CHAPTER 6

# The Exotoxin of *Shigella Dysenteriae*

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## I. Introduction

Shiga's bacillus, *Shigella dysenteriae* (or *shigae*) (1898), occurs less frequently in outbreaks of bacillary dysentery than does Flexner's bacillus (*Shigella paradysenteriae*), but the disease that it gives rise to is much more severe; as well as the abdominal pain, diarrhea and tenesmus common to all forms of bacillary dysentery, there is often a "profound toxemia," ending in collapse (Wilson and Miles, 1964). These additional symptoms in Shiga dysentery may be due to the so-called "dysentery toxin" or "neurotoxin" which is produced by *Sh. dysenteriae* in addition to the polymolecular endotoxin common to all gram-negative enteric organisms, including the other types of dysentery bacilli (i.e., *Sh. paradysenteriae, Shigella sonnei,* and *Shigella ambigua*).

Conradi (1903) was the first to observe that Shiga's bacillus contained a poison that caused paralysis when injected (in the form of a sterile concentrated autolyzate of the bacillus) into rabbits. This was soon confirmed by Todd (1904), who found that guinea pigs, mice, and monkeys were far less susceptible to the poison than rabbits. He showed that Flexner's bacillus did not produce such a poison. Krauss and Dörr (1905) concluded (in the event, correctly) that there were two toxins, (1) a soluble toxin, present after 8–10 days in filtrates of cultures, fatal to rabbits but not guinea pigs, and capable of giving rise to a specific neutralizing antibody, and (2) an insoluble toxin present in the cell bodies, fatal to both rabbits and guinea pigs. These conclusions were supported by the work of many others. Flexner and Sweet (1906) described symptoms of intoxication in rabbits that have since been observed with highly purified toxin, i.e., flac-

cid paralysis first appearing in the front legs and then spreading to the hind limbs with the animals sometimes living as long as 10 days after onset of paralysis. On autopsy, small hemorrhages in the brain and softening of the grey matter of the spinal cord were observed.

Pfeiffer and Ungerman (1909) defined the two toxins as an exotoxin producing neurological symptoms in the rabbit and an endotoxin producing hyperthermia and peritoneal exudation in the guinea pig. Bessau (1911) showed that antiserum neutralized the exotoxin but not the endotoxin. Olitsky and Kligler (1920) clearly demonstrated the presence of two different toxins: (1) an exotoxin which appeared in culture filtrates in the early phase of growth, which was relatively heat labile, which had a special affinity for the nervous system, and which was neutralized by antiserum, and (2) an endotoxin which appeared in culture filtrates only after autolysis, which was relatively heat stable, which exerted a typical action on the intestinal tract, and which was not neutralized by antiserum. Although other workers, notably McCartney and Olitsky (1923), had succeeded in obtaining preparations of either toxin comparatively free of the other by manipulating the conditions of culture, it was Boivin and Mesrobeanu (1937a,b,c) who first succeeded in actually separating the two toxins by chemical methods. They showed that the neurotoxin was protein in nature and precipitable by trichloroacetic acid, while the enterotoxin (*substance glucidolipidique*) was soluble in trichloroacetic acid. They found that the neurotoxin was produced by both rough and smooth variants of Shiga's bacillus, whereas the enterotoxin was produced only by smooth variants.

Thus it is now clear (despite some arguments to the contrary, e.g., Waaler, 1936; Boroff, 1949; Boroff and Macri, 1949) that while all types of dysentery baccilli produce the enterotoxic polymolecular endotoxin, both rough and smooth variants of *Sh. dysenteriae* differ from other dysentery types in also producing a protein toxin which, though not readily secreted by the organism, is found in culture filtrates earlier than the endotoxin, which is particularly toxic to rabbits (in which it produces neurological symptoms), and which is neutralized by its homologous antibody. This toxin of Shiga's bacillus (dysentery toxin, neurotoxin, exotoxin, true toxin, soluble toxin, rabbit toxin) is the subject of this essay. In this introduction, many names distinguished in the history of bacteriology have been recorded; in the list of references it will be seen that very few papers on the subject have been published since the mid-1950's.

## II. Toxicity

Culture filtrates of *Sh. dysenteriae* are not very toxic, even to the highly susceptible rabbit, despite the fact that the toxin itself is extremely potent.

This is because the organism produces very little toxin—about 1 mg/gm dry weight (see van Heyningen and Gladstone, 1953a), and only a fraction of the toxin passes from the cells to the medium. Thus, the total amount of toxin in a whole culture grown under optimal conditions for toxin production would be equivalent to about 250 mouse $LD_{50}$/ml or about 2500 rabbit $LD_{50}$/ml, but not all this toxin would be in the filtrate.

Table I shows that there are great differences in the susceptibility of different animal species to dysentery toxin, and different workers have found different degrees of difference. For example, Halapine and Basilevskaia (1937) found the rabbit to be 250-2500 times more susceptible per unit weight than the mouse; Dubos and Geiger (1946) found a difference of 75-150 times; van Heyningen and Gladstone (1953a) found a 1500-fold difference.

The highest values for the toxicity of dysentery toxin are those reported by van Heyningen and Gladstone (1953a) for a highly purified preparation of the toxin. For mice injected intraperitoneally, the $LD_{50}$ was 0.027 $\mu$g, or 1.35 $\mu$g/kg mouse; for rabbits injected intravenously, the $LD_{50}$ was 0.0023 $\mu$g, or 0.00087 $\mu$g/kg rabbit. On a basis of equal weight of experimental animals, the toxicity of dysentery toxin for rabbits is about the same as that of tetanus and botulinum toxins for guinea pigs, and about 350 times that of diphtheria toxin.

## III. Production and Purification

### A. Growth of the Organism

McCartney and Olitsky (1923) showed that aeration of cultures of *Sh. dysenteriae* was necessary for the production of the "exotoxin." This can be achieved by growing the organism on agar media in Roux bottles or by

TABLE I
RELATIVE LETHAL DOSES OF DYSENTERY TOXIN PER UNIT WEIGHT OF VARIOUS ANIMAL SPECIES[a]

| Species | Dose |
|---|---|
| Rabbit | 1 |
| Monkey (*Macacus mulatta*) | 5 |
| Hamster | 40 |
| Mouse | 700 |
| Rat | 5,000 |
| Guinea pig | > 10,000 |

[a]From Cavanagh *et al.* (1956).

aerating the culture by shaking it or passing air through it. Dubos and Geiger (1946) showed that the presence of iron above a small optimal value (about 0.15 μg Fe/ml) inhibited toxin production. For toxin production on a large scale, van Heyningen and Gladstone (1953a) used the casein hydrolyzate (CCY) medium of Gladstone and Fildes (1940) in which the iron content had been reduced by adsorption on calcium phosphate to a level of 0.05 μg Fe/ml (van Heyningen and Gladstone, 1953b). This procedure also reduced the magnesium content to a level insufficient for optimal growth. Iron and magnesium were added to the de-ferrated medium to raise their respective levels to 0.15 μg Fe/ml and 20 μg Mg/ml. The level of 0.15 μg Fe/ml gave the greatest yield of toxin per unit volume of culture; below this level, the toxin yield per organism was greater but the organism yield was smaller. The cultures (2 liters) were grown semicontinuously in a vertical glass tube of 5 cm diameter and 183 cm length. The culture was aerated by passing air through a sintered glass filter disk at the rate of 100 ml/minute at 20 cm Hg pressure. Every 12 hours, all but 50–100 ml of the culture was withdrawn and replaced with fresh medium. For larger-scale (2 kg dry weight of organisms) production, cultures were grown continuously and organisms were harvested when 1 hour old (Macsween, private communication).

## B. Extraction of Toxin

The exotoxin of *Sh. dysenteriae* can be obtained in soluble form by allowing the organism to autolyze, but the yield obtained is low and variable. Dubos and Geiger (1946) precipitated chilled cultures at pH 4.2 and extracted the toxin from the washed precipitate at pH 7.5 with dilute sodium carbonate or with saline buffered with phosphate. Disintegration of the organisms by mechanical and ultrasonic devices or by grinding with aluminum oxide powder (see van Heyningen and Gladstone, 1953a; Vicari, *et al.*, 1960) may result in quite good yields of toxin, but this method is not reliable or convenient. Van Heyningen and Gladstone (1953a) extracted the toxin as follows: The cells were spun down, washed with water, and suspended in potassium phosphate buffer pH 6 to a concentration of 50–60 mg dry weight organisms/ml. The organisms were then killed by heating at 56–59°C for 30 minutes and the suspension was adjusted to pH 11 with 1 *N* KOH. After standing at room temperature for 3 hours, the suspension was neutralized and spun down. The residue was resuspended in half the original volume and reextracted, and the turbid greenish extracts were combined. The yield of toxin (as judged by a comparison of the toxicity of the organisms and the extract) appeared to be 50–120%. When much more highly diluted suspensions were extracted, the yield of

toxin was 2–3 times as high. The killing of the organisms by heating was an essential stage of the extraction process, because no detectable toxin was released when the stage was omitted. It was found, confirming an earlier observation of Boivin *et al.* (1940), that 30% of the toxin passed from the cells into the suspending medium on heat-killing, and this may be relevant to Li's finding (1966) that dead, acetone-dried *Sh. dysenteriae* organisms released exotoxin freely into the medium under conditions in which living organisms did not release toxin. Although the toxin, even in a crude state, is inactivated under alkaline conditions (Dubos and Geiger, 1946), it survives treatment at pH 11 under the conditions of extraction.

### C. Purification of Toxin

Van Heyningen and Gladstone (1953a) purified the toxin in the following way: The viscous, turbid, yellow-green solution obtained by extracting the heat-killed organisms (50–60 mg dry weight/ml) at pH 11 was dialyzed overnight against running tap water and clarified by adding 0.1 volume 0.2 *M* $Na_2HPO_4$ and 0.05 volume 1 *M* $CaCl_2$. The pH was adjusted to 7.6, the solution allowed to stand for 30 minutes and then was spun down to remove the precipitate of calcium phosphate. The clear supernate, which was now free of nucleic acids, was freeze-dried, and the powder was dissolved in 1% NaCl solution to a concentration of 5 gm/100 ml. Advantage was now taken of the insolubility of the toxin in the absence of salt. The solution was dialyzed at 4°C against several changes of distilled water, and, after 10–20 days, most of the toxin precipitated out. It was dissolved in 0.1 the original volume of 1% NaCl, leaving an appreciable insoluble residue which was discarded, and the solution was again dialyzed against distilled water. A copious precipitate started forming after 30 minutes, and, after overnight dialysis, the precipitate was collected, redissolved, and redialyzed. After two further dialyses, a preparation was obtained which still contained some impurities. The remaining impurities were removed by adsorption on calcium phosphate, precipitated as in the early stage. The supernate was dialyzed and freeze-dried to yield a product which moved as a single symmetrical peak by paper electrophoresis and in the ultracentrifuge, and which, on immunodiffusion, gave two precipitation lines, both of which were apparently derived from a single antigen (Li, 1966; see Section VII, below). Although the material gave a single symmetrical boundary in the ultracentrifuge, it was not homogeneous with respect to its sedimentation coefficient. This heterogeneity could have resulted from contamination of a homogeneous toxin with irrelevant material, from a degree of heterogeneity having been produced in the course of purification (see above, the double immunodiffu-

sion lines derived from a single line in unprocessed material), or from the active toxin having been heterogeneous in the natural state.

Sŏurek and Răska (1963) purified the toxin by preparative electrophoresis and gel filtration on Sephadex.

## IV. Nature

*Shigella dysenteriae* exotoxin is a protein with properties as shown in Table II. The toxin is insoluble in distilled water and soluble in aqueous salt solution. The solubility of the toxin in salt solution has a high temperature coefficient. If a 1% solution of toxin in 1% NaCl is cooled to −5°C, a precipitate forms and redissolves on warming (van Heyningen and Gladstone, 1953a).

## V. Synthesis

Very little is known about the biosynthesis of the exotoxin by *Sh. dysenteriae*. Dubos and Geiger (1946) showed that exotoxin production by rough and smooth strains was inhibited when more than a very low concentration of iron was present in the culture medium. This discovery accounted for earlier observations that more toxic organisms were produced when they were grown in moderately alkaline broth, since in the preparation of alkaline broths, calcium phosphate is likely to precipitate out and adsorb iron; in de-ferrated media toxin can be produced at neutral pH. The finding of Dubos and Geiger was confirmed by van Heyningen (1955b; van Heyningen and Gladstone, 1953b), who found that at very

TABLE II
PROPERTIES OF *Shigella dysenteriae* EXOTOXIN[a]

| | |
|---|---|
| $LD_{50}$/mg (mouse) | 40,000 |
| $LD_{50}$/mg (rabbit) | 400,000 |
| Lf/mg | 5,500 |
| L+/mg (5 unit level) | 4,200 |
| N content | 15.7% |
| Electrophoretic mobility, $-\mu_{30}$ (barbital buffer pH 8.8, $\mu = 0.1$) | $2.78 \times 10^{-5}$ cm²/second/V |
| Weight average sedimentation coefficient, $Sm_{20,w}$ | 4.80 $S \pm 1\%$ |
| Diffusion coefficient, $D_{20,w}$ | $5.7 \times 10^{-7}$ cm²/second ± 5% |
| Frictional coefficient, $f/f_0$ | 1.26 |
| Average molecular weight | 82,000 |

[a]From van Heyningen and Gladstone (1953a) and Baldwin (1953).

low concentrations of iron, cell growth and toxin production per unit volume of culture increased while the toxin content per unit weight of cell remained high and constant, but that above an iron concentration of 0.10–0.15 μg Fe/ml medium, toxin production per cell and per unit volume of culture decreased (see Table III). The decrease in toxin production started after growth had reached its maximum and the iron in the culture medium had ceased being qualitatively incorporated in bacterial hemes. The non-heme iron in the cells which inhibited toxin production was reactive to oxine. At the lower end of the inhibitory range of iron concentration, there was a fall in production of 1 molecule of toxin for every 40 atoms of iron, but at about 0.2 μg Fe/ml medium, this ratio abruptly changed to 1 molecule of toxin for 320 atoms of iron. At 0.4 μg Fe/ml medium, toxin production was abolished, and this coincided with the appearance in the cell of iron, which (like heme iron) was not reactive to oxine. Above 0.8 μg Fe/ml, iron ceased to be incorporated quantitatively in the organism. The relation between heme and exotoxin in *Sh. dysenteriae* is quite different from that in *Corynebacterium diphtheriae*. In the dysentery bacillus, toxin production falls when available iron is not being incorporated in bacterial heme; in the diphtheria bacillus, toxin production falls when available iron is being incorporated in bacterial heme (see Vol. IIA).

Van Heyningen (1955a) found that the growth rate of *Sh. dysenteriae*, but not that of *Shigella flexneri* or *Sh. sonnei*, was retarded in metal-deficient medium, while the growth yield of all three organisms was retarded. Iron, but not cobalt or nickel, increased the growth rate of *Sh. dysenteriae*, but all three metals increased the growth yield of all three organisms. All three organisms, when grown in metal-deficient media, showed lags in the oxidation of Krebs cycle intermediates (but not of other substrates), and these lags were abolished when iron was added to the growth medium, but not when cobalt or nickel were added. However, all three metals inhibited exotoxin production by *Sh. dysenteriae*. The conclusion drawn was that there was unlikely to be a connection between the effects of these metals on the growth and respiratory characteristics of these organisms and exotoxin production by *Sh. dysenteriae*, since, for example, cobalt inhibited toxin production but had no effect on the growth rate or respiratory activity of *Sh. dysenteriae*.

The exotoxin can be released from young cultures of *Sh. dysenteriae* by phage. Li *et al.* (1961) reported that a complex of phage and *Sh. dysenteriae* released various biologically active antigens not ordinarily detected as extracellular products. Li (1966) showed that the exotoxin was one of the antigens released (see Section VII).

TABLE III

EFFECT OF IRON IN THE CULTURE MEDIUM ON THE GROWTH YIELD AND IRON, HEME, AND TOXIN CONTENT OF *Shigella dysenteriae*[a]

| Property | Effect at iron content (μg Fe/ml medium) | | | | |
|---|---|---|---|---|---|
| | 0-0.1 | 0.1-0.2 | 0.2-0.4 | 0.4-0.8 | 0.8 |
| Bacterial growth yield | Rises | Constant (3 mg/ml) | Constant | Constant | Constant |
| Incorporation of iron in organisms | Complete | Complete | Complete | Complete | Incomplete |
| Iron content of cells | Constant (0.06 μg/mg) | Rises | Rises | Rises | Constant (0.6 μg/mg) |
| Heme content of cells | Constant | Constant | Constant | Constant | Constant |
| Incorporation of iron in heme | Complete | Incomplete | Incomplete | Incomplete | Incomplete |
| Iron in excess of heme requirements | 0 | + | + | + | + |
| Oxine-reactive iron in cells | 0 | + | + | + | + |
| Oxine nonreactive iron in cells (other than heme iron) | 0 | 0 | 0 | + | + |
| Toxin content of cells | Constant (10 Lf/mg: 800 rabbit $LD_{50}$/mg) | Falls 1 molecule per 40 atoms Fe | Falls 1 molecule per 320 atoms Fe | 0 | 0 |
| Toxin content of culture | Rises | Falls | Falls | 0 | 0 |

[a]From van Heyningen and Gladstone (1953b) and van Heyningen (1955b).

## VI. Mode of Action

The exotoxin of *Sh. dysenteriae* has been called a neurotoxin ever since its discovery because it produces unmistakable neurological symptoms in the rabbit, the animal that is most susceptible to its action. A rabbit that receives an intravenous injection of one or two fatal doses of toxin shows no overt signs of injury for the first 2 days. Muscular weakness then first appears in the forelimbs and causes them to splay out. During the next 24-48 hours, the paralysis increases and extends to the hind limbs and the abdominal muscles. By the fourth or fifth day the animals are entirely paralyzed and prostrated with complete loss of muscle tone; often there is a marked retraction of the head, or, alternatively, the animal is unable to hold up its head. A remarkable feature of the paralysis, which sets it apart from tetanus or botulism, is that frequently an animal which is completely prostrated at the height of intoxication may make a rapid and complete recovery and within 2 days show little or no sign of the former paresis.

These neurological symptoms are accompanied by changes in the central nervous system. Soon after the discovery of the toxin, Dopter (1905) showed that there were histological changes in the spinal cord of intoxicated rabbits involving chromatolysis of the anterior horn cells and interstitial hemorrhages. Similar observations have since been made by many workers, but since crude preparations of the toxin were always used, it could not be certain whether these effects were due to the exotoxin, the endotoxin, or to other active substances present in the crude preparations. Bridgwater *et al.* (1955) used a highly purified sample of *Sh. dysenteriae* toxin prepared by van Heyningen and Gladstone (1953a) for a study of the effect of the toxin in the rabbit. They found in rabbits that died after receiving 2 $LD_{50}$ intravenously, or were killed at the height of intoxication, that there were macroscopic changes in the spinal cord, especially in the cervical enlargement, but sometimes also in the lumbar enlargement. The cord was swollen, and on section it could be seen that there were extensive areas of softening with numerous petechial hemorrhages in the gray matter. The white matter was unaffected and sometimes formed the wall of a tubelike structure filled with pultaceous contents. Histological examinations were made of the central nervous systems of rabbits killed at intervals after receiving 2 $LD_{50}$ of toxin. After 24 hours, before there were external signs of intoxication, there were small hemorrhages from capillaries and small veins but no morphological signs of injury to nerve cells in the gray matter of the cervical enlargement. After 48 hours, even though there were not yet any signs of functional deterioration, the vascular disturbances were more pronounced and a small proportion of the large motor perikaryons in the anterior horns

showed signs of injury. After 72 hours, when paresis of the forelimbs was evident, the hemorrhages were conspicuous and many large motor neurons were damaged. After 120 hours, the animals were profoundly intoxicated and evidence of destruction was widespread, involving all types of cells, the region of the gray matter being occupied only by the debris of necrosed neurons, neuroglia, and blood vessels.

Bridgwater *et al.* (1955) came to the conclusion that *Sh. dysenteriae* exotoxin was not, in fact, a neurotoxin, but a vascular toxin attacking the endothelium of blood vessels, and that the neurological symptoms were a secondary effect resulting from vascular damage in the neural tissue. The action of the toxin seemed to be confined to the blood vessels in the spinal enlargements, particularly in the cervical enlargement. That the effect was due to vascular damage was further demonstrated when the toxin was injected in the right vertebral artery. On postmortem examination of rabbits intoxicated by this route, longitudinal section of the cervical enlargement of the spinal cord showed a sharply demarcated unilateral distribution of necrosis, restricted to the side on which the injection was made.

Howard (1955) also came to the conclusion that the toxin was a vascular toxin rather than a neurotoxin. He found that the toxin was more lethal to mice intravenously than intraperitoneally or intramuscularly, and, in rabbits, it was more effective intravenously than intramedullarly. This is the converse of what is found with tetanus toxin.

The neurological symptoms that follow the action of the toxin in the blood vessels in the spinal cord and the subsequent destruction of motoneurons probably do not result directly from the destruction of these neurons, because prostrated rabbits may recover completely; yet these neurons probably cannot regenerate. It is more likely that the edema and swelling of the spinal cord within the confines of the spinal column result in pressure on nerves and that this brings about the paralysis.

The rabbit is the species most susceptible to *Sh. dysenteriae* exotoxin, and the neurological consequences of the localized action of the toxin in the spinal cord predominate in the toxemia, but the toxin acts on other tissues as well. Seneviratne (1948) found that intravenous injection of (unrefined) toxin into rabbits leads to a reduction in plasma volume accompanied by a fall in plasma protein and an increase in interstitial fluid, due partly to edema of the cecum, where the toxin appears to localize. This suggested an increase in capillary permeability.

Cavanagh *et al.* (1956) pointed out that the neurological effects of *Sh. dysenteriae* exotoxin in the rabbit tended to obscure intestinal effects which were frequent, though less constant. Previous workers had found, besides profound neurological disturbances, intestinal changes, in particular in the cecum, which showed changes varying from gelatinous edema to

hemorrhagic necrosis (Flexner and Sweet, 1906; Istrati, 1938; Boivin, 1940).

Cavanagh *et al.* studied the effect of the highly purified toxin of van Heyningen and Gladstone (1953a) on a number of animal species. In the rabbit, they observed diarrhea, and, on necropsy, they found severe gelatinous edema of the cecum, with the mucosa frequently speckled with petechial (and sometimes confluent) hemorrhages. Histologically, there was severe edema of the loose submucosal areolar tissue and of the mucosa. In mice, the effects of toxemia were comparable with those in rabbits. There was flaccid paralysis and sometimes convulsions accompanied by distinct focal changes in the histology of the brain and the spinal cord, scattered at random through the neuraxis. In the hamster, which was more susceptible than the mouse, there was no paralysis or convulsions and no diarrhea. The most prominent feature of intoxication in this animal was a profound bilateral serous effusion associated with congestion and edema of the lungs. There were no lesions in the central nervous system. In the rat, which was very resistant, intravenous injection of the toxin lead to diarrhea and oral regurgitation of the stomach contents; there was no flaccid paralysis. On necropsy, it was seen that the stomach was dilated with liquid. The small intestine was similarly dilated, and there was vascular damage in the middle and lower thirds. There were areas of intense congestion in the ileum, with ecchymotic hemorrhage in the submucosa or more extensive hemorrhagic necrosis. There were no lesions in the central nervous system. The guinea pig was highly resistant. Animals injected with 200 mouse $LD_{50}$ showed no signs of disturbance, and when killed after 5 days showed no histological abnormality in the central nervous system or the viscera. It may be concluded that the species specificity in susceptibility and reaction to the exotoxin of *Sh. dysenteriae* is probably related to species-related differences in blood vessels.

Unlike many toxins (e.g., diphtheria toxin, *Clostridium welchii* $\alpha$-toxin), *Sh. dysenteriae* exotoxin does not produce any reaction when injected into the skin, and therefore is probably not generally histotoxic, but it does destroy a number of cells cultivated *in vitro*. Vicari *et al.* (1960) showed that a preparation of the toxin, purified by the method of van Heyningen and Gladstone (1953a), exerted specific cytopathogenic effects on cells in tissue cultures. KB cells, normal human liver cells, and monkey kidney cells, when subjected to the toxin at a concentration of 1 pg/ml, showed similar degenerative processes, starting with thickening of the cell membrane and ending with complete destruction of the cytoplasm and pyknosis of the nucleus. The toxin was fixed to the cells instantly. Thus if toxin and antitoxin were mixed before being added to the cells, the toxin was neutralized, but if toxin were first added to the cells

and immediately followed by antitoxin, the cells were destroyed. Howard (1955) had observed that rapid fixation of the toxin also took place *in vivo*. He injected antitoxin intravenously into mice at various intervals after injection of toxin by intravenous, intramuscular, and intraperitoneal routes and found that intoxication became irreversible within 0.5 hour after intravenous injection of toxin, and within 6 hours after intramuscular or intraperitoneal injection. I. Mesrobeanu *et al.* (1962a,b) confirmed that the exotoxin of *Sh. dysenteriae* had a cytotoxic action on tissue cultures of human embryonic and KB cells. Their unrefined preparation of toxin was active at a concentration of 0.2 $\mu$g per tube, bringing about a massive detachment of cells. They confirmed that the toxin was rapidly fixed by the cells.

## VII. Immunology

Although exotoxin and endotoxin are distinct and separable entities, there are suggestions of an interrelationship and immunological cross reaction (see Boroff, 1949; Boroff and Macri, 1949; Engley, 1952; L. Mesrobeanu *et al.*, 1962). These suggestions may be relevant to recent work of Li (1966), which suggests that the exotoxin may be the product of depolymerization of a larger molecule. Li showed that the purified preparation of van Heyningen and Gladstone (1953a) showed two lines on immunodiffusion against refined polyvalent antitoxin, corresponding to antigens 1a and 1b (Fig. 1). When *Sh. dysenteriae* organisms in a 20-hour culture were infected with a T-phage (i.e., lysed from within?) they released a number of antigens, one of which on immunodiffusion showed a reaction of identity with both antigens 1a and 1b of the van Heyningen and Gladstone preparation. Apparently, the phage-released antigen was depolymerized into antigens 1a and 1b during the purification process.

Further experiments showed that antigen 1a was apparently the exotoxin and that antigen 1b had a common determinant with somatic antigen. Antigen 1a showed a reaction of identity with the apparently sole antigen (probably the exotoxin) released when washed cells from an 18-hour culture were resuspended in de-ferrated casein hydrolyzate and shaken at 35°C for 18 hours. The antibody to antigen 1b was removed when the polyvalent antitoxin was absorbed with whole cells of *Sh. dysenteriae*. An antigen similar to the antigen released by phage lysis from within could be extracted from acetone-dried cells with phosphate buffer, i.e., on immunodiffusion it also gave a reaction of identity with both antigens 1a and 1b in the van Heyningen and Gladstone preparation. When young organisms (4 hours in the exponential phase) were washed and treated for one hour with a high concentration of phage (i.e., lysed from

without?), antigen 1a (the exotoxin) appeared to be depolymerized into two smaller antigenic parts (antigens 1a,a and 1a,b, see Fig. 1). It is not known whether either or both of these antigens were toxic.

*Shigella dysenteriae* exotoxin is readily toxoided with formaldehyde and other reagents (see Engley, 1952), and antisera are available. The toxin can be estimated by flocculation with antitoxin (Lf determination) or titration against antitoxin, using mouse killing as indication of excess toxin ($L_+$ determination; see van Heyningen and Gladstone, 1953a,c).

## VIII. Pathogenesis

It has already been noted that Shiga's bacillus causes a more severe disease than the other dysentery bacilli. The disease is characterized by "profound toxemia" and "collapse" (whatever is meant, exactly or inexactly, by these expressions). Involvement of the nervous system is very rare in human Shiga dysentery; Cavanagh *et al.* (1956) noted that only 2 instances in 3800 cases were described by Dumas in 1938, and they suggested that the nervous system of man may be as resistant to the toxin as is that of the rat, and that the possibility of an extraneural site should be considered. Antitoxin has no therapeutic value

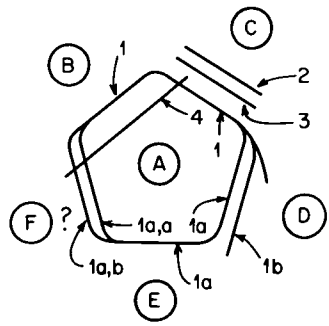


FIG. 1. Diagrammatic interpretation of immunodiffusion lines of antigens obtained from *Sh. dysenteriae* by various means. A = antitoxin to crude exotoxin; B = phosphate buffer extract of acetone-dried organisms; C = 20-hour organisms lysed (from within?) by T-phage; D = purified exotoxin preparation of van Heyningen and Gladstone (1953a); E = exotoxin released by resuspending washed 18-hour culture in de-ferrated casein hydrolyzate for 18 hours; F = 4-hour exponential phase organisms lysed (from without?) by T-phage in 1 hour—the question mark signifies that the presence or absence of other antigens was not mentioned. Line 1 represents common antigen from which antigens 1a and 1b are derived. (With apologies to Li, 1966).

in dysentery, but it is doubtful whether the antitoxin to any toxin produced by any organism has any therapeutic value. Active immunization with toxoid has not been tried with humans, presumably because of the low incidence of Shiga dysentery.

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