Status of Bacterial Toxins and Their Nomenclature:  
Need for Discipline and Clarity of Expression

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INTRODUCTION

In view of the exponential growth of scientific literature, one might justifiably ask why this paper should be published, thereby adding to the overwhelming task of perusing the current literature. But it is just this growth of scientific publication which prompted us to assume the task of focusing attention on the complexity and growing confusion surrounding the classification and nomenclature of bacterial toxins. Since the Ehrlichian era the field has grown more or less like the proverbial "Topsy," and because there have not been any hard or fast rules governing where a particular toxin fits in a general nomenclatural scheme or how one should refer to it, a rather confused situation now exists. As increasing numbers of individuals direct their activities toward the study of toxins, and as biochemical sophistication increases our awareness of the diversity and complexity of materials which may legitimately be classified as toxins, the inadequacies and inconsistencies of currently employed terminology will become magnified. One might argue that this situation has existed for decades and that terms are so ingrained in our minds and the literature that it is futile to do anything about it. Indeed, in a survey which we took of approximately 20 active workers in the field of bacterial toxins, this opinion was voiced by several of the workers. The others were of the opinion that something should be done to clarify the situation, but there was no agreement as to how this could be accomplished. Obviously, then, if those who are most knowledgeable in the field of bacterial toxins have no simple solution, there is none. We, however, cannot sympathize with those who adopt the "why bother" attitude. What we have attempted to do is to discuss toxins in historical perspective and show that older systems do not encompass new concepts and knowledge. It is not our purpose to lay down any hard and fast rules which the scientific community is asked to adopt. On the contrary, we hope to show the need and advantages of clarity of thought and
expression, and to begin the dialogue which will hopefully end as a universally agreed upon nomenclature of toxins. The "why bother" attitude may be tolerated at the moment, but a more rational approach is mandatory for the next generation of toxinologists.

The specific objectives of this communication are threefold: (i) to describe the old and currently employed nomenclature of bacterial toxins and to suggest some ideas to make the terminology clearer and more uniform; (ii) to stimulate thought and awareness of the problem in the scientific community with the hope that this will lead to analysis of a constructive nature and ultimately to the adoption of a consistent terminology; (iii) to suggest where older nomenclature has been and remains useful when employed in the proper context.

The material covered is restricted primarily to bacterial toxins, but there is no reason on theoretical grounds why the concepts and suggestions made do not apply equally to all toxins of plant and animal origin.

**NOMENCLATURE OF TOXINS**

Toxins are usually referred to in two senses. In common parlance, a toxin refers to any poisonous material derived from living organisms. In the scientific literature, notably of bacteriology, the term toxin has been a label for a specific class of poisons. Poisons are any substances, either organic or inorganic in nature, which when ingested, inhaled, adsorbed through the skin, or injected parenterally produce damage to tissues or disruption of normal physiological functions. On the other hand, as a specific class of poisons, toxins are distinct from the simple chemical poisons by their cellular origin, high molecular weight, and antigenicity. Since scientific nomenclature should contribute to clear thinking by insistence on precise definitions of terms, the term toxin should be restricted to a particular defined class of poisons of animal or plant origin. The existence of poisonous substances as a class of proteins demands a word to categorize these proteins. The fact that substances traditionally called toxins have proven to be proteins is the justification for restricting the term toxin to poisonous proteins. This does not imply that living organisms do not produce other kinds of poisons which are not proteins. It simply means that these other kinds of poisons should not be called toxins. Toxins should not be confused with venoms. The term venom is derived from the Latin *venenum*, meaning a drink of the Goddess Venus (a love potion!), and should be restricted to animal poisons inflicted either by a sting or bite. Venoms are usually mixtures of distinct chemically unrelated entities, and they can include one or more toxins among their constituents (50).

The property of antigenicity is a subtle one. It would be inappropriate to call an antigen or a hapten a toxin if it causes lesions by participation in a harmful antigen-antibody reaction and of itself is not toxic (32). This restriction on the use of the term toxin would eliminate calling the traditional bacterial endotoxins a class of true toxins, if in the future the scientific community should become convinced that these complex lipopoly saccharides of bacterial origin cause damage exclusively through participation in antigen-antibody reactions. In that case, the invention of a new term such as "endobacterial poisons" could be employed legitimately to describe the endotoxins, so that they would not be included among the true toxins. At the present time, however, the endotoxins should not be disqualified, since there is sufficient evidence that they are directly harmful and do not depend upon antigen-antibody reactions for all of their toxic properties (27). Newborn piglets, free from immunoglobulins, have been found to be extremely sensitive to the lethal effect of endotoxin. The development of tolerance to endotoxin (68), on the other hand, suggests that some of the toxic reactions evoked by endotoxins are antigen-antibody mediated. Therefore, it is likely that the complex effects elicited by endotoxins are due to both their inherent toxicity and antigenicity.

Implied, if not always stated, in the definition of a toxin is the property of specificity in mode and sites of harmful activities. Experience suggests that the great majority and possibly all of the toxins are not general cellular poisons, because, in those cases where information is available, the primary anatomical (morphological) or functional (biochemical) lesions have been found to be restricted to a limited spectrum of related tissues or organ systems; e.g., neurotoxin,cardio toxin, etc. As will be discussed, these descriptive terms, though useful, hardly suffice in the characterization of all bacterial toxins.

Generally speaking, bacterial toxins possess no recognized function in the metabolism or structure of the organisms producing them (excluding endotoxins which represent a portion of the bacterial soma). The hypothesis that diphtheria toxin was part of the cytochrome system of *Corynebac terium diphtheriae* (43) has never been substantiated. It seems highly improbable, however, that all of these complex molecules would be synthesized as waste products and nonsense molecules. Rather than being a general characteristic of bacterial toxins, the absence of known function for these materials may be an indictment of our
scientific ignorance. Some toxins in venoms serve to immobilize and prevent the escape of potential food, and in some reptilian venoms several toxins act to immobilize prey prior to and during ingestion (11).

Any kind of disturbance of normal function or structural change may be employed for the detection of a toxin. Oakley (39), who feels that the term toxin has outlived its usefulness and might be replaced by "soluble bacterial antigen," has reviewed various methods employed to detect toxins, most of them of an immunological or serological nature. Although immunological methods have the desirable elements of specificity and sensitivity, they require that specific antiserum of high titer be available. This will become increasingly a more difficult logistic problem for the average laboratory as new and more complex toxins are characterized. Immunological assays also have the disadvantage that a toxin may retain its immunological specificity while having lost all or part of its biological activities. The lethal toxins are usually detected by the death of suitable laboratory animals. Methods of varying degrees of sophistication have been devised to quantitate lethal toxins, but they all depend on a statistical treatment of one kind or another. They all assume a typical "normal" host population which may or may not always be true (35). Several excellent reviews on this subject are available (7, 61). For reasons of economy and convenience, many investigators prefer in vitro assays for detection and quantification of toxins. Unfortunately, in many cases, the animal assay is the only reliable method available; e.g., botulinum and anthrax toxins. In recent years, tissue culture methods have been explored as a substitute for observation of whole animals. Although reasonable success has been achieved with diphtheria toxin (18), it has not been useful for many other bacterial toxins because of the absence of any visible cytopathic effect specifically induced by the toxins. The enthusiasm for tissue culture assays is understandable, but it should be tempered by a recognition of two possible pitfalls. Tissue cultures can be extraordinarily sensitive to impurities present in toxin preparations. Therefore, unless a highly purified toxin or antiserum with antibody directed against only the true toxic component is available, extreme caution must be exercised in attributing observed cytological changes to the toxin itself. Secondly, a cytopathic effect on a tissue culture may not be meaningful when extrapolated to an intact animal. Since tissue cultures tend to dedifferentiate, in vitro cells are often not identical metabolically with the in vivo cells from which they were originally derived. At the very minimum, tissue cultures are removed from nervous system and endocrine stimuli and restraints. Therefore, it should be kept in mind that prominent effects of toxins on tissue culture cells can be of minor or no significance for the whole organism. An area of research which might improve the utility of tissue culture assays is the formulation of environmental conditions required to prevent dedifferentiation in vitro.

Different Bases of Nomenclature

Historically, the nomenclature of toxins has developed along three major paths. This occurred in response to three distinct points of emphasis which investigators placed on their studies: (i) the intracellular or extracellular nature of the toxin, i.e., whether it is associated with a structural component of the bacterium or found primarily in the extracellular menstruum; (ii) concern with the structural or biochemical lesions caused by the toxin; and (iii) attempts to understand the relationship between chemical structure and toxicity. Nomenclatures based on each of these different foci of interest are not mutually exclusive, and each of them can be individually and uniquely useful in communication of concepts and data. Which system an investigator or author employs should be related to the major theme of his effort or discussion. The nomenclature based on the relation of chemical structure to toxicity has been least developed and requires thoughtful consideration, since this is a field in which we can expect the most rapid development of knowledge. The following commentary attempts to summarize present usage and, wherever deemed necessary, suggests new terms or ideas which might serve to fill recognized gaps in the currently employed nomenclature.

Nomenclature by Anatomical Location

The classical nomenclature of toxins based on their intracellular or extracellular nature spawned the concept of exotoxins as opposed to endotoxins. Although this is useful as an operational classification, it is by no means completely accurate. The exotoxins were considered to be metabolic products excreted into the growth menstruum during active growth of the bacterium or alternatively as a result of autolysis (12). This has given rise to the idea that little or no toxin of this kind can be obtained from intact cells. It has been found, however, that tetanus toxin (54) and botulinum toxin (8) can be extracted from intact cells in considerable quantities. In the latter case, it was observed that, if young cultures of Clostridium botulinum (type A) were employed, as much as nine times more toxin could be obtained from the cells than from the extracellular menstruum. Indeed, in recent years it has become routine
procedure to use intact bacilli rather than culture filtrates for the purification of type E botulinum toxin (58, 59).

The second class of toxins according to the classical scheme is the endotoxin. These complex materials are considered to be derived from an intrinsic part of the cellular structure, and, although they are currently defined as lipopolysaccharides, the exact nature of the toxic moiety is still uncertain. At the moment, the nature and properties of endotoxins are being investigated by many researchers, and, rather than becoming involved in polemics of the day, it may be judicious to postpone consideration of where the endotoxins should be placed in a general scheme until more information concerning their chemistry and biological properties becomes available. For the time being, however, a useful differentiation between exotoxins and endotoxins can be made primarily on their chemical and biological properties rather than on their association with bacterial structures. The classical exotoxins which have been purified are pure unconjugated proteins, heat-sensitive, highly antigenic, and specific regarding their biological activities and the bacterial species producing them. Purified endotoxins apparently do not contain protein in the toxic complex (57), are heat-stable, are antigenic but do not appear to stimulate classical neutralizing antibodies (5), and possess biological activities which are identical regardless of the bacterial species from which they are derived. It can be speculated that the antigenic nature of the endotoxins depends on their association with proteins in the in vivo situation.

Although none has been described to date, another class of toxins based on anatomical location is conceivable. These would be materials which are synthesized on the cell membrane and which can be found only in the extracellular environment, or which form a portion of the membrane. The former is analogous with specific exoenzymes, such as the penicillinase of Bacillus cereus (44). Although no bacterial toxin has been shown to fall into this category, the lethal toxin produced by B. cereus may prove to be an example. This toxin is found only extracellularly, and lethality is not expressed by whole cells or extracts derived from them (Bonventure, unpublished data). Toxins which might belong to the membrane-bound category have also not been described with any certainty. The plague murine toxin, however, has been demonstrated both intracellularly and closely associated with the cell membrane (37). It has not been possible to determine as yet whether the intracellular protein and the membrane-bound material are identical, or whether the demonstration of two distinct molecules is an artifact of purification. A self-explanatory term which could be applied to any membrane-bound toxins to differentiate them from the exo- and endotoxins is "ectotoxin."

Nomenclature by Mode or Site of Action

A second system of toxin nomenclature in use is one based on tissue or organ affinity. This classification describes a toxin in terms of either its apparent site or mode of action in a sensitive host. In practice, because of our limited knowledge of the biochemical mode of action, most toxins are referred to in terms of the tissues affected. For example, the toxins of C. botulinum and C. tetani and several others are described as neurotoxins. The accuracy of this terminology is based on sound clinical and pharmacological evidence (78). A more specific description of the botulinum and tetanus neurotoxins based on this type of nomenclature must await the elucidation of the exact biochemical lesions they induce in the nervous tissues. Although there is good evidence that tetanus toxin acts primarily at the level of the central nervous system after it is bound by specific sphingolipids of the brain (71), and that botulinum toxin acts primarily on peripheral nerve elements (13), it probably would not be justifiable to include this information in the descriptive terminology. If the toxins, for example, were shown conclusively to affect the metabolism of acetylcholine or other nerve cell components in a specific fashion, then it would be logical to broaden their description from the generic term, neurotoxin, to include the mode of action.

One example of a true bacterial toxin being shown to possess enzymatic activity is the lecinthase (phospholipase) of Clostridium perfringens. The toxin is an enzyme which cleaves phospholipid substrates to yield phosphorylcholine and diglycerides and, according to currently accepted terminology, is a phospholipase C. Although this is a perfectly acceptable description, because we feel strongly that a toxin should be described in biochemical or enzymatic terms whenever possible, it is not known whether the enzymatic activity of the clostridial lecinthase contributes significantly to the pathogenesis of gas gangrene infections. Proof of enzymatic action would reduce the problem to a proper use of enzyme nomenclature. To ensure recognition of the relationship of the enzymatic activity to toxicity, a compound name representing a marriage of enzymatic and toxin nomenclature could be employed. The phospholipase C produced by B. cereus is neither hemolytic nor lethal, although the organism produces a lethal toxin and a hemolysin as well (24). Therefore, it may be that
description of the clostridial α toxin (alas, another classification!) as a lecithinase is accurate but also misleading in that its enzymatic activity may have nothing to do with its in vivo mode of action. In such a case, the term lecithinase describes a property but not a toxin as toxin.

The hemolytic toxins include many examples of descriptive terms which do not clarify their true nature. As already pointed out, the phospholipase C of *C. perfringens* is most often designated by the Greek letter α. The term α toxin of course has precedence, and it is understandable that it would be accepted and used at a time when none of its biochemical properties was known. There are several reasons why we feel that such a name is no longer useful. First, in the light of our current knowledge, there are several descriptive terms which are preferable. Second, one of the staphylococcal hemolysins is also referred to as α toxin, and this at times may be a source of confusion if, as often happens, the Greek letter is not coupled with the generic or specific name of the organism which synthesizes the toxin. Since the hemolytic nature of the staphylococcal and *C. perfringens* α toxins is coincidental with their lethal property, there is no question as to the correctness of calling them true toxins. Many of the other hemolysins produced by microorganisms, however, probably are not true toxins, since their activity seems to be restricted to hemolysis of erythrocytes of one or more animal species and probably has little or no significance in vivo (34).

Diphtheria toxin is an example where no other descriptive term has been applied. Although the toxic protein has been characterized extensively both chemically and immunologically (31, 42, 47, 72), its mode and site of action have been extremely difficult to pinpoint. A recent study in the sensitive guinea pig has provided strong evidence that the action of the toxin in vivo may be restricted to inhibition of protein synthesis in cardiac tissue only (10). This biochemical lesion correlates well with clinical findings in which fatal cases of diphtheria have been ascribed to cardiac failure (2). If and when this evidence is accepted by the scientific community at large, it will be possible to include diphtheria toxin in the nomenclature based on mode and site of action. Since a phrase describing its capacity to inhibit cardiac protein synthesis would be cumbersome, it might be referred to simply as diphtheria cardiotoxin.

The classification of toxins associated with food poisoning within the framework of mode or site of action is difficult. The toxin associated with staphylococcal food poisoning has been called enterotoxin, a term implying direct action on the alimentary tract. To be able to differentiate clearly between directly and indirectly acting toxins, we recommend restricting the term enterotoxin to substances acting directly on the alimentary tract. Focusing on this distinction presents another challenge to the pathophysiologist in the fundamental understanding of site and mode of action. Enterotoxins are proteins synthesized by specific strains of *Staphylococcus aureus* in several immunologically distinct forms (6, 14).

When a toxin is ingested or formed within the gastrointestinal tract by infectious organisms, its harmful effects can be due to direct action on the intestinal tissues. In this case, the toxin is properly spoken of as an enterotoxin, since by direct contact it specifically affects the normal behavior of cells which comprise the intestinal tract. Observations of gastrointestinal disturbances, however, are not of themselves conclusive evidence of a direct intestinal site of toxic activity. The alimentary tract is notorious for its sensitivity to nervous-system stimuli. When gastrointestinal signs are prominent but it is not certain that the primary site of action is the alimentary tract, it might be logical to employ the term "nutriotoxin" rather than enterotoxin. This would specify the source of the toxin without nomenclatural commitment to site of action.

Three experimental approaches can be considered to determine whether a toxin acts directly on the gastrointestinal tract. If toxic activity is direct and limited to the intestinal tissue, parenteral injection might be expected to be without effect or of reduced effect in producing gastrointestinal disturbances. Since the absorption of proteins from the intestine is often by way of the lymphatics (23, 33), another approach would be to cannulate the thoracic duct or cisterna chyli of animals exposed to the toxin by the oral route. Theoretically, such a procedure should prevent toxin from entering the general circulation. Therefore, if the primary site of toxin action is remote from the intestinal tract, the intestinal symptoms should either not be seen or the illness of the cannulated animals should be of a milder nature. A third experimental possibility would be to observe the effects of the toxin on isolated gut segments or primary tissue cultures derived from intestinal tissues (60).

The nomenclature of toxins based on site and mode of action is potentially a very useful one. However, from the examples given of current usage, it should be clear that the classification may be misleading when it is used indiscriminately. We would recommend that toxins be described in this fashion whenever possible, but only when there is good experimental evidence to support it.
Nomenclature by Structure of the Toxin Molecule

A nomenclature based on the chemical structure and related physicochemical properties of toxin molecules is not well developed at the present time. In view of the intense effort being devoted to the biochemical characterization of toxins, it would appear that such a system will become needed and most useful in the future. The following discussion considers examples of toxins, both real and theoretical, which can be described on the basis of the types of protein molecular structures related to their specific biological activities.

General categories based on molecular structure.

On the basis of present knowledge, three general types of toxins can be distinguished. We have chosen to call them simple toxins, complex toxins, and toxic mixtures. A fourth kind of possible toxin is a conjugated protein: a protein to which is attached a biologically active nonprotein prosthetic group. Classical endotoxin would have to be considered a conjugated toxin if lipopolysaccharide were proven to be associated with a protein, and when in the conjugated state to possess specific toxic activity absent in the protein-free state.

Two kinds of simple toxins can be described. The first is a toxin which when purified consists of one molecular species. In addition, it is always synthesized in the fully biologically active form. The toxin molecule may polymerize to form dimers or other poisonous aggregates. Tetanus toxin would legitimately fit into this category, since it is a homogeneous protein, is synthesized as a completely active molecule, and dimerization has been described (55). The second type of simple toxin is one which also is unimolecular but which may exist in an inactive or partially active precursor form. The biological inactivity may be the result of polymerization or tertiary structure, either of which may mask toxophoric chemical groups. Examples of toxins which can be put into this category are the type E botulinum toxin and the ε toxin of C. perfringens type D. The maximal biological activities of these toxins can be obtained by a brief exposure to a proteolytic enzyme, such as trypsin (15, 69). That a similar process may occur endogenously during growth has been suspected for type A botulinum cultures (9). The mechanism by which activation occurs is not certain, but it may be either a fragmentation of the molecule into toxic smaller units (21) or merely a change in configuration of the polypeptide chain. In either case, the end result would be an exposure of chemical groups responsible for toxicity. Though neither term has achieved universal acceptance, the partially active and nonactive states of simple toxins have been called protoxins or prototoxins. It may be desirable to limit these terms to the completely inactive state and to use another as yet uninked term for the partially active stage convertible to full toxicity. The universality of the phenomena of activation for toxins remains unexplored.

Complex toxins and toxic mixtures are similar in that more than one molecular species is necessary for toxicity to be expressed. A complex toxin is one which consists of two or more components which must bond in the chemical sense to form a biologically active entity. The individual components by themselves do not demonstrate the toxicity of the complex, and in a true complex the bond is easily broken to yield unaltered original components. Toxic mixtures, on the other hand, also require more than one component to form an active toxin, but the components retain their molecular identity and do not undergo a chemical union. The toxins in both of these categories can be considered multicomponent toxins. Although several cases of multicomponent toxins are known, it is not yet possible to say with certainty whether they are complex toxins or toxic mixtures. As with the simple toxins, the possibility exists that the individual components of toxic mixtures and complex toxins might exist in a precursor prototoxic state. In addition, it is conceivable that one or more components of a multicomponent toxin might be nonprotein in nature. At least one component must be a protein for the substance to be classified as a true toxin.

Theoretical consideration of three classes of toxins and means available for their differentiation. A schematic representation and possible nomenclature of the multicomponent toxins and simple toxins are shown in Fig. 1. This overly simplified diagram illustrates the unique manner in which the three toxin types would interact with a sensitive host.

With the simple toxin, there is a direct effect of the toxic material on sensitive host cells or tissues. The host responds in a predictable manner and the symptoms or signs of the specific toxemia are manifested.

The complex toxins consist of two or more components which must have the opportunity to form a chemical entity before biological activity of the toxin is expressed. The complex conceivably could be formed in vitro before coming into contact with the host, or in vivo during the course of an infection or intoxication. In a hypothetical experimental in vivo situation, the sequence in which the components are introduced into the host would probably have no effect on
toxic activity, providing that the complex is formed.

The toxic mixture presents a somewhat more complicated situation since two alternative mechanisms are possible. In the first case, the individual components of the mixture might act independently in the host, with each component exerting a necessary function before full toxicity is expressed. A component by itself would not elicit the symptoms elicited by the complete toxic mixture, even though it still retains the unique activity associated with that particular component. If one component is inactivated in some fashion, biological activity of the mixture is lost. A second mechanism by which a toxic mixture might express its biological activity in vivo is dependent upon the sequence in which the individual components come into contact with sensitive host tissues. The first component might act so that the result is an altered host not obviously showing signs of poisoning. The second component then is able to act on the altered host, in which case the effects of the multi-component toxin are expressed as characteristic toxic signs. In the normal host, the introduction of the second component alone would not be toxic since the first had not prepared the host. The introduction of the first component after the second might or might not result in overt toxic activity. Observable toxicity would depend in this case on the length of time elapsed between the exposure of the host to the individual components. The same rationale would hold if the reverse situation occurred (i.e., introduction of the first component followed by the second component). Since this is the sequence in which the toxic mixture must act, however, overt toxicity would more likely be expressed in this case than the other.

The terms syntoxin and mixtoxin can be considered as synonyms for complex toxin and toxic mixture, respectively. They have the advantages of being simple and etymologically provocative terms. Conceptually, it is not difficult to visualize the difference between a syntoxin and a mixtoxin, but a problem does exist in dis-
istinguishing them in practice. The criteria for judging whether a particular toxin is a complex or mixture can be physiological, serological, or biophysical. When injected separately, though concurrently, components constituting a complex toxin should cause a slower or less intense toxic reaction than when injected after prior mixing in vitro. This result is expected, since separate injection of components would result in dilution and decreased opportunity for complex formation. On the other hand, the response to a toxic mixture would be predicted to be independent of whether or not the individual components were mixed together prior to their injection, provided that they were injected simultaneously. This physiological test would be limited to cases where individual components of a toxin have been separated. This requirement does not exist for serological and biophysical tests. When the Oudin (41) and Ouchterlony (40) techniques are used, a syntoxin should show a specific precipitin line for the complex in addition to any lines for individual components of the complex. A toxic mixture would show a number of specific precipitin lines corresponding to the number of component precipitins in the mixture. Biophysical measurements, such as sedimentation, diffusion, and gel filtration, can also be applied. Unlike a mixture, a complex with each of these techniques would be expected to reveal a larger-sized component (the complex itself) than recorded for any individual component in the complex. In addition, a complex would have either an additive or reduced electrophoretic mobility relative to individual components, since charged groups could be expected to be exposed, neutralized, or masked by the formation of the complex.

Theoretically, the characterization of simple toxins would not appear to be as difficult as that of the multicomponent toxins. Yet, they may present considerable difficulties. Since the unimolecular toxins are proteins, the problem is one of protein purification as well as the criteria employed as indices for determining purity or homogeneity (3). As in the case of the multicomponent toxins, physicochemical and immunological methods are applicable. These include electrophoretic mobility, sedimentation rates, diffusion coefficients, viscosity, amino acid analysis, agar-gel diffusion, immunoelectrophoresis, and neutralization of biological activity with specific antisera. As these methods and others become more readily available and are put to use for this purpose, it should be possible in the not too distant future to purify and characterize all of the toxins.

**Examples of "Problem" Toxins**

At the present time, there are only two bacterial toxins which on the basis of sound experimental evidence can be classified as multicomponent toxins: the anthrax toxin and staphylococcal leucocidin. However, there are several others which possess some properties which deserve comment within this context.

**Diphtheria toxin.** On the basis of biophysical and biochemical criteria, diphtheria toxin is a homogeneous protein which warrants its classification as a simple toxin. Immunological evidence, on the other hand, suggests that even the highly purified, crystalline preparations do not satisfy the requirements for a homogeneous preparation. The extensive studies of Pope and Stevens have shown that solutions of crystalline diphtheria toxin yield several distinct precipitin lines by gel diffusion when reacted with specific antitoxin (45). In addition, they have shown that the components differ in stability to phosphate salts and to the action of pepsin and trypsin (46, 48, 49). In spite of the apparent heterogeneity, it has not been possible to associate toxicity with individual fractions, nor has it been shown that the fractions must act in tandem for toxicity to be expressed. Poulik and Poulik (51) showed that diphtheria toxin could be separated into several components by means of electrophoresis, but that all of the protein fractions retained toxicity to a greater or lesser degree. Therefore, although there is some evidence that diphtheria toxin is not a single protein species and therefore may not be a simple toxin, its known properties are such that at this time it cannot be considered to be a complex toxin or toxic mixture. The immunological heterogeneity may merely reflect a group of closely related but not identical protein molecules which all possess the same chemical groupings responsible for the unique biological activity of diphtheria toxin. In such a case, the diphtheria toxin could be considered to be a number of closely related simple toxins.

**Botulinum toxin.** The botulinum toxins also demonstrate several peculiar characteristics which make it difficult to assign them to the ranks of the simple unimolecular toxins. The early work done concerning the purification and characteristics of botulinum toxins type A (1, 29) and B (30) revealed that they were pure proteins of typical amino acid composition. Originally, it was estimated that the molecular weight of type A approached 1 million (52), and that of type B, approximately 60,000 (30). It has been observed by several investigators, however, that type A toxin can be separated into fractions...
either by ultracentrifugation (73, 74), column chromatography (56), or high-voltage electrophoresis (28), and that toxicity is distributed (not necessarily equally) throughout the fractions. The molecular weights of toxic components have been re-evaluated recently (20, 22), and the much lower estimates of between 9,000 and 16,000 have been assigned.

In the light of these reports, several interesting questions can be asked. Are the low and high molecular weight toxic units of a particular type identical in amino acid composition and, therefore, likely to be immunologically identical as well? Is it conceivable that a botulinum toxin type is composed of nonidentical polypeptide units each of which has the specific toxic moiety incorporated within its three-dimensional structure? Must each such unit be considered a simple toxin in its own right? Depending on environmental conditions, these units may or may not aggregate and thus account for the fact that different methods of purification are reported to yield toxins of different molecular weights. These questions may be raised, but it should be pointed out that no experimental evidence suggests the botulinum toxins to be either complex toxins or toxic mixtures.

Plague murine toxin. The plague murine toxin also possesses several properties which make categorization within the chemical system of nomenclature difficult.

At the present time, it is not possible to say whether it is composed of two distinct unmolecular simple toxins or whether it is a single molecular species associated with the cell membrane and cytoplasm. Pasteurella pestis bacilli have been extracted, and two antigenically distinct toxic proteins have been isolated by disc electrophoresis (38). Each of the purified toxins is highly toxic for mice, and there is no evidence that the mixture of both proteins is a necessity for toxicity to be expressed. Toxin A (molecular weight, 240,000) appears to be associated with the cell membrane, whereas toxin B (molecular weight, 120,000) is associated with the cytoplasmic fraction. Kadis et al. (25) have speculated that, if the demonstration of the two toxins is not an artifact of purification, it may be possible that the smaller molecular weight toxin B found in the cytoplasm is a precursor of the toxin A which is incorporated enzymatically into the cell membrane after dimerization. A. J. (personal communication) is of the opinion that the two proteins are distinct toxins which act together to elicit the toxic signs observed in mice. What is not certain at present is whether the toxic response engendered by the individual components is identical to the response elicited by both proteins injected simultaneously. If the toxic response is found to be the same in both instances, then the plague murine toxin is most likely composed of two distinct molecular forms of the same toxin (e.g., a monomer and dimer). On the other hand, if the individual proteins do not elicit the same physiological responses in the sensitive host, then it is likely that the plague toxin consists of two distinct unmolecular toxins which act independently of each other in vivo.

Anthrax toxin. The anthrax toxin is undoubtedly a multicomponent toxin. As such, it is an example of a situation in which nomenclatural problems can be discussed in a specific manner. The situation can best be appreciated by describing the historical development of the anthrax toxin's nomenclature.

The initial demonstration of an in vivo toxin produced by Bacillus anthracis was accomplished by the fine efforts of the English group at Porton (26, 62). Their observations also established that the toxin was the major contributing factor leading to anthrax death in guinea pigs.

Subsequent investigations established that the toxin was composed of three components, which the English group (66) named factors I, II, and III. American scientists, on the other hand, assigned the descriptive terms of edema factor (EF), protective antigen (PA), and lethal factor (LF), respectively, to the three components (4). The English usage has historical precedence, but this in and of itself is not sufficient cause to continue such a nomenclature if a better one can be found. Although historical precedence is a useful concept, it should not be an inviolate rule when new knowledge makes a change desirable and useful.

Each component of anthrax toxin by itself is nontoxic, but is immunogenic. In the terminology used by Americans, components EF plus PA produce an edema when injected intracutaneously into the skin of the guinea pig; PA plus LF cause death of several animal species, with the Fisher 344 rat being the most uniformly susceptible host. The three components together produce all of these effects, which parallel the symptoms of the infectious disease in many ways. Although there are certain discrepancies in the published literature regarding the antigenicity and protective ability of each of the components, there seems little doubt that the Sterne strain vaccine results in the production of antibodies for each of the three components (65).

We recommend that the three components be called an edema component, a protective-antigen
component, and lethal component. The edema and protective-antigen components have possibly been separated or converted into a second order of molecular species. The edema component was shown to be converted spontaneously during purification to a form designated as fraction Y (64). It was shown by serological means that more than one component might be present in "Factor I" (edema component). Unfortunately, the interpretation of these findings is difficult since the sample was contaminated with guinea pig serum. Working with the protective-antigen component, Strange and Thorne (67) observed multiple lines of precipitation on Ouchterlony plates and suggested that these were due to products of enzymatic degradation. Wright and Lukas (77) showed that protective-antigen component, after purification, contained three components that were closely related serologically but differed in electrophoretic mobility. Fish (19), who purified and separated the three components, presented evidence that the toxin exists as a molecular aggregation of variable composition. Toxin produced in vivo demonstrated an increase in the number of protective-antigen component bands after the serum was stored at 4°C, whereas all the purified components developed multiple lines of precipitation on Ouchterlony plates. The identity of the subcomponents described by these investigators is not clear, but at this time the components appear to be related molecular species and might be identified by Arabic numbers as edema component 1 and edema component 2. For completeness, it should be noted that several authors have described a biologically inactive toxin or inactive components (36, 65, 67).

Although it can be stated unequivocally that the anthrax toxin is multicomponent in nature, the manner in which the components interact to elicit toxicity in the sensitive host is not sufficiently understood so that a choice between complex toxin or toxic mixture can be made. On the basis of the fact that death can be brought about in the absence of the edema component, present evidence suggests that the complete toxin is a mixture rather than a complex. On the other hand, the possibility that the protective-antigen component and the lethal component must complex before full toxicity is expressed cannot be ignored. If this proves to be the case, then, what has been called the anthrax toxin might be both a toxic mixture and a complex toxin.

**Staphylococcal leucocidin.** Staphylococcal leucocidin (Panton-Valentin leucocidin) is the other example of a known multicomponent toxin. Called the "true" leucocidin to distinguish it from the α hemolysin and Δ hemolysin of *Staphylococcus aureus*, it is nonhemolytic in nature but produces extensive morphological changes of both rabbit and human leukocytes (72). The studies of Woodin have shown that the leucocidin causes an extrusion of lysosomes after their fusion at or on the cell membrane (76). The toxin consists of two components which have been separated by fractionation on Dowex and Amberlite columns. The two fractions are immunologically distinct and have been designated as the F (fast) and S (slow) components according to their respective rates of elution from the columns. Since the molecular weights of the components are quite similar (75), the differences in their rates of elution can probably be attributed to differences in the net charge of the two proteins. Although it is known that either or both of the leucocidin components are adsorbed out of solution onto cell surfaces (75), and that both must be present for poisoning to occur, the manner in which they interact to exert toxicity is unknown. Therefore, it is not possible to say whether the staphylococcal leucocidin is a complex toxin or a toxic mixture. It would appear on the basis of the availability of purified components that investigation directed toward making such a distinction is now feasible. Such an effort might provide guidelines which could be used in determining how other multicomponent toxins exert their toxicity in vivo.

**Toxoids**

Ehrlich originated the term toxoid to identify diphtheria toxin preparations which upon aging lost their biological potency without concomitant loss of antigenicity (16). In practice, the term has been extended to any toxin retaining antigenicity in the face of loss of toxicity by any mechanism whatsoever. Commercial toxoids prepared by formaldehyde treatment have been the most successful and widely employed for immunization. Therefore, the suggestion has sometimes been made that toxoid refer only to formaldehyde-treated toxins. Indeed, several of the experts queried in our survey felt that this should be the case. Others, however, saw no necessity for formaldehyde treatment as a requisite for inclusion of a material in the toxoid league, providing that it satisfied the criteria of biological inactivity and antigenicity. We tend to agree with the latter viewpoint. Ramon (53), the discoverer of the superior toxoiding properties of formaldehyde, employed the term anatoxin for such preparations. It would be in keeping with historical precedent and systematic usage of language to retain the word toxoid for the entire class of antigenic, nontoxic preparations of toxin, and anatoxins for the special class of formaldehyde-
treated preparations. There appears to be no compelling need, however, to make a terminological distinction between naturally occurring and experimentally induced toxoids.

We believe that it is incorrect to consider a component of a complex toxin as a toxoid when there has been no change in the fundamental molecule either in configuration or number of atoms. Toxoiding could be proven for the components of a complex toxin if it were shown that they were inactive when mixed with the other component or components necessary for lethality or other expected biological activity, and yet remained immunologically active.

**SYMBOLIC NOTATION FOR IDENTIFICATION OF TOXINS**

Although symbolic notation of toxin nomenclature has not been discussed as such, several examples have been given in other contexts (e.g., $\alpha$ toxin, type A, factor 1, etc.). It is clear that such a terminology has inherent weaknesses but can be useful if used intelligently and in a consistent manner. Inconsistencies do occur in the symbolic notations used today, and they are likely to become more pronounced as more toxins are characterized. We would like to point out several instances in which symbolic notation is used inconsistently and finally make some recommendations which might help in the clarification and standardization of toxin nomenclature.

**Capital Letter Notation**

It has become common practice to identify the botulinum toxins by capital letters. The classification of *C. botulinum* types A, B, C, D, E, and F is based on the immunological specificity of toxic proteins produced by the individual strains. Although the proteins are distinct in terms of amino acid composition and antigenicity, they evoke essentially the same neurological disease in sensitive animal hosts. Although there is no objection to the use of the letters A through F to identify the toxins of botulism, it is an arbitrary classification. Once a system of classification is used, however, it should no longer remain arbitrary but must be guided by specific rules. These rules should be neither too rigid nor too flexible so that they can accommodate various situations while remaining meaningful. No one has ever suggested that capital letters be used only to identify immunologically distinct toxins having the same biological activity, as is the case for the botulinum toxins. In retrospect, however, this appears to be a reasonable suggestion. Indeed, the rule, without having ever been stated, was applied in the case of the staphylococcal enterotoxins, and, therefore, no inconsistency in nomenclature resulted. As previously noted, enterotoxins A, B, and C have been identified as immunologically distinct proteins (6, 14) and presumably have the same mode of action in vivo. Therefore, the use of capital letters to identify the staphylococcal enterotoxins is valid and presents no difficulties, even if in the future other immunological species are discovered. However, in the absence of rules, inconsistencies can and will arise. The two toxic proteins extracted from *Pasteurella pestis* have been designated as toxin A and toxin B (25). In view of the uncertain status of the relationships among the plague toxins, the use of capital letters may prove to be inconsistent with their use as applied to the botulinum toxins and staphylococcal enterotoxins. As more and more toxins are discovered and characterized, there is no doubt that further inconsistencies will arise if guidelines concerning the use of capital letters are not applied consistently by present and future investigators.

**Greek Letter Notation**

The origin of the Greek letter system for the identification of bacterial toxins is obscure but has gained considerable popularity. As the system has developed, Greek letters have been assigned to different toxins produced by either one bacterial species or a strain within a species. This system of nomenclature is used for the staphylococcal toxins and for the toxins of the gas-gangrene group of clostridia. If this classification is to retain any validity, then it should be restricted to the case where a number of different toxins are produced by the same organism. It may very well be that the murine plague toxins, if they are found to be separate and distinct, should be placed within this category (i.e., $\alpha$ and $\beta$ toxins) rather than being designated as toxin A and toxin B.

One overt weakness of the Greek letter system is that it has been applied indiscriminately for all of the bacterial products without taking into consideration whether they are true toxins. This is misleading, since many of the $\alpha$ to $\mu$ products of the *C. perfringens* group may be completely irrelevant to pathogenicity, or at most only auxiliary virulence mechanisms (Miles, personal communication). The same situation may exist for the $\beta$ and $\Delta$ hemolysins of *S. aureus*. In our opinion, materials of this kind should not be classified as toxins. It should be pointed out, however, that some investigators feel just as strongly that they be considered toxins for want of a better niche in which to put them (70). We do not intend to reopen the old aggressin versus toxin polemic, since no constructive pur-
pose would be served. Suffice it to say that the existence of the controversy proves that there has been genuine doubt about including those materials which have been labeled aggressins as true toxins. We feel that only those materials which exert a direct toxic effect upon sensitive host cells and tissues should be classified as toxins. Materials such as hyaluronidase, deoxyribonuclease, and several others should be differentiated in some manner and should not be referred to as true toxins unless evidence can be adduced for their role in generating signs of illness. Until a better term is found, these substances might be classified as auxiliary virulence factors, a phrase employed originally by A. A. Miles. Within this context, Miles (34) referred to the enzyme-substrate fallacy in a discussion concerning the relevance of these substances to pathogenicity. He pointed out quite correctly that the mere presence of the appropriate substrate in host tissues for enzymatic products of pathogenic bacteria does not necessarily imply that the enzymes contribute to the pathogenesis of the infections. We would like to broaden this concept to the antigen-antibody fallacy as well. Although the bacterial aggressins for the most part are antigenic and elicit specific antibodies in mammalian hosts, these substances do not necessarily contribute significantly, or at all, to the diseases with which they are associated.

Symbolic Notation for Multicomponent Toxins

It is likely that the multicomponent toxins will eventually present the most difficult nomenclatural problems of all. At this point, however, the anthrax toxin is the only one which is appropriate as a model for discussion and recommendation. The English investigators, who were instrumental in originating and perpetuating in the literature the terms factors I, II, and III of the three components of anthrax toxin, still feel strongly that this is the simplest and best nomenclature and that there is no reason for changing it (Smith, Keppie, Belton, Strange, and Stanley, personal communication). We have already stated that their terminology has historical precedence, but that this should not constitute a barrier if a more suitable nomenclature were available. In the case of the multicomponent toxins, descriptive names based on sound experimental evidence are preferable to symbolic notation. Readers of the anthrax literature, especially those who are not experts in the field, would be much better informed if the terms edema component, protective-antigen component, and lethal component were used rather than factors I, II, and III. We prefer to use the term component rather than factor even in the descriptive terminology. Factor is defined as one of the facts, circumstances, or influences which leads to a result. Implied in this definition is the element of dependence on other factors for the result to be manifested. Component, on the other hand, may be defined as a distinct element or constituent of a larger, complex entity. In this case, each component may act independently to bring about the final result. Admittedly, the distinction is a fine one but nevertheless real. At best, a descriptive terminology should create a mental image which is accurate, and only by the proper choice of words is this possible.

Experimental evidence will not always be available to allow a satisfactory descriptive terminology. In this case, we should be prepared to adopt a uniform set of symbols to identify the multicomponent toxins. It is obvious that no one system will appeal to all scientists, and we are not suggesting that the following possibilities are the best ones. To reiterate, our primary objective is to stimulate thought along these lines and even to provoke some healthy controversy (preferably of the nonvindictive variety).

Two possibilities might be considered. With the anthrax toxin again as an example, the multicomponent toxin might be referred to as anthrax toxin component 1, component 2, component 3. The Arabic numerals are suggested because of their universal use and recognition by scientists throughout the world. Another advantage which cannot be ignored is their suitability for programming in computer systems. It may be argued that the suggested nomenclature is too cumbersome, but it could be simplified by choosing an appropriate abbreviation for the component terms.

Another system which was suggested by A. A. Miles (personal communication) might also be considered. A system similar to the one used for serum complement could be adopted. If the symbol MT were accepted as a symbol for a multicomponent toxin, the individual components could be designated as MT'1, MT'2, etc. The description of the activities and even the sequence of action in vivo could be expressed if this information were available. This system would also facilitate the addition of another number for a newly discovered component of the toxin or, alternatively, the deletion of a numbered component in the symbolic notation if it was proved to be irrelevant to toxin activity in vivo. The anthrax toxin according to current knowledge would be identified as anthrax toxin - MT'1, MT'2, MT'3. By use of the same system, the staphylococcal leucocidin would be formulated as leucocidin - MT'1, MT'2.
RECOMMENDATIONS

In conclusion, we would like to suggest that our colleagues in microbiology recognize the parallelism which exists in the inherent difficulties of toxin nomenclature with the problems faced by the biochemists and enzymologists. It should be realized that inevitably confusion arises from poorly conceived and ill-defined systems of nomenclature. Thus, something should be done to clarify and systematize the nomenclature of toxins while the confusion is still relatively minor. Recently, the International Union of Biochemistry (17) published its recommendations on the nomenclature of 875 enzymes and, by virtue of its authority and recognition, provided a workable scheme for the overall nomenclature of enzymes. In spite of the completion of this monumental effort, general acceptance will not be immediate, and the confusion caused by the earlier names for enzymes will make itself felt for many years to come. As our knowledge of the biochemistry of toxins increases, the toxin nomenclature will by necessity be forced to change. The ultimate nomenclature which at present is only a distant goal is one based on knowledge of amino acid sequence of both the entire molecule and the active sites within the molecule. For the time being, however, a logical step that can be taken is for a scientific society, such as the American Society for Microbiology, to initiate a study (i) from within the Society's membership, (ii) in cooperation with international microbiology organizations, or (iii) in cooperation with organizations outside of the microbiological specialty (e.g., American Chemical Society, American Association for the Advancement of Science, Federation of American Societies for Experimental Biology), with the purpose of examining the growing problem of toxin nomenclature and making concrete recommendations for a rational solution. It is a project worthy of the concern of the American Society for Microbiology, and one which thoughtful analysis will show it has an obligation to initiate.

LITERATURE CITED
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