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 α and the collagenase κ) 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 egens, zoodyserteriace and vitilitoxicus, and W. agni with its varieties
paludis, wilsoni 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 distinct-
ness as types is emphasized not only by differences in size and biochemical
activity, but also by the fact that their lecinthinases (γ and β) are immuno-
logically 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. oede-
matiens types A and B, and the pathological picture it produces is different.
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 C. 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 δ 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.
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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.