Bacterial Toxins and Classification

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To be widely useful in classification, a character should be reasonably easy to demonstrate, it should be consistent, and its incidence should be known over a wide range of organisms. On all three points the production of a particular soluble bacterial antigen is hardly satisfactory as a diagnostic criterion, as it is inconstant in a particular species, or even in a particular strain, its demonstration often requires complex immunological procedures, and far too little is known about its distribution. I have been asked to speak about bacterial toxins, but prefer to consider soluble bacterial antigens in general; the killing power of some bacterial antigens is, of course, practically important, but since it is dependent on concentration as well as on existence, it is hardly a true discriminant.

The degree to which the soluble antigens of bacteria have been examined depends very largely on their real or imagined importance in human or veterinary medicine, and on the personal interests and opportunities of those who have examined them. So it is not very surprising that the soluble antigens that have received the greatest attention are the bacterial toxins, particularly those of the genera Corynebacterium, Staphylococcus, Streptococcus, Shigella and Clostridium, while the soluble antigens of non-pathogenic organisms have hardly been seriously examined at all.

Our ignorance of the distribution of soluble bacterial antigens is matched by our ignorance of the mode of action of the few we know something about; for apart from the lecithinases, collagenases and hyaluronidases, whose action is to some extent understood, we have as a rule little idea of the fundamental actions of bacterial toxins. Thus Clostridium welchii produces eight or nine lethal substances, only two of which (the lecithinase \( \alpha \) and the collagenase \( \kappa \)) have known modes of action; the action of the rest is unknown, and there seems no reason to suppose that it is the same for any two of them. So that any attempt to use bacterial antigens in classification is limited by our ignorance, as well as by the regrettable habit bacteria have of ceasing to produce antigens that are regarded as characteristic of them, or of producing them only in circumstances that are very complex and difficult to repeat. Moreover, concentration of bacterial filtrates may show that traces of active material are present, though they cannot be demonstrated in the unconcentrated material.

As far as I can see, bacterial toxins are chiefly of value, and of very considerable value, too, in examining the relationships within a genus, and deciding whether a particular classification is useful and convenient or not. Thus Clostridium welchii produces a set of soluble substances, mainly dis-
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criminated by immunological means, by the use of which it is possible to divide the strains of this species into six groups. All the groups possess certain soluble antigens in common, and it is obviously convenient that the antigen should be called by the same name whichever group it occurs in. For this reason I prefer to divide *C. welchii* into six types, so that whether I am speaking of *C. welchii* type B, or *C. welchii* type C, or *C. welchii* type F, I can speak of the β toxin of *C. welchii* without ambiguity. Prévot's classification, by which *C. welchii* is divided into two species; *Welchia perfringens* with its varieties *elegans, zoodyenteriae* and *vitulitoxicus*, and *W. agni* with its varieties *paludis, wilsdoni* and *hominitoxicus* seems to me to be inconvenient in this respect—what are we to call the toxins?—and to have little backing from other methods of classification, as the organisms are far too much alike, colonially and biochemically, to justify their separation into species.

The lethal toxins of *Clostridium oedematiens* and *C. gigas* are immunologically indistinguishable; this supports the other colonial and biochemical characters by which they are classified together as types of *C. oedematiens*; their distinctiveness as types is emphasized not only by differences in size and biochemical activity, but also by the fact that their lecithinases (γ and β) are immunologically distinct. *C. oedematiens* type C from the Dutch East Indies does not certainly produce any identifiable soluble antigens, and its relationship to *C. oedematiens* has been demonstrated by other means. *C. haemolyticum* is closely related colonially and biochemically to *C. oedematiens*, but is usually differentiated from it because its lethal toxin is distinct from that of *C. oedematiens*. Immunological investigation of its toxin shows, however, that its lethal toxin is antigenically equivalent to the β toxin of *C. oedematiens* type B, but that no α toxin is present. It seems convenient therefore to call *C. haemolyticum*, *C. oedematiens* type D, and to differentiate the types by their production of αγ (A), αβ (B), no toxin (C) or β (D).

Similar arguments of convenience apply to *Corynebacterium diphtheriae* and *C. ulcerans*. Some strains of *C. ulcerans* produce diphtheria toxin, and it seems reasonable therefore to call the *ulcerans* strains *C. diphtheriae* var. *ulcerans*, rather than to separate them as a species.

A useful case to consider here is *Clostridium botulinum*. As far as I know, the toxins of all toxigenic strains of *C. botulinum* act in the same way, or at any rate on the same structures, but the only ones that show any antigenic relationship are those of types C and D. Now it has been suggested that *C. botulinum* should be divided into *C. parabotulinum* (proteolytic) and *C. botulinum* (non-proteolytic). Luckily this brings types C and D into the same species (*C. botulinum*), which seems to me very much more convenient than separating them, and to be a case in which antigenic structure does not oppose conclusions based on biochemical activity.

One more use of the minor antigens of a species is to identify degraded strains; *Clostridium welchii* strains occasionally lose their capacity to produce their main lethal toxins, and one may have to fall back on the others. Thus a *C. welchii* strain producing β and κ is very likely to belong to type C, though
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it does not produce $\beta$, while one producing $\beta$, $\lambda$ and $\mu$ is very likely to be a type B, though it does not produce $\epsilon$.

Can we apply our knowledge of soluble bacterial antigens to larger groups than species? Not, I think, even in the most tentative way. Thus Clostridium oedematiens, C. welchii, C. bifermentans, all produce lecithinases that act in the same way, by splitting unsaturated lecithin into stearyloleylglyceride and phosphorylcholine. It is true that there are slight differences in small matters, but the striking fact is the equivalence of the main activity. Notwithstanding this, the C. oedematiens lecithinases differ antigenically from one another and from the lecithinase of C. welchii. C. bifermentans lecithinase, on the other hand, though it differs in some points from the lecithinase of C. welchii, has some antigenic relationship with it. But this antigenic relationship between their lecithinases can hardly be taken as evidence that C. bifermentans and C. welchii are very closely related, for they differ very markedly in their biochemical activities, while C. welchii and C. oedematiens, whose lecithinases have little, if anything, in common, appear in other respects to be fairly closely related. Perhaps the worst case for the use of antigenic differences is C. oedematiens itself, for two of its types, A and B, though they share the lethal antigen $\alpha$, produce antigenically distinct lecithinases $\gamma$ and $\beta$. This does not encourage the use of antigens having the same enzymic activity to define a group, and though C. welchii, C. oedematiens and C. bifermentans are usually classed together in the genus Clostridium for what appear to me to be valid reasons, I cannot imagine that removal of Bacillus cereus and B. mycoides to the genus Clostridium because they produced a lecithinase would be very popular, or even justifiable, even though the lecithinases of B. cereus and B. mycoides are antigenically related.

The collagenases of Clostridium welchii and C. histolyticum are antigenically distinct, though they probably act in a similar way; C. septicum and C. welchii hyaluronidase are distinct; but in both cases the differentiation between the species has been made by other means.

I do not think that the oxygen-labile lysins give much information. They all have certain properties in common, and there is some evidence that they are antigenically related, but a consideration of the species known to produce them—Clostridium welchii, C. septicum, C. histolyticum, C. tetani, streptococci and pneumococci—hardly inspires much confidence in their usefulness for classification.

In the present state of our knowledge, soluble bacterial antigens can be used, it seems to me, only to divide species, devised on other criteria, into types or similar smaller groups. As far as I know, no example exists of the same soluble antigen being shared by two obviously unrelated bacteria, but even if it did occur, we could get over it by insisting that in classification all characters ought, in theory at least, to be taken into account, and not only those that seemed important to the investigator, possibly only because he was working on them. Obviously far more work is necessary to make soluble antigens more than adjuncts to the usual means of classification.

Finally, I should like to emphasize the value of the minor soluble antigens.
They can often be worked on with very little apparatus, and no animals, and I feel that they would well repay investigation, if only to clear up discrepancies in testing for the 'major' ones.

DISCUSSION

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It would have a most salutary effect if the good example set by Oakley's cautious assessment of the taxonomic significance of soluble antigens (toxins) were followed by workers concerned with non-soluble bacterial antigens.