TAXONOMY OF THE FAMILY ENTEROBACTERIACEAE

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Most approaches to the problem of bacterial taxonomy have arisen from either of two viewpoints, one derived from phylogenetic and the other from practical considerations. The former viewpoint too frequently arises from some major premise which has little practical connotation. The latter viewpoint often leads to the submergence of large groups of bacteria, not known to be of economic importance, because of an attitude of impatience toward any system which does not reflect the methods used in the specialized laboratory where steps in the identification of an unknown organism must be measured in terms of utility and speed.

Regardless of personal viewpoints, it must be realized that precise delineation of species cannot be the primary aim of bacterial taxonomy at present. It is seldom possible and often it may not even be desirable. We must compromise by recognizing the necessity for the organization within a taxonomic system of a selected body of knowledge of important differential characters which may be applied when practical considerations demand that phylogenetically related organisms be distinguished, one from another. In addition, the taxonomic system should suggest, by implication if not by statement, the fields and directions in which further investigation is necessary or may prove fruitful. This implies that taxonomic systems must undergo periodic revision with the advent of new knowledge.

This discussion will be concerned with the taxonomic system followed for the *Enterobacteriaceae* in the fifth edition of "Bergey's Manual of Determinative Bacteriology" (Bergey, Breed, Murray and Hitchens, 1939).

THE FAMILY *Enterobacteriaceae* Rahn

(Zentr. Bakt. Parasitenk., II, 96, 280, 1937)

The difficulties besetting the taxonomist in devising and revising a classification are well illustrated by the family *Enterobacteriaceae*. New knowledge of this group has accumulated to such an extent that incorporation of it into our present system produces a confusingly top-heavy structure. Furthermore, satisfactory labeling of many forms with intermediate characteristics is not now possible even though those forms are commonly encountered in practical work. Not only is simplification needed but also reconstruction of the general framework of the system so that it will provide more effectively than it now does for the absorption of newly described types.

This family, while not discontinuous with other bacterial families, is so well delineated by tradition and usage that no radical change in its definition is

needed. The accepted definition, however, fails to point out the manner in which the almost innumerable forms comprising the family intergrade with each other to form an almost continuous series. The degree of interrelationship suggests that we are dealing with organisms having a common phylogeny but existing in a state of evolutionary flux. A definition of the family should prepare the student to deal with its subdivisions as arbitrary concepts, utilitarian in purpose, not as sharply defined, entirely homogeneous categories. The following description is suggested:

Gram-negative, non-sporogenic rods widely distributed in nature. Grow well on artificial media. All species attack glucose, forming acid or acid and visible gas (H_2 present). Characteristically, nitrites are produced from nitrates. When motile, the flagella are peritrichous. The family consists of innumerable, intergrading races, types or varieties, divisible into genera only because of the relative stability of certain characters which appear to represent "way-stations" along the path of evolution of this family, wherein the composite picture suggests an evolutionary trend toward commensal or parasitic existence, and is characterized by increasing specialization and the phenomenon of loss variation. Certain types must be regarded as transitional forms. Antigenic composition is best described as a mosaic which results in serological interrelationships among the several genera, even extending to other families.

PRINCIPAL OBJECTIONS TO CURRENT SYSTEM

Although the current system for subdividing the *Enterobacteriaceae* is a considerable improvement over previous ones, it has certain faults. Much confusion existing in the specialized fields is directly traceable to the taxonomic system.

The tribe *Eschericheae* is a case in point. Sanitary bacteriologists have for many years tried to find a suitable label for the organisms of the tribe which are useful in determining safety or quality of water, milk, etc., and have devised the term "coliform group." Obviously, the genera *Escherichia*, *Aerobacter* and *Klebsiella* intergrade so closely (cf Stuart, Griffin and Baker, 1938; Edwards, 1929; Osterman and Rettger, 1941) that generic distinctions become highly artificial. There can be little doubt that the term "coliform group" likewise includes certain forms commonly classified in still another tribe, the *Erwineae*. In fact, when many organisms listed in that tribe are subjected to tests commonly applied to coliform organisms without regard to source, the artificial nature of these tribal boundaries becomes apparent (Stanley, 1939; Elrod, 1942). Thus it appears that, into the classification of the lactose-fermenting forms of the family, the current system has introduced theoretical subdivisions which cannot be substantiated.

Perhaps the most striking example of the confusion resulting from inconsistencies in our taxonomic system is in the field of enteric diseases. In this field knowledge has progressed far beyond its practical application. Paracolon types, aerogenic and anaerogenic, are repeatedly mistaken for known pathogens and, conversely, when these organisms are properly recognized, they are too frequently dismissed as of no significance. Marked confusion is caused by the inclusion of dubious species in the genera *Shigella* and *Eberthella*. In fact, the

latter genus is productive of misconceptions by its very existence, since the worker must assume that there is a very close phylogenetic relationship between the typhoid organism and some dozen other *Eberthella* "species" which by modern standards cannot be distinguished from anaerogenic coliforms. Furthermore, practical diagnostic procedures are complicated by the inclusion of species of doubtful status in the genus *Shigella*. Insufficient stress is laid upon antigenic analysis in defining the genus *Salmonella* and too much stress is laid upon it in defining "species" within the genus. The very multiplicity of names used for the *Salmonella* types is a deterrent to progress in the field of medical bacteriology and particularly to recognition of the importance of these organisms in public health. The sections of the *Enterobacteriaceae* which include natural pathogens of man and animals require a revision which can be based upon practical considerations, fortunately consistent with phylogeny.

In general, further comment may be summarized by stating that delineation of "species" has been permitted to go so far that the taxonomic structure is becoming top-heavy and is in danger of falling of its own weight; that insufficient thought has been given to the possibility of using varietal designations or symbols of comparable rank; and that varietal terminology, when used as for *Salmonella*, has been used with little regard for its descriptive potentialities.

THE TAXONOMIC UTILITY OF DIFFERENTIAL CRITERIA WITHIN THE FAMILY

Before discussing specific recommendations concerning subdivisions within the family it is pertinent to comment briefly on differential criteria which have been used to show systematic relationships within the group. These may be included in the following categories: Source, habitat, pathogenicity, host specificity, morphology, staining affinities, colonial form, chromogenesis, biochemical activity, optimal growth temperatures, and antigenic structure.

The source of a given culture certainly should be included in any description of a new organism. It is of no intrinsic taxonomic value, however, unless the organisms cannot survive outside of that restricted environment. Too frequently, source is used as a synonym for habitat and, while the source of a culture is suggestive of its habitat, it may by no means represent the characteristic habitat for the species. The idea of restricted habitat is often based upon incomplete investigation and oftentimes overlooks the marked adaptability that a given organism may exhibit. In the *Enterobacteriaceae*, experience has shown that a restricted habitat is characteristic only for those forms which have become specialized for a parasitic existence. Commensals should be considered as having a restricted habitat only after it has been proved that they cannot compete with saprophytic forms in a free environment.

Association with disease is often confused with pathogenicity. Many factors are involved in infection, and part of the success of an organism in establishing infection is dependent upon properties of the host, specific or non-specific. Instances of infection with usually saprophytic species can often be explained by a peculiarity of the host such as a general "lowered resistance" which may be due to many causes. Pathogenicity thus may be shown at times by a variety

of commensal and saprophytic forms but, unless an organism is capable of attacking the normal healthy host, little taxonomic significance can be afforded to this property. In view of the frequency of subclinical and carrier infections by the proven pathogens in this family, even less taxonomic weight can be placed on the property of pathogenicity.

Host specificity among the pathogenic *Enterobacteriaceae* is more apparent than real. The typhoid organism and those forms of *Shigella* pathogenic for man are exceptions as well as certain of the animal strains of *Salmonella* such as *choleraesuis* and *pullorum*. The use of specific epithets which imply host specificity (e.g. *typhimurium*) when the organism manifests pathogenicity for a wide variety of hosts is unfortunate.

Morphological, staining and colonial characteristics have been, so far, of limited value in differentiating groups within the family. Occurrence of flagella, for instance, is useful for certain groups, but the number of exceptions restricts its value. Chromogenesis is shown to a marked degree only by one section of the *Enterobacteriaceae*.

Biochemical reactivity presents wide possibilities for bacterial taxonomy. It is possible in a taxonomic system to oversimplify by restricting the number of tests or to complicate to the point of absurdity by adding test after test. This is especially true of the *Enterobacteriaceae* which range in activity from the complex enzyme systems of *Aerobacter* to the relatively inert organisms such as *Shigella dysenteriae*. The extent of differentiation on biochemical grounds should be considered from the viewpoint of practical utility, and criteria selected which express the phylogenetic relationships within the family. Growth temperature is an important factor in evaluating biochemical reactivity.

Antigenic structure is an important basis for determining phylogenetic relationships. In the *Enterobacteriaceae*, except for one or two groups, little knowledge is available to the taxonomist. What is known about the antigenic relationships of the genus *Salmonella* to coliform types, for instance, is enough to show that antigenic structure must be used with reserve in attempting to define these genera.

In any system attempting to show the natural relationships between the various groups of the *Enterobacteriaceae* the result is, of necessity, a compromise. When one set of differential criteria is applied to a sufficiently representative group of cultures a continuous series of types can be assembled which appears to express the phylogeny of the family. With other criteria a quite different series may result. When, however, a series of criteria of practical utility results in a logical picture of readily apparent phylogenetic relationships, those criteria, weighted in the order of importance, provide the means of producing a usable taxonomic scheme.

THE GENERA OF THE ENTEROBACTERIACEAE

A subdivision of this family into tribes presupposes that we are prepared to make distinctions which become more and more precise as we progress to genera and thence to species. This does not seem desirable for two reasons: (1) The great majority of forms within the family have not been studied more than

superficially and (2) the most thoroughly studied forms (*Salmonella*) present such a complexity of possible subdivisions that confusion results when the most precise criterion (antigenic structure) is used for species differentiation. It therefore seems advisable to dispense with tribes and to progress directly to the genera which may be defined on the basis of a relatively few differential criteria.

The first possible grouping within the family may be made by separating the chromogenic from the non-pigmented types. There does not appear to be any reason for dividing the chromogenic types into more than one genus, *Serratia*.

Utilitarian reasons decree that non-chromogenic types shall be further differentiated. Avidity for lactose is a primary differentiating character which has the weight of tradition behind it. In addition, it is the basis for many practical procedures in the fields of sanitary and of diagnostic bacteriology. Procedures of practical and theoretical importance require consideration of more than the simple ability to ferment lactose or the lack of it. The production of gas and even the rate of its production are thus magnified in importance. Since the majority (and the most important) of the organisms in this family which are pathogenic for man and animals do not attack lactose, such forms must be differentiated whenever possible from those saprophytes which are likewise inactive. These requirements render the task somewhat difficult. However, we can set apart not only those forms which produce gas from lactose with relative rapidity but also certain entirely inactive forms which can be sharply defined, leaving a host of intermediate forms in a third group justified primarily by a need for further investigation.

There is more than the reason of practical utility for stressing the rate of lactose fermentation as an important differential characteristic. Examination of the characteristics of the family as a whole suggests that loss or retardation of the ability to split lactose marks one of the "way-stations" along the evolutionary path of these organisms—one of the transition points between commensal and parasitic existence. Some pathogens (including the Sonne dysentery organism and perhaps some of the paracolon group) retain a certain degree of their original lactose-splitting ability, and other pathogens, notably the *Salmonella* types, occasionally yield lactose-fermenting variants which apparently have regained a lost ancestral capability. Conversely, the generally accepted view that an organism which rapidly attacks lactose is non-pathogenic has proved to be so nearly correct that it is seldom questioned.

Before proceeding with a separation based upon avidity for lactose it is necessary to create an arbitrary standard by the application of which an organism may be classed as a rapid lactose fermenter. Some organisms ferment lactose readily between 25 and 30C whereas the reaction proceeds slowly or not at all at 37C. For such forms, our observations indicate that for practical purposes two days of incubation at the lower temperature are equivalent to one day at 37C for those having a higher optimal temperature. We therefore suggest that rapid fermentation of lactose be defined as fermentation within 24 hours at 37C or within 48 hours at 30C or below, depending upon the temperature found optimal for the organism under study.

Among the rapid lactose fermenters there is no practical need for more than

one genus. As pointed out previously, these forms intergrade so closely that any separation into genera is highly artificial. Merging of all these forms into one genus has the added advantage of making the more inclusive genus practically synonymous with the coliform group used in sanitary bacteriology.

Lactose fermentation by many forms is frequently delayed for many days. An organism which, however, *consistently* fails to ferment the sugar within 30 days either at 37°C or at a lower temperature more nearly optimal may safely be regarded as a non-fermenter and treated as such.

For clear-cut presentation to the student and for practical diagnostic work, the non-lactose-fermenters must be further differentiated. One group, *Proteus*, is clearly separable from the others upon the basis of hydrolysis of urea (Rustigian and Stuart, 1941, 1943). The remaining forms consist of *Salmonella*, *Shigella* and certain other organisms related in one or more ways to the "paracolons." Since it is possible to define *Salmonella* largely upon the basis of antigenic structure, and the majority of the pathogens are now listed as *Shigella* on the basis of both biochemical and serological characteristics, these two groups may be sharply circumscribed.

There remains to be considered a large group of organisms, most of which are poorly defined at present. These include late lactose fermenters which are aerogenic, the anaerogenic lactose fermenters, and those non-lactose-fermenters which are excluded by definition from *Proteus*, *Salmonella* and *Shigella*. A primary division of these forms into a group producing gas from carbohydrates and a group entirely anaerogenic seems the only practical step which can be taken at present. A very few species are clearly described and well-known, including one pathogen of man, the dysentery bacterium of Sonne.

This view of the *Enterobacteriaceae* enables us to develop a workable key to the genera of the family into which many forms will fit which today occupy an uncertain position. We propose the following:

- I. Ordinarily chromogenic at 25–30°C, producing pink, rose, red or orange-red pigment, but occasionally non-pigmented. Genus I *Serratia*
- II. Non-chromogenic; infrequently, types or variants show yellow to orange pigment.
 - A. Lactose fermented with visible gas formation within 24 hr at 37°C or within 48 hr at 25–30°C Genus II *Colobactrum*
 - B. Lactose not fermented within 30 days either at 37°C or at 25–30°C (Infrequent variants serologically identical with normal strains ferment lactose)
 1. Urea decomposed within 48 hr Genus III *Proteus*
 2. Urea not decomposed within 48 hr
 - a. Major somatic antigens of S forms or flagellar antigens of at least one phase identical with those of the Kauffmann-White schema Genus IV *Salmonella*
 - aa. Major somatic antigens of S forms not identical with those of the Kauffmann-White schema; flagellar antigens lacking (non-motile); do not produce H₂S or acetylmethylcarbinol; do not utilize citrate; very rarely reduce trimethylamine oxide; inocula of young cells from broth cultures do not produce acid and usually do not grow in glucose broth at 45°C Genus V *Shigella*
 - C. Lactose fermented slowly (occasionally not at all); intermediate or transitional forms not conforming to those described above
 1. Glucose fermented with formation of visible gas Genus VI *Paracolobactrum*

2. Anaerogenic in all carbohydrates

a. Salicin fermented

Genus VII *Erwinia*

aa. Salicin not fermented

Genus VIII *Proshigella*GENUS I *Serratia* Bizio *emend* Breed and Breed

(Bizio, Biblioteca italiana o sia Giornale de lettera, scienze ed arti, **30**, 288, 1823;
Breed and Breed, Zentr. Bakt. Parasitenk., II, **71**, 435, 1927)

The description given in Bergey's Manual, 5th ed., needs little change. It is suggested that reference be made to the effect of temperature upon pigment production since incubation at a temperature near 37 C often fails to demonstrate pigment which is produced in abundance at temperatures of 25–30 C. The emended description would then read as follows:

Small, aerobic, rapidly liquefying, nitrate-reducing, gram-negative, non-sporogenic, peritrichous rods which produce pink, rose, red or orange-red pigment at 25–30 C (occasionally non-pigmented). Coagulate and digest milk. Liquefy blood serum. Typical species produce CO₂ and frequently H₂ from glucose and other carbohydrates; also acetic, formic, succinic and lactic acids, acetylmethylcarbinol and 2,3 butylene glycol. The type species is *Serratia marcescens* Bizio.

The description of species as listed in the Manual seems to provide a satisfactory scheme of differentiation. No change is suggested at present. Studies of antigenic relationships may prove of taxonomic significance.

GENUS II *Colobactrum* *gen nov*

(From the Greek, large intestine and rod)

Establishment of a genus, *Colobactrum*, to include the majority of forms now listed under *Escherichia* and *Aerobacter* as well as some included in *Klebsiella* and *Erwinia* is suggested. It has been shown (Stuart *et al.*, 1938; Parr, 1939) that the coliform group is composed of a series of forms intergrading biochemically in such fashion that recognized generic boundaries are not clear-cut. Hence, the terms *Escherichia* and *Aerobacter* no longer have significance. In fact, this genus has been anticipated by the work of Stuart *et al.* (1940, 1943b) who suggested a single genus.

Much the same may be said for the rapidly aerogenic forms listed as *Klebsiella* and *Erwinia*, genera which are delineated primarily upon the ground of pathogenicity although there is no sure evidence that most organisms listed in these genera are not "free-living" forms which invade their "hosts" only under favorable conditions. These forms appear to be in the process of evolution from saprophytic to commensal or parasitic existence, a trend apparently evident throughout the family. For the present, therefore, no benefit is gained from giving them generic status which cannot be just as logically achieved by regarding them as species or varieties.

The name *Colobactrum* is suggested for the entire group of rapid lactose fermenters to avoid the confusion which would result from the retention of one of the generic names in current use because of the special significance now attached to them. Thus the authors consider older names (*Klebsiella*, *Aerobacter*, *Escherichia*) *nomina ambigua* for the genus. As pointed out previously,

this genus will to all intents and purposes be synonymous with the broad "coli-form group" used in sanitary bacteriology. A description follows:

Aerobic, non-sporogenic, gram-negative, short rods. Fermentation of glucose and lactose with formation of visible gas characteristically occurs within 24 hr at 37 C or within 48 hr at 25–30 C. Widely distributed in nature as saprophytes; some forms commensals or parasites of plants and animals.

The type species is *Colobactrum aerogenes* (Kruse) *comb nov.*

The type species given above has been selected because it may be regarded as most nearly like the progenitor of the group when considered from an evolutionary viewpoint which recognizes a probable retrograde direction of evolution within the family. This principle will be invoked for the other new genera to be discussed.

No useful purpose can be served by establishing specific status for each biochemical type possible within this genus. Furthermore, a common antigenic pattern is not discernible within any one biochemical type (Stuart *et al.*, 1940; Wallick and Stuart, 1943). It seems advisable, therefore, to rely upon those well-established criteria which will serve as a nucleus about which the specialist may arrange his varieties. The production of acetylmethylcarbinol and the utilization of citrate appear to be significant. Based upon these two criteria, we suggest a maximum of three species as follows:

I. Acetylmethylcarbinol produced.

1. *Colobactrum aerogenes* (Kruse) *comb nov.* (In Flüge, Die Mikroorganismen, **2**, 340, 1896)

II. Acetylmethylcarbinol not produced.

- A. Citric acid utilized as sole source of carbon.

2. *Colobactrum freundii* (Braak) *comb nov.* (Thesis (Delft), 140, 1928).

- B. Citric acid not utilized as sole source of carbon.

3. *Colobactrum coli* (Migula) *comb nov.* (In Engler and Prantl, Natürlichen Pflanzenfamilien, **1**, 27, 1895).

GENUS III *Proteus* Hauser

(Sitzber. physik. med. Sozietät Erlangen, 156, 1885)

Although there has been considerable disagreement over the criteria for the genus *Proteus*, Rustigian and Stuart (1941, 1943) have shown that ability to hydrolyze urea in the medium they have described is the most constant characteristic of the genus. The following description is suggested:

Aerobic, non-sporogenic, gram-negative, somewhat pleomorphic, proteolytic rods. Generally actively motile at 25 C; motility may be weak or absent at 37 C; flagella peritrichous when present. Motile forms produce characteristic swarming colonies on moist media. Ferment glucose but not lactose with the formation of acid and usually visible gas; gas volumes small even after prolonged incubation. Most forms attack sucrose, some slowly. One species ferments mannitol. Hydrolysis of urea marked and rapid (within 48 hours).

The type species is *Proteus vulgaris* Hauser.

There does not appear to be any sound reason for retaining many forms now assigned to this genus. The following key to the species of the genus is suggested:

- I. Urea hydrolyzed in 8 to 12 hours at 37 C
 - A. Mannitol not fermented
 - 1. *Proteus vulgaris* Hauser (Sitzungsber. d. phys.-Med. Sozietät zu Erlangen, 156, 1885)
 - B. Mannitol fermented (usually acid only)
 - 2. *Proteus rettgeri* (Hadley *et al.*) Rustigian and Stuart (Hadley, Elkins and Caldwell, R. I. Agr. Exp. Sta. Bull. 174, 169, 1918; Rustigian and Stuart, Proc. Soc. Exptl. Biol. Med., 53, 241, 1943)
- II. Urea hydrolyzed less rapidly, but within 48 hours.
 - 3. *Proteus morganii* (Winslow *et al.*) Rauss. (Winslow, Kligler and Rothberg, J. Bact., 4, 429, 1919; Rauss, J. Path. Bact., 42, 183, 1936)

GENUS IV *Salmonella* Lignières

(Rec. med. vet., Ser. 8, 7, 389, 1900)

Before discussing the genus *Salmonella* it is first necessary to discuss certain forms now included in *Eberthella* and *Shigella*. The only justification for retention of the genus *Eberthella* is the typhoid organism, since the other forms listed are incompletely described and of doubtful significance, even of doubtful identity. Clearly, the typhoid organism is a *Salmonella* in spite of anaerogenic fermentation of carbohydrates or of marked host specificity. The inclusion of the Sendai type among the species of *Eberthella* was not justified since it is normally an active gas producer. The status of *gallinarum* as a species of *Shigella* is likewise unsatisfactory since it is clearly a *Salmonella*. Anaerogenic *Salmonella* organisms are by no means rare.

Kauffmann (1941) has recently included certain coliform antigens in his antigenic schema of the *Salmonella* group. This has little practical value in a schema which was originally designed to serve as an aid to the practical bacteriologist. Such trends should be discouraged even though it is recognized that there is a considerable overlapping of antigens among coliforms and *Salmonella*. Nevertheless, the antigenic analysis of White (1926) as extended by Kauffmann (1941), herein called the Kauffmann-White schema, provides a means of circumscribing the *Salmonella* group which makes its use in a taxonomic scheme essential. We therefore propose the following description:

Aerobic, non-sporogenic, gram-negative rods, the majority motile by means of peritrichous flagella. Except for infrequent variants, do not attack lactose, sucrose or salicin and do not produce indole or acetylmethylcarbinol. Liquefaction of gelatin is confined to a few types. Do not hydrolyze urea within 48 hours. Glucose is fermented, usually with the formation of visible gas. Mannitol and arabinose are fermented and H₂S is produced by nearly all varieties. All are parasites of warm-blooded animals although invasiveness is frequently manifested only in young hosts. The carrier state is frequent in adults. Motile forms may or may not show natural phase variation with respect to flagellar antigens. Somatic antigens of smooth forms and flagellar antigens are characteristic of those described in the Kauffmann-White schema. Certain varieties warrant specific designation because of host specificity. The type species is *Salmonella choleraesuis* Weldin.

The delineation of species within this genus presents a difficult problem. Certain species such as *Salmonella choleraesuis* and *Salmonella typhosa* are so well established by tradition and usage that their preservation becomes almost mandatory if any revision is to gain wide acceptance. However, continued separation into species upon the basis of antigenic structure creates confusion which is detrimental to the practical application of knowledge of this important group of pathogens. Biochemical distinctions are likewise confusing. Simplification of specific designations must be effected and will still permit the specialist to designate varieties for epidemiological purposes by names or symbols. The following plan is proposed:

- I. Somatic antigen VII of the Kauffmann-White schema present in S cultures accompanied, when motile, by flagellar antigens c or 5 or both; arabinose not fermented.
 1. *Salmonella choleraesuis* Weldin (Iowa State Coll. J. Sci., 1, 155, 1927)
- II. Somatic antigen IX of the Kauffmann-White schema, sometimes masked by the presence of Vi antigen, present in S cultures; motile forms are monophasic and are characterized by flagellar antigen d; anaerogenic.
 2. *Salmonella typhosa* (Zopf) White. (Zopf, Die Spaltpilze, 3 Aufl.: 126, 1885; White, J. Hyg., 29, 443, 1930)
- III. Major somatic antigens of S cultures identical with one or more described in the Kauffmann-White schema, or flagellar antigens of at least one phase identical with flagellar antigens of strains possessing such somatic antigens.
 3. *Salmonella kauffmannii* sp nov. (Named for F. Kauffmann)

The species *S. choleraesuis* is retained principally to provide a well delineated type species. Otherwise it might well be considered a variety of *S. kauffmannii*.

The specialist and the epidemiologist will find the bare designation, *Salmonella kauffmannii*, inadequate to describe all *Salmonella* types except *S. choleraesuis* and *S. typhosa*. However, the use of that term, when accompanied by suitable varietal designations, is no more clumsy, and is considerably less confusing, than the use of more than one hundred and forty specific or pseudospecific names which are of no significance with respect either to host specificity or to geographical distribution. The term in no way invalidates the recognition of the various types as biologic entities.

The circumscription of *Salmonella kauffmannii* may delay the incorporation of an occasional type into the genus until a connecting link is found. A similar situation obtained for some time with regard to the Cerro type (XVIII; z₄ . . . , —) until the Duesseldorf type (VI, VIII; z₄ . . . , —) was described. This does not appear to be a particularly serious objection.

GENUS V *Shigella* Castellani and Chalmers

(A Manual of Tropical Medicine, 936, 1919)

The genus *Shigella* as currently constituted includes a number of forms which cause enteric infection of man, others of doubtful pathogenic significance, some species incompletely described and at least one form probably not a member of the family. Much confusion results in practical diagnostic work. It is proposed that this genus be restricted to the non-lactose-fermenting *Shigella* organ-

isms which cause dysentery in man. This proposal is based in part upon studies by Wood *et al.* (1943a, 1943b) and by Stuart *et al.* (1942, 1943a). The following description will narrowly circumscribe this genus:

Aerobic, non-sporogenic, gram-negative, non-motile rods, anaerogenic in glucose. (Infrequently, variants antigenically identical with normal forms are micro-aerogenic.) Do not grow on bismuth sulfite agar. Uniformly do not form acetylmethylcarbinol or H₂S, ferment lactose, utilize citrate, hydrolyze urea; very rarely reduce trimethylamine oxide. Inocula of young cells from broth cultures do not produce acid and usually do not grow in glucose broth at 45 C. Part of the antigens of certain varieties may be identical with those of the genus *Salmonella* but in no case are the major antigens of an S form of this genus identical with those of the Kauffmann-White schema. In nature, parasites of man.

The type species is *Shigella dysenteriae* (Shiga) Castellani and Chalmers.

The type species given above is cited because of priority only. We believe that *S. paradysenteriae* (Collins) Weldin would make a much more descriptive type species.

The following description of species is suggested:

- I. Mannitol not fermented
 - A. Indole not produced
 - 1. *Shigella dysenteriae* (Shiga) Castellani and Chalmers (Shiga, Zentr. Bakt. Parasitenk., I Abt., 24, 817, 1898; Castellani and Chalmers, A Manual of Tropical Medicine, 936, 1919)
 - B. Indole produced
 - 2. *Shigella ambigua* (Andrewes) Weldin (Andrewes, Lancet, 194, 560, 1918; Weldin, Iowa State Coll. J. Sci., 1, 177, 1927)
- II. Mannitol fermented
 - 3. *Shigella paradysenteriae* (Collins) Weldin (Collins, J. Infectious Diseases, 2, 620, 1905; Weldin, Iowa State Coll. J. Sci., 1, 178, 1927)

This scheme omits from the genus lactose-fermenting strains (such as *S. sonnei* and those in the *S. dispar* group) and, among others, Andrewes' *S. alkalescens*. In our opinion these are all more readily classified in the proposed genus *Proshigella*. There will probably arise soon the need for designating varieties in the non-mannitol-fermenting group but it is as yet too early to determine the place of recently described organisms (Sachs, 1943; Christensen and Gowen, 1944; Gøber *et al.*, 1944).

There is need in this genus, as in *Salmonella*, for consistent and concise use of a varietal terminology. The studies of Boyd (1938, 1940) and of Wheeler (1944a) suggest antigenic structure as the logical basis.

GENUS VI *Paracolobactrum* gen nov

(From the Greek, near or resembling *Colobactrum*)

The term "paracolon" has come to occupy an established place in the literature as a result of the inadequacies of taxonomic systems. There is no doubt that many of these forms are allied to *Colobactrum* and others to *Salmonella*. There is good evidence that some are pathogenic for man and animals. The

"paracolon" forms deserve generic rank (Stuart *et al.*, 1943b) if only to direct attention to this group and to enable those who work with them to talk a common language, a difficulty previously discussed by Stuart, Mickle and Borman (1940). The following description is proposed for the classification of those aerogenic strains excluded from other genera in the key previously given:

Aerobic, non-sporogenic, gram-negative rods characterized by *consistently* delayed fermentation of lactose (occasionally negative). Glucose is fermented with formation of visible gas. Certain forms attack carbohydrates characteristically at 25–30 C but not at 37 C. Antigenic relationships to other genera in the family are common, even with respect to major antigens.

The type species is *Paracolobactrum aerogenoides* *sp nov.*

There is as yet no way of determining the relative importance of biochemical characters, antigenic structure, habitat, or pathogenicity in the classification of organisms grouped here. For the present, we suggest that a plan similar to that used for *Colobactrum* be used and that an earnest attempt to apply varietal names be made when further subdivision is required by the specialist. The following species are suggested:

- I. Acetylmethylcarbinol produced. (Other characteristics as for the genus.)
 - 1. *Paracolobactrum aerogenoides* *sp nov.*
(Latinized, resembling *aerogenes*).
- II. Acetylmethylcarbinol not produced.
 - A. Citric acid utilized as sole source of carbon.
(Other characteristics as for the genus.)
 - 2. *Paracolobactrum intermedium* *sp nov.*
(From the Latin, intermediate.)
 - B. Citric acid not used as sole source of carbon.
(Other characteristics as for the genus.)
 - 3. *Paracolobactrum coliforme* *sp nov.*
(Latinized, resembling *coli*.)

GENUS VII. *Erwinia* Winslow *et al.*

(J. Bact., 2, 560, 1917)

Until the relationships of the anaerogenic plant pathogens of the family are more thoroughly investigated this genus should be retained. Those forms appear to be differentiated from those organisms herein termed *Proshigella* by their ability to split salicin. The following definition is proposed:

Gram negative, non-sporogenic, aerobic rods pathogenic for plants. Produce acid but not gas from glucose and salicin; may or may not attack lactose. Generally motile by means of peritrichous flagella.

The type species is *Erwinia amylovora* (Burrill) Winslow *et al.*

Two species have been described. Since one of these has not been available to the authors, the continued separation of these forms largely upon the basis of host specificity is recommended. Thus the species of this genus may be described as follows:

- I. Nitrates not reduced to nitrites but occasionally to ammonia.
 1. *Erwinia amylovora* (Burrill) Winslow *et al.* (Burrill, Illinois Indust. Univ., 11th Rept: 142, 1882; Winslow *et al.*, J. Bact., **5**, 209, 1920)
- II. Nitrates reduced to nitrites.
 2. *Erwinia salicis* (Day) Chester (Day, Oxford For. Mem., **3**, 14, 1924; Chester in Bergey *et al.*, Manual of Determinative Bacteriology, 5th ed: 406, 1939)

GENUS VIII *Proshigella* gen nov(From the Latin, before or prior to *Shigella*)

Certain anaerogenic organisms, some of importance, have been excluded from preceding genera. This group contains some forms sufficiently far along in the evolutionary chain, and sufficiently well stabilized, to justify a departure from the differential criteria used for *Colobactrum* and *Paracolobactrum*. For the present it is sufficient to list as species those forms which are important because of prevalence or of pathogenicity. The following is a generic description:

Aerobic, non-sporogenic, gram-negative rods. Glucose is fermented anaerogenically. Lactose may or may not be attacked. Salicin not fermented. This genus is reserved for forms, apparently transitional, which do not conform to the descriptions of other genera of the *Enterobacteriaceae*.

The type species is *Proshigella dispar* Andrewes *comb nov*.

This genus, like *Paracolobactrum*, becomes a receptacle for forms not conforming to narrower categories. However, the genus, while one of convenience, is given a certain dignity by the inclusion of well-known species. The organisms which may in the future be allocated to this genus are, also like *Paracolobactrum*, deserving of thorough study. It is hoped that the proposed genus will direct attention to the need for such study. A suggested list of species follows:

- I. Indole produced
 - A. Lactose fermented, usually late
 1. *Proshigella dispar* (Andrewes) *comb nov*. (Lancet, **194**, 560, 1918)
 - B. Lactose not fermented
 2. *Proshigella alkalescens* (Andrewes) *comb nov*. (Lancet, **194**, 560, 1918)
- II. Indole not produced
 - A. Lactose fermented, usually late
 3. *Proshigella sonnei* (Levine) *comb nov*. (J. Infectious Diseases, **27**, 31, 1920)

Re-establishment of the species *S. dispar* in this genus seems advisable. Reasons for splitting this species into two or more do not appear adequate at present (Carpenter, 1943) except in so far as the Sonne dysentery organism is concerned.

The status of the species now known as *Shigella equirulis* is somewhat in doubt. If that organism is to be retained in the family *Enterobacteriaceae* it belongs in this genus but should be treated as a *species inquirenda*.

Forms not conforming to the species listed above are not uncommon. Several such species now listed in the genera *Eberthella* and *Shigella* are so inadequately described that no useful purpose will be served by assigning them a specific rank.

Newly encountered forms should be studied thoroughly before admission as named species.

CIRCUMSCRIPTION OF SPECIES

The outline presented above is a plan for the logical presentation of differential characteristics. It does not, however, adequately circumscribe certain species. A sufficiently detailed description of each species, supplementing the key, should be given whenever ambiguity might otherwise result. This is particularly true of *Shigella dysenteriae* and the several *Proshigella* species. Advantage should be taken of recent serological investigations which afford a knowledge of the antigenic structure of *Proshigella alkalescens* (Stuart *et al.*, 1943c) and *P. sonnei* (Wheeler, 1944b). Descriptions of morphological, tinctorial, colonial and biochemical characteristics should, however, be kept to the minimum required for clarity.

SUB-SPECIFIC NAMES OR SYMBOLS

In the taxonomy of the *Enterobacteriaceae* the bacteriologist is faced with a problem no less complex than that which confronts anyone attempting to catalogue the varieties of certain higher plants such as those of the genus *Rosa*. If we wish to secure a certain kind of hybrid tea rose, we ask for it using an informal name, such as "Talisman," which serves in place of a more formal varietal designation. The specialist in roses, amateur or professional, has no great difficulty in determining what is wanted even though the name is not at all a descriptive one. Surely, epithets of varietal rank, whether formally constructed or not, can be used to the same advantage by bacteriologists.

The analogy of our problem to that of the roses does not mean that we should adopt a similar nomenclature, as in fact we have for the *Salmonella* group. Our terms should be more descriptive. Each should convey the idea of at least one distinctive peculiarity of the variety so designated.

The genus *Salmonella* offers many opportunities for the use of sub-specific epithets or symbols, some referring to biochemical characteristics but most to antigenic structure. The term "*Salmonella* sp (Eastbourne type)" or, as more commonly used, "*Salmonella eastbourne*" or even the term *Salmonella kauffmannii* var. *eastbournei* conveys little of descriptive value. We suggest that symbols designating the essential antigenic structure be appended to the binomial of this type as follows: *S. kauffmannii*: IX: eh: 5. In the case of an indole-forming variant of this type, the following is fully descriptive: *S. kauffmannii* var. *indologenes*: IX: eh: 5. A similar convention could be followed for the other types but it is conceivable that tradition and usage might sanction the use of somewhat different terms in special cases, such as *S. kauffmannii* var. *pullorum* instead of *S. kauffmannii*: IX: —: — or of *S. kauffmannii*: IX: 0.

The short manner of designating the antigenic variety of *S. kauffmannii* cited as an example would be sufficient for the student but would not convey in every case sufficient information for practical bacteriological or epidemiological work. For example, the presence or absence of an antigen not listed in the shortened

formula is often of practical importance. In such cases a more nearly complete antigenic formula would be used. For example, the presence or absence of somatic antigens I and V in different races of the organism now known as *Salmonella typhimurium* is of epidemiological importance (Edwards and Bruner, 1940) and would not be shown in the conventional designation proposed, *S. kauffmannii*: IV: i: 3.

The bacteriophage types of *Salmonella typhosa* may be treated in a similar manner. The terms *S. typhosa* W and *S. typhosa* Vi (A) are intelligible to the specialist.

The studies of Boyd (1938, 1940) and of Wheeler (1944a) have made possible the use of antigenic symbols for the varieties of *Shigella paradyseae*. Preliminary work suggests that a similar scheme will be found applicable to the non-mannitol-fermenting *Shigella*.

In using symbols to describe antigenic structure some uniform system must be used if confusion is to be avoided. It is highly important that the use of the same letters or numerals to designate different antigens be permitted only if the intent is clear. For example, the designations used for antigenic varieties of *Colobactrum* and *Paracolobactrum* by Kauffmann should be clarified. The somatic antigens XXXI, XXXII and XXXIII and the flagellar antigens z_{20} , z_{21} , z_{22} and z_{26} should be assigned new symbols *applicable only to the genus (or to the species) to which the respective varieties should be assigned*. In instances where an antigen already described and labeled for one species or genus is found in an organism of another group the antigenic formula for the second organism should not give rise to ambiguity. For example, the coliform organism (*Salmonella coli* 4) described by Kauffmann as having the antigenic formula IV; XXVII; XII: z_{21} would be better described by the term *Colobactrum freundii*: SaIV, SaXXVII; SaXII: a. The abbreviation "Sa" used for each of the several somatic antigens is suggested as a means of identifying those antigens with *Salmonella* antigens in contradistinction to others which may be described for the biochemical group to which this organism belongs. A similar convention may be used when flagellar antigens of the *Salmonella* or other group occur in another genus. The symbol "a" as used here does not refer to the *Salmonella* H antigen so designated. It is used simply to illustrate that the flagellar antigen of this variety should be given a symbol applicable either to the genus *Colobactrum* or to the species *freundii*, whichever seems the better choice.

GENERAL COMMENT

The system outlined above is not presented with any thought of advancing it as a permanent solution to taxonomic and diagnostic problems within the *Enterobacteriaceae*. That it has weaknesses is freely admitted, but those weaknesses serve to point out the fields in which investigations to augment our incomplete knowledge must be made before further progress can be attempted. For instance, adverse criticism may be directed against the reliance placed upon gas production. The authors, however, are convinced that it is important for the present to adhere in this respect to procedures which are simply applied and

readily observed. A static taxonomic system is not desirable so long as new knowledge accumulates, particularly knowledge of phylogenetic significance best exemplified by understanding of antigenic structure. The outline is given in the hope that it will be recognized as presenting a logical scheme whereby specialists in many fields may more effectively pool their knowledge toward a common end, whereby the student may receive proper orientation before specializing and whereby the practical viewpoint may be reconciled with the phylogenetic viewpoint without sacrificing the more important elements of either. The proposal is made with the hope that it will stimulate others to use it as a point of departure for more fundamental taxonomic research.

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