CLASSIFICATION AND NOMENCLATURE OF ENTEROBACTERIACEAE

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In the classification and nomenclature of the Enterobacteriaceae two different concepts exist, to which we will refer arbitrarily as the biochemical classification and as the classification by antigenic analysis, although serologic methods enter into the former and biochemical methods into the latter.

1. Biochemical Classification

In the biochemical classification, as typified in the 4th edition of Bergey's Manual, only biochemical methods were used to distinguish species. It should be said that a gradual development of serologic methods had occurred through the years and that workers relied on these methods to distinguish types within certain genera. For example, various serologically distinct types were known in the genus *Salmonella*, and these were recognized by agglutination and agglutinin absorption tests. Yet in formal classifications only a biochemical key was used to distinguish species.

That this practice has persisted to the present day is shown by the treatment of the genus *Escherichia* in the 6th edition of Bergey's Manual. Three species are recognized, as follows:

Species	Citrate Utilization	H ₂ S Production
<i>Escherichia coli</i>	-	-
<i>Escherichia freundii</i>	+	+
<i>Escherichia intermedia</i>	+	-

The above illustrated treatment of the genus *Escherichia* represents a retreat from former editions of the manual in which elaborate biochemical keys were employed and a much larger number of species was recognized. From this it is obvious that the editors felt that it was not profitable to delineate a large number of ill-defined types based upon biochemical characteristics which had no real significance. It is obvious also that the three above-mentioned species are, in fact, large groups or subgenera since they are composed of many biochemical and serologic types. In the classification of the bacteria by methods such as those outlined, the elementary units of which the species are constituted cannot be distinguished as they are by the practical use

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of antigenic analysis. If, for example, one wishes to determine the occurrence of a particular culture of *E. coli* in infantile enteritis, it is impossible to do so by biochemical methods since such methods serve to distinguish only the species of *Escherichia*, each of which makes up a large group of distinct antigenic types. Even though all biochemical methods which are presently employed are applied to the study of such cultures, only biochemical groups, each composed of numerous antigenic types, can be delineated. Such a system will not answer the present needs of the scientific worker, of the clinical bacteriologist, nor of the epidemiologist.

2. Classification by Antigenic Analysis

While great reliance had been placed in serologic tests for many years, the era of antigenic analysis in the *Enterobacteriaceae* may be said to have begun with the pioneer work of Bruce White which has been confirmed, refined, and extended by many workers. As in the older method of classification, the bacteria are divided by biochemical methods into groups comparable to the recognized genera. These groups are further divided into O-groups and serotypes through the recognition of their constituent antigens by serologic methods which have been proven to be reliable and to yield comparable results in the hands of many persons. The criticism often has been made that persons interested in antigenic analysis have failed to employ biochemical methods which would differentiate types or species. Such criticisms are not justified since all biochemical methods which have been found to yield valuable information regularly are employed in the work. After the division of the groups into O-groups on the basis of their heat stable somatic antigens, the organisms are divided into serotypes through recognition of their K (capsule or envelope) and H (flagellar) antigens. In some instances these serotypes are divided further on the basis of their biochemical properties or by their reactions to standard bacteriophages.

This division into types and subtypes is necessary to determine the epidemiological relationships of biochemically similar cultures, to investigate the pathogenic significance of individual types, and to obtain a clear picture of the composition of the genera and of the family.

As regards the separation of the family into groups or genera, it must be emphasized that no sharply defined groups exist in nature. Still, for practical purposes, it is desirable to divide the family into several groups. This can be done by biochemical methods, although such groups must be established arbitrarily and it must be kept in mind that many cultures which are studied will not conform to any of these arbitrarily defined groups. Further division of the groups should be done by serologic study of the bacteria.

Each of the groups or genera is composed of hundreds of serologic types, which from a practical standpoint are constant and which can be quickly and accurately recognized by serologic methods. It is not necessary to designate all these serotypes by special names nor to regard them as species. It is sufficient to number them or, better still, to give their antigenic formulas. These types form the elementary units established by the methods employed and are the basis of antigenic classification. It is sufficient to designate the O-groups into which the types fall with capital letters (as in the case of the genus *Salmonella*) or by arabic numerals. The alphabet does not contain sufficient letters to designate all the O-groups of some of the groups. For example, in the *Escherichia* group more than 120 O-groups now are recognized. From the foregoing it is obvious that we have two entirely different systems with which we must deal. The first of these, the biochemical classification, is a purely taxonomic concept and can be considered only as a convenience for the purposes of the orderly classification of bacteria in which each family must be divided into tribes and each tribe into genera and in which each genus must have a type species. On the contrary, the antigenic classification fulfills the needs of the scientist who is studying the phylogeny and evolution of bacteria as well as the needs of the clinical laboratory and of the epidemiologist. The two systems are in no way comparable and should not be used for the same purposes.

The question naturally arises as to whether these two systems may be correlated and whether it is possible to erect an antigenic classification. To a certain extent it is possible to do this, although it is necessary to include some groups not hitherto used in conventional classifications. It is obvious also that the groups at present recognized do not include all of the diverse strains of the enteric bacteria. Any proposed grouping must be based only on existing knowledge with the realization that further groups probably will be added. Further, it is quite possible that some groups delineated at present will be merged. With the present state of knowledge, the characterization of enteric bacteria must of necessity remain in a somewhat fluid state.

It is not believed that O-groups or serotypes constitute species. Probably species should be established at the level of the biochemically defined subgroups or subgenera. Thus each species would be made up of numerous serotypes defined by serologic analysis. To designate species at this level should cause no difficulty in any of the groups other than *Salmonella*. Fortunately, such serotypes as have been recognized in groups other than *Salmonella* have been designated only by laboratory numbers or by antigenic formulas. Examples of such labeling are *Proteus* X 2 and X 19 and *Coli* 1:1:7. In such cases the designation of biochemical species presents no problem.

Unfortunately, in the case of the *Salmonella* group, serotypes that

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were distinguished many years ago have been labeled as species, e.g. *S. bovis moribificans*, *S. typhimurium*, etc. Here we encounter the conflict between traditional usage and developing knowledge. It is now known that it is impossible to distinguish the various serotypes by biochemical methods, and such biochemical keys as have been proposed for the genus have no basis in fact. Therefore, we are left with two alternatives: (1) To state that species in the genus *Salmonella* cannot be defined, or (2) to propose a very simple biochemical key which will serve to distinguish the accepted type species (*S. choleraesuis*, *S. typhi*, and *S. kauffmannii*). Since these three species were differentiated by serologic methods and not by biochemical methods, it appears that the classification is not valid from a taxonomic standpoint. Therefore, if species are to be established in the genus the following method seems to be the least illogical:

Species	<u>Salmonella</u>			Gas Production
	Arabinose	Trehalose		
<i>Salmonella choleraesuis</i>	-	-		+
<i>Salmonella typhi</i>	-*	+		-
<i>Salmonella enterica</i>	+ or -	***		***

- = negative, + = promptly positive, * = rarely positive, ** = rarely negative

The term *S. kauffmannii* of Borman, Stuart, and Wheeler is rejected since it was not biochemically defined. In its place is substituted *S. enterica* which is biochemically defined. The result is, for all practical purposes, the same as that proposed by Borman et al. The genus, then, is divided into two biochemically defined serotypes which respectively represent the type species and the best known member of the genus, and into a large group of serotypes which cannot be biochemically differentiated one from another.

To give an idea how such a biochemical classification would look, we present a short draft in Table 1. Keys for the differentiation of groups and species are given in Tables 2 and 3. We do not recommend such a classification, but we would agree that a classification similar to that given below could be used side by side with an antigenic classification, but only with the realization that the biochemical classification was to be used for purely taxonomic purposes.

It should be emphasized that the opinions expressed herein are only the views of the writers and should not be taken as expressing the opinions of others. The terms used in the biochemical classification may or may not be valid ones and should be subjected to review by persons familiar with bacterial taxonomy.

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Table 1.

Biochemical Classification
(For taxonomic purposes)

Genus or Group	Species	
1. Salmonella	1. Salmonella choleraesuis 2. Salmonella typhi 3. Salmonella enterica	
2. Arizona	1. Arizona arizonae	eventually combined into one genus ?
3. Bethesda	1. Bethesda ballerupensis	
4. Escherichia	1. Escherichia coli	eventually combined into one genus ?(1)
5. Klebsiella	1. Klebsiella pneumoniae (or aerogenes) 2. Klebsiella rhinoscleroma- tis	
6. Shigella	1. Shigella dysenteriae 2. Shigella flexneri-boydii 3. Shigella sonnei	
7. Providencia	1. Providencia providenciae	
8. Proteus	1. Proteus hauseri a. var. vulgaris b. var. mirabilis 2. Proteus morganii 3. Proteus rettgeri	

- (1) If it is decided by the Nomenclature Committee that the generic name *Bacterium* is to be used, then the *Escherichia* and *Klebsiella* groups should be combined in one genus and called respectively *Bacterium coli* and *Bacterium aerogenes*.

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Table 2.

Biochemical Differentiation of Groups
(Typical behaviour of most important types)

	Mannitol	Lac- tose	Indole	Gel- atin	H ₂ S	Urea*	Gas	Motility
Salmonella	+	-	-	-	v	-	v	v
Arizona	+	+ or x	-	+	+	-	+	+
Bethesda	+	+ or x	-	-	+	- or x	+	+
Escherichia	+	+	+	-	-	-	+	v
Klebsiella	+	+	-	-	-	x	+	-
Shigella	v	- or x	v	-	-	-	-	-
Providencia	- or x	-	+	-	-	-	v	+
Proteus	v	-	v	v	v	+	v	+

* = Urea read after 6 hrs. incubation

+ = Promptly positive

x = Late and irregularly positive or negative

- = Negative

v = Variable

Table 3.

Biochemical Differentiation of Species

Species	Klebsiella	Ammonium Citrate	Urea	Gas Production
Klebsiella pneumoniae		+++	x	+++
Klebsiella rhinoscle- romatis		-	-	-

Species	Shigella	Mannitol	Lactose
Shigella dysenteriae		-	-
Shigella flexneri- boydii		+++	-
Shigella sonnei		+	-+

Species	Proteus	Mannitol	Maltose	Indole	Gelatin
Proteus hauseri var. vulgaris		-	+	+	+
Proteus hauseri var. mirabilis		-	-	-	+
Proteus morganii		-	-	+	-
Proteus rettgeri		+	-	+	-

+++ = rarely negative, -+ = delayed positive, x = late and irregularly positive or negative.

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Addendum - It is realized that no provision is made in the above classification for the motile forms at present classified as *Aerobacter*, nor of the forms classified by Borman, Stuart and Wheeler as *Paracolobactrum aerogenoides*. It is felt that present knowledge of these forms is insufficient to justify their placement in a definite group. It is not clear whether the genus *Klebsiella* should be expanded to embrace these forms or whether the definition of the genus *Escherichia* should be modified to allow their inclusion.

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References

- Bergey's Manual of Determinative Bacteriology, Fourth Edition, 1934, Balliere, Tindall & Cox, London.
Bergey's Manual of Determinative Bacteriology, Sixth Edition, 1948, The Williams & Wilkins Company, Baltimore.
Borman, E.K., C.A. Stuart, and K.M. Wheeler: J. of Bacter. 48, 351-367, 1944.