Mechanisms of Action of Clostridial Toxins[1]

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Study of the clostridial toxins has been irregular. During World War I the further identification and characterization of the clostridia causing gas gangrene and tetanus were ably pursued, and demonstration of the existence and harmful action of clostridial exotoxins were emphasized. Use was made of this knowledge for diagnostic purposes and for the preparation of toxoids and antitoxins (23). At the onset of World War II several important observations were at hand which permitted a more fundamental approach to the description of the modes of clostridial toxic action. It is hardly surprising to find that the more recent approach is of a biochemical character, for it was shown that many of the toxic mechanisms involved enzymatic reactions. Since knowledge of the intimate reactions resulting in toxic effects upon the host must of necessity include the characterization of *how* a toxin functions, elucidation of the enzymatic basis of toxic action constitutes a real contribution to an understanding of the problem (21, 22). With the renewed interest and activity experienced during World War II which resulted, among other findings, in the crystallization of the tetanus and botulism toxins (18), it became ever more apparent that the clostridial toxins are the most potent poisons known, indicating a catalytic rather than a stoichiometric mode of intoxication.

The intoxication of botulism was long known to be caused by an extracellular growth product of *Clostridium botulinum* and to involve the nervous system, as indicated by such symptoms as oculomotor, pharyngeal and respiratory paralysis. It was shown in 1936 by Bishop and Bronfenbrenner (4) that the toxin acted upon the myoneural junction, resulting in an interference with acetylcholine action upon the muscle, the mode of action of curare. More recent data (9, 24) indicate that acetycholine still causes muscular contraction after botulinus poisoning, an effect not obtained under the conditions of curare poisoning, but that decreased synthesis of acetylcholine occurs during botulinus poisoning. Torda and Wolff (24) found that the rate of acetylcholine synthesis by frog or mouse brain was inhibited appreciably by small amounts of botulinus toxin. Burgen, Dickens and Zatman (5) concluded that since conduction in the nerve of the poisoned muscle is unaffected by the toxin and since the muscle responds normally to direct stimulation, the mechanism of paralysis involves a neuro-muscular block at the motor end-plates which themselves remain sensitive to acetylcholine. The reduced output of acetylcholine by the poisoned muscle results, therefore, in the interference with impulse transmission from the nervous system to the muscle. Exactly how the botulinus toxin inhibits acetylcholine synthesis remains to be shown. The toxin was found to have no effect upon the enzyme catalyzing the acetylation of choline nor upon

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cholinesterase, but still to be investigated are the effects of botulinus toxin upon acetate and choline synthesis or availability, and upon the enzymatic transfer of high-energy phosphate bonds to acetate from adenosinetriphosphate to yield the acetyl phosphate apparently required for acetylcholine synthesis.

Tetanus is a toxemia due to a localized infection of injured tissues by *Clostridium tetani,* the disease being characterized by convulsive tonic contraction of voluntary muscles. The extracellular toxic product inducing the muscular spasms has been termed tetanospasmin, the resulting muscular rigidity and intermittent periods of intense motor activity clearly suggesting an imbalance in some neuromuscular regulatory mechanism. The finding of increased acetylcholine levels in the muscle of animals injected with tetanus toxin has led to the conclusion by some workers that the enzyme hydrolyzing acetylcholine, namely cholinesterase, is inhibited by the toxin **(8, 10, 25, 27).** However, other workers **(14, 20)** were unable to demonstrate the inhibition of cholinesterase activity in the nervous tissues and blood sera of tetanized and normal animals. The problem with respect to cholinesterase, therefore, remains unresolved. A rather comprehensive study of the effect of tetanus toxin upon certain energy-liberating reactions was made by Muntz **(14):** neither the glycolysis of mouse brain nor the adenosinetriphosphatase of several nervous tissues and muscle were affected by the toxin. Nor were the levels of blood glucose and lactic acid affected. Indeed, this work indicates that there is no interference with the anaerobic energy metabolism of tetanized animals although numerous other energy-liberating systems remain to be investigated. In addition, Muntz attempted to find changes in the quantities of free amino acids and ammonia excreted by mice treated with huge quantities (100,000 MLD) of tetanus toxin; again no changes over the normal values were reported. He also attempted to detect a substance which might be liberated from tetanized nerve and muscle tissues, and which might be the actual toxic agent inducing tetany; no such agent was found. In the presence of all these negative data, it can only be restated that the mechanism of tetanal toxic action remains obscure.

The toxins produced by the agents of gas gangrene, bovine hemoglobinuria and other diseases caused by related clostridia are considered together because of the existence of some overlapping pathological properties and enzymatic similarities. Several members of the genus *Clostridium* produce proteinases which are important toxic agents because of the large amounts found extra-cellularly and because of the powerful proteolytic activities of these enzymes upon native tissues. Weil, Kocholaty and their associates **(11, 12, 28),** as well as others **(3, 15),** have studied the clostridial proteinases, particularly those of *C. histolyticum,* obtaining purified enzyme preparations which require ferrous ions and cysteine for maximum activity. Since these proteinases are secreted by clostridia found in tissue infections leading to the massive disintegration and liquefaction of muscle, the proteinases have been incriminated as specific toxic agents in such infections.

Related to the proteinases is the exo-product, fibrinolysin, obtained by Reed, Orr and Brown (19) from cultures of gas gangrene clostridia. This substance is relatively thermostable (100 C/1 hr) and thus distinguishable from the usual proteolytic enzymes. As indicated by its name, fibrinolysin can act as a contributing agent in a toxic infection by dissolving fibrin and thus permitting spread of the infecting agents.

Closely related to the proteinases and fibrinolysins is the enzyme collagenase (3, 15, 16, 25). Extracellular growth products of *C. histolyticum, C. perfringens* and *C. sporogenes* have been shown to include collagenases, and the pathological effects of these substances have been ascribed to their capacity to attack the collagenous connective tissue fibers, resulting in tissue breakdown and increased diffusion of the overall infectious process. Lethality and collagenase activity have not always been found to be related directly, thus indicating only a contributory role of this enzyme in the pathological process.

Another mechanism responsible for the spreading of infections in the host as a result of the microbial elaboration of an assemblage of substances involves extracellular products previously referred to as spreading factors. The viscous mucopolysaccharide of the mesenchyme, often called the ground substance of tissues, contains hyaluronic acid, a term used more to represent a class of substances than a single compound. This substance appears to be a polysaccharide composed of disaccharide units of acetylglucosamine and glucuronic acid. The enzyme hydrolyzing this substance has been called hyaluronidase and its primary action is upon the glucosamine linkage, releasing the reducing group of the acetylglucosamine (7). In this process, the substrate is depolymerized and becomes less viscous, and with regard to its anatomical function as a supporting tissue, the hyaluronate-containing tissue becomes less rigid. Since this enzyme(s) is produced by some of the clostridia causing gas gangrene (*C. perfringens, C. novyi, C. septicum*), its toxic role has been interpreted as involving the physical enhancement of further invasion by the infective agents into the surrounding tissues from their previously localized position, and the facilitated circulation of their toxic end-products. Although many reports indicate a relationship between hyaluronidase production and virulence, potent hyalurondase producing strains have been found which are neither associated with any particular toxin type nor related directly to the lethal potency of a given toxin (23).

The last group of toxic clostridial exo-enzymes to be discussed are the lecithinases. A great deal of work has been performed in this field during the past decade, especially by British investigators (21). When it was found that the toxicity of clostridial culture filrates often parallels the capacity of the filtrates to split lecithin into a diglyceride and phosphocholine, most workers concluded that the mechanism of toxic action in gas gangrene and bovine hemoglobinuria involves the direct participation of a lecithinases (D). Lethality, necrosis and hemolysis have been attributed to the attack by this enzyme of the lecitho-proteins of cell membranes with the subsequent breakdown of vital tissues such

as nerve and cardiac muscle, leading to toxic action and death. Moreover, specific antitoxins have been shown to inhibit the lecithinase activity of *C. perfringens* toxins, and it appears valid to conclude that the antitoxins are really anti-lecithinases (30).

Related to this lecithinase is another product of lecithinase activity, described by Bard and McClung (2) in the toxins of *C. novyi* type B and *C. hemolyticum*. In the latter cases lecithinase A production by the clostridia leads to the formation of lysocethin—a powerfully hemolytic substance—or lysolecithoprotein itself is synthesized by the organisms. This hemolytic substance, an important factor in snake venom intoxication, was found in culture filtrates which also contained lecithinase D. Thus, at least two mechanisms of hemolysis are found among some of the clostridial toxins: the more common lecithinase D and the hemolytic substance, lysolecithin.

In 1948 McClung and Russell (13) found strains of *C. perfringens* yielding highly lethal toxin but with low lecthinase D activity, and vice-versa. In the case of *C. novyi* toxins, Oakley, Warrack and Clarke (17) noted that lethality and lecithinase D activity were not related, and the same conclusion was made independently by Bard (1). Wooldridge and Higginbottom showed (29) that *C. perfringens* toxin inhibited the aerobic oxidation of succinate by aqueous extracts of minced guinea pig intestine, muscle, heart, liver and kidney, the inhibition apparently interfering with the transfer of hydrogen from the substrate to the cytochrome system; specific antitoxin reduced this inhibition. On the other hand, the toxins of *C. novyi*, *C. septicum* and *C. tetani* had no effect upon succinate oxidation. These findings indicate an additional site of toxic action by members of the gas gangrene group, one apparently separate from lecithinase activity. Presented with these data, as well as others with lecithinase D producing species of the genus *Bacillus* (6), which are considerably less toxic, the possibility arises that all the toxic mechanisms attributed to lecithinase D may be in excess (23). Aside from the complication that explanation of the toxic mechanism remains incomplete to this extent, doubt arises concerning the validity of using the lecithinase test in the practical problem of assaying and controlling large-scale toxin production for the preparation of wholly effective toxoids and antitoxins.

As has been the case with the descriptions of all the clostridial toxic mechanisms presented, many gaps exist in the present knowledge of this field. A more complete comprehension of the mechanism of toxicity can only be the result of continuous study of the clostridia and their toxins and this field awaits many future contributions.

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