THE PRINCIPLES OF GROUP DIFFERENTIATION
WITHIN THE ENTEROBACTERIACEAE
BY BIOCHEMICAL METHODS

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In a recent paper on the philosophy of classification, Cowan (1955) posed two questions which are of fundamental importance in the classification of the Enterobacteriaceae:

1. "Does the hierarchial structure of a Linnaean system satisfy the requirements of microbiologists?"
2. "Can we accept the species concept, and all that this implies, or must we view our organisms as a huge spectrum composed of gradually merging forms?"

Both of these questions must be answered in the negative as the Linnaean system cannot be used and the species concept in a Linnaean sense cannot be accepted. As has been pointed out the Enterobacteriaceae must be regarded as a "series of interrelated bacterial types which do not lend themselves to sharp division into tribes or into groups" (Enterobacteriaceae Subcommittee, 1954). Further, "The family is composed of a large number of interrelated types which display almost every conceivable combination of biochemical characteristics compatible with the definition of the family. These types form such a continuous series that they are not readily susceptible to division into distinct groups. A logical method of classification would be a catalog of this continuous series of types based upon their biochemical and serological properties since any attempt at grouping results in the exclusion of strains of intermediate character which do not conform to the properties established for each of the groups. However, our knowledge of the bacteria is so fragmentary and the types are so numerous that as a matter of expediency it is necessary to divide the family into groups which form the basis of practical work" (Edwards and Ewing, 1955).

Under these circumstances and since nothing is known concerning genetic relationships and evolutionary trends within the family, it is impossible to decide whether a given classification is scientifically correct, but only whether it is more or less practical and expedient. The whole problem must be regarded from the standpoint of practical diagnosis and in the light of our limited present knowledge. This attitude is reflected in the two reports of the Enterobacteriaceae Subcommittee (Rio de Janeiro and Rome). In these reports the family is subdivided into biochemical groups, not into tribes, genera, and species. Any further subdivision of the proposed groups must rest upon the recognition of known and delineated biochemical or serological types which occur within them. Such a classification is consistent with our present knowledge and of necessity must suffice for practical and scientific purposes until such time as it can be extended as a result of further developments in the field.

At present it is not possible to define a "genus" or a "species" in the Linnaean sense. For example, endless controversies arise in any discussion as to whether the Salmonella group should be regarded as a "genus" or as a "species." To avoid all these difficulties, it is better to abandon this artificial and arbitrary classification into "genera" and "species" and to subdivide the Enterobacteriaceae into biochemical groups, subgroups, and types as proposed by the Enterobacteriaceae Subcommittee.

Since it is wished to discuss here only the principles of classification, it is neither necessary to describe the several groups individually nor to present a table of their biochemical attributes. However, since the biochemical tests are the basis of classification, it is desirable to discuss some fundamental facts which are of importance in their interpretation and the recording of results.

The biochemical differentiation of groups is based on the whole picture of biochemical behaviour as it is presently understood, and not by the results of one or several tests (Kauffmann, 1954). To obtain this complete picture 30 or more tests may be required. However, in the determination of whether a culture belongs to one or the other of two groups, not all of these tests are of equal value and often it is possible to use fewer substrates.

In reading and reporting biochemical reactions, it is necessary to differentiate between four different results:

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- 1) + = positive, 1-2 days. The majority of such cultures give a positive reaction after 1 day.
- 2) (+) = always positive, but delayed usually 3-4 days or longer. This reaction is relatively rare and the designation (+) should be used only if the constancy of this delayed reaction is proved in repeated tests with numerous cultures. A typical example of this reaction is the delayed fermentation of trehalose (+ 3-4) by Salmonella paratyphi C.

If it is not demonstrated that this delayed reaction is regularly delayed and always occurs at about the same time, it should be designated by the letter x (see below). Kristensen (1955) studied the fermentation reactions of several Salmonella types with regard to "primary slow" or "mutative" fermentation and found that a delayed fermentation was very often mutative.

3. x = late and irregularly positive or negative (usually mutative). For instance, this type of fermentation occurs in biochemical type 2 of S. typhi with regard to the fermentation of xylose. The differences between the two xylose-types of S. typhi are as follows:

Type 1 attacks xylose promptly (+¹), but type 2 ferments by mutation (= x), i. e., the fermentation of xylose is usually delayed and irregular or is lacking. It is incorrect to call this reaction a negative one, as positive reactions will be obtained when several tubes are incubated for long periods. When a loopful of a tube which became positive after some days is plated on agar containing xylose, colonies may be isolated which ferment xylose promptly and which will continue to do so. Although this mutative reaction is irregular, this irregular or variable behaviour is very constant and characteristic for the special type. The mutative reaction is the only variable reaction. The differential diagnosis between this mutative fermentation (x) and the delayed positive (primary slow) fermentation ((+)) is possible only on the basis of repeated tests of the same culture. In those instances where it has not been determined whether fermentation is primary slow or mutative, the symbol x may be used.

- 4) - = negative (the observation time should be at least 14 days).

Within many serotypes there occur several biochemical types which may give with the same substance any one of +, (+), x or - reactions. The sign "v" previously has been used to indicate "various biochemical types," but not to indicate "variable." The term "variable" should be avoided as it has caused misunderstanding and often is taken to mean "irregular." To avoid further misunderstanding it is proposed to designate "various or different biochemical types" by the letter "d" (from "different"). If, for example, one strain of S. paratyphi B ferments inositol promptly, but another strain of the same serotype does not attack this substance at all, this behaviour is not "variable," it is constant for both biochemical types which behave differently in this respect (= d). Here we have to do with two distinct types which differ in their action on inositol, but are absolutely constant in their biochemical behaviour.

When the biochemical reactions are judged correctly by +, (+), x, or -, many discrepancies in the literature and mixconceptions regarding the instability of biochemical reactions will disappear. It should be stressed that the signs x and d are different, as x means a delayed, usually mutative, reaction, and d means different biochemical types within a single serotype.

SUMMARY

Since a natural, genetic system of classification of the Enterobacteriaceae cannot as yet be established and as the artificial Linnaean system cannot be accepted, especially the Linnaean "species" concept, the Enterobacteriaceae are subdivided for practical purposes into biochemical groups and subgroups.

The biochemical tests which are the basis of this group differentiation should be judged and reported in a uniform manner and designated by + = positive, (+) = delayed positive (primary slow), x = late and irregularly positive or negative (usually mutative) and - = negative. If different biochemical types occur within a single serotype, this should be indicated by the letter "d." The word "variable" should be avoided,

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as only the mutative reaction (= x) is irregular, but the mutative reaction is a constant and characteristic property of special biochemical types.

REFERENCES

- Cowan, S. T. Jour. Gen. Microbiol. 12:314. 1955.
- Edwards, P. R. and W. H. Ewing. Identification of Enterobacteriaceae. Burgess Publishing Company, Minneapolis, Minn. 1955.
- Enterobacteriaceae Subcommittee. Intl. Bull. Bact. Nomen. and Taxon. 4:1-94. 1954.
- Kauffmann, F. Enterobacteriaceae. 2nd ed. E. Munksgaard, Copenhagen. 1954.