats, McDonel and Duncan (1976) showed that the toxin causes a significant reduction in oxygen consumption by the sacs. Cellular glycolysis was unaffected but oxidative metabolism (e.g., oxygen consumption by isolated mitochondria) was impaired. Such damage to the respiratory control within the intestinal cells may or may not be secondary to the structural
damage caused, especially at the villus tips (McDonel et al., 1978) but nevertheless may result in the fluid and electrolyte changes that culminate in diarrhoea (fig. 6).

An amphipathic structural model has been proposed for the enterotoxin, with one tightly formed hydrophobic domain and another, less rigid, hydrophilic domain (Granum and Whitaker, 1980). Limited proteolysis of the toxin has been reported to activate the enterotoxin about threefold (Granum, Whitaker and Skjelkvåle, 1981). In particular, trypsination yields a polypeptide of 16 000 mol. wt that can inhibit cell-free protein biosynthesis (Granum, 1982) and intracellular protein biosynthesis may be inhibited after the entry of part of the complete enterotoxin molecule into the cell cytosol. It has been suggested that the hydrophilic part of the molecule is responsible for binding to cell receptors and the hydrophobic moiety is released through the membrane. Amino acid sequencing of the N-terminal end of the trypsin-activated toxin (Richardson and Granum, 1983) revealed certain similarities between C. perfringens enterotoxin and the corresponding region of sub-unit B of cholera toxin which is, itself, associated with membrane binding.

**Staphylococcus aureus**

Several antigenically distinct staphylococcal enterotoxins are produced by a significant percentage of clinical isolates of *Staph. aureus* (Bergdoll, 1976). A summary of the physicochemical features of the different serotypes of toxin is presented in table XI. Symptoms of intoxication in man include vomiting, diarrhoea and nausea and all serotypes produce similar clinical features.

However, although much is known about the structural features of the various

![Diagram](image_url)

**Fig. 6.**—Fluid and electrolyte changes in intestinal mucosa induced by *C. perfringens* enterotoxin. Modified from McDonel (1979). A represents the normal flow of electrolytes across the intestinal epithelium; B represents the electrolyte flow induced by *C. perfringens* enterotoxin.
enterotoxin types the biochemical mechanism by which the toxins act is largely unknown. One suggestion for their activity has been direct cytotoxicity causing changes in intestinal transport of fluids and electrolytes (Sullivan, 1969; Huang, Chen and Rout, 1974). Contrary evidence has recently been presented by Buxser and Bonventre (1981) who studied the putative leakage of several radioactively-labelled markers from human embryonic intestinal epithelial cells (Henle 407) by enterotoxin. Enterotoxin failed to produce any "functional holes" in the cell membranes and, even in the presence of several staphylococcal cytotoxins, no synergistic cellular damage was found. Nor did the enterotoxin affect the ability of the Henle cells to take up amino acids or to synthesize protein, RNA or DNA.

**Bacillus cereus**

Culture filtrates of isolates of *B. cereus* from cases of gastroenteritis contain, among other exo-products, an enterotoxin of mol. wt 55 000 (Spira and Goepfert, 1975) which resembles several of the enterotoxins of gram-negative bacteria. It causes fluid accumulation in the ligated rabbit ileum, activates adenylate cyclase and enhances vascular permeability in rabbit skin. It is distinct from a low mol. wt (5000) heat-stable product of other strains of *B. cereus* associated with food poisoning (Turnbull et al., 1979) which is responsible for vomiting but not diarrhoea in the affected individuals.

**Clostridium difficile**

It is only within the last 10 years that our understanding of the pathogenesis of antibiotic-associated diarrhoea and pseudomembranous colitis (PMC) has been developed. In particular *C. difficile* has been identified as the aetiological agent. It produces two antigenically and biologically distinct toxins; toxin A (an enterotoxin) causes the accumulation of haemorrhagic fluid in rabbit ileal loops and caecitis in hamsters, and toxin B (a cytotoxin) can damage a wide spectrum of tissue culture cells.

Toxin A has been purified (Sullivan, Pellett and Wilkins, 1982) and shown to be a hydrophobic protein of mol. wt 550 000. Its primary action is to cause fluid accumulation within the intestine but not by activation of adenylate cyclase. In addition it has some cytotoxic activity but very much less than seen with toxin B. Toxin

### Table XI

**Staphylococcal enterotoxins**

<table>
<thead>
<tr>
<th>Serological type of enterotoxin</th>
<th>Isoelectric point (pI) of main form</th>
<th>Number of electrophoretic forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.3</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>8.6</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>8.6</td>
<td>3</td>
</tr>
<tr>
<td>C2</td>
<td>7.0</td>
<td>3-8</td>
</tr>
<tr>
<td>E</td>
<td>7.0</td>
<td>not known</td>
</tr>
<tr>
<td>F</td>
<td>7.2</td>
<td>1</td>
</tr>
</tbody>
</table>

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\[C. G. GEMMELL\]
B is also a hydrophobic protein with a mol. wt of 350 000. PMC is caused by the production of large amounts of both toxins in the adult colon. In the somewhat similar disease in hamsters, both toxins seem to be of equal importance because the animals have to be immunised against both to survive (Libby, Jortner and Wilkins, 1982). In man, toxin A perpetuates the diarrhoea and increases the permeability of the intestinal epithelium while toxin B causes areas of necrosis and haemorrhage. Both toxins might thereafter enter the circulation where they could cause lethal effects far removed from the intestinal tract.

Summary

It is apparent that there are considerable similarities between many of the enterotoxins produced by enteric pathogens. Although the effect of most of these toxins is restricted to the intestine in vivo, many cells are also sensitive to intoxication in vitro. The resultant in-vitro biochemical changes may have no pathological significance but serve to underline the central role of cyclic nucleotides in cellular fluid regulation. The biological activity of these enterotoxins is the result of interaction with membrane-bound adenylate cyclase, leading to persistent elevation of intracellular levels of cAMP. Stimulation of adenylate cyclase occurs consistently after a characteristic lag phase which varies somewhat between toxins. The duration and degree of stimulation of adenylate cyclase by the various toxins may point to possible differences in affinity, dissociation and mechanism of activation of the cyclase molecule. Subtle events at, or within, the cell membrane must occur during intoxication and may include complex associations of toxin with membrane lipid and protein components.

The heat-labile toxins of V. cholerae, E. coli, Salmonella spp., A. hydrophila and Y. enterocolitica have much in common in their structures, membrane receptors and biochemical modes of action. Similarly the heat-stable toxins of E. coli and Y. enterocolitica, match each other in their biological activities. Classified along with the enterotoxin of C. perfringens, the enterotoxin produced by Sh. dysenteriae (and possibly some strains of E. coli) appears to differ from the other enterotoxins by acting on protein biosynthesis primarily and not on the nucleotide cyclase activation systems. In another category must be placed the various enterotoxins produced by Staph. aureus until more is known. Surprisingly little research has been directed towards the elucidation of their mode of action, although much is known of their serological and structural differences. Evidence to date suggests that staphylococcal enterotoxins differ from the other diarrhoeagenic agents discussed in this review.

The structural and immunological similarities between the various heat-labile enterotoxins suggest a common genetic origin with gene transfer between the different bacterial species being responsible for the spread of enterotoxigenicity. It is possible that many of the “newer” enterotoxins owe their origin to genetic recombination with the “older” enteropathogens like V. cholerae.

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