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The Use of Serology in the Classification of Micro-organisms

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In considering the classification of micro-organisms, and of bacteria in particular, one is confronted by two opposing points of view; one, that they should be classified according to their natural relationships; the other, that a natural classification is unimportant, and that all that is needed are keys by which micro-organisms can be identified simply and unequivocally. In agreeing that a natural classification is desirable it must be admitted that this cannot be achieved until there is available a great deal more information upon which to base it. I therefore consider that the first aim should be to build up a system of determinative keys which take full account of the broader fundamental relationships and from these a natural classification might in time be evolved. With this approach in mind, I am convinced that serological techniques could, with great benefit, be more generally used in the systematic study of all groups of micro-organisms. The high specificity of serological reactions is determined by the chemical nature of the antigen, and it is possible to detect differences between complex molecules, particularly proteins, which cannot yet be distinguished by chemical analysis. Serology is therefore a delicate tool for comparing and contrasting antigenic components of the microbial cell, providing information of use both in identification and classification.

This is well illustrated by reference to the genus *Streptococcus*. The classification of the members of this genus, or indeed their unequivocal identification had been extremely difficult. Some species had been well characterized by cultural and physiological tests, but it was not until Lancefield (1933) used serological methods on a collection of strains from a wide variety of sources that the beginning of some kind of order was established. In this genus, *Streptococcus*, we have an example of serological studies which not only detect minute differences between strains but give a broader picture which shows the division of the members into well-defined groups on the basis of specific group antigens. Finally, there is revealed a nucleoprotein antigen shared by streptococci, staphylococci and pneumococci, and one must assume that this wider association is no accident and that the common nucleoprotein does in fact represent a close and possibly natural relationship between these Gram-positive cocci.

A further important example of the use of serology in elucidating broad relationships is provided by the large group of bacteria classified (*Bergey's Manual*, 1948) as the family Enterobacteriaceae. The serological reactions of this family, particularly of the intestinal and allied members, have been more widely studied than any other group of comparable size. Not only did the

pioneer work of Weil & Felix (1920) and of Bruce White (1926) on *Salmonella* provide a background for the later very extensive serological investigations in this genus and with other members of the family, but it was in this family that the classical work of Smith & Reagh (1903), Weil & Felix (1917) and Arkwright (1921) demonstrated the possibility of using serological methods to reveal something of the architecture of the bacterial cell. From studies mainly initiated by epidemiological considerations, a picture of the antigenic structure of the Enterobacteriaceae is being built and although not yet complete, a general pattern is becoming evident. The primary division into genera is made on physiological characters (*Bergey's Manual*, 1948). Whilst serological analysis of the more superficial antigens associated with flagella and capsules has detected slight differences between strains, broader groupings within each genus is possible on the basis of somatic antigens. Furthermore, the sharing of some antigens between genera, e.g. *Escherichia* and *Klebsiella* (Kauffmann, 1949), supports the view that not only are the members of this family closely related but they do in fact form a continuous series.

By analogy with serological observations on the organisms of the family Enterobacteriaceae and on streptococci, pneumococci and staphylococci, the wider or group relationships are generally attributed to the less superficial antigenic components of the cell. This appears to be the case with the genus *Clostridium* in which it has been established that motile species possess flagellar (H) antigens and somatic (O) antigens similar to those described for many aerobic bacteria and it is possible that here also wider divisions may be based on somatic antigens, e.g. *C. tetani* can be divided by flagellar antigens into at least ten types all of which possess a common somatic antigen. However, there is as yet no complete serological survey of this genus, and the picture is so complicated that no definite conclusions can be drawn. Many members of the genus *Clostridium* are characterized by the toxins they produce and serological techniques have been widely used in their identification. However, as Prof. Oakley has already pointed out in this discussion, toxin production is not a sufficiently constant character to provide a basis for classification.

Serological methods have been used to solve practical problems in various fields of microbiology, but comprehensive studies upon which classifications could be based, have been made in only a small minority of bacterial groups. In some cases serological studies have failed to provide a basis for classification and there are no doubt many reasons for such failures. In this connexion the choice of serological techniques may be an important factor. The early success of agglutination reactions in effecting divisions within the genus *Salmonella* encouraged their wide application, but the broad divisions obtained with *Salmonella* on the basis of somatic agglutination were not paralleled for example in the genus *Streptococcus*. Here agglutination techniques, whilst providing a means of identifying individual strains and being of particular use in epidemiological studies of collections of organisms from limited sources (e.g. Griffith, 1927, 1934; Stableforth, 1932), failed as a basis for broader studies (Hucker, 1932). Lancefield (1933), however, showed that antigens

upon which broader groupings could be based were indeed present in streptococci. In agglutination reactions these group antigens are masked by surface antigens which possess narrow specificities. Group antigens can readily be extracted from the cell and their group specificity demonstrated by precipitin techniques.

The agglutination methods generally used in the antigenic analysis of *Salmonella* species do not always give satisfactory results even with other Gram-negative motile rods. Difficulties have been encountered for example with *Pseudomonas aeruginosa* in the preparation, by ethanol treatment, of suspensions for somatic (O) agglutinations (Mayr-Harting, 1948; van den Ende, 1952). Van den Ende solved this problem of technique by preparing trichloroacetic acid extracts, and was able by precipitin tests to make a serological grouping of his strains.

The importance of the choice of antigenic material can also be illustrated by reference to the genus *Bacillus*. Whilst there has been no published systematic study of the serology of this genus, it seems that in the vegetative cell neither somatic nor flagellar antigens form a suitable basis for species differentiation. However, there is a strong indication that spore antigens may well form the basis for a division of species in agreement with physiological studies (e.g. Lamana 1940*a, b*; Davies, 1951). Davies found spore antigens of *Bacillus polymyxa* to be species-specific and later unpublished work by Miss S. N. Davies & Mr H. Proom (personal communication) with various *Bacillus* species, clearly defined by other methods, appears to confirm the species-specificity of the spore antigens.

Identification of species within the genus *Lactobacillus* has been another notoriously difficult problem, and until recently serological studies have done little to clarify the picture. The reasons for these failures are those so often found in investigations of this description. Most workers have confined their studies to investigating the more superficial antigens by agglutination techniques, and mainly with strains from a limited number of sources, thus making it impossible to assess the wider significance of their results. Recently Sharpe (1955), who used precipitin techniques and HCl extracts of lactobacilli, has succeeded in grouping a large and comprehensive collection of named strains and fresh isolates. The serological groups thus defined are in general agreement with certain groupings based on physiological characters (Briggs, 1953), thus emphasizing the significance of the serological findings.

In considering the application of serological techniques to problems of bacterial taxonomy one is faced with the question of deciding what weight should be given to antigenic structure as compared with morphology and physiology. In my opinion the answer must await the results of a greater number of comprehensive studies on a wide variety of micro-organisms; it might then be possible to draw general conclusions on the taxonomic significance of antigenic structure. Nevertheless, it would be well to make use of all the information at present available in an attempt to piece together the jig-saw puzzle. The great difficulty in organizing a logical nomenclature is the lack, even now, of an unequivocal definition of species. This has resulted over

the years in an illogical granting of species rank even within the same genus. Most people who have tried to assess the place of serological studies in the study of bacterial taxonomy would agree that the greatest significance should be placed on broad groupings, and that the large numbers of highly specific reactions often elicited by surface antigens in particular, should not alone be a basis for naming species. The consigning of species names to an almost infinite number of serotypes in the genus *Salmonella* is an extreme example of a great lack of discrimination in this respect. The Enterobacteriaceae subcommittee of the Nomenclature Committee of the International Association of Microbiologists seek to retrieve this position by recommending (*Int. Bull. Bact. Nomen. Tax.*, 1954) that all new serological types of *Salmonella* should be described by formula only and not by name.

Whilst deploring the giving of species names solely on the basis of type antigens, a knowledge of the distribution of serotypes within a particular group may well help the taxonomist to retain or reject some species names already established by other criteria. In this connexion serological typing of streptococci of group D provides information by which species names originally given on physiological grounds can be assessed (Sharpe & Shattock, 1952).

With micro-organisms such as viruses, rickettsias and the pleuropneumonia group where morphological or physiological characters cannot readily be used as a basis for classification, serological studies play an even more important role than they do in the classification of those organisms in which both morphology and physiology can be used more easily. Serological reactions of animal viruses have been very extensively studied in relation to diagnosis and immunity and many antigenic relationships have been discovered which are in agreement with other properties. At the 5th International Congress of Microbiology the Virus subcommittee, in agreeing on the criteria upon which the classification of animal viruses should be based, placed immunological properties high on this list (Andrewes, 1952). With plant viruses, also, serological studies have helped in elucidating relationships, and Bawden (1950) thought that serological criteria would form a sound basis for their classification. With bacteriophages there is no doubt that serology is an excellent basis for classification. For example, Burnett (1933) demonstrated the value of serological relationships in classifying the coli-dysentery group of phages and this classification agreed well with other characters, namely size of phage particle and plaque morphology. The T-series of coli-phages has also been clearly divided, the original seven phages of this series falling into four distinct and unrelated serological groups in agreement with morphological differences (Delbrück, 1946). The reliability of serological reactions in the classification of phages is such that Adams (1953), in discussing criteria for a biological classification of bacterial viruses, regarded serological criteria as of first importance.

In this meeting many methods of approach to the problem of microbial classification have been discussed and it will be recognized that it is not easy to assess the relative value of these various methods in the classification of micro-organisms. Indeed it is all too evident that one comprehensive set of

rules to meet all requirements cannot be formulated. I would submit, however, that the use of serological methods, particularly when supplemented by the chemical characterization of antigens, cannot fail to provide invaluable information to help in the gradual building up of a classification based on fundamental relationships.

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DISCUSSION

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My work on the serological classification of the lactobacilli, mentioned by Dr Shattock, was undertaken not only for its scientific interest, but because the identification of these organisms is of great practical importance.

The collection of organisms used in the serological work comprised strains of all available species, and freshly isolated strains from many different sources. Antisera were prepared against a number of strains. Using HCl extracts and precipitin tests it was possible to place 70 % of the 442 strains examined in six groups and one subgroup. Briggs (1953), working independently on the same collection of organisms, classified most of the strains in a number of physiological groups, with which my serological groups agreed. Wheeler (1955*a, b*) has since extended the work on the physiological characteristics of lactobacilli, and her findings also substantiate the serological groups. A serological classification of many strains of lactobacilli correlated with their physiological characteristics has thus been established.

The lactobacilli appear to possess antigens analogous to those of the streptococci, and this serological classification has been based on them (Sharpe, 1955).

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DISCUSSION

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We have heard various bacteriological criteria discussed in relation to their value in classification. It is obvious that these criteria, which we will refer to as disciplines, have been used for all families of bacteria, but that the importance of any one discipline varies with the family under investigation,

and that all disciplines fail to give a clear-cut answer at one time or another. The next point is that the disciplines are used in a recognized order, an order which for all practical purposes repeats the history of bacteriological research, a sort of phylogeny of the science of bacteriology. This orderly approach is exemplified in the family Enterobacteriaceae where the colonial appearance and morphology are noted, followed by the biochemical tests, then the serology giving the antigenic analysis which may be followed by special biochemical tests and by phage typing. The results of the disciplines used in this order are the basis of classification of the Enterobacteriaceae. For various reasons, we have found it necessary to make certain investigations in which we have not followed the usual order of disciplines. We have noted morphology and colonial appearance, jumped to serology, then returned to the biochemical tests. This approach has given some interesting results, of which the following is an example.

In 1949 a strain, E. 1073₍₂₎, was isolated from a case of infantile gastro-enteritis, and used for the preparation of an antiserum. The antiserum was then used in the investigation of faeces and other material, particularly from cases of gastro-enteritis of infants. By this means, five strains of the same serotype were isolated. In 1952 Dr Patricia Carpenter sent three cultures isolated from the faeces of adults with diarrhoea, which were found to have the same surface and somatic antigens as E. 1073₍₂₎. One was used for the preparation of an antiserum. Some time later, Dr H. Seeliger sent four strains, and the homologous antiserum of one, which he had isolated from an outbreak of gastro-enteritis in adults, and which he labelled 'Katwijk' (Seeliger, 1954). Through the kindness of Brigadier J. S. K. Boyd we also received strains isolated by Dr J. H. Bekker in Holland, which had been investigated by Dr W. H. Ewing, of Chamblee, Georgia, U.S.A. Table 1 shows the results of

Table 1. *Agglutinin titres before and after cross-absorption tests*

Serum	Antigens*	
	'K'	'O'
E. 1073 ₍₂₎		
Unabsorbed	1,600	25,000
Absorbed with E. 1073 ₍₂₎ , or Katwijk, or Carpenter	< 50	< 100
Katwijk		
Unabsorbed	400	3,200
Absorbed with E. 1073 ₍₂₎ , or Katwijk, or Carpenter	< 50	< 100
Carpenter		
Unabsorbed	1,600	6,400
Absorbed with E. 1073 ₍₂₎ , or Katwijk, or Carpenter	< 50	< 100

* Identical results were obtained with antigens E. 1073₍₂₎, Katwijk and Carpenter.

direct titration and agglutinin absorption of the three sera by the three strains, which proves that all three have identical somatic and surface antigens.

A total of fifteen strains has been examined, all of which belong to this serotype. The biochemical reactions of these strains, which fall into three

groups, are given in Table 2. All strains, other than E. 1073₍₂₎ and two strains of the same biochemical type, were non-motile. The biochemical type of Carpenter (Table 2) might well be classified as a shigella. In fact, Seeliger points out that a serologically identical strain isolated in Italy was described as such by Cefalù & Gullotti (1953). The latter also demonstrated that their

Table 2. *Biochemical results*

Strain	Lactose	Glucose, maltose, mannitol	Sucrose, salicin, dulcitol	MacConkey broth 44°	Country of origin and no. of strains
E. 1073 ₍₂₎	AG	AG	AG late	AG	Germany 1 Great Britain 5
Katwijk	A	A	—	A	Germany 3 Great Britain 1
Carpenter	— (21 days)	A	—	—	Holland 2 Great Britain 3

All strains were MR +, VP —, indole +, urease —, citrate —, inositol —. A, acid; AG, acid + gas.

organism, Ca/792, was related antigenically to both *Shigella* serotype 425 and *Escherichia coli* 0.28. Seeliger found an additional relationship to *E. coli* 0.42. We were able to confirm the relationships to the *E. coli* 'O' antigens, and also found that two of the motile strains had the flagella antigens H₄ and H₃₂, respectively, of *E. coli*, but were unable to identify the 'H' antigen of the third strain. These results show that these organisms can be classified within the *Escherichia* group (Kauffmann, 1954). This example is cited as a plea for a reasonable approach to classification, and to show that the value attached to a particular discipline may vary in relation to the results from other disciplines.

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