



Bacterial Exotoxins: General Characteristics and Mode of Action (Article Review).

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ABSTRACT

The idea of the harmful effects of pathogenic bacteria via toxin production has existed since ancient times. In 1872, Klebs hypothesized that "sepsins" were responsible for the formation of toxins produced by staphylococci, although he was unable to prove this hypothesis with evidence. In 1884, Robert Koch discovered that cholera was caused by toxins produced by bacteria rather than the bacteria themselves, demonstrating that bacterial pathogens could grow in the digestive tract without damaging the intestinal wall or nearby organs. However, research and studies have shown that injecting cholera filtrate through the gastrointestinal tract leads to results similar to those shown in real cases of the disease. In 1889, Loeffler revealed that the diphtheria bacillus remained localized at the injection site and was not present in the internal organs, indicating that the toxic substance produced at the injection site had spread into the blood circulation. According to Roux and Yersin, who have isolated several highly toxic bacteria, toxins are the reason behind all the bad things that pathogenic bacteria do. During the nineteenth century, studies widely demonstrated that bacterial toxins were the primary cause of the negative effects of all infectious diseases. As a result, medical research focused primarily on developing antitoxin serums. Exotoxins mostly consist of proteins that cause the immune system to work normally against them, and these toxins are produced by Gram-positive bacteria. However, endotoxins are secreted or released as a result of bacterial decomposition, especially the bacterial cell wall. Exotoxins are more sensitive to heat and may be susceptible to the effects of conditions and substances that do not affect endotoxins. This study represents an overview of most investigations that dealt with the properties of protein-nature exotoxins secreted by pathogenic bacteria, with examples of their production and mode of action.

Keywords: Toxin, Exotoxins, Pathogenic Bacteria, Endotoxin..

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INTRODUCTION

The concept that pathogenic bacteria may exert their detrimental effects via the production of toxins is almost as ancient as the concept of pathogenic bacteria themselves [1]. In 1872, Klebs hypothesized that chemical molecules known as "sepsins" were accountable for the lesions created by staphylococci. However, Klebs failed to provide any evidence to support the existence of these chemicals [2]. In 1884, Robert Koch determined that cholera is a toxicosis since the pathogenic bacterium multiplies inside the gastrointestinal contents without showing signs of invading or harming the intestinal wall or adjacent organs. Nevertheless, the administration of cholera filtrate by injection into the body did not result in any harmful consequences in laboratory animals, leading to the abandonment of the concept of an external cholera toxin [3]. Nevertheless, subsequent studies have shown that the introduction of cholera filtrate via the digestive system induces similar effects to those seen in the actual sickness [4]. This suggests that Koch's initial hypothesis may indeed have been accurate. In the same year that Koch expressed his perspective on cholera toxicosis, Loeffler put up a comparable proposition on the diphtheria bacillus [5]. He had identified this bacillus and noticed that in animals that died following deliberate infection, the organism stayed at the site of injection and was completely absent from the internal organs, despite the fact that they were significantly harmed. He determined that this unequivocally indicated that a toxin generated at the site of injection must have circulated within the bloodstream [6]. In 1887, he tried to show the existence of a toxic substance in extracts derived from a culture of the diphtheria bacillus. However, due to reasons that may now be understood, he was unsuccessful in his effort [7]. Roux and Yersin were responsible for isolating a preparation the next year that was deadly to guinea pigs at a dosage of 0.5 milligrams. This was achieved by using cadmium chloride precipitation on old alkaline cultures of the diphtheria bacillus [8]. They reached the conclusion, maybe accurately, that this material (which they designated, for the first time, as a "toxin") was a "type of enzyme" [9]. The identification of diphtheria toxin in 1889 prompted Roux to propose that the detrimental effects of all disease-causing bacteria were caused by toxins. He hypothesized that by gradually exposing animals to increasing amounts of these toxins, it would be possible to make the animals resistant to both the toxins and the bacteria themselves. His view was partly validated.

Shortly thereafter, two other bacterial toxins were identified: tetanus toxin, which was found by Faber in the same year, and botulinum toxin, which Van Ermengem discovered in 1896 [10]. In late 1890, Vod Behring, and Kitasato reported that rabbits and mice who had received modest amounts of weakened tetanus and diphtheria toxins exhibited immunity to these toxins. They showed that the serum without cells from these animals could neutralize the toxins. Additionally, the serum's ability to counteract toxins was long-lasting, since it retained its action even when transplanted to additional animals. Using antitoxic serum, they successfully shielded mice against 300 least-lethal doses of toxin [11].

It is unsurprising that throughout the transition to the nineteenth century, the prevailing belief was that bacterial toxins were responsible for the harmful consequences of all infectious illnesses and that the concept of antitoxic serum treatment should be the dominant focus of medical study [12]. During that time period, there was a significant amount of bacterial injection, along with the injection of their byproducts and other chemicals, into animals. Additionally, the ensuing immunological sera was mixed with other preparations [13]; [14].

There is likely a significant connection between the fact that the initial three toxins identified are the only ones that have been definitively proven to cause the harmful effects associated with the diseases they are associated with, specifically diphtheria, tetanus, and botulism (although it may be appropriate to include cholera in this limited group) [15]; [16]. The poisons that have been mentioned thus far are known as exotoxins. Boivin and Mesrobian published a study in 1935 on a novel category of toxins known as endotoxins, which they isolated from gram-negative microbes [17]. However, it is beneficial at this juncture to provide a concise overview of the distinctions between endotoxins and exotoxins [18]. All endotoxins are created by gram-negative species, whereas gram-positive organisms mostly produce exotoxins. However, it is worth noting that at least two exotoxins, namely those produced by *Shigella dysenteriae* and *Vibrio cholerae*, are produced by gram-negative organisms [19]. The prefixes endo- and exo- are now maintained mostly for historical purposes because the primary distinction between exotoxins and endotoxins lies not in their location outside or within the bacterial cell but rather in their structural characteristics. The exotoxins are proteinaceous substances, likely devoid of any non-protein components, and are inherently immunogenic [10]. On the other hand, endotoxins are immunogenic compounds consisting of a combination of proteins, polysaccharides, and lipids. Among these components, the protein component determines the immunogenicity, the polysaccharide component determines the immunological specificity, and some of the lipid component may contribute to the toxicity [20]. Endotoxins originate from the bacterial cell wall and are exclusively discharged during bacterial degradation. Exotoxins may be detected in the culture medium either during the logarithmic or decreasing growth stages. However, in some instances, they are only produced during bacterial degradation [21]. Homologous antibodies effectively neutralize exotoxins; however, they do not neutralize endotoxins due to their specificity towards the harmless protein and polysaccharide constituents. Exotoxins are more heat-labile compared to endotoxins. They can be detoxified by agents that do not have an effect on endotoxins. Exotoxins are significantly more toxic than endotoxins, and each exotoxin has its own distinct pharmacology, whereas all endotoxins share the same pharmacology [22].

1.Toxicity

Table 1: Properties of Purified Bacterial Toxins

Toxin	Toxic dose (μg)	Lethal toxicity compared strychnine	Isoelectric point (pH)	Diffusion constant 20° ($\text{cm}^2 \times 10^7 / \text{sec}$.)	Sedimentation constant $\text{sw}20^\circ$	Frictional coefficient (f/f_0)	Molecular weight	References
Botulinum A (crystalline)	2.5×10^{-5}	1000000	5.5	2.14	14.5	1.76	900000-1130000 approx.	[27]
Botulinum B	2.5×10^{-5}	1000000	-	-	14.9	-	1000000 approx.	[28]
Botulinum D	0.8×10^{-5}	3000000	-	-	14.6	-	1000000	[29]
Tetanus (crystalline)	4×10^{-5}	1000000	5.1	-	4.5	-	67000	[30]
<i>Cl. perfringens</i> ϵ -toxin	8×10^{-5}	300	-	6.76	2.48	1.68	40,500	[31]
Diphtheria (crystalline)	6×10^{-5}	2000	4.1	6.0	4.6	1.22	72000	[32]
Dysentery neurotoxin	2.3×10^{-5}	1000000	approx. 4.0	5.7	4.8	1.26	82000	[33]
Staphylococcal leukocidin:	4×10^{-3} per ml	-	-	-	-	-	-	-
F component (crystalline)	approx. 4×10^{-4}	-	-	8.5	3.05	-	32000	[34]
S component (crystalline)	per ml (kills macrophages)	-	-	7.5	3.3	-	38000	-
Staphylococcal enterotoxin	approx. 2.5 (emesis, monkey)	-	8.6	-	2.45	-	24000	[35]
Staphylococcal α -toxin	5 (lethal, rabbit)	350	-	-	3.0	-	44000	[36]

Staphylococcal - toxin	2.5 per ml (50% hemolysis 1% red cells)	-	-	-	6.1	-	68000	[37]
Staphylococcal erythrogenic toxin	5×10^{-6} (skin reaction, man)	-	-	9.5	2.7	1.1	27000	[38]

Table 1 shows a compilation of the characteristics of bacterial exotoxins that have undergone purification. Meaningful comparisons of poison toxicities cannot be made due to the variations in animal breeds, species, sexes, ages, weights, and susceptibilities. Additionally, the use of different treatment routes, workers, laboratories, and countries, as well as the diverse effects of poisons on different issues, further complicates the comparison. The data presented in column 3 of Table 1, which aims to provide a rough comparison of the toxicities of bacterial toxins and other poisons, is derived from two unfounded assumptions. Firstly, it assumes that all animals have equal susceptibility to these toxins. Secondly, it assumes that the lethal dose is directly proportional to the weight of the animal. The first assumption is indefensible due to reasons that will be elaborated upon later. For an examination of the indefensibility of the second assumption [23]; [24]. It is evident that there is a significant difference in toxicity between bacterial exotoxins and nonprotein poisons, with the former being orders of magnitude more lethal. For the sake of theoretical analysis, it may be determined that the deadly dosage of bacterial neurotoxins such as tetanus, botulinum, and dysentery is 1 mg per 1 million kg of living matter, or 100 gm for the whole global human population [25]; [26]

A. ORAL TOXICITY

The previously mentioned fatal dosages for animals specifically pertain to doses that are supplied via non-oral routes. It is often believed that bacterial exotoxins, excluding enterotoxins such as Botulinum and *Staphylococcus*, are not orally poisonous. It has been hypothesized that the poisons are eliminated in the gastrointestinal tract via the action of digestive enzymes or due to changes in pH levels [39]; [40]. demonstrated that toxins such as diphtheria toxin and tetanus toxin may indeed be harmful when ingested orally, as long as a sufficient dosage is administered. The oral fatal dosage is much higher than the parenteral dose, making it challenging to administer such a big dose orally to experimental animals [41]. The oral-to-parenteral fatal dosage ratio of botulinum toxin is less compared to that of tetanus toxin, which exhibits similar toxicity when taken parenterally. There is data suggesting that botulinum toxin may traverse the gastrointestinal barrier, either in its original form or as toxic pieces [42].

B. SPECIES SUSCEPTIBILITY

Different species of animals exhibit significant differences in their sensitivity to a specific bacterial toxin. Therefore, the guinea pig is far more vulnerable to neurotoxic dysentery compared to the mouse, with a susceptibility that is 1000 times higher. Furthermore, several specimens of the same toxin may exhibit differences in their comparative toxicity [43]. According to Rossetto and Montecucco [42], some samples of botulinum toxin are 6,000 times more poisonous to guinea pigs compared to mice. However, Hall et al. [44] found that other samples only had a threefold difference in toxicity. According to Llewellyn Smith's research from 1942–43, some samples of tetanus toxin are 350 times more harmful to guinea pigs compared to mice, while other samples are just three times as dangerous. These variations are also observable at the cellular level. The *Clostridium perfringens* α - and *Clostridium oedematiens* toxins are phospholipase C enzymes. The α -toxin causes rapid lysis of sheep red blood cells and slow lysis of horse red blood cells. Conversely, the γ -toxin exhibits the opposite behavior [45]. However, both toxins hydrolyze the phospholipids extracted from sheep and horse red blood cells at an equal rate. Phospholipase toxins from three strains of *Cl. bifermentans* have in vitro enzymatic activity that is 9, 60, and 70 times less toxic and less hemolytic than the phospholipase from *Cl. perfringens* [46].

C. POTENTIATION

Several professionals have seen a rise in the toxicity of tetanus toxin when diluted with broth or serum. This phenomenon does not seem to be only a result of the protective influence of broth or serum on highly diluted toxin, but rather a true enhancement [47]. Masuyer et al. [48] discovered a 64-fold increase in potency in some tetanus toxin preparations, but no increase in potency was seen in others. Potentiation was detected after the injection of the toxin in mice and guinea pigs, but not in cats and rabbits. Hence, there is a potential correlation between the enhancement of toxins and the occurrence of variations in the vulnerability of different species. The presence of accompanying compounds may influence the toxin's capacity to reach its target in the animal, and these substances may have varying effects in different species. In their study, Nigam and Nigam [49] discovered a significant increase of 5000 times in the potency of botulinum type D toxin when it was diluted in gelatin buffer. This resulted in a fatal dosage of 2.5×10^{-8} mg of pure toxin for mice. This intriguing topic warrants more investigation.

D. ASSAY

Toxins may be evaluated by quantifying their biological efficacy or their ability to bind with antibodies. Excluding the assessment of enzymatic activity in specific cases of toxins that are confirmed to be enzymes, the evaluation of biological potency typically involves determining the minimal dosage required to induce a specific effect within a specified timeframe. This effect could include causing the death of a certain percentage of test animals, breaking down a certain proportion of red blood cells, or generating a specific area of tissue necrosis on the skin, among other possibilities [50]. These assays have the benefit of assessing the biological activity of toxins, but they also have the drawback of inherent inaccuracy due to variations in individual susceptibilities within the group of animals or red cells being measured [51]. During antibody-combining power

experiments, the toxin is measured against antitoxin, and the biological impact is used subjectively rather than quantitatively as a sign of an excess of toxin. When toxin and antitoxin are combined in certain ratios, many toxin-antitoxin complexes tend to form clumps. Interestingly, in the majority of situations, combinations that are neutral or close to neutral in nature form clumps more rapidly compared to mixes that are more hazardous or unclear. The quantification of flocculating toxins may be achieved without the use of external markers by measuring the first flocculation of toxin when exposed to a certain quantity of antitoxin. Assays of antitoxin-binding capacity provide the benefit of enhanced precision, but the drawback is that they assess the overall antigenic potency of the toxin preparation, which is likely to include toxoid [52]. Bacterial toxins are sometimes used for the purpose of identifying and classifying microorganisms, particularly in the context of anaerobic clostridia [53].

2. Toxigenicity

The generation of toxins by a certain organism may be influenced by many variables, mostly related to the strain of the organism, the culture medium, and the culture circumstances.

A. ORGANISM

There may be significant variances among various strains of the same organism in their ability to manufacture toxin on a certain medium, even if it is ideally toxigenic. This is shown in the synthesis of α -toxin by *Cl. perfringens* [54]. There is only a limited knowledge of the reasons for these variances, especially in the case of some lysogenic and nonlysogenic species. Through repeated cultures, the majority of toxigenic strains of organisms gradually lose their capacity to generate toxin. Consequently, it is often imperative to revert to a freeze-dried sample of a culture that is confirmed to be toxigenic. Workers reinstate the toxigenicity of a strain by deliberately infecting animals and then isolating the bacterium again [55]. However, this approach is no longer popular in current times. For diphtheria toxin and certain other toxins (such as *Staphylococcus* toxin, *Streptococcus* toxin, *Bacillus cereus* toxin, and dysentery neurotoxin), it seems that the ability to produce toxins depends on the presence of lysogeny in the organism [56]; [57].

B. CULTURE MEDIUM

Media that support the favorable development of certain bacteria capable of producing toxins may not always possess toxic properties themselves. In order to maintain control, many parameters need to be regulated, including pH, organic and inorganic components, and the gas phase [58]. When examining the biological components, it is crucial to understand the sources of energy. For instance, Méndez et al. [59]. demonstrated that glucose had toxic effects on both the α and β toxins of *Cl. perfringens*, in addition to stimulating the organism's development. This was not just a consequence of the pH of the medium, since it was regulated. Under conditions where glucose is not present and the pH is kept at 7.5, only 0-toxin is generated. Kokubo et al. [60] discovered that using dextrin as the carbohydrate instead of glucose resulted in much higher amounts of α -toxin. However, this finding was only seen in select American strains of *Cl. perfringens* and did not apply to British strains. Assessing the toxic effects of carbs is relatively straightforward; however, identifying the toxic components in culture mediums other than carbohydrates is challenging and time-consuming, resulting in their infrequent identification [61]. which is crucial for the synthesis of tetanus toxin. However, without studying the physiology of toxin generation, there is no apparent need to use a culture medium with a known composition. To optimize toxin generation and purification, it is advisable to use "rich" complex media that provide high yields, as long as these media are reproducible in the given environment. Given that the process of purifying toxins involves isolating them from other proteins and large-molecular compounds, it is recommended to steer clear of these contaminants. This may often be accomplished by subjecting a concentrated, complicated medium to dialysis and using the resulting diffusate [62].

However, it may be unnecessary to exclude high-molecular-weight compounds from the culture medium if the toxin remains confined inside the bacterial cells until a later stage of rapid development. Under such circumstances, the cells may be collected prior to autolysis, therefore being isolated from the growth medium. They can then be floated in a little amount of hypertonic saline, and the toxin can be extracted [63]. The presence of a higher quantity of iron (0.1–1.0 mg iron per liter) in the culture medium might hinder the development of at least five bacterial toxins, including Tetanus, *Cl. perfringens* α -toxin, diphtheria, dysentery, and scarlet fever [64]. According to Caza and Kronstad [65], some strains of *Cl. tetani* exhibit the highest levels of toxin production when exposed to extremely small amounts of iron. However, other strains are not affected by iron, and, in fact, iron is intentionally supplied to enhance bacterial growth. The iron concentration in the culture medium has an impact on the metabolism and synthesis of *Cl. perfringens* α -toxin. However, there is no apparent correlation between these two effects [56]. The impact of iron on the generation of diphtheria and dysentery toxins and its connection to the synthesis of cytochrome in the parent species. Magnesium ions are potentially essential for the synthesis of staphylococcal hemolysins, whereas calcium ions have an inhibiting effect [66]. Various types of nitrogen may have diverse impacts on the generation of bacterial toxins. Yan [67] discovered that the presence of nitrate significantly enhances the development and synthesis of the cyanobacterial toxin microcystin-LR when bicine is also present. The impact of nitrogen sources on the generation of cylindrospermopsin and paralytic shellfish toxins in *Cylindrospermopsis raciborskii* CS-505 and *Raphidiopsis brookii* D9 was shown to be insignificant, according to [68]. According to İçgen [69], a study revealed that nitrogen sources had the greatest influence on the development of crystal proteins in *Bacillus thuringiensis*. Among the nitrogensources tested, peptone was determined to be the most effective organic nitrogen source for toxin production. In a study conducted by Hui in [70], it was shown that ammonium nitrogen had a substantial impact on the growth, biochemical composition, and toxin

generation of *Microcystis aeruginosa* FACHB905. These findings indicate that the correlation between nitrogen and bacterial toxin production is intricate and may vary based on the particular bacterium and toxin being studied.

C. CULTURE CONDITIONS

The generation of toxins by a strain of bacteria that is capable of producing toxins in a medium that supports toxin synthesis may be influenced by several additional conditions. Physical parameters, such as the culture volume and the level of aeration and agitation, may have a significant role [71]. According to Oliveira et al. [72], it seems that oxygen and carbon dioxide stimulate the generation of staphylococcal toxins. As an illustration. In their study, Sundaran et al. [73] achieved very large quantities of diphtheria toxin (180-250 Lf/ml) by using 60-liter batches of medium in 80-liter aluminum tanks. This was accomplished by the use of vortex stirring, agitation of the medium, and the introduction of air. Comparable amounts of toxin are acquired in highly agitated flasks equipped with baffles, and the toxin obtained in this manner seems to constitute about half of the total protein generated, with around 1500 Lf/mg of protein nitrogen compared to roughly 3000 Lf/mg of nitrogen for pure toxin [74]. The addition of detergent to some media leads to an augmentation in toxin production, whereas this effect is not seen in other media [75]. Loffredo [76] and other researchers have shown that the magnitude of the inoculum has a significant impact on the development of toxins. Demonstrated that the production of diphtheria toxin may be achieved in a 60-liter culture using a 10-liter inoculum in 24 hours, which is equivalent to the yield produced from a 200-ml inoculum in 48 hours. In some situations, it may be more expedient to extract 50% of the culture every 24 hours and substitute it with a new medium. Alternatively, one might transition from a semicontinuous culture, such as this one, to a continuous culture. In 1946, Polson and Sterne obtained a substantial amount of botulinum toxin by introducing physiological saline solution into dialyzing bags that were immersed in nutritional broth. Notably, the substitution of saline with nutritional broth in the dialyzing bags resulted in a significantly reduced production of toxin [77]. The production of toxin will be contingent upon several circumstances, such as the timing of the arrival of protease and toxin and the duration of their coexistence if these enzymes break down the toxin [78].

3. Nature

All exotoxins are proteins. The amino acid contents of some pure exotoxins are shown in Table 2. None of the pure exotoxins that have been studied so far have been documented as proteins that are chemically linked to prosthetic groups. However, the subsequent analysis will demonstrate that exotoxins are intricate in several other aspects.

A. PROTOXINS

Multiple exotoxins are released into the culture medium in the form of inactive pro-toxins, which have a resemblance to the zymogen's trypsinogen and pepsinogen. Proteolytic enzymes catalyze the conversion of these inert precursors into active enzymes by cleaving tiny pieces from them [79]. Research has shown that *Cl. botulinum* types A and B toxins, as well as E toxins, are classified as protoxins according to studies conducted by [80]. Proteolytic enzymes, found either in the culture fluids or in the digestive systems of animals that consume the toxins, transform the inactive pro-toxins into active toxins. Within the cultural environment, the protoxins may not undergo complete activation due to a lack of proteolytic enzymes in the culture fluids or the presence of proteolytic enzyme inhibitors [81]. Chapeton-Montes et al. [62] discovered data indicating that tetanus toxin may be generated as a protoxin. However, in this scenario, the protoxin is activated within the bacterial cell prior to the appearance of the toxin in the culture medium.

Table 2: Amino Acid Composition of Exotoxins (gm amino acid residue/100gm protein or 16 gm N) [82]; [10]

Amino Acid	Botulinu m type A	Tetanus	<i>Cl. perfrin gens</i>	<i>Staphylococcus</i> α -toxin	<i>Staphylococcus</i> enterotoxin	Diphtheria	Streptococcus erythrogenic
Alanine	3.9	3.4	3.0	2.5	1.3	5.1	1.8
Arginine	4.6	3.8	2.6	3.9	2.5	4.1	2.1
Aspartic acid	20.3	15.6	17.3	13.2	19.1	12.6	15.3
1/2Cystine	0.8	0.9	0.6	0	-	0.8	2.2
Glutamic acid	15.6	10.7	10.0	7.2	9.3	13.2	15.7
Glycine	1.4	3.2	3.1	3.9	2.8	4.7	3.6
Histidine	1.0	1.2	1.3	1.4	3.0	4.9	3.5
Isoleucine	11.9	8.8	3.7	3.8	4.0	6.7	5.9
Leucine	10.3	8.7	5.8	4.3	7.3	7.7	9.5
Lysine	7.7	9.8	14.2	7.7	17.1	9.1	12.2
Methionine	1.1	2.1	1.9	1.9	2.5	1.9	1.7
Phenylalanine	1.2	5.1	1.7	3.6	5.7	4.4	5.3
Proline	2.6	3.9	3.8	1.9	1.7	3.6	4.6
Serine	4.4	5.7	6.1	5.2	6.1	8.5	5.7
Threonine	8.5	5.2	10.0	6.2	5.0	6.1	6.1
Tryptophan	1.9	0.9	0.5	-	0.8	1.0	0.8
Tyrosine	13.5	7.0	6.0	3.8	10.7	4.8	11.3
Valine	5.3	4.6	7.0	3.2	5.8	7.4	5.8

Total	116.0	100.6	98.6	73.7	104.7	105.6	113.1
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B. DISSOCIATION AND AGGREGATION

The molecular weight of botulinum toxin ranges from around 12,000 to 1,000,000. In their study, Gillespie [83] discovered the presence of two separate hazardous units in unprocessed cell extracts and culture fluids of *Cl. Botulinum* Type A. These poisonous units had molecular weights ranging from 150,000 to 200,000 and 400,000 to 450,000. showed that the type A molecule with a molecular weight of about 1,000,000 would dissociate under certain conditions of pH and ionic strength into particles with a molecular weight of about 70,000, some of which were a toxic and some more toxic than the original preparation (with no gain in total toxicity). Whaler [84], demonstrated that rats who swallowed botulinum type A toxin, which had a sedimentation coefficient of 17.9 S, exhibited the presence of particles with a sedimentation coefficient of 7.9 S in their lymph. There are indications of even more minute bits of botulinum toxin. However, the trypsin-activated toxin included pieces that were unable to separate from the meniscus with an ultracentrifugal force of 260,000 g. They observed that part of the toxicity of the preparation would pass across dialyzable membranes. a harmful component known as a category A toxin, which has a molecular weight of 12,000. In addition, Goff [85], discovered that there were harmful peptic digestion byproducts of type A toxin that were able to pass through dialyzing membranes. These minute, poisonous particles lacked tyrosine. Thaysen-Andersen et al. [86], first documented that the sedimentation constant of tetanus toxin is 4.6 S, which corresponds to a molecular weight of 67,000. These authors discovered that the refined toxin spontaneously lost its toxicity (but not its antigenicity, i.e., it was toxoided; see below) while its sedimentation constant climbed to 7.0 S. They argued that the poison formed a dimer and that, in doing so, the coalescing molecules mutually hindered biological action. Veronese et al. [87] reached a similar result, with the sedimentation constants of the monomer and dimer being 3.9 S and 7.6 S, respectively, according to their findings. Chang et al. [88] isolated a protein from the tetanus bacillus that had a sedimentation constant of 7 S, yet exhibited the same level of toxicity as Pillemer's monomer. The material was depolymerized by subjecting it to treatment with cysteine followed by moniodoacetate, resulting in the production of a hazardous preparation with a sedimentation constant of 4.5 S. The depolymerization of the aggregated forms of tetanus toxins seems to be achieved by rupturing disulfide bonds [48]. Diphtheria toxin may also manifest as a dimer. Pitard and Malliavin [89] showed that certain preparations of diphtheria toxin contain two forms of the toxin, one with a sedimentation constant (uncorrected) of 4.6 S, corresponding to the familiar form of toxin with a molecular weight of 64,500, and another with a sedimentation constant (uncorrected) of 6.8 S, presumably a dimer. The dimer may be transformed into the monomer by treatment with the sulfhydryl reagent dithiothreitol, which implies the breaking of a disulfide link. The dimerized version of the toxin is seen in certain cultures of the diphtheria bacillus when ingested orally. It is hypothesized that this dimer form may arise due to a mutation occurring either in the bacteria itself or in its genetic material. A lysogenic phage may arise due to the unusual growth circumstances of the organism.

C. COMPLEXITY

Certain bacterial toxins have a structure consisting of several distinct proteins.

1. Staphylococcal leukocidin

An exemplary instance is staphylococcal leukocidin, which induces the death of rabbit and human leukocytes, accompanied by distinct alterations in their morphology. Breibeck and Rompel [90] demonstrated that it comprises two proteins, namely the F and S components, both of which he successfully crystallized (see Table I). Individually, these components are inert, but when combined, they exhibit synergistic behavior.

2. Staphylococcal α -toxin

The hemolytic staphylococcal α -toxin is likely a combination of proteins that cannot be differentiated based on their antigenic and biological properties. Habermehl [91], got a seemingly uniform sample of the compound, which they were able to separate into four distinct components using electrophoresis on a density gradient. These components did not exhibit significant differences in their biological characteristics. One of these components exhibited significantly lower hemolytic activity per unit weight compared to the others. However, its hemolytic activity could be enhanced to a similar level by subjecting it to dialysis against a solution of ethylenediamine - tetra acetic acid. This indicates that the component primarily consisted of an inactive toxin that could be activated.

4. Anthrax toxin

The anthrax toxin is composed of three separate protein components that work together in a synergistic manner. Factor I have no toxicity when administered alone. However, when administered in conjunction with Lactor L, it induces edema in the rabbit's skin and results in the mortality of mice. The combination of factors I and II is less deadly per unit of edema-inducing action compared to the unrefined toxin, which resulted in the identification of a third component. Factor III had distinct serological differences from factors I and II. It did not possess deadly properties on its own or when combined with factor I, but became lethal when combined with factor II. Additionally, it enhanced the lethality of combinations containing factors I and II per unit of edema-producing activity [92].

4. Diphtheria toxin

There is evidence suggesting that the diphtheria toxin may not consist of a single protein. Ben David [93], demonstrated that the purified toxin exhibited many antigenic determinants or was a composite, or a consistent blend, of various antigens, with perhaps just one being lethal. The crystalline toxin preparation of Pope and Stevens exhibited a solitary line when tested

against antisera from horses that were hyperimmunized against the crude toxin. However, when the toxin was subjected to partial denaturation by heating or treatment with alkali, the single diffusion line would divide into four distinct lines. The newly divided lines all exhibited a sense of individuality in relation to the original single line. Beretta [94] saw comparable results when studying human and bovine serum albumin. They attributed the presence of several diffusion lines to the existence of various antigenic determinants inside a single protein molecule, which interacted with distinct particular antibodies. The crystalline diphtheria toxin was analyzed using an interferometric technique, revealing a single immunodiffusion line consisting of a minimum of four closely spaced, highly distinct lines. Čapek [95] noted that although a single line was seen when the crystalline toxin was tested against most antitoxic sera using immunodiffusion, this line was not found with all antitoxic sera. Out of the total of thirty-seven antitoxic sera, five of them exhibited two distinct lines. The aforementioned data may be explained if the diphtheria toxin were a monomeric protein with many antigenic determinants, each capable of inducing the production of distinct antibodies. However, Pope maintains that the crystalline poison comprises many autonomous antigens. Kragh-Hansen [96] conducted investigations on the antigenic determinants of albumin after partial digestion of the albumin with proteolytic enzymes. In such conditions, it is anticipated that peptide links within a single protein molecule would be cleaved. However, Pope successfully achieved the separation of the several forms of diphtheria toxin by subjecting them to certain conditions (such as treatment with sulfite, urea, phosphate, and heating), even though it was implausible for the peptide bonds to be hydrolytically cleaved. A crystalline preparation of diphtheria toxin, which is immunologically and kinetically homogeneous, may actually be a mixture of multiple molecular species, similar to crystalline protamine insulin. However, for such a mixture to appear as a single immunodiffusion line (only resolvable by interferometry), there must be a force holding the components together. This force is necessary to maintain a highly consistent and integrated balance between the concentrations, diffusion coefficients, and optimal precipitation ratios of the various separate antigens and their corresponding antibodies. The crystalline preparations of diphtheria toxin, which have been consistently obtained in several labs worldwide, exhibit a high degree of similarity. Despite being composed of several molecular species; these mixes have a consistent nature that is likely important in the physiology of the diphtheria bacillus. The whole complex consists of diphtheria toxin. The toxicity of the complex may potentially be attributed to one of its constituents; however, a specific toxic component has not yet been identified.

5. Activity

A. BIOLOGICAL ACTIVITY

For each toxin, it is feasible to identify a specific chemical or an unspecified component of tissue or plasma, a cell, tissue, or organ that is targeted by the toxin with varying degrees of accuracy. Some toxins have the ability to target a wide range of cells, whereas others specifically affect just one kind of cell or tissue. Several toxins, such as diphtheria, *staphylococcus* α -toxin, and *CL. perfringens* α -toxin, cause necrosis when injected into the skin and are often cytotoxic. When different toxins are injected into the skin, they cause edema and/or bleeding instead of necrosis, likely by directly or indirectly impacting blood vessels [63]. This category includes scarlet fever toxin, anthrax toxin, maybe cholera toxin, and *Cl. sordellii* toxins. The dysentery neurotoxin is hemorrhagic but does not affect the skin [97]. Several bacterial exotoxins may lyse red blood cells and are categorized as oxygen-labile or oxygen-stable hemolysins. The oxygen-sensitive hemolysins consist of the O-toxin from *Cl. perfringens*, the tetanolysin from *Cl. tetani*, the pneumolysin from the pneumococcus, and the streptolysin O from the hemolytic *Streptococcus*. These hemolysins have immunological relationships and shared features despite their various origins, indicating a potential common hemolytically active prosthetic group that functions as a hapten. Oxygen-stable hemolysins cause hemolysis after an initial period of action on red blood cells without actual cell destruction [98]. The hemolysis occurring after the induction phase is spontaneous and cannot be inhibited by a titoxin; it is unrelated to the hemolysin. *Staphylococcal* B and *Cl. perfringens* α -toxins are hemolysins that exhibit "hot-cold" properties. Red blood cells exposed to low toxin concentrations at 37°C do not lyse at that temperature but lyse upon cooling. Cells treated with staphylococcal β -toxin are sensitive to lysis at 37°C by various compounds such as glycerol, broth components, lipases, and proteases. However, they are no longer vulnerable to the α -toxin. The staphylococcal leukocidin specifically targets white blood cells known as polymorphonuclear leukocytes in particular animals. Multiple exotoxins target cells in tissue culture, protoplasts, and spheroplasts of bacteria, as well as iviosomes from various origins, releasing degradative enzymes. Cells: polymorphonuclear leukocytes Multiple exotoxins target cells in tissue culture, protoplasts, and spheroplasts of bacteria, as well as lysosomes from various origins, releasing degradative enzymes. Diphtheria toxin disrupts protein synthesis in both individual cells and cell extracts [99]. Certain toxins have been shown to affect the gastrointestinal tract. The staphylococcal enterotoxin induces vomiting when ingested by humans and rhesus monkeys but does not have any effect on the skin. Cholera toxin induces diarrhea in susceptible animals when administered into the stomach and may cause edema when injected into the skin. The very powerful neurotoxins from *Clostridium botulinum* and *Clostridium tetani* do not show any visible signs of harm to cells or tissues. However, their distinct paralyzing effects, which are mostly flaccid and spastic, indicate that they affect neural tissue without causing degradation [100].

B. RELATION BETWEEN TOXIC AND ENZYMATIC ACTIVITIES

There are several justifications for hypothesizing that bacterial exotoxins may possess enzymatic properties. These proteins are present in cultures with other proteins that function as enzymes. They exhibit the same level of instability as enzymes. The extreme toxicity of the substance may be attributed to its catalytic activity, whereas it is hard to see a protein having any

biological function other than as an enzyme. In addition to the enzymatic example, there are only two more recognized precedents: trypsin inhibitors and γ -globulin. A trypsin inhibitor is a protein that hinders the activity of trypsin by forming a complex with it. Globulin is a kind of protein that binds with antigens, including protein antigens, and as a result, triggers various biological responses. One of the impacts might be the suppression of the enzymatic activity of certain toxins. Therefore, it is conceivable that certain exotoxins may function by inhibiting enzymes. A further hypothesis proposes that proteins might operate as effectors, meaning they can influence the activity of enzymes by binding to them and altering their function. Alternatively, two inactive proteins could potentially unite to create an active enzyme. The relevance of staphylococcal leukocidin in this context lies in the fact that it is composed of two proteins, which are individually inactive but become active when working together [101]. Defining "toxic activity" is a challenge when examining the correlation between hazardous and enzymatic activities. Several pathogenic organisms secrete extracellular enzymes that have biological effects, and several of these enzymes are referred to as toxins. Certain bacterial agents target the soluble components of bodily fluids, the interstitial substance of cells, and the reticular framework of muscle [102]. These enzymes, streptokinase and staphylokinase, are proteolytic in nature and have the ability to transform the inactive harmful activity that is difficult to describe. Several pathogenic organisms secrete extracellular enzymes that have biological effects, and a subset of these enzymes are referred to as toxins. Certain bacterial agents target the soluble components of bodily fluids, the interstitial substance of cells, and the reticular framework of muscle. These enzymes, such as streptokinase and staphylokinase, convert the inactive plasminogen into the active enzyme plasmin. Plasmin then digests fibrin and prevents blood clotting. Another enzyme, hyaluronidase, depolymerizes hyaluronic acid, which is the substance that holds cells together. Collagenase, such as the κ -toxin of *Cl. perfringens*, breaks down the reticular scaffolding of muscle without damaging the muscle fibers themselves. Determining whether these enzymes should be classified as poisons in the traditional sense poses a challenge. The early discoverers of bacterial toxins most likely did not consider them significant, since their contribution to the toxicity of bacterial filtrates is rather little compared to other toxins present in such filtrates [103].

C.MODE OF ACTION

The α -toxin of *Cl. perfringens* was the first bacterial toxin to have its mode of action defined at the molecular level. This means that the chemical structure(s) of some of its susceptible substance(s) were identified, as well as the specific changes that occurred in these substances. Macfarlane and Knight [104], made this groundbreaking discovery.

1. General Mechanism of Action

Take the structure of the *Cl. perfringens* Alpha Toxin (CPA) toxin as an example (Figure 1). The amount of phosphatidylcholine (PC) to sphingomyelin (SM) and the presence of toxins in the area are two things that determine how complex and varied CPA's work is in different types of cells. It is hydrolysis that breaks down phosphatidylcholine (PC) and sphingomyelin (SM), which makes diacylglycerol (DAG) and ceramide (CER). Working with Gi-GTP-binding proteins, CPA can make host enzymes work better in a roundabout way. It also makes it easier for tropomyosin receptor kinase A (TrkA) to interact with cell membranes, which starts the MEK/ERK pathway. Cells lacking gangliosides have a heightened sensitivity to the effects of CPA, since it may decrease the flexibility of their cell membranes and induce alterations in their electrical charge. Sialidases have the ability to enhance the responsiveness of cells to CPA, which might possibly work along with other toxins produced by *Cl. perfringens* [105].

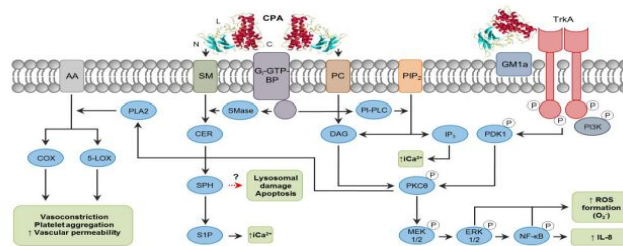


Figure 1: Intracellular mechanisms implicated in the intracellular activity of α -toxin [105]

CPA has the ability to attach to membrane PC (phosphatidylcholine) and SM (sphingomyelin), which leads to the activation of Gi-GTP-BPs (G-protein coupled receptors) and the initiation of various pathways depending on the specific cell type. Although the inherent CPA phospholipase activity causes the breakdown of horse erythrocytes, CPA predominantly impacts sheep erythrocytes via affecting SM metabolism through GTP-BPs. Superoxide anion production triggered by CPA occurs in rabbit neutrophils and DonQ cells via the activation of endogenous phospholipase C (PLC) and phosphorylation of PI3K via the TrkA receptor. This pathway is also crucial in the production of IL-8 by A549 cells. The limited presence of neutrophils in gas gangrene-affected tissues and their buildup on the vascular endothelium may be attributed to increased levels of IL-8, which facilitates strong attachment to extracellular matrix proteins. Cyclophosphamide (CPA) causes a decrease in the ability of neutrophils to mature and be replaced in the bloodstream. The stimulation of arachidonic acid metabolism via phospholipase A2 (PLA2) caused by CPA continues to occur during gas gangrene [106].

2. Mechanisms of Cell Death

The phytochemical CPA may cause the plasma membrane to break down significantly and reactive oxygen species (ROS) to be released, which is a characteristic of necrosis. Small amounts of CPA may speed up the metabolism of SM, which can lead to the production of substances that help cells die, like CER, N-acyl ethanolamine, and saturated fatty acids. This process also causes mitochondrial cytochrome C to be released, caspase-3 to be turned on, and more phosphatidylserine to be seen in GM95 cells. Going through some steps turns CER into sphingosine (SPH), which can cause lysosomes to break open and then release proteases that play a role in apoptosis. ROS generation may cause damage to lysosomes when CPA is taken up via caveolae that contain cholesterol. Elevated concentrations of intracytoplasmic calcium ions (iCa^{2+}) often play a role in the first stages of both apoptosis and necrosis, which may lead to cell death. Sphingosine-1-phosphate (S1P) and inositol trisphosphate (IP3) are made when CPA is activated. These chemicals help move and raise intracellular calcium (iCa^{2+}) [107]. Immunology

The exotoxins have antigenic properties, meaning they induce the formation of antibodies when delivered into appropriate animals in a certain manner. Consequently, the antitoxins produced by these exotoxins counteract their effects.

NEUTRALIZATION BY ANTITOXIN

The neutralization of a physiologically active protein antigen by its homologous antibody is not always guaranteed. Therefore, the antisera targeting various enzymes exhibit limited or incomplete neutralization of their enzymatic activity. For instance, urease-anti urease floccules, catalase-anti catalase, tyrosinase-anti tyrosinase, papain-anti papain, and ribonuclease-anti-ribonuclease exhibit enzymatic activity retention rates of 80%, 73%, 100%, 30%, and 78%, respectively [108]. In vitro, homologous antibodies effectively block many enzymes, such as bacterial phospholipases. Hence, the ability of an antibody to counteract the activity of an enzyme seems to be coincidental. The antibody-combining site and the "active center" of an enzyme are distinct entities, and their proximity may vary. If they are in close proximity, the enzyme's activity may be neutralized as a result of the antibody physically obstructing the active center. The inhibition of the effects of all known bacterial exotoxins by their corresponding antitoxins is an established fact. The aforementioned discovery, along with the fact that several enzymes remain unaffected by their respective antibodies, prompted some researchers to posit the existence of a basic immunological distinction between poisons and enzymes. Nevertheless, the impact of antitoxins on toxins is often shown in living organisms, and in such situations, other elements outside the toxin, substrate, and antitoxin are involved. The hazardous enzyme urease serves as a compelling illustrative example. Anti-urease exerts a 20% inhibition on urease; however, it effectively protects rabbits against the deadly consequences of the enzyme [109]. The expeditious eradication of an antigen is facilitated by the existence of circulating antibodies. The phagocytosis of a toxin-antitoxin complex, even in the absence of a visible precipitate, may have significance; nevertheless, it should not be seen as the only determinant, since antitoxin has the potential to counteract toxin in scenarios when phagocytes are absent. Therefore, it has the potential to impede the activity of hemolytic toxins on red blood cells that have been washed. In such instances, it is conceivable that the toxin-antitoxin complex can have difficulties traversing obstacles that are accessible to the unhindered toxin. Cataudella et al. [110] demonstrated that diphtheria toxin hinders protein synthesis in HeLa cells and extracts derived from these cells. They also observed that diphtheria toxin-antitoxin floccules maintained a notable toxicity against cell-free extracts, despite the toxin's neutralizing effect on intact HeLa cells (and the entire animal).

TOXOIDS

Toxins have the potential to undergo spontaneous toxicity loss while retaining their antigenicity. This means that they may still elicit the generation of antibodies that counteract the toxicity of the toxins and interact with both the toxic and non-toxic proteins. The term "toxoid" was first used by Ehrlich to refer to the detoxified antigen. Toxoids often exhibit greater stability compared to toxins, and their solubilities vary. The process of toxoiding extends beyond poisons and may also occur with enzymes. It is likely that the structural alterations seen during toxoiding also occur in proteins that lack recognized hazardous or enzymatic characteristics. The toxoidation of toxin by formaldehyde treatment was first observed by Lowenstein in 1909. Subsequently, a multitude of toxoiding agents have been identified, including hexamethylenetetramine, ketone, nitrous acid, iodine, ascorbic acid, carbon disulfide, and pepsin. Typically, the process of toxoiding involves the incubation of a toxin solution with formaldehyde at concentrations ranging from 0.1% to 0.2%. A pH range of 6 to 9 was maintained at a temperature of 37°C for a duration of many weeks. As the pH decreases, the reaction rate decreases. At a pH range of 8-9, a significant amount, 90% or more, of the toxin undergoes toxoidation within a few days. However, a longer treatment period is required to achieve the appropriate level of detoxification required for antigens intended for human consumption. Extended toxoidation often diminishes the ability to first promote antibody synthesis and subsequently to bind with antibodies [86]; [111].

PASSIVE AND ACTIVE IMMUNIZATION

Passive or active vaccination against bacterial toxins may provide protection for animals against their harmful effects. Immunization against toxins does not guarantee protection against infection by toxinogenic organisms. In several instances, such as with staphylococci, it does not provide any protection. Passive immunization is the hyperimmunization of appropriate animals, often horses, with a toxoid until a significantly high concentration of antitoxin is achieved in the serum. Subsequently, the serum, or the purified γ -globulin fraction obtained from it, is directed towards the human or animal recipient for the purpose of immunization. Although passive vaccination may provide preventive benefits, its therapeutic efficacy remains uncertain. The rationale for this phenomenon is that exotoxins exhibit a high degree of fast fixation to their susceptible

locations far in advance of the manifestation of any apparent indications of intoxication. Once fixed, these exotoxins are resistant to displacement or inhibition by antitoxin agents. However, there are drawbacks associated with prophylactic passive immunization. Firstly, the foreign protein that is introduced by this method is quickly removed. Secondly, individuals who have already received a single dosage of serum are at danger of experiencing allergic responses and anaphylactic shock if they are required to get a second dose of serum. Contemporary inclination is to completely forsake antiserum and instead depend on active vaccination. Active immunization is the administration of a toxoid to those who are at risk, prior to their exposure to that danger. Children are proactively given immunizations to protect against diphtheria and tetanus toxins, while troops get immunizations specifically targeting tetanus toxin. Individuals who have been actively vaccinated not only possess stable antibodies in their bloodstream when they are infected, but they also promptly react to any additional antigenic stimulation caused by the infection by generating significant amounts of antitoxin [112]. The eradication of tetanus and diphtheria may be achieved in places where there is widespread implementation of active vaccination against the corresponding toxins. Individuals who have successfully survived an episode of diphtheria often have lifelong immunity to the illness, but survivors of tetanus do not possess the same level of immunity. This is because the lethal dose of tetanus toxin is far lower than the quantity required to generate functional immunity. Significant quantities of toxoid have the potential to provide protection to animals against future injections of identical toxin within a 24-hour period, prior to the formation of any antibodies. The observed early protective effect of toxoids might perhaps be attributed to the competitive interaction between significant quantities of weakly binding toxoids and minimal quantities of actively binding toxin for the vulnerable substance [113].

PREVENTION OF TOXIN-PRODUCING BACTERIA

Bacterial toxins Presenting a notable hazard in the food sector, it is essential to avoid its existence in order to guarantee food safety [114] through the following preventive measures:

Handwashing: It is important to properly wash your hands after handling raw meat, poultry, shellfish, flour, or eggs.

Utensils and Surfaces: Clean utensils, cutting boards, and surfaces using hot water and soap after handling each food item.

Fresh Produce: Thoroughly cleanse fresh fruits and vegetables by rinsing them with running water.

Storage Temperatures: Frozen meals should be stored at temperatures lower than -18°C, while hot cooked foods should be kept above 60°C until they are served.

Prevent Re-Freezing: Abstain from re-freezing prepared food.

Effective Cooling: Employ suitable cooling techniques to inhibit the proliferation of bacteria.

References

- [1] Do Vale, A., Cabanes, D., and Sousa, S. 2016. Bacterial toxins as pathogen weapons against phagocytes. *Frontiers in microbiology*, 7, 42.
- [2] Alouf, J. E. 2006. A 116-year story of bacterial protein toxins (1888–2004): from “diphtheritic poison” to molecular toxicology. *The Comprehensive Sourcebook of Bacterial Protein Toxins*, 3-21.
- [3] Van Heyningen, W. E. 2019. *Cholera: The American scientific experience, 1947-1980*. Routledge.
- [4] Baldauf, K., Royal, J., Hamorsky, K., and Matoba, N. 2015. Cholera Toxin B: One Subunit with Many Pharmaceutical Applications. *Toxins*, 7(3), 974–996. doi:10.3390/toxins7030974.
- [5] Adler, R., 2017. *Robert Koch and American Bacteriology*. McFarland and Company; Illustrated edition.
- [6] Logan, N. A., Hoffmaster, A. R., Shadomy, S. V., and Stauffer, K. E. 2011. *Bacillus and other aerobic endospore-forming bacteria*. *Manual of clinical microbiology*, 381-402.
- [7] Dworkin, M. 2011. *Robert Koch—From Obscurity to Glory to Fiasco*.
- [8] Tiwari, T. S., and Wharton, M. 2012. *History of the disease*. *Vaccines E-Book*, 153.
- [9] Mora-Obando, D., Díaz, C., Angulo, Y., Gutiérrez, J. M., and Lomonte, B. 2014. Role of enzymatic activity in muscle damage and cytotoxicity induced by *Bothrops asper* Asp49 phospholipase A2 myotoxins: are there additional effector mechanisms involved. *Peer J*, 2, e569.
- [10] Cavaillon, J. M. 2018. Historical links between toxicology and immunology. *Pathogens and disease*, 76(3), fty019.
- [11] Beeman, E. A. 2007. *Charles Armstrong, MD: A Biography*. National Institutes of Health.
- [12] Magner, L. N. 2009. *A history of infectious diseases and the microbial world*.
- [13] Brown, L. R. 2010. Microbial enhanced oil recovery (MEOR). *Current opinion in Microbiology*, 13(3), 316-320.
- [14] Abdulmajeed, M. A., Jafar, N. B., & Hamada, Y. H. 2023. Investigation and molecular characterization of Shiga toxin-producing *E. coli* O157: H7 from meat and dairy products in Kirkuk province, Iraq. *Journal of Hygienic Engineering & Design*, 42.
- [15] Van Dolah, F. M. 2000. Marine algal toxins: origins, health effects, and their increased occurrence. *Environmental health perspectives*, 108(suppl 1), 133-141.
- [16] Taylor, D. L., Kahawita, T. M., Cairncross, S., and Ensink, J. H. 2015. The impact of water, sanitation and hygiene interventions to control cholera: a systematic review. *PLoS one*, 10(8), e0135676.
- [17] Cavaillon, J. M. 2022. From bacterial poisons to toxins: the early works of Pasteurians. *Toxins*, 14(11), 759.
- [18] Bolon, B., Uzal, F. A., and Schutten, M. 2023. *Bacterial Toxins*. In Haschek and Rousseaux's *Handbook of Toxicologic Pathology*, Volume 3 (pp. 629-678). Academic Press.

- [19] Kadis, S., Weinbaum, G., & Ajl, S. J. (Eds.). 2016. Bacterial Endotoxins: A Comprehensive Treatise.
- [20] Williams, K. L. 2007. Endotoxin structure, function, and activity. *DRUGS AND THE PHARMACEUTICAL SCIENCES*, 167, 67.
- [21] Sheehan, J. R., Sadlier, C., and O'Brien, B. 2022. Bacterial endotoxins and exotoxins in intensive care medicine. *BJA education*, 22(6), 224.
- [22] Yanagisawa, C., Hanaki, H., Natae, T., and Sunakawa, K. 2007. Neutralization of staphylococcal exotoxins in vitro by human-origin intravenous immunoglobulin. *Journal of Infection and Chemotherapy*, 13(6), 368-372.
- [23] Peterson, M. E., & Talcott, P. A. 2006. Small animal toxicology. Elsevier Health Sciences.
- [24] Mahdi, N. B. 2023. Prevalence of panton–Valentine leukocidin and toxic shock syndrome toxin-1 genes in methicillin-resistant *Staphylococcus aureus* isolated from nose of restaurant workers in Kirkuk city. *Journal of Advanced Pharmaceutical Technology & Research*, 14(1), 34-38.
- [25] Emmeluth, D. 2010. Botulism. Infobase Publishing. Second Edition.
- [26] Aljamali, N. M., El-Taei, F. W., and Alasady, D. 2021. Review on Types of Toxins (Pharmaceutical, Biological, Chemical). *Glob Acad J Pharm Drug Res*, 3.
- [27] Gregory, K. S., Mojanaga, O. O., Liu, S. M., & Acharya, K. R. 2022. Crystal structures of botulinum neurotoxin subtypes A4 and A5 cell binding domains in complex with receptor ganglioside. *Toxins*, 14(2), 129.
- [28] Grimstad, Ø., Kvammen, B. Ø., & Swartling, C. 2020. Botulinum toxin type B for hidradenitis suppurativa: a randomised, double-blind, placebo-controlled pilot study. *American Journal of Clinical Dermatology*, 21, 741-748.
- [29] Souillard, R., Grosjean, D., Le Gratiot, T., Poezevara, T., Rouxel, S., Balaine, L., ... & Le Maréchal, C. 2021. Asymptomatic carriage of *C. botulinum* type D/C in broiler flocks as the source of contamination of a massive botulism outbreak on a dairy cattle farm. *Frontiers in microbiology*, 12, 679377.
- [30] Okidi, R., Sambo, V. D. C., & Eyul, J. 2020. Neonatal tetanus in St. Mary's Hospital Lacor: A case report. *Clinical Case Reports*, 8(11), 2234-2236.
- [31] Grenda, T., Jarosz, A., Sapała, M., Grenda, A., Patyra, E., & Kwiątek, K. 2023. *Clostridium perfringens*—Opportunistic Foodborne Pathogen, Its Diversity and Epidemiological Significance. *Pathogens*, 12(6), 768.
- [32] Gupta, A., Gujral, M., Singh, A., & Chawla, K. 2020. Diphtheria resurgence in India: A case study from south Karnataka. *Journal of Family Medicine and Primary Care*, 9(11), 5776.
- [33] Cai, S., Kumar, R., and Singh, B. R. 2021. Clostridial neurotoxins: Structure, function and implications to other bacterial toxins. *Microorganisms*, 9(11), 2206.
- [34] Toor, H. G., Banerjee, D. I., and Chauhan, J. B. 2021. In silico evaluation of human cathelicidin LL-37 as a novel therapeutic inhibitor of panton-valentine leukocidin toxin of methicillin-resistant *Staphylococcus aureus*. *Microbial Drug Resistance*, 27(5), 602-615.
- [35] Etter, D., Schelin, J., Schuppler, M., & Johler, S. 2020. Staphylococcal enterotoxin C—an update on SEC variants, their structure and properties, and their role in foodborne intoxications. *Toxins*, 12(9), 584.
- [36] Spaan, A. N., Neehus, A. L., Laplantine, E., Staels, F., Ogishi, M., Seeleuthner, Y., ... and Casanova, J. L. 2022. Human OTULIN haploinsufficiency impairs cell-intrinsic immunity to staphylococcal α -toxin. *Science*, 376(6599), eabm6380.
- [37] Francis, R., Singh, P. K., Singh, S., Giri, S., and Kumar, A. 2020. Glycolytic inhibitor 2-deoxyglucose suppresses inflammatory response in innate immune cells and experimental staphylococcal endophthalmitis. *Experimental eye research*, 197, 108079.
- [38] Schlievert, P., Kilgore, S., and Leung, D. 2023. Agr Regulation of Streptococcal Pyrogenic Exotoxin A in *Staphylococcus aureus*. *Micropublication Biology*, 2023.
- [39] Chaveli-López, B. 2014. Oral toxicity produced by chemotherapy: A systematic review. *Journal of clinical and experimental dentistry*, 6(1), e81.
- [40] Simpson, L. L. 2004. Identification of the major steps in botulinum toxin action. *Annu. Rev. Pharmacol. Toxicol.*, 44, 167-193.
- [41] Turner, P. V., Brabb, T., Pekow, C., & Vasbinder, M. A. 2011. Administration of substances to laboratory animals: routes of administration and factors to consider. *Journal of the American Association for Laboratory Animal Science*, 50(5), 600-613.
- [42] Rossetto, O., & Montecucco, C. 2019. Tables of toxicity of botulinum and tetanus neurotoxins. *Toxins*, 11(12), 686.
- [43] Padilla-Carlin, D. J., McMurray, D. N., & Hickey, A. J. 2008. The guinea pig as a model of infectious diseases. *Comparative medicine*, 58(4), 324-340.
- [44] Hall, A. P., Elcombe, C. R., Foster, J. R., Harada, T., Kaufmann, W., Knippel, A., ... & York, M. J. 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes conclusions from the 3rd International ESTP Expert Workshop. *Toxicologic pathology*, 40(7), 971-994.
- [45] Ochi, S., Oda, M., Matsuda, H., Ikari, S., & Sakurai, J. 2003. *Clostridium perfringens-Toxin Activates the Sphingomyelin Metabolism System in Sheep Erythrocytes. *Journal of Biological Chemistry*, 279(13), 12181–12189. doi:10.1074/jbc.m307046200.*

- [46] Fiester, S. E., Arivett, B. A., Schmidt, R. E., Beckett, A. C., Ticak, T., Carrier, M. V., ... Actis, L. A. 2016. Iron-Regulated Phospholipase C Activity Contributes to the Cytolytic Activity and Virulence of *Acinetobacter baumannii*. PLOS ONE, 11(11), e0167068. doi: 10.1371/journal.pone.0167068.
- [47] Hassel, B. 2013. Tetanus: Pathophysiology, Treatment, and the Possibility of Using Botulinum Toxin against Tetanus-Induced Rigidity and Spasms. Toxins, 5(1), 73–83. doi:10.3390/toxins5010073.
- [48] Masuyer, G., Conrad, J., & Stenmark, P. 2017. The structure of the tetanus toxin reveals pH-mediated domain dynamics. EMBO Reports, 18(8), 1306–1317. doi:10.15252/embr.201744198.
- [49] Nigam, P. K., & Nigam, A. 2010. Botulinum toxin. Indian journal of dermatology, 55(1), 8.
- [50] Lahiani, A., Yavin, E., & Lazarovici, P. 2017. The Molecular Basis of Toxins' Interactions with Intracellular Signaling via Discrete Portals. Toxins, 9(3), 107. doi:10.3390/toxins9030107.
- [51] Tarrant, J. M. 2010. Blood Cytokines as Biomarkers of In Vivo Toxicity in Preclinical Safety Assessment: Considerations for Their Use. Toxicological Sciences, 117(1), 4–16. doi:10.1093/toxsci/kfq134.
- [52] Singh, G., Yadav, M., Ghosh, C., & Rathore, J. S. 2021. Bacterial toxin-antitoxin modules: classification, functions, and association with persistence. Current Research in Microbial Sciences, 2, 100047. doi: 10.1016/j.crmicr.2021.100047.
- [53] Forbes, J. D. 2020. Clinically Important Toxins in Bacterial Infection: Utility of Laboratory Detection. Clinical Microbiology Newsletter, 42(20), 163–170. doi: 10.1016/j.clinmicnews.2020.09.003.
- [54] Rood, J. I., Adams, V., Lacey, J., Lyras, D., McClane, B. A., Melville, S. B., ... Van Immerseel, F. 2018. Expansion of the *Clostridium perfringens* toxin-based typing scheme. Anaerobe. doi: 10.1016/j.anaerobe.2018.04.011.
- [55] Molnar, A., Lakat, T., Hosszu, A., Szebeni, B., Balogh, A., Orfi, L., ... Hodrea, J. 2021. Lyophilization and homogenization of biological samples improves reproducibility and reduces standard deviation in molecular biology techniques. Amino Acids. doi:10.1007/s00726-021-02994-w.
- [56] Parveen, S., Bishai, W. R., & Murphy, J. R. 2019. *Corynebacterium diphtheriae*: Diphtheria Toxin, the tox Operon, and Its Regulation by Fe²⁺ Activation of apo-DtxR. Microbiology Spectrum, 7(4). doi: 10.1128/microbiolspec.gpp3-0063-2019.
- [57] Abdul-Hussein, S. M., & Shawkat, S. M. 2023. Effect of adding curcumin and betacyclodextrin nanocomplex on some of the physicochemical, microbiological and sensory traits of yogurt. Journal of Kirkuk University for Agricultural Sciences, 14(4).
- [58] Hernández-Cortez, C., Palma-Martínez, I., Gonzalez-Avila, L. U., Guerrero-Mandujano, A., Solís, R. C., & Castro-Escarpulli, G. 2017. Food Poisoning Caused by Bacteria (Food Toxins). Poisoning - From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis. doi:10.5772/intechopen.69953.
- [59] Méndez, M. B., Goñi, A., Ramirez, W., & Grau, R. R. 2012. Sugar inhibits the production of the toxins that trigger clostridial gas gangrene. Microbial Pathogenesis, 52(1), 85–91. doi: 10.1016/j.micpath.2011.10.008.
- [60] Kokubo, E., Sonoki, H., Aizawa, K., Takagi, H., Takada, M., Ito, A., ... and Miyaji, K. 2022. In Vivo Digestibility of Carbohydrate Rich in Isomaltomegalosaccharide Produced from Starch by Dextrin Dextranase. Journal of Applied Glycoscience, 69(3), 57-63.
- [61] Kroemer, G., López-Otín, C., Madeo, F., & de Cabo, R. 2018. Carbotoxicity—Noxious Effects of Carbohydrates. Cell, 175(3), 605–614. doi: 10.1016/j.cell.2018.07.044.
- [62] Chapeton-Montes, D., Plourde, L., Deneve, C., Garnier, D., Barbirato, F., Colombié, V., ... Popoff, M. R. 2020. Tetanus Toxin Synthesis is Under the Control of A Complex Network of Regulatory Genes in *Clostridium tetani*. Toxins, 12(5), 328. doi:10.3390/toxins12050328.
- [63] Nash, A. A., Dalziel, R. G., & Fitzgerald, J. R. 2015. Mechanisms of Cell and Tissue Damage. Mims' Pathogenesis of Infectious Disease, 171–231. doi:10.1016/b978-0-12-397188-3.00008-1.
- [64] Lax, A. J. 2005. Toxin: the cunning of bacterial poisons. Oxford University Press, USA.
- [65] Caza, M., and Kronstad, J. W. 2013. Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. Frontiers in Cellular and Infection Microbiology, 3. doi:10.3389/fcimb.2013.00080.
- [66] Xie, Y., & Yang, L. 2016. Calcium and Magnesium Ions Are Membrane-Active against Stationary-Phase *Staphylococcus aureus* with High Specificity. Scientific Reports, 6(1). doi: 10.1038/srep20628.
- [67] Yan, H., Pan, G., Zou, H., Song, L., & Zhang, M. 2004. Effects of Nitrogen Forms on the Production of Cyanobacterial Toxin Microcystin-LR by an Isolated *Microcystis aeruginosa*. Journal of Environmental Science and Health, Part A-Toxic/Hazardous Substances and Environmental Engineering, 39(11-12), 2993–3003. doi:10.1081/lesa-200034799.
- [68] Stucken, K., John, U., Cembella, A., Soto-Liebe, K., & Vásquez, M. 2014. Impact of Nitrogen Sources on Gene Expression and Toxin Production in the Diazotroph *Cylindrospermopsis raciborskii* CS-505 and Non-Diazotroph *Raphidiopsis brookii* D9. Toxins, 6(6), 1896–1915. doi:10.3390/toxins6061896.
- [69] İçgen, Y., İçgen, B., and Özcengiz, G. 2002. Regulation of crystal protein biosynthesis by *Bacillus thuringiensis*: II. Effects of carbon and nitrogen sources. Research in Microbiology, 153(9), 605-609.

- [70] Tang, Q. M., Chen, F., Xiang, W. Z., Peng, J., & He, H. (2008). Effect of ammonium nitrogen on the growth, biochemical constituents and toxin production of *Microcystis aeruginosa* FACHB-905. *Journal of Jinan University (Natural Science)*, 29(3), 290-294.
- [71] Ghosh, S., Lahiri, D., Nag, M., Dey, A., Sarkar, T., Pathak, S. K., ... Ray, R. R. 2021. Bacterial Biopolymer: Its Role in Pathogenesis to Effective Biomaterials. *Polymers*, 13(8), 1242. doi:10.3390/polym13081242.
- [72] Oliveira, D., Borges, A., & Simões, M. 2018. *Staphylococcus aureus* Toxins and Their Molecular Activity in Infectious Diseases. *Toxins*, 10(6), 252. doi:10.3390/toxins10060252.
- [73] Sundaran, B., Rao, Y. U. B., and Boopathy, R. 2001. Process optimization for enhanced production of diphtheria toxin by submerged cultivation. *Journal of bioscience and bioengineering*, 91(2), 123-128.
- [74] Doshi, J. ., Ravetkar, S. ., Ghole, V. ., and Rehani, K. 2003. Comparative quantitation for the protein content of diphtheria and tetanus toxoids by DC protein assay and Kjeldahl method. *Biologicals*, 31(3), 187–189. doi:10.1016/s1045-1056(03)00038-1.
- [75] Manceva, S. D., Pusztai-Carey, M., Russo, P. S., & Butko, P. 2005. A Detergent-like Mechanism of Action of the Cytolytic Toxin Cyt1A from *Bacillus thuringiensis* var. *israelensis*†. *Biochemistry*, 44(2), 589–597. doi:10.1021/bi048493y.
- [76] Loffredo, M. R., Savini, F., Bobone, S., Casciaro, B., Franzzyk, H., Mangoni, M. L., & Stella, L. 2021. Inoculum effect of antimicrobial peptides. *Proceedings of the National Academy of Sciences*, 118(21), e2014364118.
- [77] Monheit, G. D., & Pickett, A. 2017. AbobotulinumtoxinA: A 25-Year History. *Aesthetic Surgery Journal*, 37(suppl_1), S4–S11. doi:10.1093/asj/sjw284.
- [78] Ohtani, K., and Shimizu, T. 2016. Regulation of toxin production in *Clostridium perfringens*. *Toxins*, 8(7), 207.
- [79] Kumar, R., Feltrup, T., Kukreja, R., Patel, K., Cai, S., & Singh, B. 2019. Evolutionary Features in the Structure and Function of Bacterial Toxins. *Toxins*, 11(1), 15. doi:10.3390/toxins11010015.
- [80] Steward, L., Brin, M. F., & Brideau-Andersen, A. 2020. Novel native and engineered botulinum neurotoxins. *Botulinum Toxin Therapy*, 63-89.
- [81] Palma, L., Muñoz, D., Berry, C., Murillo, J., & Caballero, P. 2014. *Bacillus thuringiensis* Toxins: An Overview of Their Biocidal Activity. *Toxins*, 6(12), 3296–3325. doi:10.3390/toxins6123296.
- [82] Su, M. G., Huang, C. H., Lee, T. Y., Chen, Y. J., & Wu, H. Y. 2014. Incorporating amino acids composition and functional domains for identifying bacterial toxin proteins. *BioMed research international*, 2014.
- [83] Gillespie III, E. J. 2011. A Chemical Genetic Approach to Studying Anthrax Lethal Toxin (Doctoral dissertation, University of California, Los Angeles).
- [84] Whaler, B. C. 2016. VI. NEUROTOXINS. *The Nervous System: Autonomic Nervous System Drugs*, 399.
- [85] Goff, J. P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *Journal of Dairy Science*, 101(4), 2763–2813. doi:10.3168/jds.2017-13112.
- [86] Thaysen-Andersen, M., Jørgensen, S. B., Wilhelmsen, E. S., Petersen, J. W., & Højrup, P. 2007. Investigation of the detoxification mechanism of formaldehyde-treated tetanus toxin. *Vaccine*, 25(12), 2213–2227. doi:10.1016/j.vaccine.2006.12.033.
- [87] Veronese, P. K., & Lucius, A. L. 2010. Effect of temperature on the self-assembly of the *Escherichia coli* ClpA molecular chaperone. *Biochemistry*, 49(45), 9820-9829.
- [88] Chang, M. J., Ollivault-Shiflett, M., Schuman, R., Nguyen, S. N., Kaltashov, I. A., Bobst, C., ... and Lees, A. 2022. Genetically detoxified tetanus toxin as a vaccine and conjugate carrier protein. *Vaccine*, 40(35), 5103-5113.
- [89] Pitard, I., and Malliavin, T. E. 2019. Structural Biology and Molecular Modeling to Analyze the Entry of Bacterial Toxins and Virulence Factors into Host Cells. *Toxins*, 11(6), 369. doi:10.3390/toxins11060369.
- [90] Breibeck, J., and Rempel, A. 2018. Successful amphiphiles as the key to crystallization of membrane proteins: Bridging theory and practice. *Biochimica et Biophysica Acta (BBA) - General Subjects*. doi:10.1016/j.bbagen.2018.11.004.
- [91] Habermehl, G. 1993. Microbial toxins in foods and feeds. *Toxicon*, 31(1), 91. doi:10.1016/0041-0101(93)90362-m.
- [92] Liu, S., Moayeri, M., & Leppla, S. H. 2014. Anthrax lethal and edema toxins in anthrax pathogenesis. *Trends in Microbiology*, 22(6), 317–325. doi:10.1016/j.tim.2014.02.012.
- [93] Ben David, A., Diamant, E., Barnea, A., Rosen, O., Torgeman, A., & Zichel, R. 2013. The Receptor Binding Domain of Botulinum Neurotoxin Serotype A (BoNT/A) Inhibits BoNT/A and BoNT/E Intoxications In Vivo. *Clinical and Vaccine Immunology*, 20(8), 1266–1273. doi:10.1128/cvi.00268-13.
- [94] Beretta, B., Conti, A., Fiocchi, A., Gaiaschi, A., Galli, C. L., Giuffrida, M. G., ... Restani, P. 2001. Antigenic Determinants of Bovine Serum Albumin. *International Archives of Allergy and Immunology*, 126(3), 188–195. doi:10.1159/000049513.
- [95] Čapek, P., & Dickerson, T. 2010. Sensing the Deadliest Toxin: Technologies for Botulinum Neurotoxin Detection. *Toxins*, 2(1), 24–53. doi:10.3390/toxins2010024.
- [96] Kragh-Hansen, U. 2018. Possible Mechanisms by Which Enzymatic Degradation of Human Serum Albumin Can Lead to Bioactive Peptides and Biomarkers. *Frontiers in Molecular Biosciences*, 5. doi:10.3389/fmolb.2018.00063.

- [97] Fieldhouse, R. J., Turgeon, Z., White, D., & Merrill, A. R. 2010. Cholera- and Anthrax-Like Toxins Are among Several New ADP-Ribosyltransferases. *PLoS Computational Biology*, 6(12), e1001029. doi: 10.1371/journal.pcbi.1001029.
- [98] Sierig, G., Cywes, C., Wessels, M. R., & Ashbaugh, C. D. 2003. Cytotoxic Effects of Streptolysin O and Streptolysin S Enhance the Virulence of Poorly Encapsulated Group A Streptococci. *Infection and Immunity*, 71(1), 446–455. doi:10.1128/iai.71.1.446-455.2003.
- [99] Nishida, H. 2020. Factors That Affect the Enlargement of Bacterial Protoplasts and Spheroplasts. *International Journal of Molecular Sciences*, 21(19), 7131. doi:10.3390/ijms21197131.
- [100] Argudín, M. Á., Mendoza, M. C., & Rodicio, M. R. 2010. Food Poisoning and *Staphylococcus aureus* Enterotoxins. *Toxins*, 2(7), 1751–1773. doi:10.3390/toxins2071751.
- [101] Upadhyay, A., Mooyottu, S., Yin, H., Nair, M., Bhattaram, V., & Venkitanarayanan, K. 2015. Inhibiting Microbial Toxins Using Plant-Derived Compounds and Plant Extracts. *Medicines*, 2(3), 186–211. doi:10.3390/medicines2030186.
- [102] Dietrich, R., Jessberger, N., Ehling-Schulz, M., Märklbauer, E., & Granum, P. E. 2021. The Food Poisoning Toxins of *Bacillus cereus*. *Toxins*, 13(2), 98. doi:10.3390/toxins13020098.
- [103] Tam, K., and Torres, V. J. 2019. *Staphylococcus aureus* Secreted Toxins and Extracellular Enzymes. *Microbiology Spectrum*, 7(2). doi:10.1128/microbiolspec.gpp3-0039-2018.
- [104] Macfarlane, M. G., & Knight, B. C. J. G. 1941. The biochemistry of bacterial toxins: The lecithinase activity of *Cl. welchii* toxins. *Biochemical Journal*, 35(8-9), 884.
- [105] Navarro, M., McClane, B., & Uzal, F. 2018. Mechanisms of Action and Cell Death Associated with *Clostridium perfringens* Toxins. *Toxins*, 10(5), 212. doi:10.3390/toxins10050212.
- [106] Brandenburg, L.-O., Pufe, T., & Koch, T. 2014. Role of Phospholipase D in G-Protein Coupled Receptor Function. *Membranes*, 4(3), 302–318. doi:10.3390/membranes4030302.
- [107] Villalpando-Rodriguez, G. E., & Gibson, S. B. 2021. Reactive oxygen species (ROS) regulates different types of cell death by acting as a rheostat. *Oxidative Medicine and Cellular Longevity*, 2021.
- [108] Hirota, K., Nagata, K., Norose, Y., Futagami, S., Nakagawa, Y., Senpuku, H., ... Takahashi, H. 2001. Identification of an Antigenic Epitope in *Helicobacter pylori* Urease That Induces Neutralizing Antibody Production. *Infection and Immunity*, 69(11), 6597–6603. doi:10.1128/iai.69.11.6597-6603.2001.
- [109] Deutscher, J., Francke, C., & Postma, P. W. 2006. How Phosphotransferase System-Related Protein Phosphorylation Regulates Carbohydrate Metabolism in Bacteria. *Microbiology and Molecular Biology Reviews*, 70(4), 939–1031. doi:10.1128/mmbr.00024-06
- [110] Cataudella, I., Trusina, A., Sneppen, K., Gerdes, K., & Mitarai, N. 2012. Conditional cooperativity in toxin–antitoxin regulation prevents random toxin activation and promotes fast translational recovery. *Nucleic Acids Research*, 40(14), 6424–6434. doi:10.1093/nar/gks297.
- [111] Botana, L. M., & Alfonso, A. (Eds.). 2015. *Phycotoxins: chemistry and biochemistry*. John Wiley and Sons.
- [112] Zaragoza, Orellana, Moonen, Moutafis, & Marcellin. 2019. Vaccine Production to Protect Animals Against Pathogenic Clostridia. *Toxins*, 11(9), 525. doi:10.3390/toxins11090525.
- [113] Murata, M., Kovba, A., Kaneko, A., Morimoto, M., Ishigami, A., Natsume, T., ... & Akari, H. 2023. Annual two-dose tetanus toxoid vaccination induces protective humoral immunity to all age groups of rhesus macaques. *Experimental Animals*, 72(4), 490-495.
- [114] Shaw, I. C. 2018. *Food safety: the science of keeping food safe*. John Wiley and Sons.

السموم الخارجية البكتيرية: خصائصها العامة وآلية عملها (مقالة مراجعة).

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الخلاصة

فكرة التأثيرات الضارة للبكتيريا المسببة للأمراض عن طريق تخليق السموم موجودة منذ العصور القديمة. في عام 1872، افترض *Klebs* أن "sepsins" مسؤولة عن تكوين السموم التي تنتجها المكورات العنقودية، مع أنه لم يتمكن من إثبات هذه الفرضية بالدليل. في عام 1884، اكتشف روبرت كوخ أن مرض الكوليرا ناتج عن سموم تنتجها البكتيريا، وليس البكتيريا نفسها، موضحاً أن مسببات الأمراض البكتيرية يمكن أن تنمو في الجهاز الهضمي دون الإضرار بجدار الأمعاء أو الأعضاء المجاورة. بعدها أظهرت الأبحاث والدراسات أن حقن راسح بكتيريا الكوليرا عبر الجهاز الهضمي يؤدي إلى نتائج مماثلة لتلك التي تظهر في حالات المرض الحقيقية. وفي عام 1889 كشف *Loeffler* أن عصيات الخناق ظل موضعياً في موقع الحقن، ولم يكن موجوداً في الأعضاء الداخلية مما يشير إلى أن المادة السامة التي أُنتجت في موقع الحقن قد انتشرت في الدورة الدموية. ووفقاً لكل من *Yersin* و *Roux* اللذان عزلوا العديد من البكتيريا شديدة السمية، فإن السموم هي السبب وراء كل الأعراض السيئة التي تسببها البكتيريا المسببة للأمراض. خلال القرن التاسع عشر، أظهرت الدراسات على نطاق واسع أن السموم البكتيرية هي السبب الرئيسي للآثار السلبية لجميع الأمراض المعدية. ونتيجة لذلك، تركزت الأبحاث الطبية في المقام الأول على تطوير الأمصال المضادة للسموم. والسموم الخارجية في الغالب تتكون من البروتينات التي تجعل الجهاز المناعي يعمل على نحو طبيعي ضدها، وهذه السموم يتم إنتاجها بواسطة البكتيريا الموجبة لصبغة كرام. على أن السموم الداخلية تفرز أو تتحرر جراء التحلل البكتيري، ولا سيما جدار الخلية البكتيرية. تعد السموم الخارجية أكثر حساسية للحرارة، وقد تكون عرضة لتأثير عوامل ومواد لا تؤثر في السموم الداخلية. وهذه الدراسة تمثل استعراضاً لمجمل الأبحاث التي تطرقت إلى خصائص السموم الخارجية ذات الطبيعة البروتينية التي تفرزها البكتيريا المرضية مع إيراد الأمثلة على آلية إنتاجها وتأثيرها.

الكلمات المفتاحية: السموم، السموم الخارجية، البكتيريا المرضية، السموم الداخلية.