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# THE COLON-TYPHOID GROUP OF BACTERIA AND RELATED FORMS. RELATIONSHIPS AND CLASSIFICATION*

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THE classification and nomenclature of the organisms of the so-called "intestinal" or "colon-typhoid" group of bacteria are still in an unsatisfactory state. There are included in the group a large number of species of economic importance. This has led to an intensive study of a number of individual organisms, but comparatively few attempts have been made to formulate a complete classification. Among such attempts should be mentioned those of Castellani and Chalmers (1919), who outlined a classification and described a number of species, and of Bergy *et al* (1923, 1925), who have attempted with a measurable degree of success to bring together descriptions of species listed in the literature and to differentiate them. A general review of the group has been given by Winslow, Kligler and Rothberg (1919) and a dichotomous key to most of the species has been prepared by Weldin and Levine (1923).

The present paper embodies a review of the literature on classification of the group, as well as the results of studies of a large number of organisms particularly of the colon subsection. Brief descriptions of some species are given. Keys to their classification and discussion of the validity of the names are particularly stressed. It is expected that this paper will be followed by a more extensive one which will more adequately monograph the group.

## DESIGNATION OF THE GROUP

The first question which arises in a discussion of the generic designation to be used is whether the group should be considered as a single genus (with or without subgenera) or whether sufficient differences exist among the various subgroups to warrant the recognition of a number of genera. There seems to be at the present time considerable diversity of opinion with regard to this question. The committee on classification of the American Society of Bacteriologists (1920) included two genera, *Proteus* and *Bacterium,* in the tribe *Bactereae.* Castellani and Chalmers (1919) distributed the organisms generally recognized as being in the group among 14 genera. Weldin and Levine (1923) included in the tribe *Bacterieae* two genera, *Bacterium* (not known to be plant pathogens) and *Erwinia* (known to be plant pathogens). The former was then subdivided into six subgenera. The classification of Bergey *et al* (1923) is similar to that outlined by Weldin and Levine (1923), except they discarded the generic name *Bacterium* and recognized the six subgenera as of generic rank, adding the genus *Encapsulatus* of Castellani and Chalmers.

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There seems to be little doubt but the colon-typhoid series of organisms constitutes a natural group, and the various species are closely related. In most cases proposed subdivisions of the group have been upon the basis of physiological characters. The propriety of using such characters in the differentiation of genera has been questioned (as by Hall, 1926), though apparently universally accepted for the separation of species. The group, however, contains such a large number of species that some method of subdivision is highly desirable. Such subgroups may be characterized with a considerable degree of satisfaction. The difficulty facing the student is whether to recognize a single genus with subgenera, or to recognize a group of genera.

If the entire group is to be included in a genus, the choice of a generic name is difficult. Strangely enough, apparently no new generic name has ever been proposed for the group as a whole. The only name which has found any degree of general acceptance is *Bacterium*. There is good reason to regard this name as invalid for this group under any strict interpretation of the Code of Nomenclature. It could, of course, be validated by international agreement, if thought desirable. (See discussion of *Bacterium*, p. 128.

There is an increasing tendency, particularly in America (especially since the publication of Bergey's Manual), to recognize a group of genera. This is the procedure here adopted. However, in the discussion of each species, the alternative name, if included in the genus *Bacterium*, is given. It is believed that in course of time the recognition of a number of genera will be common practice.

## CHARACTERISTICS OF THE GROUP

This group contains a large number of species and varieties, many of which are normally found in the intestinal tract of man and animals; others are found in a variety of habitats. The organisms are all Gram-negative, non-spore-forming short rods. They are rarely found in chains. Involution forms are not uncommon. Some species are motile, the flagella when present being peritrichic. They all grow well upon ordinary laboratory media. The metabolism is complex, amino-acids being utilized and generally carbohydrates. Most of the species produce acid from carbohydrates and some form gas composed mostly of $CO_2$ and $H_2$.

It should be noted the line of demarcation between this and some other groups of bacteria is not distinct. Especially is this true with *Pasteurella* forms, whose distinguishing feature is ordinarily given as bipolar staining. Many of the organisms of the colon-typhoid group will, on occasion, exhibit bipolar staining though perhaps not so distinctly as the true *Pasteurella* species. While the members of the intestinal group are not ordinarily considered as pigment formers, some strains, particularly of the colon subsection, exhibit yellow pigmentation even on agar slants.

The following characteristics have been found useful in differentiation of the members of the group: Motility, capsule formation, liquefaction of gelatin, fermentation of carbohydrates, acetyl-methyl-carbinol production, production of indol and hydrogen sulphide, ultilization of uric acid as the sole source of nitrogen and citric acid as the sole source of carbon, behavior in litmus milk, agglutination and agglutinin-absorption reactions, and pathogenicity.

## PROPOSED GENERIC AND SUBGENERIC NAMES NOT RECOGNIZED

1. *Actinobacter.* This name was proposed by Duclaux in 1882 for a group of milk bacteria. The first species named was *Actinobacter polymorphum.* The genus was recognized as valid by Maggi (1886). No species have been included in the genus other than the type. From the description it would seem that the organism was probably related to the species usually termed *Aerobacter aerogenes* or *Bacterium aerogenes,* (the *Bacillus lactis aerogenes* of many authors). As Buchanan (1925) observes, it is doubtful if the type species could ever be determined from its descriptions and it would, therefore, be inadvisable to accept as valid a genus with such an uncertain type species. It may be considered as a possible synonym of the genus *Aerobacter.* To accept it as adequately described would cause it to replace *Aerobacter* as generic designation.

2. *Alkaligenes.* A variant spelling of *Alcaligenes* used by Castellani and Chalmers (1919).

3. *Balkanella.* Used by Castellani and Chalmers (1919) for the seventh genus of their tribe *Ebertheae.* Definition: *Ebertheae* which ferment glucose completely with the production of acid and gas; lactose not fermented. Milk clotted. Type species: *Balkanella coagulans* Castellani, 1916.

Only three species were listed. The genus has not been recognized by other authors.

The clotting of milk would hardly seem to be of sufficient importance to warrant separation of this group from the genus *Salmonella.*

4. *Brucella.* Meyer and Shaw (1920) proposed this name for a genus to include the organisms causing Malta fever and infectious abortion of cattle. They did not give a generic diagnosis for the genus and considered only the two organisms mentioned.

Evans (1923) discussed the name in detail. She pointed out that Castellani and Chalmers had previously described a genus which they called *Alcaligenes,* whose definition would include the *melitensis-abortus* group, although they did not discuss these organisms. She was, herself, of the opinion that a generic distinction should be made between *Alcaligenes* and *Brucella* on the ground that the organisms included in the former are characteristically saprophytes, while the *Brucella* types are characteristically tissue invaders. The latter are also slightly different morphologically, being somewhat smaller and producing great numbers of coccoid cells. She gave a general description of *Brucella melitensis,* the type species for the genus.

The morphological dissimilarities between *Brucella* and *Alcaligenes* are hardly definite enough to serve in themselves for differentiation. It is true that as regards habitat and pathogenicity, *Br. abortus* and *Br. melitensis* are distinct from the *Alcaligenes* organisms. Bacteriologists have in general avoided these characters for generic differentiation. It should be noted that Evans would include in the genus an organism (*Bacillus abortus* var. *lipolyticus* Evans 1916), which she isolated from freshly drawn cow's milk and for which no pathogenicity is recorded. On the whole, it is felt that no sufficiently conclusive reasons for the creation of a genus *Brucella* have yet been advanced. The organisms suggested as belonging to it will be considered as members of the genus *Alcaligenes.*

5. *Cloaca.* Castellani and Chalmers (1919) used the name *Cloaca* for the second genus of their tribe *Proteae,* a tribe distinguished from the rest of the non-spore-forming, Gram-negative rods by not producing pigment and by liquefying gelatin. The genus was defined as: "Slow gelatin liquefiers; ferment lactose; Gram-negative." Type species was *Cloaca cloacae* (Jordan, 1890). Only one other species (*Cloaca levans*) was listed.

Weldin and Levine (1923) included these species in their subgenus *Acrobacter* and Bergey *et al* (1923) in the genus *Aerobacter.*

Since liquefaction of gelatin by these organisms is frequently slow and relatively difficult to recognize, and since their characters conform to the definition of the genus *Aerobacter,* it seems best to include them with the latter.

6. *Dysenteroides.* Used by Castellani and Chalmers (1919) for the fourth genus of their tribe *Ebertheae.* Definition: *Ebertheae* partially fermenting glucose and lactose, with the production of acid, but no gas. Milk not clotted. Type species: *Dysenteroides metadysentericus.*

The genus has not been recognized by other authors.

All motile forms which ferment glucose with acid but not gas are sufficiently related to be in a single genus. To break up this group into three genera on the basis of acid production from lactose and clotting of milk is considered unwarranted. Castellani and Chalmers differentiated the genus *Dysenteroides* from their genus *Eberthus* on its ability to produce acid from lactose and further, from their genus *Lankoides* by its inability to coagulate milk. These characters are considered inadequate for generic differentiation. The members of this genus will be included here among those of the genus *Eberthella.*

7. *Eberthus.* The name given by Castellani and Chalmers (1919) to the second genus of their tribe *Ebertheae.* It was described as follows: *Bacillaceae* motile, partially fermenting glucose with the production of acid but no gas. Lactose not fermented. Milk not clotted. Type species: *Eberthus typhosus* (Zopf, 1885).

This definition conforms in large part to that given for the *Eberthella* group; the type species used is the same. What has been said with regard to *Dysenteroides* applies equally well here. The genus *Eberthus* is included, therefore, in the genus *Eberthella,* which has priority.

8. *Encapsulatus.* This term was first used by Castellani and Chalmers (1919) for the single genus of their tribe *Encapsulateae.* Its characters were those of the tribe, which were given as follows:

> *Bacillaceae* growing well on ordinary laboratory media without endospores, neither fluorescent nor chromogenic aerobes, not liquefying gelatin, possessing capsules in animal tissues.

The type species of their genus was *Encapsulatus pneumoniae* (Friedlaender, 1883). Besides their type species they included two other organisms, *Encap. acidi lactici* and *Encap. lactis aerogenes.*

Bergey *et al* (1923) recognized both the tribe *Encapsulateae* and the genus *Encapsulatus,* with a somewhat modified characterization, but with capsule formation as the significant character for differentiation from other tribes and genera. Their type species was *Encapsulatus pneumoniae.* They did not, however, include either *Encap. acidi lactici* or *Encap. lactis aerogenes* in the genus.

Perkins (1925) is strongly in favor of a genus *Encapsulata* to include *Bacillus aerogenes, B. duodenale* (*B. acidi lactici*), *B. pneumonicum* (*B. pneumoniae*), and *B. rhinoscleromae,* all of which he says are constant capsule formers.

Considerable difficulty is foreseen in the use of capsules as a character for generic differentiation. Capsule formation is often demonstrated only with difficulty. The writer knows of no organism which constantly produces evident capsules on all media on which the organism grows. Many of the so-called capsule-formers produce their capsules only when grown in the animal body or upon some special medium such as milk or serum media. *Escherichia acidi lactici,* for example, is listed by some authors—notably by Perkins and by Castellani—as producing easily demonstrated capsules. The original descriptions by Hueppe and by Grotenfelt make no mention of capsules, nor do any of the earlier descriptions. MacConkey (1905), a recognizedly careful worker, who considered capsule formation an important character, studied a Kral transfer of the organism and did not anywhere indicate its ability to produce capsules. Perkins (1904) studying a Kral transfer emphasized the presence of capsules. As both of these investigators worked at about the same time, and with presumably the same culture from the Kral laboratory, the natural conclusion is that capsule formation for this organism is at best an indistinct and unreliable characteristic.

By some authors, capsules are considered a purely morphological characteristic of certain organisms, the capsule being considered a thickened cell wall. Buchanan (1924) says, "a thin capsule or layer of capsular material is present in most bacteria, but in only a few does it become very thick and conspicuous." There is a possibility that almost any organism may be made to develop thick capsules if grown under proper conditions. The argument for capsule formation as a basis of differentiation on the ground that capsules are a morphological character thus becomes a contention for the employment of the relative thickness of the cell wall as a fundamental criterion for generic differentiation. If, on the other hand, we take the viewpoint that capsules are a physiological product of certain organisms, a response to certain chemical stimuli, the character possesses no advantage over other more constant and more easily determined physiological characters. In any case, in order to make the character of value, a certain set of conditions should be defined under which all the so-called capsule formers regularly produce capsules and all others of the intestinal group not included in the genus, do not produce them. Such a set of conditions has not been defined to date.

Further, there is considerable evidence that with proper technique all or nearly all the organisms of the colon-typhoid series may be shown to be capsule formers. Marrasini (1913) described a capsulated typhoid bacillus. Fletcher (1918) found mucoid types of *Bact. aertrycke* and of the paratyphoid B organism. Recently Cooper (1925) has demonstrated capsules on cells of "*Bacillus typhosis, B. coli, B. enteritidis, B. dysenteriae-Shiga, B. paratyphosus A, B. paratyphosus B, B. fecalis-alkaligenes, B. proteus-vulgaris* and *B. cloacae.*"

Indeed, there seem to be no other reasons for grouping the capsulated forms together other than that they have capsules; they do not have a common source, do not agree as to pathogenicity, nor as to their ability to attack certain carbohydrates. It would seem to be fully as logical and much

safer to include them with the subgroups to which they are most clearly related on the basis of other characters.

If the genus were retained its name should be changed to *Klebsiella*. This name was used by Trevisan in 1885 for a genus based upon the same type species as *Encapsulatus*.

9. *Enteroides*. Castellani and Chalmers (1919) use the term *Enteroides* for the ninth genus of their tribe *Ebertheae*. They describe it as follows:

> *Ebertheae* which ferment glucose and lactose completely with the production of acid and gas. Milk not clotted. Type species: *Enteroides entericus* (Castellani 1907).

The only character which differentiates this genus from their genus *Escherichia* is the inability to clot milk, a character which perhaps needs some explanation in view of the statement that lactose is completely fermented with production of acid and gas. Clotting of milk is often dependent to considerable extent upon the treatment which the milk has undergone before inoculation and sometimes occurs only when the cultures are heated. It is felt that this character is unreliable, at least without qualifications, for a differential character for genera or subgenera.

The members of the genus *Enteroides* of Castellani and Chalmers will be included here in the genus *Escherichia*.

10. *Erwinia*. Winslow *et al* (Committee S. A. B., 1917) used this term for a generic name with the following description:

> Plant pathogens. Growth usually whitish, often slimy. Indol generally not produced. Acid usually formed in certain carbohydrate media, but as a rule no gas.

The type species was later (1920) given as *Erwinia amylovora* (Burrill) Committee.

Weldin and Levine (1923) recognized the genus, separating its members from those of the genus *Bacterium* solely on the basis of pathogenicity for plants, *Erwinia* species being designated as known plant pathogens. Both genera were included in one tribe, *Bactereae*.

Bergey *et al* (1923, 1925) retained the genus, but placed it in the tribe *Erwinieae* with the genus *Phytomonas*, the latter being non-motile or motile with polar flagella, while *Erwinia* species are motile with peritrichous flagella. The only characters given by Bergey *et al* which serve to differentiate *Erwinia* from their tribe *Bacterieae* are those of habitat and pathogenicity.

Pathogenicity is an unreliable and difficult character to use for differentiation. The information at hand indicates that the members of this genus are very closely related to the colon-typhoid group; some of them, perhaps, should be included in that group. Unfortunately, however, the emphasis has been placed by students of the plant pathogens on a different set of characters for these organisms than those used for the intestinal bacteria and it is practically impossible at present to definitely locate them with the latter. Undesirable as pathogenicity may be considered for a differential character, its use is practically unavoidable until further work has been done on the plant pathogens.

Since pathogenicity, then, is to be considered a primary character for subdivision, it appears the logical procedure should be to group these organisms with the other plant pathogens, as Bergey *et al* (1923) have done.

Many of these latter—polar flagellates, pigment producers, etc.—are evidently more closely related to other tribes than to the *Bactereae*. The most expedient plan, then, is to keep them, temporarily at least, in a separate tribe (*Erwinieae*), with the idea that they may be later redistributed to the tribes and genera to which their morphological, cultural and physiological characters more properly allocate them.

11. *Flexnerella.* Castellani and Chalmers (1919) divided their genus *Shigella* into two subgenera, *Flexnerella,* fermenting mannitol, and *Shigella,* not fermenting mannitol. The mannitol-fermenting organisms, associated with bacillary dysentery, do form a comparatively distinct group on the basis of their serological and physiological activities and might be considered a subgenus of the genus *Shigella.* It is felt that mannitol fermentation is not adequate to warrant subdivision into genera.

12. *Graciloides.* Castellani and Chalmers (1919) used this name for the single genus of the tribe *Graciloideae.* The characters were those of the tribe, which were as follows:

*Bacillaceae* growing very slowly and scantily on ordinary and blood media, without endospores or capsules; neither fluorescent nor chromogenic. Type species of the genus: *Graciloides albofaciens* Castellani, 1904. Two species, *albofaciens* and *tardus*, are recognized.

Relative vigor of growth can hardly be considered a reliable character for differentiation, at least unless the composition and character of the medium used is definitely stated. This is, however, the only distinguishing character of the tribe and genus. It is significant to note that Castellani and Chalmers have included the descriptions of the two organisms of this genus in the table entitled "Aerobic asporogenous intestinal bacilli." Evidently they belong with the colon-typhoid series.

The genus is considered insufficiently characterized to warrant its retention. The species listed as belonging to it by Castellani and Chalmers will be included here in the genus *Shigella.*

13. *Klebsiella.* Trevisan (1885) first used this name for a genus with the type species, *Klebsiella crouposa* (*Bacterium pneumoniae-crouposae* Zopf).

Since the pneumobacillus of Friedlander is the type species, this name, as pointed out by Buchanan (1925) has priority over the term *Encapsulatus* used by Castellani and Chalmers (1919) and Bergey *et al* (1923) with the same type species. Bergey *et al* (1925) substituted *Klebsiella* for *Encapsulatus.*

For the reasons discussed under the heading of *Encapsulatus,* this genus is not recognized here.

14. *Lankoides.* Used by Castellani and Chalmers (1919) for the fifth genus of the tribe *Ebertheae.* Their description is as follows:

*Ebertheae* fermenting glucose partially with the production of acid, but no gas; lactose not fermented or only partially, without gas production. Milk clotted. Type species: *Lankoides pyogenes* (Passet, 1902).

What has been said with regard to the genus *Dysenteroides* of Castellani and Chalmers may be said of *Lankoides.* Like the former, it will be

rejected and the organisms listed as belonging to it included among those of the genera *Eberthella* and *Shigella.*

15. *Wesenbergus.* Used by Castellani and Chalmers (1919) for the eighth genus of their tribe *Ebertheae.* It was defined as follows:

> *Ebertheae* which ferment glucose completely and lactose partially, producing acid, but no gas. Milk not clotted. Type species: *Wesenbergus wesenbergi* Castellani, 1913.

Three species were included.

It has been the experience of most bacteriologists that organisms of the colon-typhoid group which attack glucose with production of gas produce gas from all other carbohydrates attacked. Sometimes the amount of gas produced is very small and may even be absorbed by the liquid. Nevertheless, if the proper medium be used, the gas production can be demonstrated. It seems best, therefore, until further work is reported on these organisms, to include them with members of the genera *Salmonella, Proteus,* etc.

KEY TO GENERA

a. Fermenting glucose with the production of acid or acid and gas.
  b. Acid and gas formed from glucose.
    c. Gas formed from lactose.
      d. Acetyl-methyl-carbinol not produced from glucose; reversion of reaction in 0.5 percent glucose-phosphate, peptone solution negative or very slow; generally not able to utilize uric acid as the sole source of nitrogen nor citric acid as the sole source of carbon.....Genus 1. *Escherichia.*
      dd. Acetyl-methyl-carbinol produced from glucose; reversion of reaction in 0.5 percent glucose-phosphate-peptone relatively rapid; generally able to use uric acid as the sole source of nitrogen or citric acid as the sole source of carbon .....Genus 2. *Aerobacter.*
    cc. Gas not formed from lactose.
      d. Gas formed from sucrose.....Genus 3. *Proteus.*
      dd. Gas not formed from sucrose.....Genus 4. *Salmonella.*
  bb. Acid but not gas formed from glucose.
    c. Motile .....Genus 5. *Eberthella.*
    cc. Non-motile .....Genus 6. *Shigella.*
aa. Neither acid nor gas produced from glucose....Genus 7. *Alcaligenes.*

Genus *BACTERIUM* Ehrenberg em. Orla-Jensen.

SYNONYMY. In case it should be decided to include the "colon-typhoid" group in a single genus with the name *Bacterium,* all the generic names discussed in this paper would be considered synonyms in part.

Buchanan (1925) has thoroughly and adequately discussed the validity of the use of the name *Bacterium.* His conclusion is that there is, at present, no strictly valid definition of a genus *Bacterium* with a recognizable type species. Ehrenberg (1828) first used this generic name, naming the single species *Bacterium triloculare.* His description of the organism, how-

ever, was inadequate for its later recognition. Breed, Conn and Baker (1918) suggested that the name be dropped. Buchanan (1925) seemed inclined to agree. Castellani and Chalmers (1919) and Bergey *et al* (1923) have followed the suggestion and split the colon-typhoid group into a number of genera, for none of which have they used the name *Bacterium*.

The Committee, S. A. B. (1920), however, retained the name including it in their list of names recommended for adoption as approved genera. Their description of the genus was as follows:

*Bacterium* Ehrenberg 1828, emended Orla-Jensen (1909), p. 315. Gram-negative, evenly staining rods. Often motile, with peritrichic flagella. Easily cultivable, forming grape-vine leaf or convex whitish surface colonies. Liquefy gelatin rarely. All forms except *Bact. alcaligenes* and the *Bact. abortus* group attack the hexoses and most species ferment a large series of carbohydrates. Acid formed by all, gas ($CO_2$ and $H_2$) only by one series. Typically intestinal parasites of man and the higher animals although several species may occur on plants and one (*Bact. aerogenes*) is widely distributed in nature. Many species pathogenic.

Type species, *Bact. coli* Escherich 1885, p. 518.

The reason advanced by the committee for recommending the adoption of this generic name as well as a number of others, was that, "it seems desirable to preserve in this way a number of generic names which have come into such general use that their abandonment would cause confusion, particularly in dealing with the large number of medical bacteriologists who are not familiar with the principles of botanical taxonomy." An opinion has already been expressed with regard to the advisability of including the whole colon-typhoid group in a single genus. The term *Bacterium* apparently is the only name which has been suggested for such a genus. The name is familiar, and in the minds of a great many bacteriologists, especially since the Committee Report (1920) has, along with the genus *Proteus*, stood for the group of bacteria under discussion. General use has tended more than ever to establish the name and it is possible that its abandonment may result in more bacteriologists using the term *Bacillus* for all rod-shaped organisms regardless of their other characters, morphological or physiological.

Strict adherence to the rules of botanical nomenclature, however, apparently would make it incorrect to use the term without formal adoption by some authoritative body such as an international Botanical Congress. If such were done, and *Bacterium* approved, the diagnosis for the amended genus *Bacterium* to include the whole of the "colon-typhoid" group might well be as follows:

*Bacterium* Ehrenberg, 1828, emended Orla-Jensen, 1909.

*Gram-negative, non-spore-forming rods growing well on artificial media. Generally forming acids from carbohydrates and often gas. Non-motile or motile by means of peritrichous flagella. Generally found in the intestinal tract of man and the higher animals; some species widely distributed in nature.*

Type species: *Bacterium coli* Escherich, 1885.

Genus 1. *ESCHERICHIA* Castellani and Chalmers, 1919.

SYNONYMY: *Aerobacter* (in part) Beijerinck, 1900; *Enteroides* Castellani and Chalmers, 1919.

Castellani and Chalmers (1919) used this for the tenth genus of their tribe *Ebertheae*, characterizing it as follows:

*Ebertheae* which ferment glucose and lactose completely; milk clotted. Type species: *Escherichia coli* (Escherich, 1886).

This definition would include all of the colon subgroup of the colon-typhoid series with the exception of those organisms which do not coagulate milk. These they placed in the genus *Enteroides*. (See discussion of *Enteroides*, p. 120).

Weldin and Levine (1923) split the colon subgroup or lactose-fermenters into two subgenera, *Escherichia* and *Aerobacter*. Their description of the former was essentially as follows:

Members of the genus *Bacterium* fermenting glucose and lactose with acid and gas. Acetyl-methyl-carbinol not produced from glucose (Voges-Proskauer negative); reversion of reaction in 0.5 percent glucose-phosphate-peptone solution negative or very slow (methyl red reaction positive); not able to utilize uric acid as a source of nitrogen.

Bergey *et al* (1923) used the term for a genus with the following characterization:

Motile or non-motile rods, commonly occurring in the intestinal canal of normal animals. Attack carbohydrates forming acid and frequently acid and gas. Do not produce acetyl-methyl-carbinol. Type species: *Escherichia coli* (Escherich) Castellani and Chalmers.

It is to be noted that in their key to the genera of the tribe *Bactereae*, *Escherichia* is distinguished by gas production in dextrose and lactose, as well as lack of ability to produce acetyl-methyl-carbinol from dextrose. Their complete description, therefore, agrees essentially with that of Weldin and Levine.

We have here a distinct section of the intestinal group of organisms with correlated characters clearly differentiating it from the rest of the colon-typhoid series and making it worthy of generic recognition. The name *Escherichia* is valid and may properly be used for this section.

**Generic Diagnosis:** *Motile or non-motile, Gram-negative, non-spore-forming rods fermenting glucose and lactose with both acid and gas. Do not produce acetyl-methyl-carbinol from glucose (Voges-Proskauer negative); reverse the reaction in 0.5 percent glucose-phosphate-peptone solution very slowly or not at all (methyl red reaction positive); generally not able to utilize uric acid as the sole source of nitrogen. Gelatin not liquefied. Pathogenicity usually slight.*

**Type species:** *Escherichia coli* (Escherich) Castellani and Chalmers, 1919.

KEY TO SPECIES OF THE GENUS *ESCHERICHIA*

a. Neither acid nor gas formed from sucrose.
  b. Motile.
    c. Acid and gas formed from dulcitol.
      d. Acid and gas formed from salicin; indol produced.
        1. *Escherichia coli.*
      dd. Neither acid nor gas formed from salicin; indol not produced.
        2. *Escherichia vekanda.*
    cc. Neither acid nor gas formed from dulcitol.
      3. *Escherichia grünthali.*

bb. Non-motile.

c. Acid and gas formed from dulcitol and salicin.

4. *Escherichia enterica.*

cc. Neither acid nor gas formed from dulcitol nor salicin.

d. Acid and gas formed from adonitol.

5. *Escherichia acidi-lactici.*

dd. Neither acid nor gas formed from adonitol.

6. *Escherichia vesiculosa.*

aa. Acid and gas formed from sucrose.

b. Motile.

c. Acid and gas formed from dulcitol.

7. *Escherichia communior.*

cc. Neither acid nor gas formed from dulcitol.

8. *Escherichia pseudo-coloides.*

bb. Non-motile.

c. Acid and gas formed from dulcitol and salicin.

9. *Escherichia neapolitana.*

cc. Neither acid nor gas formed from dulcitol nor salicin.

d. Indol produced.

10. *Escherichia coscoroba.*

dd. Indol not produced.

11. *Escherichia astheniae.*

SPECIES OF *ESCHERICHIA*

1. *Escherichia coli* (Escherich) Castellani and Chalmers, 1919.

Alternative: 1. *Bacterium coli* (Escherich) Lehmann and Neumann, 1896.

SYNONYMY: *Bakterium coli commune* Escherich, 1885; *Bacillus cavicida* Flügge, 1886; *Bacillus C* Booker, 1887; *Bacillus Escherichii* Trevisan, 1889; *Bacillus Schafferi* von Freudenrich, 1890; *Bacillus coli communis* Sternberg, 1892; *Bacillus coli* (Escherich) Migula, 1895; *Bacterium cavicida* (Brieger) Migula, 1900; *Aerobacter coli* Beijerinck, 1900; *Bacillus coli verus* Ford, 1901; *Bacillus coli communis verus* Durham, 1901; *Bacillus mustelae septicus* Matzuschita, 1902; *Bacillus communis* (Escherich) Jackson, 1911; *Escherichia shaefferi* (von Freudenrich) Bergey *et al*, 1923; *Escherichia cavicida* (Brieger) Castellani and Chalmers, 1919.

This organism was first described by Escherich (1885) under the name *Bakterium coli commune*. He isolated it from the stools of children. He described it as a short rod 1-5$\mu$ long by 0.3-0.4$\mu$ wide, with rounded ends, single or in pairs, motile and without spores. It stained well with anilin dyes and was Gram-negative. On gelatin it showed involution forms. It formed white, spreading surface colonies on gelatin and yellowish sub-

surface colonies. On agar and serum the growth was white and on potato yellowish and spreading. Gelatin, agar and serum were not liquefied. It produced sufficient acid in milk to produce coagulation and it fermented dextrose. It was pathogenic for guinea pigs and rabbits when injected intravenously and for guinea pigs when injected subcutaneously.

In a later and more detailed description (1886) he stated that it would grow anaerobically on glucose, but not in milk nor in lactose solution. Its motility was here described as slow and pendulum-like, "langsam pendelnde Bewegungen." Migula (1895) described it as having peritrichous flagella though not as many as the typhoid bacillus and in his System der Bakterien (1900) stated that organisms having polar flagella cannot be included in the species.

Migula (1895) noted that "*B. coli* (Escherich)" is undoubtedly a collective species—"Sammelspecies"—which will be separated into its component species as their individual characters are more fully determined. This has been the case; as the use of physiological characters for differentiation have come into use, the species has become more restricted. However, the essential characters used by Escherich have not been changed and the organism may be properly considered as the type species for the group.

*Bacillus schafferi* von Freudenreich. This organism was first described and named by von Freudenreich (1890). He isolated it from spoiled cheese and from potatoes. It corresponded culturally and morphologically with Escherich's *Bact. coli commune* except that it was a little smaller. It was definitely motile; did not show spores and had little resistance to desiccation, heating or the action of antiseptics. It produced acid and gas from "sugars," and grew anaerobically. Milk was not coagulated, though some acid was found in it. It was not pathogenic for guinea pigs. Von Freudenreich distinguished it from Escherich's organism on the basis of its smaller size, *greater motility,* ability to grow anaerobically in sugar solutions and its lack of pathogenicity.

McConkey (1909) listed *Bacillus schafferi* as being *non-motile* and many authors have followed his lead in this respect. Bergey *et al* (1923) listed it as motile, differentiated from *Esch. coli* by its failure to coagulate milk, a questionable characteristic for specific differentiation.

The original description was not very complete and did not sufficiently differentiate the organism from *Esch. coli.* Later descriptions have added little of value. The name is considered a synonym of *Esch. coli* (Escherich) Castellani and Chalmers.

*Bacillus cavicida* Flügge. This organism as originally described by Flügge (1886) is practically identical with *Esch. coli* (Escherich) both morphologically and culturally. It was isolated by Brieger (1884) from human faeces. Castellani and Chalmers (1919) described it as not fermenting maltose; there may be some question as to this character since they list it as producing acid and gas from dextrin. There does not seem to be sufficient reason for identifying it as a definite species.

**Specific Diagnosis:** ***A motile rod 0.4 to 0.6μ broad by 1 to 2μ long, conforming to the generic diagnosis. Dulcitol and salicin are fermented with acid and gas production; sucrose and adonitol are not attacked. Indol is produced. Litmus milk becomes acid and coagulated, but is not peptonized. Commonly found in the intestinal tract of man and vertebrates generally. Intestinal pathogenic varieties rare. Sometimes pathogenic when associated with other organisms.***

2. *Escherichia vekanda* (Castellani) Bergey *et al*, 1923.

Alternative: 2. *Bacterium vekanda* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus vekanda* Castellani, 1917; *Enteroides vekanda* (Cast.) Castellani and Chalmers, 1919.

This organism was isolated by Castellani from cases of enteroidea and appendicitis encountered in the Balkans. It was described as a member of the colon-typhoid series; its physiological characters were given in comparative detail. Castellani and Chalmers placed it in their genus *Enteroides*, which was separated from *Escherichia* by inability to clot milk. There may be some skepticism with regard to this character (see discussion of *Enteroides*); an organism which attacks lactose as well as a number of other carbohydrates with production of acid and gas ordinarily would be expected to produce sufficient acid to coagulate milk, although whether it does or does not often depends upon the treatment of the milk before or after inoculation.

Perkins (1925) feels the species should not be recognized, inasmuch as it has been described only by Castellani. This contention, however, is not valid, for a number of investigators, including Savage (1907), Nicoll (1911), Buchan (1910), Stewart (1917) and Azzi (1917), have described organisms which are apparently identical with Castellani's *vekanda*.

**Specific Diagnosis:** *A motile rod, similar to Escherichia coli in its morphological and cultural characters; conforming to the generic diagnosis. Dulcitol, salicin and adonitol are fermented with acid and gas; sucrose is not attacked. Indol is not produced. Litmus milk becomes acid. Found in the intestines.*

3. *Escherichia grünthali* (Morgan) Castellani and Chalmers, 1919.

Alternative: 3. *Bacterium grünthali* (Morgan) Weldin and Levine, 1923.

SYNONYMY: Das Grünthaler Bacterium, (?) Fischer, 1902; *Bacillus Grünthal* Morgan, 1905; *Bacillus acidi lactici* var *grünthali* Levine, 1918; *Escherichia para-Grünthali* Castellani and Chalmers, 1919; *Bacterium coli* var. *para-Grünthali* Weldin and Levine, 1923.

Fischer in 1902 isolated from "liver paste" and "liver wurst" which had caused some cases of food poisoning a bacterium, "welches morphologisch sowie culturell von dem *Bacterium coli commune* nicht zu unterschieden war, aber bei der Verfütterung an Mäuse den Tod derselben unter den Erscheinungen der hamorrhagischen Enteritis herbeiführte." While Fischer's description is not very detailed as to cultural and physiological characters, his organism would seem to be more closely related to the *enteritidis* type than to the colon organism, both because of its apparent pathogenicity and its reversion of the reaction of milk from a definite acidity to a marked alkalinity. Fischer, however, did not name the organism, simply designating it as "das Grünthaler Bacterium."

Morgan (1905) gave in tabular form the characters, presumably, of the same organism, which he calls "*B. Grunthal.*" He described it as a motile bacillus, producing on agar and gelatin a creamy, raised, moist, translucent growth, not liquefying gelatin and producing a general turbidity in broth. Indol was produced, acid and curd in milk and acid and gas in glucose, lactose, mannitol, but not in saccharose nor in dulcitol. Mac-

Conkey (1906) added more carbohydrates to the list attacked and still others have been added by subsequent authors.

The species is adequately characterized and has been recognized by most recent systematists. It may easily be differentiated from *Esch. coli* by its inability to ferment dulcitol.

*Escherichia paragrunthali* Castellani and Chalmers. This organism as described by Castellani and Chalmers is almost identical with *Escherichia grunthali* so far as the characters of the two organisms are known. They make the statement that it differs from the latter in fermenting maltose. However, in their description of the Grunthal organism, and in all available descriptions by other authors, including Castellani (1912), no statement is made as to the behavior of the organism in maltose one way or the other. It would be rather startling if *Esch. grunthali,* which is able to produce acid and gas from dextrin, should fail to attack maltose.

Weldin and Levine (1923) used the term *paragrünthali* for a non-motile variety of *Bacterium coli;* Bergey *et al* (1923) used it for a species of *Escherichia.*. While it differs but slightly from *Esch. coli,* it differs still less from *Esch. grünthali,* in neither case being sufficiently distinct to warrant specific rank. It will be considered synonymous with *Escherichia grünthali.*

**Specific Diagnosis:** *A motile rod, similar in its morphological and cultural characters to Escherichia coli, and conforming to the generic diagnosis. Does not produce acid or gas from sucrose, salacin, dulcitol or adonitol. Indol is produced. Litmus milk is coagulated. Originally isolated from liver paste and liver wurst. Frequently found in the intestines of man and animals.*

4. *Escherichia enterica* (Castellani and Chalmers) Comb. nov.

Alternative: 4. *Bacterium entericum* (Castellani and Chalmers). Comb. nov.

SYNONYMY: *Bacillus coli immobilis* Kruse, Flügge, 1896; *Bacterium coli immobilis* Chester, 1897; *Bacillus Schafferi* MacConkey, 1909; *Bacillus entericus* (Castellani) Castellani and Chalmers, 1910; *Enteroides entericus* (Castellani) Castellani and Chalmers, 1919; *Escherichia Schaefferi* (MacConkey) Castellani and Chalmers, 1919; *Bacillus coli* var *immobilis* Winslow, Kligler and Rothberg, 1919; *Bacterium* (*Escherichia*) *schafferi* Weldin and Levine, 1923; not *Bacillus entericus* Ford, 1903; *Eberthella enterica* (Ford) Bergey *et al,* 1923.

This organism was first described by Kruse (1896) under the name *Bacillus coli immobilis.* The only significant difference listed between it and Escherich's *Bacterium coli commune* was the lack of motility. Kruse's name for the organism was trinomial and should be discarded. *Bacterium immobile* was used by Chester (1901) for *Bacillus fluorescens-immobilis* Kruse (1896).

MacConkey (1909) described an organism practically identical with *Escherichia coli* except for motility, giving it the name *Bacillus schaefferi.* This name is invalid because of the previous use for another organism by Kruse. As has been stated before (See *Escherichia coli*), *Bacillus schaefferi* von Freudenreich was distinctly motile. This name cannot be used for the organism under question.

Castellani and Chalmers (1910) used the term *Bacillus entericus* for a non-motile species, otherwise corresponding to *Escherichia coli.* Later

(1919), the name was changed to *Enteroides entericus* and additional description given. Although Ford in 1903 used the name *Bacillus entericus* for an entirely different species, his name does not invalidate *Escherichia enterica* or *Bacterium entericum.*

**Specific Diagnosis:** *A non-motile rod, otherwise similar to Escherichia coli. Dulcitol and salicin are fermented with acid and gas production; sucrose and adonitol are not attacked. Indol is produced. Its normal habitat is the intestinal tract of man and animals.*

5. *Escherichia acidi lactici* (Grotenfeldt) Bergey *et al,* 1923.

Alternative: 5. *Bacterium grotenfeldtii* Migula, 1900.

SYNONYMY: *Bacterium acidi lactici I,* Grotenfeldt, 1889; *Bacillus acidi lactici* (Hueppe) Morgan, 1905; *Encapsulatus acidi lactici* (Hueppe) Castellani and Chalmers, 1919; *not* "Die Milchsaurebacterien" Hueppe, 1884; *Bacterium acidi lactici* Zopf, 1883, 1884; *Bacillus acidi lactici* Zopf, 1885.

Hueppe (1884) has been cited by many as the authority for the species name *acidi lactici.* While the organism which Hueppe isolated from sour milk and described in exceptional detail for his time conforms in most of its characters to the definition of the genus *Bacterium,* his descriptions showed very definitely that he was working with a spore former. In the cells in unstained mounts Hueppe observed, "stark lichtbrechenden" bodies; these could not be stained with his dyes and, he says, resisted heating. "Dass diese stark lichtbrechenden Körperchen, welche keinen Farbstoff annahmen, wirkliche Sporen waren, konnte dann nicht weiter ermittelt werden dadurch, dass kurzes Aufkochen, welches zum Vernichten der lebensfähigen Bacillen ausreicht, nicht genugte alles Leben in diesen Zuckerlösungen zu vernichten, sondern dass sich aus derselben wieder die unzweifelhaften Milchsäurebacillen entwickelten." Zopf (1885), Schroeter (1889), DeToni and Trevisan (1889), Kramer (1892), Sternberg (1892), Migula (1895, 1900) have all, quoting Hueppe, listed spore formation as a character of this organism. Migula (1895) was the first to describe it under the generic name *Bacterium.* Chester (1901) and Lehmann and Neumann (1896), though giving Hueppe as authority for the name, have omitted any reference to spore production, and practically all authors since 1901 have followed their example. If the term *acidi lactici* is to be applied to the organisms described by Hueppe and we assume that he was working with a pure culture, his *Bact. acidi lactici* must be considered to be a spore former.

Hueppe, however, did not use the term *acidi lactici* at all; he referred to his lactic acid producing organisms only by the term "die Milchsaurebakterien."

Apparently the first use of the species name *acidi lactici* was by Zopf (1883, 1884) for an imperfectly described milk-souring organism which he called *Bacterium acidi lactici.* It was isolated from sour milk, sauerkraut, beer mash, old cheese, sugar solutions, etc. Its description is too brief to permit of its later recognition. The organism showed rods, chains and spherical forms. It was a lactic acid producer, but grew best at 50° C., an optimum growth temperature too high for a member of the colon-typhoid group. It is probable that Zopf either had an impure culture or a member of the *Lactobacillus* group. A few later systematists (DeToni and Trevisan, 1889; Chester, 1901; Frost, 1903) gave Zopf credit for the

name *acidi lactici*, but their descriptions followed that of Hueppe. Zopf later (1885) used the name *Bacillus acidi lactici* for Hueppe's "milchsaure-bakterien," which he said was different from his own *Bacterium acidi lactici*.

Grotenfeldt in 1889 described an organism which he isolated from sour milk and which he called *Bacterium acidi lactici* I. The organisms were short rods, 1.0-1.4$\mu$ long by 0.3-0.4$\mu$ wide, non-motile and non-spore forming. On gelatin plates they produced porcelain-like, white, glistening, round colonies; on agar, a whitish-yellow, thick, pulp-like layer, with gas bubbles; on potato, a grey or greyish-yellow layer. They were facultative anaerobes. In milk, lactic acid was produced, casein precipitated, gas ($CO_2$) and alcohol formed. Gelatin was not liquefied.

This description, as far as it goes, is satisfactory for the organism now commonly known as *Bact. acidi lactici*. Winslow, Kligler and Rothberg (1919) cite Grotenfeldt as the author of the specific name *acidi lactici* for non-spore-forming, short rods. We likewise find a *Bacterium acidi lactici* Grotenfeldt in Eisenberg (1891), Kramer (1892) and Chester (1897, 1901). Migula (1900) changed the name to *Bacterium Grotenfeldtii*.

Zopf's prior use of the combination prohibits *Bacterium acidi lactici* being used for Grotenfeldt's organism. It, however, does not invalidate the use of *Escherichia acidi lactici* (Grotenfeldt) Bergey. This would seem to be the proper designation of the species under discussion, with the alternative of *Bacterium Grotenfeldtii* Migula.

Castellani and Chalmers (1919) list this organism in their genus *Encapsulatus*. Whether Hueppe or Grotenfeldt is considered as having first described the species, neither of the authors listed capsule formation as one of its characters, nor have any subsequent authors with the exception of Perkins (1904) and Castellani and Chalmers (1919). It is very probable that capsules are produced, this characteristic being quite common for many if not all members of the "colon-typhoid" group under suitable conditions, but the fact that very few of the authors who have used the term noted capsule formation would indicate that this characteristic is not evident under ordinary conditions.

**Specific Diagnosis:** *A non-motile rod, 0.3 to 0.4$\mu$ broad and 1.0 to 1.4$\mu$ long, conforming to the generic description. It produces acid and gas from adonitol but fails to ferment sucrose, salicin or dulcitol. Indol is produced. Litmus milk is acidified and coagulated. Has been isolated from milk, cheese and faeces.*

6. *Escherichia vesiculosa* (Henrici) Castellani and Chalmers, 1919.

Alternative: 6. *Bacterium vesiculosum* Henrici, 1894.

SYNONYMY: *Bacillus vesiculosus* MacConkey, 1909; *Escherichia vesiculosa* (Henrici) Bergey *et al*, 1923.

Henrici (1894) described an organism which he isolated from cheese under the name *Bacterium vesiculosum*. It was a short rod, single or in pairs, sometimes in short chains, non-motile. Growth on gelatin was slimy and dirty-white in color, later becoming brown. On agar the growth was slimy, smooth and glistening. Broth was turbid, with a white sediment and gas production. The organism was aerobic and facultative. There is nothing in the description which might serve to identify the organism on later isolation.

MacConkey (1909) adopted the name for an organism isolated by him from various sources and gave a comparatively complete description of its physiological characters. Perkins (1925) states that in so doing the later author is ascribing characters to an organism which it might or might not have had. That is true enough. However, MacConkey finding his organism identical with that of Henrici so far as the characters are given by the latter, assumed that they were the same and ascribed additional characters to the species. These characters then became amplifications or emendations of the original. This is a logical and recognized procedure and the emended description may be properly accepted as valid for the species in question providing no pure cultures of the original strain have been preserved and proved to have other characters.

**Specific Diagnosis:** *A non-motile rod, conforming to the generic description. Sucrose, dulcitol, salicin and adonitol are not attacked. Indol is formed. Acid and curd are formed in milk. The organism was first isolated from cheese, but is common in the intestinal tract.*

7. *Escherichia communior* (Durham) Bergey *et al*, 1923.

Alternative: 7. *Bacterium communior* (Durham) Holland, 1920.

SYNONYMY: *Bacillus coli communior* Durham, 1901; *Bacillus communior* Ford, 1903; *Bacillus paraentericus* (Cast.) Castellani and Chalmers, 1910; *Bacillus pseudo coli* Castellani, 1912; *Escherichia pseudo-coli* Castellani and Chalmers, 1919; *Bacterium coli communior* (Durham) LeBlaye and Guggenheim, 1914; *Escherichia meta-coli* Castellani and Chalmers, 1919; *Escherichia pseudo-coliformis* Castellani and Chalmers, 1919; *Enteroides para-entericus* (Cast.) Castellani and Chalmers, 1919.

Durham (1901) isolated a coli-like organism from animal faeces, which he called *Bacillus coli communior*. He says of it, "Characters and morphology like those of group *Bacillus coli communis verus* except that sucrose is fermented and acid freely formed from it. Mutual serum reactions not frequently met with within the group. I am inclined to think that this is a commoner inhabitant of human faeces than members of the last group, but have not made any direct experiments. Should this prove to be the case, it might be distinguished from the Escherich type as *B. coli communior*."

Subsequent investigators have agreed with Durham as to the existence of a large group of organisms in faeces, differing from *Bact. coli* of Escherich in ability to attack sucrose and the species has been generally accepted. Durham's name, however, being a trinomial, was not valid. It was shortened to *Bacillus communior* by Ford (1903).

**Specific Diagnosis:** *Morphologically and culturally like Escherichia coli. Motile. Sucrose and dulcitol fermented with acid and gas production; adonitol not attacked. Indol is formed. Litmus milk is acidified and coagulated. Found in the intestines of man and animals.*

8. *Escherichia pseudo-coloides* Castellani and Chalmers, 1919.

Alternative: 8. *Bacterium pseudo-coloides* (Castellani and Chalmers) Weldin and Levine, 1923.

According to Castellani and Chalmers (1919) this organism is identical with their *Escherichia pseudo-coli* (see *Esch. communior*) except that it fails to ferment dulcitol. Weldin and Levine (1923) included the organ-

ism as a species of their genus *Bacterium* and Bergey *et al* (1923) also accepted it as a recognized species.

If Castellani and Chalmers had been the only ones to describe such a species, there would hardly be justification for differentiation on the basis of a single character such as dulcitol fermentation. Quite a long list of unnamed organisms has been found, however, which are identical with *pseudo-coloides* as described by Castellani and Chalmers. Evidently organisms of this type are fairly widespread. It is felt the species should be recognized. A partial list of the unnamed organisms referred to is as follows:

Cathcart (1906) Organism No. 1.

Savage (1907) Organisms Nos. 7, 20, 30, 46 and 60.

Bergey and Deehan (1908) Bacillus No. 18, No. 20 and No. 116.

MacConkey (1909) Bacillus No. 100, No. 106, No. 109.

Buchan (1910) Organisms Nos. 5, 10, 15, 21, 24, 26, 29, 38, 43, 50, 55, 57 and 58.

Nicoll (1911) Bacillus No. 106, No. 109.

Kligler (1914) *Bacillus communior* No. 36.

Rogers, Clark and Davis (1914) Cultures m, bl, bv, bw, cl, cu, cz, dy and aj.

Logan (1914) Organisms Nos. XI 2, X 20, XLI 1, XLV 3, 4, X 18, XLVI 4.

Azzi (1917) Organisms Nos. 16, 2, 9, 43, 28 and 39.

Nankivell and Stanley (1920) Organism No. 10.

Redman (1922) Organisms Nos. 49, 38, 50 and 61.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Acid and gas are produced from sucrose and generally from salicin and adonitol; dulcitol is not fermented. Indol is usually formed. Acid and curd are formed in litmus milk. Found in the alimentary tract of man.*

9. *Escherichia neapolitana* (Flügge) Castellani and Chalmers, 1919.

Alternative: 9. *Bacterium neapolitanum* (Flügge) Lehmann and Neumann, 1896.

SYNONYMY: *Bacillus neapolitanus* Flügge, 1886; *Escherichia neapolitana* Bergey *et al*, 1923.

This organism was first described by Emmerich (1884). He isolated it from cholera cadavers in Naples and once from the blood of a patient and thought it to be the cause of cholera in man. Other investigators soon showed that he was mistaken. Emmerich described it as a short rod with rounded ends, single or in pairs, about one and a half times as long as broad, non-motile. It grew on gelatin as an opalescent layer, deep colonies a yellowish brown; gelatin was never liquefied. On agar, a moist white layer; on potato, yellowish brown. It was aerobic and facultative, non-spore-forming and Gram-negative. It was pathogenic for laboratory animals when injected in large amounts. Emmerich called the organism the "Neapel bacillus." Flügge (1886) seems to have been the first to give it a specific name, *Bacillus Neapolitanus.*

There is little in Emmerich's description, or in the descriptions given by any author up to 1905 (Flügge, 1886; Trevisan, 1889; Eisenberg, 1891; Lehmann and Neumann, 1901, etc.), which might serve to identify the organism. MacConkey, however, working with a culture obtained from Kral, studied its action on a number of carbohydrates and added sufficient information in this respect definitely to differentiate it from related species. He separated it from *Bact. coli commune* Escherich on its non-motility and ability to produce acid and gas from cane sugar. Its characters have been studied in still greater detail by subsequent investigators until today it stands as a well-defined and easily recognized species.

**Specific Diagnosis:** *A non-motile rod, conforming to the generic diagnosis. Acid and gas are produced from sucrose, dulcitol and salicin, but not from adonitol. Indol is produced. Litmus milk is acidified and coagulated. Found in the intestinal tract of man.*

10. *Escherichia coscoroba* (Tretop) Comb. nov.

Alternative: 10. *Bacterium coscoroba* (Tretop) Holland, 1920.

SYNONYMY: *Bacillus coscoroba* Tretop, 1900; MacConkey, 1906; *Bacillus communior* var. *coscoroba* Winslow, Kligler, Rothberg, 1919; *Escherichia pseudocoscoroba* Castellani and Chalmers, 1919.

Tretop (1900) has generally been given credit for the term *coscoroba*. He used it for an organism which he had isolated from diseased swans. Castellani and Chalmers (1919) contended that his organism was not a member of the colon-typhoid series, but belonged rather to the *Pasteurella* group. Tretop did, indeed, emphasize polar staining—"les coccobacilles des organes retiennent fortement la matiere colorante aux poles, le centre restant clair"—and the pathological symptoms noted in swans dead of the disease correspond closely to those ordinarily given for hemorrhagic septicemias. No mention was made of its ability to attack any carbohydrate. The evidence, so far as Tretop's description goes, perhaps does favor assignment to *Pasteurella*.

However, MacConkey (1906) studied an organism labelled *B. coscoroba* which he had secured from Dr. Binot at the Pasteur Institute and which presumably was a subculture of Tretop's bacillus. He found it to be a non-motile rod, which fermented (with acid and gas production) glucose, lactose, sucrose, galactose, laevulose, mannose, arabinose, raffinose, mannitol, and did not liquefy gelatin. Indol was produced. Whether or not this organism was really a lineal descendant of Tretop's bacillus, of course, cannot be proven. Such evidence as we have does not indicate that it was not. Polar staining has often been described for members of the colon-typhoid series. If it could be shown that MacConkey was mistaken in identifying his organism with that of Tretop's, the name *pseudocoscoroba* proposed by Castellani and Chalmers (1919) would replace *coscoroba*. Until this is shown, however, *Bacillus coscoroba* Tretop emended MacConkey must be considered a valid species, clearly a member of the "colon-typhoid" group.

**Specific Diagnosis:** *A non-motile rod, conforming to the generic description. Sucrose is fermented with acid and gas; dulcitol, adonitol and salicin are not attacked. Indol is produced. Litmus milk becomes acid and coagulated. Originally described from an epidemic of swans. Found in the intestinal tract and in sewage.*

11. *Escherichia astheniae* (Dawson) Bergey *et al*, 1923.

Alternative: 11. *Bacterium astheniae* Dawson, 1898.

Dawson (1898) described this organism briefly as follows: A rod, 1-1.3$\mu$ long and 0.5$\mu$ wide, often in pairs. Does not stain with acid or alkaline methylene blue, carbol fuchsin, or any alcoholic solutions of stains. Stains well with aqueous solutions of fuchsin, methylene blue, Bismarck brown, night blue and Gram's stain. Aerobic and facultative anaerobic. Bouillon, turbid, with pellicle and ring on glass; putrefactive odor and yellowish green color. Produces acid and gas ($H_2$ and $CO_2$) from glucose, lactose and sucrose. Coagulates milk, with acid and whey. Growth on gelatin, spreading, brownish, deeply dentated. Agar growth, luxuriant, white, opaque, with wavy margins. Potato growth, yellowish, creamy, spreading, with gas containing blisters, and pungent, disagreeable odor. Does not produce indol nor phenol. Isolated from duodenal contents of chickens in an asthenic condition. Pathogenic for guinea pigs and rabbits.

The organism was included by Weldin and Levine (1923) in the genus *Bacterium* (subgenus *Escherichia*) and subsequently by Bergey *et al* in the genus *Escherichia*.

The fact that it is Gram-positive would tend to throw it out of the colon-typhoid series. The production of a yellowish green pigment in acid bouillon might indicate the genus *Pseudomonas* as its proper group; the members of this genus, however, should also be Gram-negative. In fact, there is no described genus to which it might properly belong. Perkins (1925) suggests the organism was really Gram-negative. Its other characters, namely ability to attack carbohydrates, its normal habitat and its cultural and morphological characters, show it to be more closely related to the colon-typhoid organisms than to any other recognized group, and it seems, therefore, that it might be included tentatively with them.

**Specific Diagnosis:** *A non-motile rod, reported Gram-positive, but otherwise conforming to the generic diagnosis. Ferments sucrose with acid and gas production; does not attack dulcitol nor salicin. Indol is not produced. Litmus milk is acidified and coagulated. Isolated from the intestines of chickens in "asthenia."*

**Genus 2. AEROBACTER** Beijerinck 1900, Emend. Weldin and Levine, 1923.

SYNONYMY: *Actinobacter* (?) Duclaux, 1882; *Cloaca* (in part) Castellani and Chalmers, 1919; *Encapsulatus* (in part) Castellani and Chalmers, 1919.

The name *Aerobacter* was used by Beijerinck (1900) to designate a part of the so-called "colon" group. The organisms of his genus were considered to be facultative anaerobes in sugar solutions. They fermented dextrose and levulose with production of acid and usually of gas ($CO_2$ and $H_2$). Sulphates were never reduced. Nitrates were reduced to nitrites, but not to ammonia. No spores were found. Flagella, when present, were either peritrichous or monotrichous. In a list of organisms included in the genus the first was *Aerobacter aerogenes* (*Bacillus lactis aerogenes* Escherich). While Beijerinck's genus would include many more organisms than are included, for instance by Bergey *et al* (1925) under the same name, his description is not broad enough to take in the whole "colon-typhoid" group.

Buchanan (1918) used the term for a subgenus of the genus *Bacterium*. He included all the organisms of the colon-typhoid group which produce gas from lactose, erroneously giving *Bacterium* (*Aerobacter*) *coli* Escherich

as the type. Enlows (1920) recognized the genus with a definition closely resembling the original definition of Beijerinck. Weldin and Levine (1923), using *Aerobacter* as a subgeneric name, restricted it to the lactose-fermenters which produce acetyl-methyl-carbinol from glucose. While they did not definitely designate a type species, they listed *Bacterium aerogenes* first in their key to species.

Bergey *et al* (1925) used the name for the twelfth genus of the family *Bacteriaceae*. The genus as characterized by Weldin and Levine (in subgeneric sense) is clearly differentiated from other subdivisions of the colon-typhoid group upon correlated characters. *Aerobacter* is apparently the valid name if the group is to be accorded generic recognition.

**Generic Diagnosis:** *Motile or non-motile, Gram-negative, non-spore-forming rods, fermenting both glucose and lactose with both acid and gas. Produce acetyl-methyl-carbinol (Voges-Proskauer reaction positive); reverse the reaction of 0.5 percent glucose-phosphate-peptone solution relatively rapidly; generally able to utilize uric acid as an available source of nitrogen. Pathogenicity usually slight or absent.*

**Type species:** *Aerobacter aerogenes* (Escherich, 1885), Beijerinck, 1900.

KEY TO SPECIES OF THE GENUS *AEROBACTER*

a. Non-motile. (Acid and gas formed from gycerol; starch, adonitol and inositol usually fermented with acid and gas; gelatin rarely liquefied.)

b. Acid and gas formed from sucrose.

c. Neither acid nor gas formed from dulcitol.

1. *Aerobacter aerogenes.*

cc. Acid and gas formed from dulcitol.

2. *Aerobacter oxytocum.*

bb. Neither acid nor gas formed from sucrose.

3. *Aerobacter chinense.*

aa. Motile. (Glycerol, starch, adonitol and inositol rarely fermented; gelatin usually liquefied.)

b. Acid and gas formed from sucrose.

4. *Aerobacter cloacae.*

bb. Neither acid nor gas formed from sucrose.

5. *Aerobacter levans.*

SPECIES OF *AEROBACTER*

1. *Aerobacter aerogenes* (Escherich) Beijerinck, 1900.

Alternative: 12. *Bacterium aerogenes* (Escherich) Chester, 1897.

SYNONYMY: *Bacterium lactis aerogenes* Escherich, 1885; *Actinobacter polymorphum* (?) Duclaux, 1883; *Bacterium aceticum* Babinsky, 1888; *Bacillus capsulatus* Pfeiffer, 1889; *Bacillus guillebeau* C. Freudenreich, 1890; *Bacillus lactis aerogenes* Sternberg, 1892; *Bacterium capsulatum* Migula, 1895; *Bacillus aerogenes* Kruse, Flügge, 1896; *Bacillus lactantium* Trevisan, 1889; *Encapsulatus lactis aerogenes* (Escherich) Castellani and Chalmers, 1919; *Encapsulatus capsulatus* Castellani and Chalmers, 1919; *Encapsulata aerogenes* Perkins, 1925.

This organism was first described by Escherich in 1885 and again in greater detail in 1886 under the name *Bacterium lactis aerogenes.* Like his *Bakterium coli commune* it was first isolated from the stools of milk-fed children. It was described as a short rod with rounded ends, 1.4-2$\mu$ long by 0.5$\mu$ broad, usually in groups of two. According to his first description, spores were produced in sugar solution, but in 1886 Escherich says he was evidently mistaken about spore production. It was non-motile, easily stained, but Gram-negative. The colonies on gelatin were round, arched, viscid ("saftig") and glistening; gelatin was not liquefied. On agar it formed a luxuriant white layer; on blood serum a white strip. On potato it formed a yellowish white layer with some gas bubbles. It produced sufficient acid for coagulation in milk, the principal acid being lactic; whey was squeezed from the coagulated casein. Glucose, lactose and sucrose were fermented with acid and gas ($CO_2$ and $H_2$); it grew anaerobically in the presence of sugars. It was considered slightly pathogenic when injected into experimental animals. Escherich found it in intestinal contents of milk-fed animals and children, in the faeces of the same and once in unheated milk.

There seems to be but little question but that the species known today under the specific name "aerogenes" is the same one that Escherich described, though later authors have added much to our knowledge of its physiological characters. Castellani and Chalmers have placed the species in their genus *Encapsulatus,* and Perkins lays great stress on its ability to produce capsules. While Escherich did not definitely give capsule formation as a character of the species, his description of its growth on gelatin suggests it. Many other authors have described capsule formation, especially in milk. As stated elsewhere, however, capsule formation is not considered a desirable character for group differentiation. (See *Encapsulatus.*)

The name was changed by Chester (1897) to *Bacterium aerogenes* to eliminate the trinomial form. Beijerinck (1900) placed it in his newly created genus, *Aerobacter.*

**Specific Diagnosis:** *A non-motile rod, 0.5 to 0.8$\mu$ broad by 1.0 to 2.0$\mu$ long, conforming to the generic diagnosis. Acid and gas are formed from sucrose, glycerol, inositol, adonitol and usually from starch; dulcitol is not attacked. Gelatin is rarely liquefied. Indol is rarely formed. Litmus milk is made acid and coagulated. The organism is found in the alimentary tract of man and animals and widely distributed in nature.*

2. *Aerobacter oxytocum* (Flügge) Bergey *et al,* 1923.

Alternative: 13. *Bacterium oxytocum* (Flügge) Migula, 1900.

SYNONYMY: *Bacillus oxytocus perniciosus* (Wyssokowitsch) Flügge, 1886; *Escherichia oxytocus perniciosus* (Wyssokowitsch) Castellani and Chalmers, 1919.

This organism was described under the name *Bacillus oxytocus perniciosus* (Wyssokowitsch) in Flügge, Die Mikroorganism, 1886. Flügge states that it was isolated by Wyssokowitsch, a student in his laboratory. No publication of the name by the latter has been found, and it is presumed that such publication does not exist. Flügge rather than Wyssokowitsch should, therefore, be given credit for the specific name.

The organism was isolated from old milk. It was a short bacillus with rounded ends. On gelatin, deep colonies were small and yellowish; surface

colonies, grayish-white, round and arched. Milk was coagulated with acid reaction in 24 hours. Large doses injected into the veins of rabbits caused severe and fatal diarrhoea.

MacConkey (1906) obtained what was persumably a subculture of the original strain from Kral. He found it able to produce acid and gas from every carbohydrate tested (including glucose, lactose, sucrose and dulcitol) except erythritol. It was Voges-Proskauer positive, did not liquefy gelatin, did not produce indol and was non-motile.

It seems that an organism exhibiting such exceptional fermentative ability should be recognized as a species. Migula (1900) reduced the trinomial to *Bacterium oxytocum.*

**Specific Diagnosis:** *Non-motile rods, conforming to the generic diagnosis, Sucrose, dulcitol, glycerol, adonitol and inositol fermented with acid and gas production. Gelatin not liquefied. Indol is usually produced. Litmus milk is acidified and coagulated. Was first isolated from old milk. Found in dairy products, soil and the alimentary tract. Is pathogenic for rabbits on intravenous injections.*

3. *Aerobacter chinense* (Hamilton) Bergy *et al,* 1923.

Alternative: 14. *Bacterium chinense* (Hamilton) Migula, 1900.

SYNONYMY: *Bacillus capsulatus chinensis* Hamilton, 1898; *Bacterium duodenale* (?)Ford, 1903; *Aerobacter chiense* (Hamilton) Bergey *et al,* 1923.

This organism was isolated by Hamilton (1898) from Chinese ink. She described it under the name *Bacillus capsulatus chinensis* as a capsulated rod, 4-6$\mu$ long by 0.5-0.75$\mu$ broad, with two or three members in capsule, involution forms common, non-motile, non-spore-forming, Gram-negative. Capsules are more readily formed on nutrient media than in the animal body. On gelatin the colonies were white, glistening, hemispherical with sharp edges; deep colonies a yellowish color. Gelatin was not liquefied. On agar the growth was quicker and more abundant, the colonies appearing as large slimy drops. On glycerin agar and sucrose agar a thick slimy layer covered the whole surface. Blood serum did not support as good a growth as agar; it was not liquefied. On potato the growth was creamy and distinct ammonia odor was produced; at 37° C. gas bubbles were formed and the reaction was distinctly alkaline to litmus. Milk was coagulated by acid production. Glucose, lactose and maltose were fermented, glycerin slightly and sucrose not at all, with production of acid and gas; the latter was found to be $CO_2$, $H_2$, $CH_4$ and a trace of $N_2$. The organism was aerobic and facultative. It was decidedly pathogenic for mice and guinea pigs.

The organism seems to be a well characterized, distinct species, which should be recognized.

Migula (1900) reduced the name from trinomial form to *Bacterium chinense.* Bergey et al (1923) first placed the species in the genus *Aerobacter.* (Note: In the Manual of Determinative Bacteriology (1923) by Bergey *et al,* the name is incorrectly spelled "chiense".)

**Specific Diagnosis:** *A non-motile rod, conforming to the generic diagnosis. Acid and gas are produced from glycerol, but not from sucrose. Gelatin is not liquefied. Litmus is slowly acidified and coagulated. Was first isolated from Chinese ink. Is pathogenic for white mice and guinea pigs.*

4. *Aerobacter cloacae* (Jordan) Bergey *et al*, 1923.

Alternative: 15. *Bacterium cloacae* (Jordan) Lehm and Neum., 1896.

SYNONYMY: *Bacillus cloacae* Jordan, 1890; *Clocoa cloacae* (Jordan) Castellani and Chalmers, 1919.

Jordan (1890) isolated this organism from sewage at Lawrence, Mass. He described it as a short ,plump, oval bacillus, with rounded ends, about 0.8-1.9$\mu$ long by 0.7-1.0$\mu$ broad, non-spore-forming, motile, aerobic and facultative. On gelatin, the deep colonies were round and yellowish; surface colonies slightly bluish with irregularly notched edges. Gelatin was liquefied quickly. In gelatin tubes the growth was rapid. Growth was good along the line of inoculation, a scum formed on the surface and a heavy, flocculent, whitish precipitate formed. On agar the growth was moist, slimy, porcelain white. On potato, yellowish-white, rapid growth. Milk was coagulated with a strong acid reaction. Bouillon was turbid with a slight scum and considerable whitish precipitate. Nitrates were reduced to nitrites.

Other investigators have added considerably to our knowledge of the organism. It has been found to produce indol from peptone, acetyl-methyl-carbinol from glucose and to ferment a number of carbohydrates with production of acid and gas ($CO_2$ and $H_2$).

**Specific Diagnosis:** *Motile rods, 0.5 to 1.0$\mu$ broad by 0.8 to 2.0$\mu$ long, conforming to the generic diagnosis. Sucrose is fermented with acid and gas production; glycerol, starch, dulcitol and inositol are rarely attacked and adonitol is not fermented. Gelatin is usually liquefied. Indol is usually produced. Litmus milk is acidified and coagulated. Originally isolated from sewage. Found in the alimentary tract.*

5. *Aerobacter levans* (Wolffin) Bergey *et al*, 1923.

Alternative: 16. *Bacterium levans* (Wolffin) Lehm - and Neum., 1896.

SYNONYMY: *Bacillus levans* Wolffin, 1894; *Cloaca levans* (Wolffin) Castellani and Chalmers, 1919.

This organism was isolated by Wolffin (1893), under the direction of Professor Lehmann, from sour dough. According to descriptions by Wolffin (1894) and by Lehmann (1894) the organism was identical with *Bacterium coli commune* in its morphological and cultural characters. It was aerobic and facultative, did not liquefy gelatin, did not coagulate milk, did not produce indol and fermented glucose with acid and gas ($H_2$: $CO_2$::-1:3). It also produced gas ($H_2$ and $N_2$) from sugar free bouillon. According to this description the organism could hardly be accepted in the colon-aerogenes section. However, later work by F. Frankel (1896) and by Papasoteriu (1901) with the same organism used by Wolffin and other strains isolated by themselves showed Wolffin to have been mistaken in some characters. They found indol to be produced and milk coagulated though sometimes only after 5-6 day incubation. Apparently, as Papasoteriu says, Wolffin recorded results after very short incubation. MacConkey (1906) quotes Hollinger (1902) as finding *B. levans* able to liquefy gelatin, the rate of liquefaction being very varied, sometimes taking 1-2 months. MacConkey (1906) himself studied *B. levans*, using a culture secured from Kral's laboratory, which we may assume was a descendant of the original. He found it to be motile, able to liquefy gelatin, not producing indol, Voges-Proskauer positive, producing acid and gas from glucose and lactose but

not from sucrose. In addition he gave its fermentation reaction on a long list of carbohydrates.

The species is considered adequately described, and the name valid, the accepted description being that given by Frankel and Papasoteriu and emended by MacConkey.

**Specific Diagnosis:** *A motile rod, 0.6μ broad by 1.8μ long, conforming to the generic diagnosis. Sucrose, dulcitol, inositol and adonitol are not fermented. Indol is rarely produced. Gelatin is usually liquefied. Acid and curd are formed in litmus milk. Isolated from fermented dough. Found in soil and occasionally in the alimentary tract.*

### Genus 3. *PROTEUS* Hauser, 1885

SYNONYMY: *Encapsulatus* (in part) Castellani and Chalmers, 1919; *Klebsiella* (in part) Trevisan, 1885; *Wesenbergus* (in part) Castellani and Chalmers, 1919.

*Proteus* was used by Hauser (1885) in a generic sense but without definition. Three organisms were described: *Proteus vulgaris, Proteus mirabilis* and *Proteus zenkeri.* The three agreed in being motile, non-spore-forming rods with great variability in morphology. They produced apparently motile "islands" on the moist surface of solid media. All were associated with putrefaction and decay. They were differentiated by Hauser on the basis of their relative abilities to attack gelatin.

Rogers, Clark and Lubs (1918) characterized the group as follows:

> Short, thick, Gram-negative bacillus, tendency to ferment carbohydrates to some degree; normal habitat, intestines of warm blooded animals. Liquefies gelatin and under certain conditions forms characteristic "swarming" colonies. Acid and gas from glucose, but lactose is not fermented.

Jordan (1903) in describing the "Proteus type," adds to its characteristics the ability to ferment sucrose, but says lactose may be fermented, though rarely. He placed no emphasis upon morphological or cultural characters. In fact as Buchanan (1925) points out, few authors until recently have considered the pleomorphism of these forms as distinctive.

Buchanan (1918) used the name for a genus with the following description:

> Short rods, showing great variation in morphology, filamentous and bent rods as involution forms frequent. Motile by means of peritrichous flagella. The species commonly produce motile "islands" on the surface of moist solid media. No spores. Gram-negative. Usually liquefying gelatin rapidly in absence of carbohydrates. Usually producing acid and gas from certain carbohydrates. In general the species are closely associated with decay and putrefaction, sometimes pathogenic.
>
> The type species is *Proteus vulgaris* Hauser.

Werner and Rettger (1919) have made a careful study of the group. They concluded *Proteus zenkeri* of Hauser to be identical with *B. zopfii* for which they created a new genus *Zopfius.* All of their strains fermented glucose and sucrose with acid and gas production. Pure lactose was never fermented. Maltose fermentation was variable and was the only property they found which could be used for subdivision of the group into species.

Winslow *et al* (Committee, S. A. B., 1920) used *Proteus* with almost the same description as that of Buchanan for the first of two genera composing the tribe *Bactereae.*. They added the statements that gelatin is liquefied,

and glucose and sucrose (but usually not lactose) are fermented with production of acid and gas (the latter being $CO_2$ only).

Weldin and Levine (1923) simply characterized their subgenus *Proteus* of the genus *Bacterium* by fermentation of glucose and sucrose, but not lactose, with formation of acid and gas.

The name has been used by Bergey *et al* (1923) with the following description:

> Highly pleomorphic rods. Filamentous and curved rods are common as involution forms. Gram-negative. Actively motile, possessing peritrichous flagella. Produce characteristic amoeboid colonies on moist media and decompose proteins. Ferment dextrose and sucrose, but not lactose. Do not produce acetyl-methyl-carbinol.
>
> The type species is *Proteus vulgaris* Hauser.

Little emphasis can be placed on morphological variations, especially since the work of Henrici and others on some of the organisms of the colon-typhoid series. Undoubtedly, involution forms are more easily demonstrated with these than with some of the other intestinal types. They possess peculiar cultural characters on certain media. These characteristics are comparative, however, and clear cut physiological characters would seem to be more reliable for use in differentiation. Their actions on the sugars, glucose, lactose and sucrose, constitute such characters.

Liquefaction of gelatin has been listed quite regularly as a characteristic of the *Proteus* species. Wenner and Rettger (1919) and others who have worked with such organisms point out that the property of liquefying gelatin was often lost by their cultures of *vulgaris* upon continued laboratory cultivation. In the literature many organisms are described which resemble *Proteus vulgaris* more clearly than they do any other type species, but which are gelatin non-liquefiers. In view of the unstability of gelatin liquefaction this character is not included in the generic definition.

**Generic Diagnosis:** *Gram-negative, non-spore-forming rods usually highly pleomorphic in form. Usually motile. Produce gas from both glucose and sucrose, but not from lactose. Pathogenicity slight.*

**Type species:** *Proteus vulgaris* Hauser.

KEY TO SPECIES OF THE GENUS *PROTEUS*

a. Neither acid nor gas formed from mannitol.
  b. Acid and gas formed from maltose.
    1. *Proteus vulgaris.*
  bb. Neither acid nor gas formed from maltose.
    2. *Proteus mirabilis.*

aa. Acid and gas formed from mannitol.
  b. Gelatin not liquefied.
    c. Motile.
      d. Acid and gas formed from salicin.
        3. *Proteus infantum.*
      dd. Neither acid nor gas formed from salicin.
        4. *Proteus valeriei.*
    cc. Non-motile.

d. Capsules formed; indol not formed.

5. *Proteus pneumoniae.*

dd. Capsules not formed; indol formed.

6. *Proteus asiaticus.*

bb. Gelatin liquefied.

7. *Proteus hydrophilus.*

## SPECIES OF *PROTEUS*

1. *Proteus vulgaris* Hauser, 1885.

Alternative: 17. *Bacterium vulgare* (Hauser) Lehm. and Neum. 1896.

SYNONYMY: *Bacillus proteus* Trevisan, 1889; *Bacillus Proteus vulgaris* (Hauser) Kruse in Flügge, 1896; *Bacterium vulgare* (Hauser) Chester, 1897; *Bacillus vulgaris* (Hauser) Migula, 1900; *Bacterium proteus anindologenes* von Loghem, 1918; *Proteus proteus vulgaris* (Hauser) Castellani and Chalmers, 1919.

Hauser (1885) described this organism as one of three species of a new genus *Proteus*. In his description he stressed particularly the variability of form which these organisms may assume and the amoeboid wandering colonies. He separated his three species on the basis of their relative ability to liquefy gelatin, *Proteus vulgaris* liquefying it rapidly, *P. mirabilis* slowly and *P. zenkeri* not at all. Later he decided that the last two species might be only varieties of *Proteus vulgaris*.

Early systematists accepted *Proteus vulgaris* with gradual elaboration of its description. It was soon found to be able to ferment glucose and sucrose with acid and gas production, but not lactose. The most exhaustive study of the group has been made by Wenner and Rettger (1919). These authors worked with 84 strains of *Proteus* group organisms, 58 of which were secured from other laboratories. From these and from their studies of the literature, they concluded that *Proteus vulgaris* and *Proteus mirabilis* were identical, while *Proteus zenkeri* resembled an organism previously described by Kurth (*Bacterium zopfii*). The property of liquefying gelatin they found to be too irregular and inconstant to serve as a basis for separation of species. They placed *Proteus zenkeri* in a new genus *Zopfius*. With regard to *P. vulgaris* and *P. mirabilis* they made the following suggestion:

"The *Proteus* genus comprises a large group of organisms which can be subdivided on the basis of their action on maltose into two distinct species. For the species fermenting this sugar the name *Proteus vulgaris* is suggested, and for the species failing to attack it, the name *Proteus mirabilis*. By retaining these names the nomenclature would be simplified. The differentiating characters of Hauser must be set aside, however, in order to avoid confusion."*

Their suggestion for differentiation of the two species on the basis of maltose fermentation was followed by Weldin and Levine (1923) and by Bergey *et al* (1923). It seems to be a satisfactory and convenient procedure and is here accepted.

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* The character referred to was rate of gelatin liquefaction.

The strains X2 and X19, used in the Weil-Felix reaction, agree, according to the best information at hand, culturally and morphologically, with *Proteus vulgaris*, but differ from it in their serological reactions. They should probably be considered varieties of the latter.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Sucrose fermented rapidly with acid and gas production; maltose fermented with acid and gas; mannitol and dextrin not fermented. Gelatin liquefied, at least by freshly isolated cultures. Indol is produced. Found in putrefying materials.*

2. *Proteus mirabilis* Hauser, 1885.

Alternative: 18. *Bacterium mirabile* (Hauser) Chester, 1897.

SYNONYMY: *Bacillus mirabilis* Trevisan, 1889; *Bacillus proteus mirabilis* (Hauser) Kruse, 1896.

This was one of three species first described by Hauser (1885). It was isolated by him from putrefying animal matter. For discussion, see *Proteus vulgaris*.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Sucrose slowly fermented with acid and gas production; maltose, mannitol and dextrin not fermented, Gelatin liquefied, at least when freshly isolated. Found in putrefying materials.*

3. *Proteus infantum* (Weldin and Levine) Comb. nov.

Alternative: 19. *Bacterium infantum* Weldin and Levine, 1923.

SYNONYMY: Brisbane organism, Dean, 1920.

This organism was first isolated by Dean (1920) from the urine and faeces of a child. Later it was secured from a number of faeces samples sent to him for examination for dysentery bacilli. He found it to be a motile rod, not able to liquefy gelatin, producing indol and producing acid and gas from glucose and sucrose, but not from lactose. In litmus milk it produced acid on the fifth day, clotted milk on the tenth and cleared it on the fifteenth day. In its agglutination reaction it appeared to be more closely linked to the paratyphoid B organism than to the paratyphoid A or typhoid organisms.

Weldin and Levine (1923) concluded that this organism was sufficiently described for recognition. They, accordingly, gave it the name *Bacterium infantum* and placed it in their subgenus *Proteus*, where its fermentation reactions clearly showed it to belong.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Ferments mannitol, maltose, salicin, raffinose and arabinose with acid and gas production, but does not attack inulin nor adonitol. Gelatin is not liquefied. Found in human urine and faeces.*

4. *Proteus valeriei* (Weldin and Levine) Bergey *et al*, 1923.

Alternative: 20. *Bacterium valeriei* Weldin and Levine, 1923.

SYNONYMY: Valerie 21, Boycott, 1906; *Bacillus asiaticus mobilis* Castellani, 1916; *Salmonella asiaticus mobilis* (Cast.) Castellani and Chalmers, 1919.

This organism was isolated by Boycott (1906) from the stool of a patient and described under the term "Valerie 21." It was actively motile,

did not liquefy gelatin, produced indol and acidified and clotted milk. It produced acid and gas from glucose, maltose, sucrose and mannitol as well as a number of other carbohydrates, but had no apparent action on lactose or salicin. It was pathogenic for guinea pigs when injected intraperitoneally. Agglutinin-absorption tests with "Schottmüller B" (*Salmonella schottmülleri*) and the organism of Brion and Kayser (*Salmonella paratyphi*) showed no relation to these two organisms.

Castellani (1916) described an organism found in the Adriatic-Balkan zone, which had almost identical morphological and physiological characters. He called it *Bacillus asiaticus mobilis.*

Weldin and Levine (1923) used the name *Bacterium valeriei* for Boycott's organism.

The organism was adequately described. Its ability to ferment glucose and sucrose, but not lactose, places it in the *Proteus group.*

The name *Bacillus asiaticus mobilis,* being a trinomial, must be rejected; *Proteus asiaticus* and *Bacterium asiaticum* have already been used for another recognized species (see *Proteus asiaticus*), and *Proteus mobilis* is a type of name which should be avoided as expressing a character common to all or nearly all members of the group (Recommendation XIV of the Botanical Code). *Proteus valeriei* is thus considered a valid name for the species.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Produces acid and gas from mannitol, maltose, raffinose and arabinose, but not from salicin nor inulin. Gelatin is not liquefied. Found in the intestines of man.*

5. *Proteus pneumoniae* (Zopf) Comb. nov.

Alternative: 21. *Bacterium friedlander* (Trevisan) Comb. nov.

SYNONYMY: Pneumonie Coccus, Friedlander, 1883; *Bacterium pneumoniae crouposae* Zopf, 1885; *Bacillus Pneumoniae* (Friedlander) Flügge, 1886; *Klebsiella pneumoniae* Trevisan, 1887; *Bacillus capsulatus pneumonicus* Banti, 1888; *Klebsiella friedlanderi* Trevisan, 1889; Pneumobacillus Eisenberg, 1891; *Bacterium pneumonicum* (Friedlander) Migula, 1895; *Bacterium pneumoniae* Friedlander, Lehmann and Neumann, 1896; *Bacillus Friedländer* Buchan, 1910.

This organism was first described but not named by Friedlander in 1882. He isolated it from the pleuritic and pericardial exudate from a case of acute pneumonia. It was described as a coccus, though he stated that the organisms were usually ellipsoid in shape, their length being about one micron and their breadth a little less. It was usually in pairs, though sometimes in short chains. In a later publication (1883) he described capsule formation; these capsules were always observed with organisms from the animal body, but not in cultures on gelatin or blood serum.

Later investigators working with the same organism have shown it to be unquestionably a rod, Gram-negative, with cultural characters typical of the colon-typhoid bacilli, fermenting a number of carbohydrates, including glucose and sucrose with acid and gas ($CO_2$ and $H_2$).

There is considerable disagreement among various investigators as to the ability of this organism to ferment lactose. Grimbert (1895) stated that it was able to ferment this sugar with acid production. Later (1896) he added gas production from lactose. Nicolle and Hébert (1897) con-

firmed Grimbert's findings. Lehmann and Neumann (1901) stated that it produced abundant acid, together with $CO_2$ and $H_2$ from grape- and milk-sugar. Strong (1889) working with cultures from Kral's laboratory, from the Göttingen Institute and from his own isolations, found no acid and "wenig oder gar kein Gaz bei Milchzucker gebildet." Perkins (1904) worked with a subculture from Kral's laboratory and found it produced neither acid nor gas from lactose. MacConkey (1905) also working with a culture obtained indirectly from Kral, records acid, but not gas, from lactose. Another culture obtained from H. Spitta did produce gas as well as acid. Coulter (1917) studied eleven strains of Friedlander's bacillus isolated by himself, and found none of them able to ferment lactose. It is quite evident that the investigators who were supposedly working with lineal descendants of the original strain found it incapable of producing gas from lactose. The species is, therefore, considered in the subgenus *Proteus* because it produces gas from glucose and sucrose, but not from lactose.

Zopf (1885) used the name *Bacterium pneumoniae crouposae* for the Friedlander bacillus. This name, being a trinomial, cannot stand in its entirety. Flügge (1886) used the name *Bacillus pneumoniae* for the species. The combination *Bacterium pneumoniae* is invalid for this organism, since this specific designation was first used by Migula (1895) for the pneumonia organism of Weichselbaum now generally recognized under the name *Diplococcus pneumoniae* as the type species for the genus *Diplococcus*. Trevisan (1889) used the term *Klebsiella Friedländeri* for this species; *Friedlanderi* may, therefore, be considered the valid specific name for the organism when the generic term *Bacterium* is used.

However, the combination *Proteus pneumoniae* has not been used previously, and since *pneumoniae* has priority over *friedlanderi*, this must be the accepted name when the organism is considered to be in the genus *Proteus*.

**Specific Diagnosis:** *A non-motile rod, conforming to the generic diagnosis. Maltose, mannitol, arabinose, raffinose and dulcitol are fermented with acid and gas production; inulin is not attacked. Gelatin is not liquefied. Indol is not formed. Capsules are generally produced when the organism is grown in milk. Found in saliva and exudates. Associated with pneumonia, bronchitis and various inflammations of the respiratory tract.*

6. *Proteus asiaticus* (Castellani) Bergey *et al*, 1923.

Alternative: 22. *Bacterium asiaticum* (Castellani) Weldin and Levine, 1923.

Synonymy: *Bacillus asiaticus* (No. 1 and No. 2) Castellani, 1912; *Salmonella asiaticus* (Cast.) Castellani and Chalmers, 1919; *Bacterium* (*Proteus*) *asiaticum* Weldin and Levine, 1923.

This organism was isolated by Castellani (1912) from the blood and stools of a patient suffering from a long protracted fever following anchylostomiasis. Serum reactions and the results of vaccination indicated a probable causal relationship of the organism to the fever. It was described as a short, rod-like organism, 2-5$\mu$ in length, non-motile, Gram-negative. Growth on agar was typhoid-like; in broth there was general turbidity and pellicle formation. Gelatin was not liquefied, indol production was slight,

litmus milk was acid, with reversion to alkalinity. A number of carbohydrates were attacked, including glucose, sucrose and mannitol. Lactose was not fermented. Two varieties are described, the differences largely being intensity and rate of some of the reactions. They were combined as one species in later descriptions (1916, 1919).

The species is adequately described and sufficiently distinct to warrant recognition.

**Specific Diagnosis:** *A rod, 2 to 5μ in length, conforming to the generic diagnosis. Non-motile. Ferments mannitol, maltose, sorbitol and glycerol, with acid and gas production; does not attack inulin nor adonitol. Gelatin is not liquefied. Indol is produced. Litmus milk becomes acid followed by alkalinity; marked capsule formation does not occur. Found in the intestinal tract of man.*

7. *Proteus hydrophilus* (Sanarelli) Bergey *et al*, 1923.

Alternative: 23. *Bacterium hydrophilum* (Sanarelli) Chester, 1897.

SYNONYMY: *Bacillus hydrophilus fuscus* Sanarelli, 1891; *Bacterium hydrophilus fuscus* (Sanarelli) Chester, 1897; *Bacillus hydrophilus* (Sanarelli) Chester, 1901; *Bacillus ranicida* (?) Ernst, 1890; *Bacterium ranicida* (?) (Ernst) Lehmann and Neumann, 1901.

Sanarelli (1891) obtained this organism from the lymph of frogs suffering from a fatal infectious disease. It was a Gram-negative short rod, sometimes growing into long filaments, aerobic, gelatin-liquefying and motile. On glycerin agar at 37° C. it grew luxuriantly, soon covering the entire surface and exhibiting a slight fluorescence which soon disappeared. Blood serum was liquefied. On potato it developed a yellowish to brownish growth. It was pathogenic not only for "cold-blooded" animals, but also for guinea pigs, rabbits, dogs, cats, mice, chickens and pigeons. Sanarelli called it *Bacillus hydrophilus fuscus.*

A bacillus was carefully studied and described in detail by Emerson and Norris (1905), which corresponded morphologically and culturally to Sanarelli's description, but differed slightly as to pathogenicity. They found guinea pigs susceptible, but not rabbits; no other animals were tested. In addition they described it as highly pleomorphic. Milk was acidified, coagulated and peptonized. Dextrose, sucrose and mannitol were fermented with acid and gas production, but gas was never formed from lactose. The ability to produce gas from sugars was reported lost with continued cultivation. Nitrates were reduced to nitrites and indol was slowly produced. It produced a slight yellowish pigment on some media; however, "pigment formation was so variable that no constant factors controlling its occurance were determinable." The authors concluded that their organism was identical with that of Sanarelli.

Ernst in 1890 described an organism under the name *Bacillus ranicida* which corresponds very closely in its characters to *Bacillus hydrophilus fuscus* Sanarelli, and there may be some question as to whether his name should not be used in place of Sanarelli's. However, both his and Sanarelli's descriptions were incomplete and inadequate for recognition of the species were it not for the work of Emerson and Norris. These authors definitely stated that their organism corresponded more closely to *Bacillus hydrophilus fuscus* Sanarelli than to *Bacillus ranicida* Ernst. In view of

this statement it seems best to retain the specific name *hydrophilus* and consider *B. ranicida* a possible synonym.

Its characters place it definitely in the genus *Proteus*. Chester (1901) reduced the name to binomial form (*Bacillus hydrophilus*) and Weldin and Levine (1923) placed it in their subgenus *Proteus* of the genus *Bacterium*.

**Specific Diagnosis:** ***A motile rod, conforming to the generic diagnosis. Mannitol and maltose are fermented with acid and gas production. Gelatin is liquefied. Found in the lymph of frogs dead from a disease known as "red leg." Pathogenic for frogs, salamanders, fish, guinea pigs.***

**Genus 4. *SALMONELLA* Lignières, 1909.**

SYNONYMY: Includes *Balkanella* Castellani and Chalmers, 1919; *Wesenbergus* (in part) Castellani and Chalmers, 1919.

This name was first proposed by Lignières (1900) for a genus to include the hog cholera organism of Salmon. He stated: "Le microbe de la Schweineseptikämie rentre dans le groupe des *Pasteurella;* celui du Hog-cholera ou Schweinepest est très different, il pourait servir de prototype pour la creation d'un autre groupe, celui des *Salmonella.*

Buchanan (1918) used the name for a subgenus of the genus *Bacterium* with the following description:

**Fermenting glucose but not lactose with formation of acid and gas. Frequently pathogenic. Type species: *Bacterium (Salmonella) cholerae suis.***

Castellani and Chalmers (1919) recognized it as the name of a genus of the tribe *Ebertheae.* While they included the ability to ferment mannitol (at least partially) in their characterization, they divided the genus into three groups, one of them being the Morgan group which does not attack mannitol at all.

Weldin and Levine (1923) used the name for a subgenus. They described it as including members of the genus *Bacterium* which ferment glucose, but neither lactose *nor sucrose* with acid and gas.

Bergey *et al* (1923) also recognized *Salmonella* as a genus. In their key to genera of the tribe *Bactereae,* it is separated from the other genera of the colon-typhoid series by ability to produce acid and gas from glucose, and inability to attack lactose and sucrose. Their description reads as follows:

**Motile forms occurring in the intestinal canal of animals, in various types of acute, inflammatory conditions. Attack numerous carbohydrates with the formation of both acid and gas. In general do not form acetyl-methyl-carbinol. Type species: *Salmonella schottmülleri.***

Their type species is evidently in error. In the 1925 edition it is changed to *Salmonella suipestifer* (Kruse) Lignières.

Duncan (1924) would restrict the group to exclude the Morgan group of bacilli, *Bact. paratyphi,* and a number of other species commonly included by recent authors. He suggests the following characterization for *Salmonella:*

**Gram-negative non-sporing bacilli, usually actively motile, which do not ferment lactose, saccharose or salicin, do not liquefy gelatin, and never give the indol reaction. In litmus-milk they cause a transient acidity followed after 48 hours by alkalinity They ferment glucose, mannitol and maltose with production of acid and gas.**

Such a diagnosis limits the species to be included in the genus to those which conform very closely to the type, and excludes others, which, on the basis of their serological as well as physiological reactions, are clearly related to species which Duncan would include.

The best method at present seems to be to use the term *Salmonella* for that part of the colon-typhoid series intermediate between the colon-aerogenes-proteus types and the typhoid-dysentery types. Most of this group are slightly pathogenic and many are related serologically. If further subdivision is thought necessary it might be indicated by sections such as the Gärtner section, (mannitol, acid and gas; xylose, acid and gas), the paratyphi section, (mannitol, acid and gas; xylose, negative), and the Morgan section (mannitol, negative).

**Generic Diagnosis:** ***Gram-negative, non-spore-forming rods, Usually motile. Produce gas from glucose, but not from lactose nor sucrose. Frequently pathogenic.***

**Type species:** ***Salmonella cholerae suis*** **(Smith, Th., 1894).**

KEY TO SPECIES OF THE GENUS *SALMONELLA*.

a. Acetyl-methyl-carbinol not produced from glucose.
  b. Acid produced from mannitol.
    c. Acid and gas produced from maltose.
      d. Acid and gas produced from xylose; litmus milk, slightly acid, reverting rapidly to an alkaline reaction.
        e. Dulcitol and arabinose not fermented or fermented very slowly with acid and gas production.
          f. Neither acid nor gas produced from dextrin; agglutinins from *Sal. cholerae suis* serum completely absorbed; those from *Sal. icteroides* not completely absorbed.
            1. *Salmonella cholerae suis.*
          ff. Acid and gas produced from dextrin; agglutinins from *Sal. cholerae suis* serum not completely absorbed; those from *Sal. icteroides* completely absorbed.
            2. *Salmonella icteroides.*
        ee. Dulcitol and arabinose rapidly fermented with acid and gas production.
          f. Acid and gas produced from inositol.
            g. Gas produced from mannitol; salicin not fermented; raffinose usually not fermented.
              h. Acid and gas produced from raffinose.
                3. *Salmonella psittacosis.*
              hh. Neither acid nor gas produced from raffinose.
                i. Agglutinins from *Sal. schottmülleri* serum completely absorbed.
                4. *Salmonella schottmülleri.*

ii. Agglutinins from *Sal. schottmülleri* serum not completely absorbed.

j. Agglutinins from *Sal. aertrycke* serum completely absorbed; those from *Sal. anatum* not completely absorbed.

5. *Salmonella aertrycke.*

jj. Agglutinins from *Sal. aertrycke* serum not completely absorbed; those from *Sal. anatum* completely absorbed.

6. *Salmonella anatum.*

gg. Acid, not gas, produced from mannitol; salicin usually, raffinose always fermented with acid and gas production.

7. *Salmonella veboda.*

ff. Neither acid nor gas produced from inositol.

g. Acid and gas produced from salicin; indol produced.

8. *Salmonella columbensis.*

gg. Neither acid nor gas produced from salicin; indol rarely produced.

h. Not agglutinated by *Sal. enteritidis* serum.

9. *Salmonella hirschfeldii.*

hh. Agglutinated by *Sal. enteritidis* serum.

i. Agglutinins from *Sal. enteritidis* serum completely absorbed; those from *Sal. abortivo-equinum* not completely absorbed.

10. *Salmonella enteritidis.*

ii. Agglutinins from *Sal. enteritidis* serum not completely absorbed; those from *Sal. abortivo-equinum* completely absorbed.

11. *Salmonella abortivo-equinum.*

dd. Neither acid nor gas produced from xylose; litmus milk, slightly acid, reverting very slowly if at all.

e. Neither acid nor gas produced from inositol and raffinose; indol not produced.

f. Acid and gas produced from levulose and arabinose.

12. *Salmonella paratyphi.*

ff. Neither acid nor gas produced from levulose and arabinose.

13. *Salmonella woliniae.*

ee. Acid and gas produced from inositol and raffinose; indol produced.

14. *Salmonella watareka.*

cc. Neither acid nor gas produced from maltose.

15. *Salmonella pullorum.*

bb. Neither acid nor gas produced from mannitol.

c. Neither acid nor gas produced from maltose and dextrin.

d. Acid and gas produced from levulose.

16. *Salmonella morganii.*

dd. Neither acid nor gas produced from levulose.

17. *Salmonella foetida.*

cc. Acid and gas produced from maltose and dextrin.

d. Non-motile; acid and gas produced from salicin and sorbitol.

18. *Salmonella giumai.*

dd. Motile; neither acid nor gas produced from salicin and sorbitol.

19. *Salmonella macfadyeanii.*

aa. Acetyl-methyl-carbinol produced from glucose.

20. *Salmonella archibaldii.*

## SPECIES OF *SALMONELLA*

1. *Salmonella cholerae suis* (Smith, Th.) Comb. Nov.

Alternative: 24. *Bacterium cholerae suis* (Th. Smith) Holland, 1920.

SYNONOMY: *Bacillus cholerae suis* Smith, Th., 1894; *Bacillus cholerae suum,* Migula, 1895; *Bacillus suipestifer* Kruse, 1896; *Bacterium suipestifer* Chester, 1897; *Bacterium cholerae suum* (Migula) Lehmann and Neumann, 1896; *Bacillus salmoni* (Trevisan) Chester, 1901; *Bacterium intestinale suis* LeBlaye and Guggenheim, 1914; *Bacillus suis* Krumwiede, Kohn, and Valentine, 1918; *Salmonella suipestifer* (Kruse) Castellani and Chalmers, 1919; not *Pasteurella salmoni* Trevisan, 1889.

Salmon and Smith (1885) in a study of disease of hogs which they called American swine plague, isolated an organism and described it as the "Bacterium of swine plague." The organisms appeared as elongated ovals, usually in pairs. When stained with methyl violet, many bacilli presented a center paler than the periphery. They stated further, "The darker portion is not localized at two extremities, as in the bacteria of septicemia in rabbits." The bacillus was motile and did not liquefy gelatine. It grew well on potato, blood serum, and in milk. Appearance of milk was not changed. Spores were not found. It was pathogenic for laboratory animals.

In 1886 these authors published additional information on the organism. In the meantime, it had become evident that there were two distinct infectious diseases of hogs, both of which had previously been known as

swine plague. Salmon and Smith called these two diseases swine plague and hog cholera, using the latter for the disease described in 1885, and the term, "hog cholera bacillus," for the organism previously called by them the "swine plague bacillus." The disease which they now (1886) called swine plague, had also been shown to exist in Europe, where it was likewise called swine plage or "Schweineseuche".

In 1894, Smith again emphasized that his "hog cholera bacillus" 1886, was identical with his "swine plague bacillus" 1885, and that the name "swine plague bacterium" was now being used for an organism like one similarly designated in Europe. At this time he gave the name *Bacillus cholerae suis* to the "hog cholera" bacterium. To the previous descriptions, he added that acid and gas ($CO_2$ and $H_2$) were evolved from glucose, but that lactose and sucrose were not attacked.

Kruse (1896) referring to an organism which he said was first isolated by Salmon and Smith (1885) and called by them the "Hog-cholera-Bacillus," created the name *Bacillus suipestifer*. Most of the authors who have worked on the organisms of the intermediate group have accepted Kruse's name *suipestifer;* a few have used *cholerae suis,* which evidently has priority and is the valid name. The writer has been informed of some experimental work, the results of which have not yet been made available, which indicate very strongly that there are distinct differences between American strains designated as *cholerae suis* and European cultures supposedly *suipestifer*. There is a possibility that each name should be recognized as representing distinct species.

The authors who have worked with the organisms of the intermediate group have generally recognized the species either under the name *suipestifer* or as *cholerae suis*. Ford (1905), Harding and Ostenberg (1912) and Krumwiede, Pratt and Kohn (1916) have shown it to be a xylose fermenter, thereby separating it from *Sal. paratyphi* (para A). The agglutination test was used for some time to differentiate the *hog cholera* bacillus from Gärtner's organisms. Differences in ability to attack arabinose and dulcitol were noted by Ford (1905) and Ditthorn (1913). Jordan (1917) characterized *cholerae suis* strains as fermenting arabinose and dulcitol slowly or not at all, while *schottmülleri* strains (para B) typically produced gas from these carbohydrates within 24 hours.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. It ferments maltose, mannitol and xylose with acid and gas production; dulcitol and arabinose are fermented very slowly if at all; adonitol, salicin, raffinose and dextrin are not attacked. Acetyl-methyl-carbinol is not formed from glucose. Litmus milk becomes acid, reverting rapidly to an alkaline reaction. Found in the intestines of hogs and as a secondary invader of tissues in hog cholera.*

2. *Salmonella icteroides* (Sanarelli) Bergey *et al,* 1923.

Alternative: 25. *Bacterium icteroides* (Sanarelli) Lehmann and Neumann, 1901.

Synonymy: Bacille ictéroide Sanarelli, 1897; *Bacillus icteroides* (Sanarelli) Reed and Carroll, 1900.

This organism was first described by Sanarelli (1897), who isolated it from yellow fever cadavers and believed it to be the cause of the disease. He called it bacille ictéroide. His description of its growth on ordinary

laboratory media was quite detailed, but many of the peculiarities ascribed to it were shown, by Agramonte and others, not to be constant. It fermented glucose, but not lactose nor sucrose. Sanarelli's belief that it was the etiological factor in the disease yellow fever was soon shown to be incorrect. Reed and Carroll (1900) proved its close relationship to the hog cholera bacillus.

MacConkey (1905) secured a culture of the organism from Sanarelli and determined its fermentation reactions in a number of carbohydrates. Subsequent authors have added somewhat to the list of sugars attacked, the net results showing, however, that the organism is practically indistinguishable from *Sal. cholerae suis* on the basis of its physiological reactions. Reed and Carroll (1900) found the two organisms agglutinatively identical. Krumwiede, Kohn, and Valentine (1918), however, were able to differentiate the two species by use of the agglutinin-absorption test.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Its cultural and physiological characters are the same as Sal. cholerae suis, except that dextrin and often raffinose are fermented with acid and gas production. It may be differentiated serologically from Sal. cholerae suis by the agglutinin-absorption test. It was originally isolated from yellow fever cadavers, but has been found to have no etiological relationship to the disease.*

3. *Salmonella psittacosis* (Nocard) Castellani and Chalmers, 1919.

Alternative: 26. *Bacterium psittacosis* (Nocard) LeBlaye and Guggenheim, 1914.

Synonomy: *Bacillus psittacosis* Nocard, 1893.

*Bacillus psittacosis* was isolated by Nocard (1893) from the wings of parrots which had died from pneumonia. The organism was also isolated from the blood of humans who had become infected. Its morphological and cultural characters were those of the intermediate group of the colon-typhoid series.

MacConkey (1905) secured a culture from the Pasteur Institute (presumably a sub-culture of the original) and studied its action on a number of carbohydrates. He described it as producing acid and gas from glucose, maltose, arabinose, raffinose, mannitol, sorbitol, dulcitol and dextrin, but not from lactose or sucrose. Castellani and Chalmers (1910) and Castellani (1912) agreed with MacConkey as to the carbohydrates attacked and noted further, failure to attack inulin, salicin and adonitol, and reversion from an acid to an alkaline reaction in litmus milk. Castellani (1917) quoted Bainbridge (no date given) as considering *Sal. psittacosis* identical with *Sal. aertrycke*. Up to the present the writer has not succeeded in verifying this statement.

Perry (1920) isolated from a diseased parrot an organism which was identical with Nocard's bacillus. He tested it and a subculture of Nocard's original strain secured from Dr. Besredka, by agglutinin and agglutinin-absorption tests with strains of the paratyphoid B bacillus and *Sal. aertrycke*, and found them serologically identical with the latter. Apparently the organism is very closely related to *Sal. aertrycke*, the only difference noted being its ability to attack raffinose and dextrin and its specific pathogenicity.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. It ferments levulose, maltose, raffinose, arabinose, dextrin, mannitol and dulcitol with acid and gas production; adonitol and salicin are not attacked. Acetyl-methyl-carbinol is not formed from glucose. Litmus milk becomes acid, rapidly reverting to alkalinity. Serologically identical with Salmonella aertrycke. The cause of a pneumonia of parrots; also affecting man associated with sick parrots.*

4. *Salmonella schottmülleri* (Winslow, Kligler and Rothberg) Bergey *et al*, 1923.

Alternative: 27. *Bacterium schottmülleri* (Winslow, Kligler and Rothberg), Holland, 1920.

SYNONOMY: *Bacterium paratyphi* type B, Kayser, 1904; "Bacillus paratyphoid B, Schottmüller" Morgan, 1905; *Bacillus paratyphosus* B, Wilson, 1908; *Bacterium paratyphosum* B (Schottmüller) LeBlaye and Guggenheim, 1914; *Bacillus schottmülleri* Winslow, Kligler, Rothberg, 1919; *Salmonella paratyphosus* B (Schottmüller) Castellani and Chalmers, 1919.

Achard and Bensaude (1896) described organisms similar to the psittacosis organism of Nocard, which produced gas from glucose, maltose and mannitol, but not from lactose. Milk was not coagulated. They agglutinated weakly with typhoid serum. This latter test suggests the paratyphoid B rather than the A type. The authors designated them as "Bacilles paratyphiques."

Schottmüller (1900) likewise isolated organisms intermediate in character from the typhoid bacillus and *Bacterium coli*. Kayser (1902, 1904) studied these organisms, along with a similar strain isolated by Brion and Kayser (1902), and concluded that there were two distinct species which he designated as *Bacterium paratyphi* Type A and *Bacterium paratyphi* Type B, the type A including Brion and Kayser's strain, and two of Schottmüller's strains, while type B included the rest of Schottmüller's strains (from 5 cases), as well as Achard and Bensaude's organism. (See *Salmonella paratyphi*.) The type B organisms differed agglutinatively from the type A, and in their ability to revert the reaction of litmus milk from acidity to alkalinity in two weeks time.

The organism (type B) has been isolated and studied in detail by subsequent investigators, until, at the present time, it may be clearly differentiated from related species on the basis of its cultural and serological reactions. Ford (1905) found it able to ferment xylose with acid and gas, which, as Krumwiede, Pratt and Kohn (1916) pointed out, offered a convenient method of differentiating it from paratyphoid A, which fails to attack this carbohydrate. Jordan (1917) used its ability to ferment rapidly arabinose and dulcitol to separate it from *Salmonella cholerae suis* and Weiss and Rice (1917) differentiated it from *Salmonella enteritidis* on its inositol fermentation.

The species has been commonly designated by the terms *B. paratyphoid* B and *Bacillus paratyphosus* B. Since neither of these names were in accepted form and are misleading as to the true biological relationships of the organism, Winslow, Kligler and Rothberg (1919) proposed the name *Bacillus schottmülleri* for the species.

Holland (1920) placed the species in the genus *Bacterium*, erroneously giving credit for the specific term to Winslow-Rottenberg-Parsons.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. It produces acid and gas from levulose, maltose, arabinose, xylose, dulcitol, mannitol, and inositol; but does not attack adonitol or salicin, and usually not raffinose. Acetyl-methyl-carbinol is not formed from glucose. Litmus milk becomes acid, reverting to alkalinity. Lead acetate medium is blackened. Is found in the human intestines and urine and as the causative agent of paratyphoid fever and food poisoning by use of meat from infected animals.*

5. *Salmonella aertrycke* (DeNobele) Castellani and Chalmers, 1919.

Alternative: 28. *Bacterium aertrycke* (DeNobele) Weldin and Levine, 1923.

SYNONOMY: *Bacillus aertrycke* DeNobele, 1889, 1901; *Bacillus para-aertrycke* Castellani, 1916; *Salmonella para-aertrycke* (Cast.) Castellani and Chalmers, 1919.

This organism was secured by DeNobele (1889) from an outbreak of food-poisoning. In its morphological and cultural characters it was found to be similar to the Gärtner bacillus, the paratyphoid B. organism and to *Sal. cholerae suis*. It was found to be easily differentiated from *Sal. enteritidis* by the agglutination test. Bainbridge (1909) separated it from the paratyphoid B bacillus (*Sal. schottmülleri*) by means of the agglutinin-absorption test and, apparently, by the same test showed it to be identical with *Sal. cholerae suis*. The majority of investigators since Bainbridge's work have considered *aertrycke* indistinguishable from *cholerae suis*. However, Jordan (1917) found *aertrycke* strains, like *Sal. schottmülleri*, able to ferment dulcitol and arabinose rapidly, while *Sal. cholerae suis* fermented these carbohydrates slowly or not at all. The organisms may at present be differentiated from the other members of the intermediate group except *Sal. cholerae suis* by agglutination or by agglutinin-absorption tests, and from *Sal. cholerae suis* by carbohydrate fermentations, and is considered a distinct and valid species.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Culturally, it can not be distinguished from Sal. schottmülleri, but may be differentiated from the latter by the agglutinin-absorption test. Dextrin is not fermented. The organism has been found in the intestinal tract of man and animals. It is sometimes associated with fevers of the paratyphoid type, and with cases of meat poisoning.*

6. *Salmonella anatum* (Rettger and Scoville) Bergey *et al*, 1925.

Alternative: 29. *Bacterium anatum* Rettger and Scoville, 1920.

SYNONYMY: *Bacterium anatis* Rettger and Scoville, 1919; *Escherichia anata* (Rettger) Bergey *et al*, 1923.

This organism was first described by Rettger and Scoville (1919) under the name of *Bacterium anatis*. This name had to be discarded because it had been previously used by Cornil and Toupet (1888) for an organism resembling or identical with *Pasteurella aviseptica*. Accordingly, they changed it (1920) to *Bacterium anatum*. The organism was isolated from an intestinal disease of ducklings known as "keel". Its morphological and cultural characters showed it to be a member of the colon-typhoid series. Litmus milk was made acid and then alkaline. A number of carbohydrates were fermented with acid and gas production, including glucose, dextrin,

arabinose, dulcitol, inositol, xylose and mannitol. Lactose, sucrose, inulin, raffinose, adonitol and salicin were not fermented. The organism is evidently a member of the *Salmonella* group—closely resembling *Sal. schottmülleri.* Its agglutination reactions likewise link it to this bacillus. Work done at this laboratory (results not yet published) show that the two organisms may be differentiated by means of the agglutinin-absorption test.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis, indistinguishable on the basis of morphological and cultural test from Sal. schottmülleri but differing from the latter serologically. Dextrin is fermented with acid and gas production; raffinose is not attacked. The causative agent of a disease of ducklings, known as "keel".*

7. *Salmonella veboda* (Castellani) Castellani and Chalmers, 1919.

Alternative: 30. *Bacterium veboda* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus veboda* Castellani, 1917; *Bacillus willegodai* Castellani, 1917; *Salmonella willegodai* (Cast.) Castellani and Chalmers, 1919.

Castellani (1917) characterized this organism as a motile rod, Gram-negative, not liquefying gelatine nor serum, indol negative, producing acid and gas from a number of carbohydrates, including glucose, maltose, dextrin, dulcitol, raffinose, arabinose, sorbitol and inositol. Acid but not gas was produced from mannitol and rhamnose. Lactose, sucrose, adonitol, inulin and salicin were not attacked. Castellani and Chalmers (1919) included it in their genus *Salmonella.*

Its partial fermentation of the alcohol mannitol sets it apart from the rest of the organisms of the intermediate group. On this basis, although Castellani is evidently the only author who has described such a species, it is deemed worthy of recognition. Weldin and Levine (1923) included it in their subgenus *Salmonella* of the genus *Bacterium.*

*Bacillus willegodai* of Castellani differs from *veboda* by its ability to produce acid and gas from salicin, and gas from rhamnose, its inability to produce gas from dulcitol and levulose, and by slight indol production. Until further instances of its isolation are recorded, it is thought best to regard it as a synonym of *Sal. veboda.*

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Mannitol is fermented with acid production, but no gas; acid and usually gas are produced from dulcitol; arabinose, raffinose, inositol, dextrin and usually salicin, fermented with acid and gas; adonitol is not attacked. Litmus milk is acidified, reverting to alkalinity. Found in the human intestines.*

8. *Salmonella columbensis* (Castellani) Castellani and Chalmers, 1919.

Alternative: 31. *Bacterium columbensis* Castellani, 1905.

SYNONYMY: *Bacillus columbensis* Castellani, 1917.

Castellani first described this organism in 1905 under the name *Bacterium columbense.* Later he decided it was identical with *Sal. schottmülleri,* but upon subsequent isolations and more careful study, he found it distinctly different from the latter. He isolated it from cases clinically similar to typhoid, but of medium severity. It gave distinctly negative

tests with typhoid serum, paratyphoid A serum and paratyphoid B serum. Culturally it was like paratyphoid B. Ordinarily it did not ferment lactose, though sometimes it produced a very slight amount of acid and gas. It is probable that the lactose might have been partially inverted on these occasions. It produced acid and gas from glucose, maltose, dulcitol, mannitol, arabinose, sorbitol and salicin, but not from sucrose, raffinose, adonitol, inulin or inositol. Its ability to ferment salicin separates it from *Sal. enteritidis*, and its lack of ability to attack inositol, as well as its agglutination reactions separate it from *Sal. schottmülleri*. The organism is apparently a valid species.

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Motility variable. Acid and gas produced from levulose, maltose, xylose, mannitol, dulcitol, arabinose, salicin and dextrin; raffinose, inositol and adonitol are not attacked. Indol is produced. Litmus milk becomes slightly acid, reverting to alkalinity. Acetyl-methyl-carbinol is not produced from glucose. Found in the faeces and urine, and sometimes in the blood of men suffering from a disease resembling typhoid.*

9. *Salmonella hirschfeldii Sp. nov.*

Alternative: 32. *Bacterium hirschfeldii Sp. nov.*

SYNONYMY: Paratyphoid C, Hirschfeld, 1919; *Bacillus paratyphosus* C, Castellani and Chalmers, 1919.

Uhlenhuth and Hubner (1908) proposed the term "paratyphosus C" for a race of bacilli found by them in pigs. They stated that it was culturally indistinguishable from *Sal. cholerae suis*, but was agglutinatively distinct from the latter and from the Gärtner bacillus. However, Uhlenhuth and his school did not differentiate between *Sal. cholerae suis* and paratyphoid B. It is impossible to say with certainty whether Uhlenhuth and Hubner were using *cholerae suis* or para B strains for comparison and their organism must be discarded as inadequately characterized, although there is a possibility that it may have been the same as the organism subsequently described by Hirschfeld.

Hirschfeld (1919) isolated from cases of enteric fever in the Serbian army, an organism which he labelled "paratyphoid C". This organism he considered distinct from the known paratyphoid organisms he was isolating from cases of enterica. Dudgeon and Urquhart (1921) working with the original strain of Hirschfeld's bacillus and with a strain secured by them which was similar in all respects to that of Hirschfeld, found them to be culturally indistinguishable from para B. Agglutinatively, however, they were distinct from para B, para A, the Gärtner bacillus and the Aertrycke bacillus, but serologically identical with *cholerae suis*. Mackie and Bowen (1919) and Andrewes and Neaves (1921) likewise stated that "paratyphosus C Hirschfeld" is not agglutinated by para B serum, but is serologically identical with *cholerae suis*.

Mackie and Bowen (1919), TenBroeck (1920), and Andrewes and Neave (1921) found para C distinctly separated from *cholerae suis* by its ability to ferment rapidly dulcitol and arabinose. Dudgeon and Urquhart (1921) also found differences in fermentability on these two carbohydrates for the two species. Andrewes and Neave (1921) as well as Weiss and

Rice (1917) found para C unable to attack inositol, thus furnishing a cultural method of differentiating it from para B.

The information at hand indicates that we have in *paratyphosus* C Hirschfeld, a distinct and recognizable species. Since the term "paratyphosus C" can hardly be considered in correct form, the name *Salmonella hirschfeldii* (or *Bacterium hirschfeldii*) is proposed in its place, the name to be applied to the organism originally isolated by Hirschfeld.

**Specific Diagnosis:** ***An organism conforming to the generic dignosis, and very similar to Salmonella enteritidis in its cultural and physiological characters. Levulose, maltose, arabinose, xylose, mannitol and dulcitol, but not adonitol, inositol or salicin, are fermented with acid and gas production. Acetyl-methyl-carbinol is not formed from glucose. Milk becomes acid, reverting to alkaline. Is not agglutinated by Sal. enteritidis serum. Found in the human intestines. A cause of enteric fever.***

10. *Salmonella enteritidis* (Gärtner) Castellani and Chalmers, 1919.

Alternative: 33. *Bacterium enteritidis* (Gärtner) Chester, 1897.

SYNONYMY: *Bacillus enteritidis* Gärtner, 1888; *Klebsiella enteritidis* DeToni and Trevisan, 1889; *Bacillus Gaertner* Morgan, 1905.

Gärtner (1888) isolated this organism from the flesh and organs of a cow which had been killed because of an attack characterized by mucous diarrhoea, and from the spleen of a man who died after eating some of the flesh of the animal. Its description with regard to its morphology, staining reactions, and cultural characters was typical of the colon-typhoid bacteria. Gärtner named it *Bacillus enteritidis.*

While the organism has been considered a distinct species by all authors who have described it, means of differentiating it clearly from other members of the intermediate group have been developed comparatively recently. Its quick reversion of litmus milk from an acid to an alkaline reaction was used to separate it from *Sal. paratyphi* (paratyphoid A), (Schottmüller, 1901; Kayser, 1904; Morgan, 1905). Ford (1905), Harding and Ostenberg (1912), and Krumwiede, Pratt and Kohn (1916) found that *Sal. enteritidis* was one of a group which differed from paratyphoids in their ability to produce acid and gas from xylose.*

The agglutination test served for a long time to distinguish *Sal. enteritidis* from *Sal. schottmülleri* (paratyphoid B) and from *Sal. cholerae suis.* Jordan (1917) separated *Sal. schottmülleri* and *Sal. enteritidis* from *Sal. cholerae suis* by their action on dulcitol and arabinose, the first two fermenting both sugars rapidly with acid and gas production, while *Sal. cholerae suis* fermented them slowly or not at all. In the same year, Weiss and Rice used inositol to differentiate *Sal. enteritidis* from *Sal. schottmülleri. Sal. enteritidis,* as well as *Sal. cholerae suis* and *Sal. abortivo-equinum,* were non-gas-producing on this sugar; *Sal. schottmülleri,* gas-producing. The species as it is generally recognized today is adequately defined by Winslow, Kligler and Rothberg (1919).

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* Jordan and Victorsan (1917) used blackening of lead-acetate agar for differentiation of enteritidis from paratyphi, the former giving a positive reaction, the latter, negative.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Levulose, maltose, arabinose, xylose, mannitol and dulcitol are fermented with acid and gas production; adonitol, inositol and salicin are not attacked. Acetyl-methyl-carbinol is not formed from glucose. Litmus milk becomes acid, reverting rapidly to alkalinity. Found in human and animal intestines, and sometimes in meat. One of the causes of epidemic meat poisoning.*

11. *Salmonella abortivo-equina* (Good and Corbett) Bergey *et al*, 1923.

Alternative: 34. *Bacterium abortivo-equinum* (Good and Corbett) Fitch, 1920.

SYNONYMY: *Bacillus abortivus equinus* Good and Corbett, 1913; *Bacillus abortus equi* Meyer and Boerner, 1913; *Bacillus abortivo-equinus* Good and Corbett, 1916; *Bacillus abortus equinus* Weiss and Rice, 1917; *Bacterium abortum-equi* Holland, 1920; *Salmonella abortus-equi* (Meyer and Boerner) Bergey *et al*, 1925.

The name *Bacillus abortivus equinus* was given to the organism causing infectious abortion of mares, by Good and Corbett in 1913; Meyer and Boerner in the same year proposed the name *Bacillus abortus equi*. Good and Corbett's name, however, antedates that of Meyer and Boerner by six months. They were all working with the same organism, an undoubted member of the intermediate or *Salmonella* group. The morphological and cultural characters were those of the organisms of the colon-typhoid series. Glucose, mannitol, dulcitol, xylose were among the sugars fermented with acid and gas; lactose and sucrose were not attacked. Good and Corbett (1916) and Good and Smith (1916) reported slight fermentation with gas production in both lactose and sucrose. It is significant, however, that they also found slight gas production in these sugars by *Sal. enteritidis*. This would seem to indicate a probable partial inversion of their disaccharids. No other author has reported gas production for *Sal. abortivo-equina* in these two sugars. Considerable cross-agglutination occurred with other members of the *Salmonella* group, especially with *Sal. enteritidis*. Weiss and Rice (1917) tested the organism in inositol and found it non-gas-producing.

Good and Corbett (1916) corrected the name from trinominal form by changing it to *Bacillus abortivo-equinus*.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. It produces acid and gas from levulose, maltose, arabinose, xylose, mannitol and dulcitol; dextrin, adonitol, inositol and salicin are not fermented. Litmus milk becomes acid, then alkaline. It may be differentiated from Salmonella enteritidis by means of the agglutinin-absorption test. The cause of infectious abortion of mares.*

12. *Salmonella paratyphi* (Kayser) Bergey *et al*, 1923.

Alternative: 35. *Bacterium paratyphi* Kayser, 1902.

SYNONYMY: *Bacterium paratyphi* Type A, Kayser, 1902; "Bacillus paratyphoid A. Schottmüller" Morgan, 1905; *Bacillus paratyphosus* A, Wilson, 1908; *Bacillus paratyphosus* Winslow, Kligler, Rothberg, 1919; *Salmonella para-typhosus* A, (Schottmüller) Castellani and Chalmers, 1919.

The term "paratyphosus A" has been variously accredited to Brion and Kayser (1902) and to Schottmüller (1901). The organism now commonly known under this name was described by the above authors, but was

not named by them. Kayser (1902) used for paratyphoid organisms (both A and B) the name *Bacterium paratyphi.* In 1904, he separated *Bacterium paratyphi* into types A and B, (Type A for the Brion and Kayser organism and two of Schottmüller's organisms) on the basis of their action in litmus milk and on agglutination tests. Type A produced and maintained an acid reaction in milk, while Type B reverted in two weeks to an alkaline reaction. (Boycott (1906) and others have shown that para A milk cultures eventually become alkaline.) Neither type was able to attack lactose.

The organisms have been recognized as distinct and recognizable species since the time of their first isolation. Subsequent authors working with subcultures of the original strains and with freshly isolated strains have added much to their characterization. Ford (1905) noted the inability of para A to attack xylose and suggested this character for differentiation from para B. Harding and Ostenberg (1912) and Krumwiede, Pratt and Kohn (1916) also favored xylose fermentation for differentiation of the para B-*enteritidis-cholerae suis* types from para A types. Wiess and Rice (1917) pointed out that para A fails to attack inositol while para B produces acid and gas. The species is, at the present time, very clearly distinguished from related species of the intermediate group.

In order that the species might have a name in proper Latin form, Winslow, Kligler and Rothberg (1919) proposed *Bacillus paratyphosus.* It would seem, however, that Kayser's name, *paratyphi,* might be retained for the type A since it was applied to both species before they were differentiated. This would be in accordance with the Botanical Rules of Nomenclature. (Article 47. When a species or subdivision of a species is divided into two or more groups of the same nature, if one of the two forms was distinguished or described earlier than the other, the name is retained for that form.)

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Levulose, maltose, arabinose, mannitol and dulcitol are fermented with acid and gas production; raffinose, xylose, adonitol, inositol and salicin are not attacked. Acetyl-methyl-carbinol is not produced from glucose. Indol is not produced. Litmus milk becomes acid, reverting very slowly to alkalinity. The cause of paratyphoid fever in man.*

13. *Salmonella woliniae* (Castellani) Castellani and Chalmers, 1919.

Alternative: 36. *Bacterium woliniae* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus woliniae* Castellani, 1917.

Castellani (1917) described this organism as a motile rod, Gram-negative, not liquefying gelatin nor serum, not producing indol, producing acid and gas from glucose, maltose and mannitol, acid, or acid and gas from galactose, and an acid or alkaline reaction in saccharose. Levulose, lactose, dulcitol, dextrin, raffinose, arabinose, adonitol, inulin, sorbitol, inositol and salicin were not attacked. Litmus milk was made acid and sometimes reverted to alkalinity.

The organism is distinctive by reason of its inability to attack levulose. On this account it is deemed worthy of recognition as a species. Weldin and Levine (1923) placed it in their subgenus *Salmonella* of the genus *Bacterium.*

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. It ferments maltose and mannitol with acid and gas, but does not attack levulose, raffinose, arabinose, dextrin, dulcitol, adonitol, inositol or salicin. Indol is not produced. Litmus milk becomes acid, sometimes reverting slowly to alkalinity. Found in the human intestinal tract.*

14. *Salmonella watareka* (Castellani) Bergey *et al*, 1923.

Alternative: 37. *Bacterium watareka* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus watareka* Castellani, 1917.

Castellani (1917) described this organism as a motile rod, Gram-negative, not liquefying gelatine nor serum, producing acid in litmus milk, producing indol, producing acid and gas from a number of carbohydrates, including glucose, maltose, dulcitol, mannitol, raffiinose, arabinose, sorbitol and inositol. Lactose, sucrose, dextrin, adonitol, inulin and salicin were not attacked.

The organism is evidently a member of the intermediate group. Its acid production without reversion to alkalinity in litmus milk allies it to the paratyphoid A organism. Its ability to attack inositol and raffinose, and to produce indol, however, distinctly separate it from this bacillus. Similar organisms have been described by MacConkey (1905), Cathcart (1906), Morgan (1907) and Lewis (1911).

Weldin and Levine (1923) included it in their subgenus *Salmonella* of the genus *Bacterium*.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Acid and gas are produced from levulose, maltose, raffinose, arabinose, mannitol, dulcitol and inositol, but not from adonitol or salicin. Indol is produced. Litmus milk becomes acid. Found in the intestinal tract of man.*

15. *Salmonella pullorum* (Rettger) Bergey *et al*, 1923.

Alternative: 38. *Bacterium pullorum* Rettger, 1909.

SYNONYMY: *Bacillus pullorum* Smith and TenBroeck, 1917; *Salmonella pullorum* (Rettger) Bergey *et al*, 1925.

Rettger first described this organism in 1900 and 1901. It was further described by Rettger and Harvey in 1908, but it was not named until 1909, when Rettger named it *Bacterium pullorum*. The bacillus was shown to be the cause of bacillary white diarrhoea of chicks. Its morphological and cultural characters were typical of the colon-typhoid series. At first reported motile, it was later (Rettger and Harvey, 1908) found to be nonmotile. It fermented dextrose and mannitol with acid and gas production, but not maltose, lactose, saccharose, inulin nor dextrin.

There seems to have been considerable confusion for a time between this organism and the fowl-typhoid bacillus. Various authors, notably Smith and TenBroeck (1915), Goldberg (1917), Rettger and Koser (1917), and Hadley, Caldwell, Elkins and Lambert (1917), compared the two organisms. While they found the two closely related serologically, they all found behavior in carbohydrate media an easy method of differentiation. *Sal. pullorum* produced gas as well as acid from certain carbohydrates, while the fowl typhoid organism produced acid only. Further, *pullorum*

was found to be strictly maltose-dextrin-dulcitol negative, while the other organism attacked these substances.

Hadley, Elkins and Caldwell (1918) described *Sal. pullorum* as "weakly xylose-positive" (acid and gas). Krumwiede, Kohn and Valentine (1918) recorded acid production from xylose as + or ±. Mulsow (1919) reported gas variable and acid positive for xylose. Apparently the organism should be considered as usually attacking xylose.

**Specific Diagnosis:** *A non-motile rod, conforming to the generic diagnosis. Levulose, arabinose, mannitol and usually xylose are fermented with acid and gas production; maltose, raffinose, dextrin, dulcitol, adonitol, inositol and salicin are not attacked. Acetyl-methyl-carbinol is not produced from glucose. Indol is not produced. Litmus milk is acidified, slowly reverting to neutral or alkaline reaction. The cause of "white diarrhoea" in chicks.*

16. *Salmonelli morganii* (Winslow, Kligler, Rothberg) Castellani and Chalmers, 1919.

Alternative: 39. *Bacterium morganii* (Winslow, Kligler and Rothberg) Holland, 1920.

SYNONYMY: Organism No. 1, Morgan, 1906; *Bacillus morgan* No. 1, Castellani and Chalmers, 1919; *Bacillus morgani* Winslow, Kligler, Rothberg, 1919; *Bacillus pseudo-morgani* Castellani and Chalmers, 1919.

Morgan (1906) first described this organism which he had isolated from the stools from cases of summer diarrhoea in infants. He described it (Organism No. 1) as a motile bacillus closely resembling the hog cholera bacillus of McFadyean, but differing from the latter in its alkaline reaction on litmus milk, its greater indol production and its failure to attack maltose, arabinose and dextrin. The monosaccharids were the only carbohydrates which it was able to attack. Morgan felt on account of its prevalence that it probably had some etiological significance in infant diarrhoea.

The organism has been repeatedly isolated from cases of diarrhoea in children (Lewis, 1911; Cox, Lewis and Glynn, 1912; Pirie, 1917; Stewart, 1917; Zironi and Capone, 1917; Tribondeau and Fichet, 1916; Logan, 1916; Thjotta, 1920; Levine, Ajwani and Weldin, 1925, etc.) both motile and non-motile varieties having been found. Winslow, Kligler and Rothberg (1919) concluded that "it constitutes a fairly definite type of common occurrence in the human intestinal canal" and gave it the name *Bacillus morgani.*

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Both motile and non-motile varieties. Acid and gas produced from monosaccharids, and rarely from xylose; maltose, raffinose, arabinose, dextrin, mannitol, dulcitol, inositol, salicin, adonitol and sorbitol are not attacked. Litmus milk remains neutral or becomes alkaline. Indol is produced. Found in normal and diarrheal stools, particularly of infants.*

17. *Salmonella foetida* (Perez) Bergey *et al,* 1923.

Alternative: 40. *Bacterium foetidum* (Perez) Weldin and Levine, 1923.

SYNONYMY: *Coccobacillus foetidus ozenae* Perez, 1899; *Coccobacillus (foetidus) ozaenae* Ward, 1917; *Escherichia foetida* Bergey *et al,* 1925.

Perez (1899) described this organism, isolated by him from ozena, under the name *Coccobacillus foetidus ozenae.* The bacillus was small, often coccoid in form, though it might show filaments. It was non-spore-forming, non-motile, easily cultivated and easily stained, though not by Gram's method. On ordinary media its growth was typical of the colon-typhoid series. Neither gelatin nor blood serum were liquefied. Indol was formed. Milk was never coagulated, nor was lactose fermented. It produced a pronounced and characteristic foetid odor. It was pathogenic for guinea pigs, mice, pigeons and rabbits.

Ward (1917) studied a number of strains of Perez' bacillus, both from European sources and from his own isolations. Besides confirming Perez' findings, he investigated its action on a number of carbohydrates: glucose, levulose, maltose, dextrin, lactose, saccharose, glycerol, inulin and mannitol. Of these, glucose was the only one attacked; gas production even in this sugar was slow, but might after a number of days amount to 15-75% ($CO_2$ and $H_2$).

Weldin and Levine (1923) listed the organism in the genus *Bacterium,* subgenus *Salmonella.* Bergey *et al* (1923) included it in their genus *Salmonella,* but in the second edition of their Manual (1925) moved it to the genus *Escherichia.* Just why it was placed in this group is not apparent, since its fermentation reactions as determined by Ward (1917) definitely place it with *Salmonella.*

**Specific Diagnosis:** *A non-motile rod, conforming to the generic diagnosis. As indicated above, of the carbohydrates tested, glucose is the only one which is fermented with acid and gas. Indol is produced. Litmus milk becomes slightly acid. Found in the nasal exudate from cases of ozena.*

18. *Salmonella giumai* (Castellani) Bergey *et al,* 1923.

Alternative: 41. *Bacterium giumai* (Castellani) Weldin and Levine, 1923.

Synonymy: *Bacillus giumai* Castellani, 1912; *Wesenbergus giumai* (Cast.) Castellani and Chalmers, 1919.

This organism was described by Castellani (1912) as a rather rare cause of parenteric fever in the tropics. While its cultural and morphological characters were not given in detail, they evidently were the same as those of the colon-typhoid series with which it was grouped. It was non-motile, did not liquefy gelatin or serum, produced indol, brought about an acid reaction followed by alkalinity in litmus milk and fermented a number of carbohydrates with acid and gas, including glucose, maltose, dextrin, arabinose, sorbitol, galactose, levulose, salicin and glycerol. Acid but not gas was produced from lactose. Saccharose, dulcitol, mannitol, raffinose, adonitol, inulin and inositol were not fermented.

Evidently the organism belongs in the *Salmonella* group. Its failure to attack mannitol places it in the Morgan section, but its greater fermentative ability adequately separates it from the Morgan bacillus.

**Specific Diagnosis:** *A non-motile rod, conforming to the generic diagnosis. It produces acid and gas from levulose, maltose, arabinose, dextrin, salicin and sorbitol; acid, but not gas, is produced from lactose (?); raffinose, mannitol, dulcitol, adonitol and inositol are not attacked. Litmus milk becomes acid followed by alkalinity. Indol is produced. Found in the intestines of man. A cause of parenteric fever.*

19. *Salmonella macfadyeanii* (Weldin and Levine) Comb-nov.

Alternative: 42. *Bacterium macfadyeanii* Weldin and Levine, 1923.

The writer, up to the present time, has been unable to secure McFadyean's original description of this organism. It was described by Morgan in 1905 and by Castellani in 1912, under the title "B-hog cholera, McFadyean." Morgan (1906, 1907) in discussing his organism No. 1, (*Sal. morganii*), stated that it resembled the hog cholera bacillus of McFadyean more than any other known pathogen. The organism is of interest as being, perhaps, the first described of the non-mannitol-fermenting members of the Intermediate section. It is somewhat more active than the Morgan bacillus.

**Specific Diagnosis:** ***A motile rod, conforming to the generic diagnosis. It ferments levulose, maltose, arabinose and dextrin with acid and gas production; raffinose, mannitol, dulcitol, salicin and sorbitol are not attacked. Indol is produced. Litmus milk becomes acid. Found in the intestines of hogs.***

20. *Salmonella archibaldii* (Castellani) Castellani and Chalmers, 1919.

Alternative: 43. *Bacterium archibaldii* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus archibaldi* Castellani, 1917; *Aerobacter archibaldi* (Castellani and Chalmers) Bergey *et al*, 1923.

This organism was isolated from cases of parenteric fever and described by Archibald (1912). Castellani (1917) gave it the name of *Bacillus archibald* (later changed to *archibaldi*). It evidently resembled in its morphological and cultural characters, the organisms of the colon-typhoid series. Castellani described it as a Gram-negative bacillus not liquefying gelatin nor serum. In litmus milk, it produced acidity followed by alkalinity. Glucose, maltose, dulcitol, mannitol and sorbitol were fermented with acid and gas, but lactose, saccharose, raffinose and adonitol were not attacked. It produced indol and gave a positive Voges-Proskauer reaction. This last named property sets it apart from the other members of the *Salmonella* group. Buchan (1910) has likewise described organisms of the intermediate group giving the Voges-Proskauer reaction. Although this type of organism is unusual, they evidently do exist and should, therefore, be recognized.

**Specific Diagnosis:** ***A motile rod, conforming to the generic diagnosis. Maltose, mannitol, dulcitol and sorbitol are fermented with acid and gas; raffinose and adonitol are not attacked. Acetyl-methyl-carbinol is produced from glucose. Indol is produced. Litmus milk becomes acid, followed by alkalinity. Isolated from human intestines. A probable cause of enteric fever.***

### GENUS 5. *EBERTHELLA* Buchanan, 1918.

SYNONYMY: *Dysenteroides* Castellani and Chalmers, 1919; *Eberthus* Castellani and Chalmers, 1919; *Lankoides* (in part) Castellani and Chalmers, 1919.

This name was first used by Buchanan (1918) for the third subgenus of his genus *Bacterium* with the following definition:

Not producing gas from any of the carbohydrates, acid may or may not be formed. Type species: *Bacterium (Eberthella) typhi* Flügge.

Weldin and Levine (1923) likewise used it for a subgenus of *Bacterium*, its distinguishing character being the production of acid but not gas from glucose.

Bergey *et al* (1923) elevated the subgenus to generic rank. They defined it (1925) as follows:

**Motile or non-motile rods, generally occurring in the intestinal canal of man, usually in different forms of enteric inflammation. Attack a number of carbohydrates with the formation of acid but no gas. Do not form acetyl-methyl-carbinol. Type species: *Eberthella typhi* (Eberth-Gaffky) Buchanan.**

The organisms thus characterized form a clearly defined group of the colon-typhoid series and should be recognized as such. In reality, two rather distinct types are represented: namely, typhoid and dysentery. The typhoid and dysentery bacilli produce characteristically different diseases, show little or no serological relation, the former being motile, the latter, non-motile. It is proposed to use the term *Eberthella* for the fifth genus of the colon-typhoid series, to include only the motile forms of the typhoid-dysentery section. The non-motile forms will be included in the genus *Shigella*.

**Generic Diagnosis:** ***Motile, Gram-negative, non-spore-forming rods. Produce acid but not gas from glucose. Generally occur in the intestinal tract of man or animals, usually in enteric inflammation.***

**Type species: *Eberthella typhosa* (Zopf).**

KEY TO SPECIES OF THE GENUS *EBERTHELLA*.

a. Neither acid nor gas produced from lactose.
  b. Neither acid nor gas produced from inositol.
    c. Acid, but not gas, produced from levulose, maltose and dextrin.
      d. Acid, but not gas, produced from mannitol and sorbitol; neither acid nor gas from inulin and salicin.
        1. *Eberthella typhosa.*
      dd. Neither acid nor gas produced from mannitol and sorbitol; acid, but not gas, from inulin and salicin.
        2. *Eberthella pritnitzii.*
    cc. Neither acid nor gas produced from levulose, maltose and dextrin.
      3. *Eberthella lewisii.*
  bb. Acid, but not gas, produced from inositol.
    c. Neither acid nor gas produced from salicin; indol not produced.
      4. *Eberthella kandiensis.*
    cc. Acid, but not gas, produced from salicin; indol produced.
      5. *Eberthella talavensis.*

aa. Acid, but not gas, produced from lactose.
  b. Acid, but not gas, produced from mannitol and arabinose.
    c. Acid, but not gas, produced from dulcitol.
      6. *Eberthella pyogenes.*

cc. Neither acid nor gas produced from dulcitol.

d. Neither acid nor gas produced from salicin; indol produced.

7. *Eberthella belfastiensis.*

dd. Acid, but not gas, produced from salicin; indol not produced.

8. *Eberthella wilsonii.*

bb. Neither acid nor gas produced from mannitol and arabinose.

9. *Eberthella bentotensis.*

## SPECIES OF *EBERTHELLA*

1. *Eberthella typhosa* (Zopf) Comb. nov.

Alternative: 43. *Bacterium typhosum* Zopf, 1884.

SYNONYMY: *Bacillus typhosus* Eberth, Zopf, 1885; *Bacillus typhi abdominalis* Flügge, 1886; *Bacillus typhi* (Eberth, Gaffky) Schroeter, 1889; *Vibrio typhosus* Trevisan, 1889; *Bacterium typhi,* Eberth, Gaffky-Lehmann and Neumann, 1896; *Eberthus typhosus* (Eberth) Castellani and Chalmers, 1919; *Eberthella typhi* (Eberth-Gaffky) Castellani and Chalmers, Bergey *et al,* 1923; *Eberthella typhi* (Schroeter) Buchanan, Bergey *et al,* 1925; not *Micrococcus ileotyphi* Trevisan, 1879; *Bacillus typhosus* Klebs, 1881; *Mikrokokkus typhi abdominalis* Letzerich 1881; *Metallacter ileotyphi* Trevisan, 1882.

Eberth (1880) is commonly credited as being the first to demonstrate the presence in the body of typhoid cadavers of the organism now known to be the cause of the disease. The organism was first isolated by Gaffky in 1884. Neither author gave to the bacillus any specific name. Eberth (1880) recorded finding in some cells "sporenähnlicher Körperchen" and Gaffky (1884) definitely described the organism as forming spores which were particularly abundant on potato cultures grown at 30 to 42° C. Otherwise their descriptions, though brief, were typical of the typhoid bacillus. Subsequent investigators believed Eberth and Gaffky to have been mistaken as to the presence of spores. Sternberg (1892) said, "Spherical or oval refractive granules are often seen at the extremities of the rods, especially in potato cultures kept in the incubating oven; these are not reproductive spores, as was at first supposed."

Klebs in 1881 discovered an organism which he thought was the causative agent of typhoid fever and which he called *Bacillus typhosus.* It was described as threads sometimes 50 microns long and was unquestionably a spore former. Trevisan (1879) used the name *Micrococcus ileotyphi* for an organism supposedly the cause of abdominal typhoid. His description was too brief for any possible recognition of the organism. In 1882, he transferred the species to the genus *Metallacter.* Judging from his discussion, he considered his organism identical with Klebs' *Bacillus typhosus.* In his I Generi e le Specie delle Batteriacee, 1889, we find the following:

"*Bacillus Klebsii* Trev., 1885 *(Bacillus typhosus Klebs,* 1881)—Bacillo della necrosi intestinale—", and further:

"*Vibrio typhosus* Trev. (*Bacillus typhosus Eberth*, 1880). Anaerobio facoltativo. Non liquefa la gelatina."

Letzerich (1881) used the name *Mikrokokkus typhi abdominalis* for an organism which, as described in the body of his article, might have been the typhoid bacillus. In a "Nachschrift," however, he recorded finding long spore forming filaments and concluded his organism to be identical with Klebs' *Bacillus typhosus* which had been described some months previously.

Zopf (1884) used the name *Bacterium typhosum* for Eberth's organism, with a very brief description. This was apparently the first valid name given to the true typhoid organism. In the third edition of "Die Spaltpilze," published in 1885, he changed the name to *Bacillus typhosus*.

Flügge (1886) used the term *Bacillus typhi abdominalis*. He referred to both Eberth and Klebs organisms.

*Bacillus typhi* was used by Schroeter (1889) for Eberth and Gaffky's organism. The specific name *typhi* has been used extensively for the organism, with credit given variously to Eberth, Gaffky and to Schroeter.

The organism has been carefully studied by subsequent investigators and its cultural, biochemical and serological characters fully determined. Winslow, Kligler and Rothberg (1919) gave a compact and accurate summary of its characters under the name *Bacillus typhosus* (Zopf).

**Specific Diagnosis:** *A rod, conforming to the generic diagnosis, single or in pairs, occasionally in short chains. It produces acid, but not gas, from levulose, maltose, dextrin, mannitol and sorbitol; lactose, sucrose, inulin, inositol, salicin and usually arabinose and dulcitol, are not fermented. Litmus milk is made acid, slowly reverting to neutral or slight alkalinity. Indol is not produced. Pathogenic for man and laboratory animals. The cause of typhoid fever in man.*

2. *Eberthella pritnitzii* (Castellani) Comb. nov.

Alternative: 44. *Bacterium pritnitzii* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus pritnitzi* Castellani, 1917; *Eberthus priztnitzi* (Cast.) Castellani and Chalmers, 1919; *Eberthus pristnitzi* (Cast.) Castellani and Chalmers, 1920; *Bacterium pristnitzi* Weldin and Levine, 1923.

Castellani (1917) described this organism as a motile rod, Gram-negative, not liquefying gelatin or serum, indol production negative, and producing acid in litmus milk. It produced acid, but not gas, from glucose, maltose, inulin, salicin and dextrin. Lactose, sucrose, dulcitol, mannitol, raffinose, arabinose, adonitol, sorbitol and inositol were not attacked. It was the causal organism of some cases of parenteric fever.

This organism resembles *Eberthella typhosa* very closely, differing in its action on mannitol, sorbitol, inulin and salicin. Weldin and Levine (1923) included it in their subgenus *Eberthella* of the genus *Bacterium*.

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Levulose, maltose, dextrin, inulin and salicin are fermented with acid production; it does not attack lactose, sucrose, raffinose, arabinose, mannitol, dulcitol, sorbitol or inositol. Litmus milk is acidified. Indol is not produced. A cause of paraenteric fever.*

3. *Eberthella lewisii* (Weldin and Levine) Comb. nov.

Alternative: 45. *Bacterium lewisii* Weldin and Levine, 1923.

SYNONYMY: Organism $B3_2$, Lewis, 1911.

Lewis (1911) described an organism isolated from the faeces of a normal child, as a non-chromogenic, Gram-negative, motile bacillus, not liquefying gelatin, not producing indol and making litmus milk alkaline. Of a list of 17 carbohydrates studied, only one was attacked, namely glucose, with acid production.

The fact that an organism exists of such weak fermentative ability that it is able to attack only a single sugar, would seem to be sufficient reason for its recognition as a species. Weldin and Levine (1923) created for this organism, which Lewis designated as $B3_2$, the name *Bacterium lewisii*. They included it in their subgenus *Eberthella*.

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Of the carbohydrates glucose, levulose, galactose, mannose, maltose, lactose, sucrose, raffinose, dextrin, inulin, mannitol, dulcitol, sorbitol, inositol and salicin, only glucose is fermented with acid production. Litmus milk is made alkaline. Indol is not produced. Found in human faeces.*

4. *Eberthella kandiensis* (Castellani) Bergey *et al*, 1923.

Alternative: 46. *Bacterium kandiensis* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus kandiensis* Castellani, 1912; *Eberthus kandiensis* Castellani and Chalmers, 1919.

Castellani (1912) described this organism as a motile rod, Gram-negative, not liquefying gelatin nor serum, not producing indol, and giving an acid reaction in litmus milk followed by alkalinity. Acid, but not gas, was produced from glucose and some other carbohydrates. The organism was considered to be ordinarily non-pathogenic, but, under special conditions, it might become pathogenic, causing a type of parenteric fever.

The bacillus as described resembled the typhoid organism, differing from it by its action on some of the carbohydrates, particularly inositol, sucrose, maltose and dextrin. Weldin and Levine (1923) included it in their subgenus *Eberthella* of the genus *Bacterium*.

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Acid, but not gas, is produced from levulose, sucrose (slight amount), mannitol and inositol; lactose, maltose, raffinose, arabinose, dextrin, inulin, dulcitol, sorbitol and salicin are not attacked. Litmus milk becomes acid, reverting to alkalinity. Indol is not produced. Sometimes pathogenic, causing a type of parenteric fever. Found in the human intestines.*

5. *Eberthella talavensis* (Castellani) Bergey *et al*, 1923.

Alternative: 47. *Bacterium talavensis* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus talavensis* Castellani, 1912; *Eberthus talavensis* (Cast.) Castellani and Chalmers, 1919.

This organism as described by Castellani (1912) differed from *Eberthella kandiensis* (Cast.) (which see) by its production of indol, production of acid from salicin, and inability to attack mannitol, adonitol, isodulcitol

and erythritol. It was listed as one of a number of species which are the causal agents of enteroidea.

Weldin and Levine (1923) placed it in their subgenus *Eberthella* of the genus *Bacterium.*

**Specific Diagnosis:** ***A rod conforming to the generic diagnosis. It produces acid, but not gas, from levulose, sucrose, inositol and salicin; lactose, maltose, raffinose, arabinose, dextrin, inulin, mannitol, dulcitol and sorbitol are not fermented. Litmus milk is made alkaline. Indol is produced. Found in the intestines of man. A possible cause of enteric fever.***

6. *Eberthella pyogenes* (Passet) Bergey *et al,* 1923.

Alternative: 48. *Bacterium pyogenes* (Passet) Migula, 1900.

SYNONYMY: *Bacillus pyogenes foetidus* Passet, 1885; *Lankoides pyogenus foetidus* (Passet) Castellani and Chalmers, 1919; *Bacterium pyogenes-foetidum* (Passet) Holland, 1920; *Eberthella pyogenes* (Migula) Bergey *et al,* 1925.

Passet (1885) isolated this organism from a rectal abscess. He named it *Bacillus pyogenes foetidus* and described it as a small, motile rod with rounded ends. Its growth on ordinary laboratory media was typical of the colon-typhoid organisms. MacConkey (1905) obtained a culture of the organism from Kràl and found it able to produce acid from glucose, levulose, galactose, maltose, arabinose, raffinose, lactose, sucrose, mannitol, sorbitol, dulcitol and dextrin. Castellani (1912) added to its description the ability to produce indol.

The organism is evidently a member of the genus *Eberthella,* characterized by its ability to produce acid in an exceptionally large number (for this group) of carbohydrates. Migula (1900) reduced the name to binominal form, calling it *Bacterium pyogenes.*

**Specific Diagnosis:** ***A motile rod conforming to the generic diagnosis. Acid, but not gas, is produced from levulose, lactose, maltose, sucrose, raffinose, arabinose, dextrin, mannitol, dulcitol and sorbitol. Indol is produced. Litmus milk becomes acid and coagulated. Isolated from a rectal abscess.***

7. *Eberthella belfastiensis* (Wilson) Bergey *et al,* 1923.

Alternative: 49. *Bacterium belfastiensis* (Wilson) Weldin and Levine, 1923.

SYNONYMY: *Bacillus belfastiensis* II, Wilson, 1908.

Wilson (1908) described under the name *Bacillus belfastiensis* II, an organism isolated by Mair (1906) from the urine of a case of cystitis. Mair had not completely described nor had he named the bacillus. The organism was a motile non-sporing bacillus, Gram-negative, producing a greyish-white growth on agar and uniform turbidity in broth. Gelatin was not liquefied. No gas was produced in glucose. Indol was formed. Acid was produced in glucose, levulose, maltose, lactose, sucrose, mannitol, glycerin, arabinose, raffinose and sorbitol. Dulcitol, glycerol, adonitol, erythritol, salicin, dextrin and inulin were not attacked. Acid was produced in litmus milk.

The species is clearly differentiated from the typhoid bacillus by its acid production in lactose and sucrose. Weldin and Levine (1923) included it in their subgenus *Eberthella* of the genus *Bacterium.*

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Levulose, lactose, maltose, sucrose, raffinose, arabinose, mannitol and sorbitol are fermented with acid, but not gas production; dextrin, inulin, dulcitol and salicin are not attacked. Indol is formed. Acid is produced in milk. Isolated from urine.*

8. *Eberthella wilsonii* sp. nov.

Alternative: 50. *Bacterium wilsonii* sp. nov.

SYNONYMY: *Bacillus belfastiensis* V Wilson, 1908.

*Belfastiensis* V, isolated by Wilson (1908) from urine, was similar to his *Belfastiensis* II (*Eberthella belfastiensis*) differing from it by fermenting salicin and dextrin, but not attacking sorbitol or glycerin, and by its failure to produce indol. Since the name *belfastiensis* was used for the preceding organism, it is invalid here; the specific name *wilsonii* is suggested for this species.

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Acid, but not gas, is produced from levulose, lactose, maltose, sucrose, raffinose, arabinose, dextrin, mannitol and salicin; neither acid nor gas are produced from inulin and dulcitol. Acid is produced from litmus milk. Indol is not formed. Found in urine.*

9. *Eberthella bentotensis* (Castellani) Bergey *et al*, 1923.

Alternative: 51. *Bacterium bentotensis* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus bentotensis* Castellani, 1912; *Dysenteroides bentotensis* (Cast.) Castellani and Chalmers, 1919.

This organism was described by Castellani (1912) as a motile, Gram-negative rod, producing indol, not liquefying gelatin nor serum, and giving an acid reaction in litmus milk. Acid, but not gas, was produced from glucose, maltose, lactose, sucrose, dulcitol, raffinose, inositol, salicin and glycerin. Mannitol, dextrin, arabinose, adonitol, inulin and sorbitol were not attacked.

It differs from *Eberth. typhosa* in its ability to produce acid from lactose and from *Eberth. belfastiensis* and *Eberth. pyogenes* in not attacking mannitol or arabinose. It was included by Weldin and Levine (1923) in their subgenus *Eberthella* of the genus *Bacterium*.

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Acid is produced from levulose, lactose, maltose, sucrose, raffinose, dulcitol, inositol and salicin; arabinose, dextrin, inulin, mannitol and sorbitol are not fermented. Acid is produced in litmus milk. Indol is formed. Found in human intestinal tract.*

### Genus 6. *SHIGELLA* Castellani and Chalmers, 1919.

SYNONYMY: *Graciloides* Castellani and Chalmers, 1919; *Lankoides* (in part) Castellani and Chalmers, 1919; *Eberthella* (in part) Buchanan, 1918.

Castellani and Chalmers (1919) used this term for the third genus of the tribe *Ebertheae*. The description of the genus was as follows:

*Ebertheae* non-motile, partially fermenting glucose with the production of acid but no gas; lactose not fermented. Milk not clotted. Type species: *Shigella dysenteriae* (Kruse, 1899).

Lactose fermentation and clotting of milk are not considered advisable characters to use for generic differentiation for the typhoid-dysentery group. (See *Dysenteroides* and *Lankoides*, pp. 127 and 124.)

In the discussion of the name *Eberthella*, the creation of two genera to represent the typhoid and dysentery organisms was advocated. The first was to include the motile species, producing typhoid-like infections, and was to be called *Eberthella*. The name *Shigella* appears to be the most suitable name for the non-motile types, most of which are associated with some form of bacillary dysentery.

The term *Shigella* was also used by Castellani and Chalmers (1919) for a subgenus of their genus *Shigella*. The subgenus included only the non-mannitol-fermenters, the mannitol fermenters being designated by the name *Flexnerella*. It is not felt that the name should be retained for this grouping. The mannitol fermenters and non-fermenters may be considered sections of the genus *Shigella*, under the terms *Flexner* section and *Shiga* section.

**Generic Diagnosis:** *Non-motile, Gram-negative, non-spore-forming, short rods. Produce acid, but not gas, from glucose. Generally occur in the intestinal tract of man or animals. Usually pathogenic, producing dysenteries or dysentery-like diseases.*

**Type species:** *Shigella dysenteriae* (Shiga) Castellani and Chalmers, 1919.

KEY TO SPECIES OF THE GENUS *SHIGELLA*

a. Neither acid nor gas produced from mannitol.
  b. Neither acid nor gas produced from sucrose, arabinose and dextrin.
    c. Neither acid nor gas produced from rhamnose; indol not produced.
      1. *Shigella dysenteriae.*
    cc. Acid, but not gas, produced from rhamnose; indol produced.
      2. *Shigella ambigua.*
  bb. Acid, but not gas, produced from sucrose, arabinose and dextrin.
    3. *Shigella lunavensis.*
aa. Acid, but not gas, produced from mannitol.
  b. Neither acid nor gas produced from lactose.
    c. Neither acid nor gas produced from xylose.
      4. *Shigella paradysenteriae.*
    cc. Acid, but not gas, produced from xylose.
      d. Acid, but not gas, produced from dulcitol; salicin not fermented.
        e. Acid, but not gas, produced from glycerol; indol produced.
          5. *Shigella alkalescens.*
        ee. Neither acid nor gas produced from glycerol; indol not produced.
          6. *Shigella gallinarum.*

dd. Neither acid nor gas produced from dulcitol; acid produced from salicin.

e. Acid, but not gas, produced from arabinose and maltose.

7. *Shigella pfaffii.*

ee. Neither acid nor gas produced from arabinose and maltose.

8. *Shigella rettgeri.*

bb. Acid, but not gas, produced from lactose.

c. Acid, but not gas, produced from arabinose and rhamnose.

d. Acid, but not gas, produced from xylose; indol usually produced.

e. Neither acid nor gas produced from dulcitol.

9. *Shigella madampensis.*

ee. Acid, but not gas, produced from dulcitol.

10. *Shigella ceylonensis.*

dd. Neither acid nor gas produced from xylose; indol not produced.

11. *Shigella sonnei.*

cc. Neither acid nor gas produced from arabinose and rhamnose.

12. *Shigella equi.*

## SPECIES OF *SHIGELLA*

1. *Shigella dysenteriae* (Shiga) Castellani and Chalmers, 1919.

Alternative: 52. *Bacterium dysenteriae* (Shiga) LeBlaye and Guggenheim, 1914.

SYNONYMY: Bacillus of Japanese dysentery, Shiga, 1898; *Bacillus dysenteriae* Shiga, 1898; *Bacillus Shigae* Chester, 1901; *Bacillus dysentericus* (Shiga-Kruse) Ruffer and Willmore, 1909; *Eberthella dysenteriae* Cast. and Chalm., Bergey *et al*, 1923; *Eberthella dysenteriae* (Shiga) Bergey *et al*, 1925; *not Bacillus dysenteriae liquefaciens* Kruse, 1896; *Bacterium dysenteriae liquefaciens* (Ogata) Chester, 1897; *Bacillus dysenteriae* (Kruse) Migula, 1900.

Shiga (1898) first isolated this organism from cases of dysentery in Japan. He described it as a Gram-negative, non-spore-forming rod, very similar to the typhoid bacillus. He considered it as being motile, but Kruse (1900) and all later observers agree that it is non-motile. Its growth on agar, gelatin and potato was like that of the typhoid organism. Gelatin was not liquefied, milk was not coagulated, indol was not produced and no gas was formed from glucose. Subsequent investigators have shown it to produce acid from glucose, levulose and galactose, but not from lactose, sucrose, mannitol, dulcitol, xylose, raffinose, rhamnose, arabinose, maltose, salicin, inulin, inositol nor sorbitol.

Shiga (1898) named the organism *Bacillus dysenteriae.* Ogata (1892) had isolated an organism, which was listed by Flügge (1896) under the name *Bacillus dysenteriae liquefaciens.* It was a Gram-positive, gelatin liquefying organism, evidently not a member of the colon-typhoid series.

The name, however, was shortened by Migula (1900) to *Bacillus dysenteriae*. Chester (1901) followed Migula's use of the name *dysenteriae* for the organism of Ogata and called Shiga's organism *Bacillus Shigae*, and this name has been used extensively. The proper specific designation for the Shiga organism would seem to be that given by Shiga himself.

**Specific Diagnosis:** *A short rod, 0.4 to 0.6μ broad and 1.0 to 1.5μ long, conforming to the generic diagnosis. It does not produce acid nor gas from lactose, maltose, sucrose, arabinose, xylose, dextrin, mannitol, dulcitol, salicin or rhamnose. Indol is not produced. Litmus milk becomes slightly acid, then neutral or alkaline. Found in the stools and intestines of humans. The cause of one form of dysentery.*

2. *Shigella ambigua* (Andrews) Comb. nov.

Alternative: 53. *Bacterium schmitzii* Weldin and Levine, 1923.

SYNONYMY: Schmitz bacillus, Schmitz, 1917; *Bacillus ambiguus* Andrews, 1918; *Bacillus dysenteriae* "Schmitz", Murray, 1918; *Bacterium ambiguum* Levine, 1920; *Eberthella ambigua* (Andrews) Bergey *et al*, 1923; *not Bacterium ambiguum* Chester, 1899.

Schmitz (1917) isolated from a number of cases of dysentery, an organism which he called the Schmitz Bacillus. It was similar to the Shiga Bacillus, but differed from it in being able to produce indol. Mannitol, lactose, sucrose and maltose were not attacked. Andrews (1918) described an organism under the name *Bacillus ambiguus*, which did not differ in any significant way from the Schmitz bacillus. Murray (1918) came to the conclusion that Andrews' and Schmitz' organisms were identical. Levine (1920) reporting on five strains (designated as *B. ambiguus* and secured from Andrews) found none able to ferment mannitol, lactose, glycerol, dulcitol, sucrose, xylose or dextrin. Acid was produced from rhamnose by all, and indol was produced. These last two properties served to differentiate the organism from *Shigella dysenteriae*.

Andrews' specific name *"ambiguus"* is valid when used with the generic name *"Shigella"*. *Bacterium ambiguum*, however, cannot be used as an alternative name for this species, since this name was applied by Chester (1899) to a motile, gelatin-liquefying, non-acid-producing organism. Weldin and Levine (1923) used the term *Bacterium schmitzii*.

**Specific Diagnosis:** *A short rod, conforming to the generic diagnosis. It does not attack lactose, maltose, sucrose, xylose, dextrin, glycerol, mannitol or dulcitol. Acid is produced from rhamnose. Litmus milk becomes acid, followed by a neutral or alkaline reaction. Inagglutinable by Shiga immune serum. Found in human stools. The cause of one type of dysentery of man.*

3. *Shigella lunavensis* (Castellani) Castellani and Chalmers, 1919.

Alternative: 54. *Bacterium lunavensis* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus lunavensis* Castellani, 1912.

This organism was described by Castellani (1912) as a non-motile rod, Gram-negative, producing indol and not liquefying gelatin nor serum. Acid was produced from glucose, sucrose, maltose, dextrin and arabinose. Lactose, dulcitol, mannitol, raffinose, adonitol, inulin, sorbitol, inositol and salicin were not attacked. The organism is similar to *Shigella dysenteriae*

in not fermenting mannitol, but differs in its production of indol and fermentation of sucrose, arabinose and dextrin.

Weldin and Levine (1923) included it in their subgenus *Eberthella* of the genus *Bacterium*. Similar organisms have been described by Mackie (1919) and DeBains (1917).

**Specific Diagnosis:** ***A short rod, conforming to the generic diagnosis. Acid is produced from maltose, sucrose, arabinose and dextrin, but not from lactose, mannitol, dulcitol or salicin. Indol is produced. Litmus milk becomes slightly acid, reverting to a slight alkaline reaction. Found in human stools.***

4. *Shigella paradysenteriae* (Collins) Comb. nov.

Alternative: 55. *Bacterium paradysenteriae* (Collins) Comb. nov.

SYNONYMY: *Bacillus dysenteriae* Flexner type, Strong type, Y type; *Bacillus paradysenteriae* Collins, 1905; *Shigella dysenteriae* (Flexner) (Hiss and Russell) (Strong) Castellani and Chalmers, 1919; *Bacillus flexneri* Levine, 1920; *Bacterium flexneri* Levine, 1920; *Bacterium dysenteriae* (Flexner) Holland, 1920; *Bacterium* (*Eberthella*) *flexneri* Weldin and Levine, 1923; *Eberthella paradysenteriae* (Flexner) (Hiss) (Strong) Bergey *et al*, 1923; *Eberthella paradysenteriae* (Collins) Bergey *et al*, 1925.

In March, 1900, Flexner isolated from cases of dysentery an organism which he believed to be the cause of the disease and which has since been commonly designated as *Bacillus dysenteriae* Flexner. Shiga had previously (1898) isolated a dysentery organism. At first both Flexner and Shiga believed these organisms were identical. In May, 1900, Strong found a dysentery organism which showed variations from the others and which has since borne his name. Kruse (1900) described a dysentery bacillus which was identical with that of Shiga. Three years later Hiss and Russell described still another type. To this the terms "bacillus Y", "Hiss" and "Hiss and Russell" have been variously applied.

Strong in Forscheimer's Therapeusis of Internal Disease (1917) gives a comprehensive historical review of the systematic work done with the dysentery organisms. The four types have been shown to differ from each other by agglutination and agglutinin-absorption tests, as well as by their fermentation reactions on certain carbohydrates. The Shiga type differs from the other three (Flexner-Strong-Hiss, Russell) by its lack of fermentation of a number of carbohydrates, mannitol being ordinarily the one used for differentiation. The mannitol-fermenting dysentery organisms agree in not being able to attack xylose or lactose, but differ in their behavior toward maltose and sucrose as follows:

Flexner type, maltose, acid; sucrose, negative.

Strong type, maltose, negative; sucrose, acid.

Hiss and Russell type, maltose and sucrose, negative.

Some bacteriologists regard these three as distinct species; others, as one species, with three varieties. The writer will consider them as one species for the present.

The specific name, *dysenteriae*, commonly used for these organisms, is not valid, since it was previously used by Shiga (1898) for another species. (See *Shigella dysenteriae*.) Collins (1905) used the term *Bacillus paradysenteriae*. She said, "The term paradysentery is applied to the types

which ferment mannite and differ in their agglutination reaction, but in all other respects correspond to the organism isolated by Shiga as the cause of dysentery."

There may be some question as to whether this was a proper diagnosis of the name. It will be accepted here, however, as valid. Levine (1920) used the names *Bacillus flexneri* and *Bact. flexneri* to include both the Flexner and Y types.

**Specific Diagnosis:** *A short rod, conforming to the generic diagnosis. Acid is produced from mannitol, and usually from arabinose, but not from lactose or xylose, and rarely from glycerol. Fermentation of maltose, dextrin and sucrose is variable. Indol is usually produced. Litmus milk becoms slightly acid, changing to alkalinity. Is not agglutinated by "Shiga" serum. A cause of dysentery in man.*

5. *Shigella alkalescens* (Andrews) Comb. nov.

Alternative: 56. *Bacterium alkalescens* (Andrews) Levine, 1920.

SYNONYMY: *Bacillus alkalescens* Andrews, 1918; *Eberthella alkalescens* (Andrews) Bergey *et al*, 1923.

Andrews (1918) isolated this organism from the stools of patients and named it *Bacillus alkalescens*. He described it as producing acid from glucose, maltose, mannitol and dulcitol, but not from lactose nor saccharose. Indol was formed. Litmus milk was made strongly alkaline in reaction. Levine (1920) studying twelve strains of *Shigella alkalescens* added glycerol, xylose and rhamnose to the list of carbohydrates fermented with acid. Raffinose and dextrin were not fermented.

**Specific Diagnosis:** *A short rod, conforming to the generic diagnosis. It produces acid, but not gas, from maltose, xylose, mannitol, dulcitol and rhamnose. Lactose, sucrose, dextrin and salicin are not attacked. Indol is formed. Litmus milk becomes alkaline. Is not agglutinated by specific dysenteriae or paradysenteriae sera. Isolated from the stools of dysentery patients.*

6. *Shigella gallinarum* (Klein) Comb. nov.

Alternative: 57. *Bacterium gallinarum* (Klein) Chester, 1897.

SYNONYMY: *Bacillus gallinarum* Klein, 1889; *Bacterium sanguinarium* Moore, 1895; *Bacillus sanguinarium* Krumwiede, Kohn, Valentine, 1918; *Bacterium jeffersoni* Hadley, Elkins, Caldwell, 1918; *Bacillus jeffersoni* (Hadley, Elkins and Caldwell) Winslow, Kligler, Rothberg, 1919; *Eberthella sanguinaria* (Moore) Bergey *et al*, 1923; *Eberthella jeffersonii* (Hadley) Bergey *et al*, 1923.

Klein (1889) described an organism isolated from a disease of hens, as a Gram-negative, non-motile, non-spore-forming bacillus. He named it *Bacillus gallinarum*. Its cultural characters were similar to those of the organisms of the colon-typhoid series.

In 1895, Moore isolated an organism from diseased hens which he called *Bacterium sanguinarium*. This organism was able to produce acid from glucose, but no gas. Lactose and sucrose were not attacked. Its morphological and cultural characters were typical of the colon-typhoid group. Hadley, Elkins and Caldwell (1918) compared this organism with a strain of Klein's organism secured by them from Europe and found them identical both culturally and agglutinatively. St. John-Brooks and Rhodes

(1923) found an organism labelled *B. gallinarum* Klein which they had secured from Kràl, identical culturally and agglutinatively with a strain of *B. sanguinarium* Moore received from Dr. Chas. Krumwiede. Apparently the organism under discussion should be designated by the name *Shigella* or *Bacterium gallinarum* (Klein).

The organism has been carefully studied by a number of investigators, and its cultural reactions and serological relations clearly defined. It may be differentiated from *Salmonella pullorum*, the cause of white diarrhoea in chicks, by its inability to form gas from carbohydrates. Its non-motility and ability to produce acid from mannitol place it in the Flexner section of the genus *Shigella*.

*Bacterium jeffersoni*. Hadley, Elkins and Caldwell (1918) named and described this organism which had been isolated in 1909 from a cholera-like epidemic occurring among poultry. Later work seemed to show a relation to "fowl typhoid". The organism was described as a Gram-negative, non-motile rod, which very slowly alkalined litmus milk, did not produce indol or $H_2S$ and fermented a number of carbohydrates with acid, but no gas, including dextrose, mannose, xylose, arabinose, maltose, dextrin, dulcitol and mannitol. Lactose, raffinose, inulin, adonitol, erythritol and salicin were not attacked. Agglutination tests showed only a slight antigenic relation with fowl-typhoid strains. The homologous antigen was, likewise, only slightly agglutinated.

St. John-Brooks and Rhodes (1923), by plating a culture of *Bact. jeffersoni* Hadley and picking off smooth colonies secured a strain whose anti-serum agglutinated *Shigella gallinarum* to full titre (1-6400). Biochemically, *Bact. jeffersoni* is identical with *Shigella gallinarum*.

*Bact. jeffersoni* will be considered here as a synonym of *Shigella gallinarum* (Klein).

**Specific Diagnosis:** ***A rod conforming to the generic diagnosis. It ferments maltose, arabinose, xylose, dextrin, mannitol, dulcitol and rhamnose, with acid, but not gas, production. Lactose, sucrose, glycerol and salicin are not attacked. Indol is not produced. Litmus milk becomes slightly acid, followed by alkalinity. The causative agent of fowl typhoid.***

7. *Shigella pfaffii* (Hadley, Elkins, Caldwell) Comb. nov.

Alternative: 58. *Bacterium pfaffii* Hadley, Elkins, Caldwell, 1918.

SYNONYMY: *Bacillus pfaffi* St. Johns-Brooks and Rhodes, 1923; *Eberthella pfaffi* (Hadley) Bergey *et al*, 1923.

Hadley, Elkins and Caldwell (1918) studied an organism secured from the Kràl laboratory, where it was known as the bacillus of "Kanarienvögelseuche (Pfaff)". They named it *Bacterium pfaffi*. It was found to be a Gram-negative, non-motile rod, staining well with aniline dyes, but not giving either peripheral or bipolar staining. Gelatin was not liquefied. Litmus milk was unchanged. Indol and $H_2S$ were not formed. In its fermentation reactions it differed from *Shigella gallinarum* in producing acid from salicin and failing to attack dulcitol. It differed from *Shigella rettgeri* by its acid production from arabinose, maltose and dextrin, and its inability to attack inulin and adonitol.*

*St. John-Brooks and Rhodes (1923) working with an organism secured from Kral and labelled B. der Kanarienvogelseuche (Pfaff), found it to be sluggishly motile and producing alkalinity in litmus milk.

**Specific Diagnosis:** *A short rod conforming to the generic diagnosis. It produces acid, but not gas, from maltose, arabinose, xylose, dextrin, mannitol and salicin. Lactose, sucrose and dulcitol are not attacked. Indol is not formed. Litmus milk is unchanged. A cause of typhoid in birds.*

8. *Shigella rettgeri* (Hadley, Elkins, Caldwell) Comb. nov.

Alternative: 59. *Bacterium rettgeri* Hadley, Elkins, Caldwell, 1918.

SYNONYMY: *Bacillus rettgerei* St. John-Brooks and Rhodes, 1923; *Eberthella rettgeri* (Hadley) Bergey *et al*, 1923.

This organism was isolated by Rettger in 1909 from an epidemic in chickens, resembling fowl cholera. Its characters were studied in detail by Hadley, Elkins and Caldwell (1918), who named it *Bacterium rettgerei*. It was a short, non-motile, Gram-negative rod, staining with aniline dyes and not exhibiting peripheral nor bipolar staining. It showed no distinctive characters on ordinary laboratory media. Litmus milk was made alkaline, becoming translucent. Indol was not formed* Acid was produced from glucose, mannose, xylose, adonitol, mannitol and salicin, but not from arabinose, raffinose, sucrose, lactose, maltose, dextrin, inulin, dulcitol or erythritol.

The ability to attack mannitol places the organism in the Flexner section of the genus *Shigella*. It may be differentiated from *Shigella gallinarum* by its action on litmus milk and in dulcitol and salicin.

**Specific Diagnosis:** *A short rod, conforming to the generic diagnosis. It ferments xylose, mannitol and salicin with acid, but not gas, production. Lactose, maltose, sucrose, arabinose, dextrin, and dulcitol are not attacked. Litmus milk becomes alkaline, later translucent. A cause of fowl typhoid.*

9. *Shigella madampensis* (Castellani) Comb. nov.

Alternative: 60. *Bacterium madampensis* (Castellani) Comb. nov.

SYNONYMY: *Bacillus madampensis* Castellani, 1912; *Bacillus dispar* Andrews, 1918 (in part); *Lankoides madampensis* (Cast.) Castellani and Chalmers, 1919; *Bacterium dispar* Levine, 1920 (in part); *Eberthella dispar* (Andrews) Bergey *et al*, 1923 (in part).

Castellani (1912) described a non-motile, Gram-negative, short rod, isolated from human stools, apparently a member of the colon-typhoid group. This organism produced acid, but not gas, from glucose, sucrose, mannitol, maltose and lactose, but did not attack dulcitol. It acidified and coagulated milk. Indol was produced. This organism was named *Bacillus madampensis*.

Andrews (1918) proposed the name *Bacillus dispar* for all lactose-fermenting members of the dysentery group. Of eleven strains which he had isolated and studied, all fermented glucose, maltose and mannitol, six fermented sucrose and two dulcitol. Indol formation was variable. Milk was acidified and eventually clotted. They resembled *Shig. alkalescens* in their relation to specific Flexner serum. Levine (1920) studied eleven strains and added sucrose, xylose and raffinose to the list of carbohydrates attacked. The series of organisms studied by Andrews and by Levine under the specic name "*dispar*" evidently included the type *madampensis* of

* St. John-Brooks and Rhodes (1923) reported it as forming indol.

Castellani as well as another species of Castellani, which he called *ceylonensis.*

**Specific Diagnosis:** ***A short rod conforming to the generic diagnosis. It attacks lactose, maltose, sucrose, arabinose, xylose, glycerol, mannitol and rhamnose with acid, but not gas, production; dulcitol and salicin are not attacked. Litmus milk becomes acid and is coagulated. Indol is produced. Found in the stools and intestines of men.***

10. *Shigella ceylonensis* (Castellani) Comb. nov.

Alternative: 61. *Bacterium ceylonensis* (Castellani) Comb. nov.

SYNONYMY: *Bacillus ceylanensis* B. Castellani, 1907; *Bacillus ceylonenses* B. Castellani, 1912; *Bacillus dispar* Andrews, 1918 (in part); *Lankoides ceylonensis* B. (Cast.) Castellani and Chalmers, 1919; *Bacterium dispar* Levine, 1920 (in part); *Eberthella dispar* (Andrews) Bergey *et al* 1923 (in part).

Under the name *Bacillus ceylanensis* (spelled *ceylonensis* in all subsequent descriptions) Castellani in 1907 described a rod, non-motile, Gram-negative, with cultural characters typical of members of the colon-typhoid series. It produced acid, but no gas, from glucose, sucrose, mannitol, dulcitol, maltose and lactose. Milk was acidified and clotted. The organism was pathogenic for guinea pigs and rabbits. As suggested in the discussion of *Shigella madampensis,* organisms of this type were included by Andrews in his species *Bacillus dispar.*

**Specific Diagnosis:** ***A short rod conforming to the generic diagnosis. It produces acid, but not gas, from lactose, maltose, sucrose, arabinose, xylose, mannitol, dulcitol and rhamnose; salicin is not attacked. Indol is produced. Milk becomes acid and coagulated. Found in the stools and intestines of humans.***

11. *Shigella sonnei* (Levine) Comb. nov.

Alternative: 62. *Bacterium sonnei* Levine, 1920;

SYNONYMY: Group III of Sonne, 1915; Bacillus of Sonne, Thjotta, 1919; *Bacillus dysenteriae* Sonne, Smith, J., 1924

Sonne (1915) studied a large series of organisms from faeces and urine of dysentery patients. These organisms were non-motile and glucose and mannitol fermenters (acid only). One type (Group III), which was more abundant than all the rest, did not produce indol, made litmus milk acid, and fermented maltose, sucrose, rhamnose and usually dextrin. Agglutination reactions showed no relation to other groups of dysentery bacilli.

Thjotta (1919) confirmed Sonne's conclusions as to the existence of a serologically distinct group of dysentery organisms. He proposed three groups of dysentery bacilli: Group I, the Shiga type; Group II, Flexner, Strong and Y type; and Group III, the Group III of Sonne. He characterized the latter briefly as serologically specific, producing large, uneven colonies, fermenting mannitol, maltose and saccharose with acid, not producing indol.

Levine (1920) found the Sonne organism able to produce acid from lactose and rhamnose, but not from xylose. J. Smith (1924) studied strains of the bacillus of Sonne and recorded acid production from dextrose and

the other hexoses, from arabinose, rhamnose, lactose, sucrose, mannitol and glycerol, but not from xylose, dulcitol or sorbitol.

**Specific Diagnosis:** *A short rod, conforming to the generic diagnosis. It produces acid, but not gas, from lactose, maltose, sucrose, arabinose, glycerol, mannitol and rhamnose. Xylose and dulcitol are not attacked. Indol is not produced. Litmus milk is made acid and is coagulated. Serologically distinct from the Shiga and Flexner types of dysentery organisms. Found in the faeces and urine of dysentery patients. A cause of dysentery in man.*

12. *Shigella equi* (Magnusson) Comb. nov.

Alternative: 63. *Bacterium equi* (Magnusson) Weldin and Levine, 1923.

SYNONYMY: *Bacterium viscosum equi,* Magnusson, 1919.

This organism, the cause of "joint ill" in foals, was described by Magnusson (1919) under the name *Bacterium viscosum equi*.. It was described as a small, oval, Gram-negative, non-motile bacillus, which made all liquid media in which it was grown slimy or ropy, although no capsules could be demonstrated. It fermented with acid production glucose, lactose, sucrose, maltose, raffinose, mannitol and galactose, but did not attack arabinose, rhamnose, adonitol and dulcitol. Gas was not formed from any carboyhydrate.

The organism is evidently a valid species of the genus *Shigella.* Weldin and Levine (1923) included it under the name *Bacterium equi* in their subgenus *Eberthella.*

**Specific Diagnosis:** *A short rod, conforming to the generic diagnosis. Acid, but not gas, is produced from lactose, maltose, sucrose and mannitol. It does not attack arabinose, dulcitol nor rhamnose. Colonies on solid media are mucoid; liquid media becomes slimy or ropy. The cause of "joint ill" in foals.*

Genus 7. *ALCALIGENES* Castellani and Chalmers, 1919.

SYNONYMY: *Alkaligenes* Castellani and Chalmers, 1919; *Brucella* Meyer and Shaw, 1920.

This name was first used by Castellani and Chalmers (1919) to designate a genus of their tribe *Ebertheae.* They defined it as follows:

*Ebertheae* which do not ferment glucose nor lactose, and are characterized by their general lack of fermentative power and by actually increasing the alkalinity of the media. Milk is not clotted, and is rendered alkaline. Type: *Alcaligenes faecalis* (Petruschky, 1896), *emendavit* Castellani and Chalmers, 1918.*

Their tribe *Ebertheae* possesses the general characters of the colon-typhoid organisms except with regard to capsule formation, the tribe being limited to the non-capsule formers.

Weldin and Levine (1923) used the name for a subgenus of *Bacterium,* including such members of the genus as are not known to be plant pathogens and do not produce acid or gas from glucose. Bergey (1925), using the name for his sixteenth genus of the family *Bacteriaceae,* added that

* The citation for 1918 is apparently an error, as no description for the genus can be found of this date.

acetyl-methyl-carbinol is not formed. It is felt that this character is not necessary nor hardly warranted from descriptions of the organisms available.

The lack of fermentative ability and pathogenicity sufficiently differentiates this group from the others to warrant its being recognized as a genus.

**Generic Diagnosis:** ***Motile or non-motile, Gram-negative, non-spore-forming rods, Do not ferment any of the carbohydrates with acid or gas production, but often increase the alkalinity of the medium. Pathogenicity slight.***

**Type species:** ***Alcaligenes faecalis*** **(Petruschky) Bergey *et al* 1923.**

KEY TO SPECIES OF THE GENUS *ALCALIGENES*

a. Gelatin not liquefied.
  b. Capsules not formed in milk; milk not rendered slimy.
    c. Motile.
      1. *Alcaligenes faecalis.*
    cc. Non-motile.
      d. Nitrates reduced to nitrites.
        2. *Alcaligenes metalcaligenes.*
      dd. Nitrates not reduced.
        e. Cause of Malta fever.
          3. *Alcaligenes melitensis.*
        ee. Cause of contagious abortion in cattle.
          4. *Alcaligenes abortus.*
  bb. Capsules formed in milk; milk rendered slimy.
    5. *Alcaligenes viscosum.*
aa. Gelatin liquefied.
  6. *Alcaligenes bookeri.*

SPECIES OF *ALCALIGENES*

1. *Alcaligenes faecalis* (Petruschky) Bergey *et al*, 1923.

Alternative: 64. *Bacterium alcaligenes* (Petruschky) Lehmann and Neumann, 1901.

SYNONYMY: *Bacillus faecalis alcaligenes* Petruschky, 1896; *Bacterium faecalis alcaligenes* (Petruschky) Chester, 1897; *Bacillus alcaligenes* (Petruschky) Migula, 1900; *Alcaligenes faecalis alkaligenes* (Petruschky) Castellani and Chalmers, 1919; *Alcaligenes fecalis* (Petruschky) Castellani and Chalmers, Bergey *et al*, 1923.

Petruschky (1896) first described this organism under the name *Bacillus faecalis alcaligenes*. He isolated it from typhoid stools and from spoiled beer. According to his description, the organism morphologically resembled the typhoid bacillus. It was a Gram-negative, motile rod. It did not liquefy gelatin, gave a negative indol reaction and did not produce acid or gas in sugar media. A strong alkaline reaction was produced in milk. It did not agglutinate with typhoid serum. The organism has been

isolated and studied by many subsequent investigators, but little of significant differential value has been added to its description. It has been found to be uniformly negative, both as to acid and gas production, on all carbohydrates tested.

Migula (1900) reduced the name to binomial form. Chester (1897) had placed it in the genus *Bacterium*, but had used the trinomial name. Lehmann and Neumann (1901) must, therefore, be given priority for the name *Bacterium alcaligenes*. The specific name *alcaligenes* cannot be used, however, in combination with the generic name *Alcaligenes*, (Second case, Article 55, International Rules of Botanical Nomenclature) and was, therefore, changed by Bergey *et al* (1923) to "*Alcaligenes fecalis.*" The specific name should be spelled "faecalis," however, to conform to Petruschky's original spelling.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Gelatin is not liquefied. Indol is not produced. Nitrates are reduced to nitrites. Litmus milk is made alkaline and is not coagulated. A slightly brownish growth is produced on potato. Not known to be pathogenic to man or experimental animals. Found in the intestines of man. Also isolated from spoiled beer.*

2. *Alcaligenes metalcaligenes* Castellani and Chalmers, 1919.

Alternative: 65. *Bacterium metalcaligenes* (Castellani and Chalmers) Weldin and Levine, 1923.

SYNONYMY: *Alcaligenes metalcaligenes* (Adametz) Bergey *et al*, 1923.

Castellani and Chalmers (1919) described this organism as identical with their *B. faecalis alcaligenes* except for the absence of motility. They listed it as one of the causal organisms of para-enteric fever. Weldin and Levine (1923) included it in their subgenus *Alcaligenes* of the genus *Bacterium*.

Similar, but unnamed, organisms have been described by Ford (1901), Lewis (1911), Cox, Lewis and Glynn (1912), Ritchie (1914) and Pirie (1917).

**Specific Diagnosis:** *A rod conforming to the generic diagnosis and similar in its morphological, cultural and biochemical properties to Alcaligenes faecalis except that it is non-motile. Pathogenicity, slight if any. Found in the intestines of man and animals and in milk.*

3. *Alcaligenes melitensis* (Bruce) Bergey *et al*, 1923.

Alternative: 66. *Bacterium melitensis* (Bruce) Evans, 1918.

SYNONYMY: *Micrococcus melitensis* Bruce, 1893; *Brucella melitensis* Meyer and Shaw, 1920; *Bacillus melitensis* Khaled, 1921.

Bruce (1893) gave the name *Micrococcus melitensis* to the organism which he discovered in 1887 and concluded to be the cause of the "fièvre méditerraneénne" (now known as Malta fever). He described it as being round or oval, non-motile and Gram-negative. It developed slowly on all laboratory media at 37° and hardly at all at room temperature. Gelatin was not liquefied. Subsequent investigators have found it unable to produce acid or gas from any carbohydrate.

Evans (1918) studied six strains of varied origin. She showed it to be a Gram-negative, non-motile, non-spore-forming rod and called it *Bacterium*

*melitensis*. She found it to be biochemically and agglutinatively identical with *Bacterium abortus* Bang. Meyer and Shaw (1920) found *abortus* and *melitensis* morphologically and biochemically identical. They proposed the generic name *Brucella* for these two organisms. (See Brucella.) Khaled (1921) likewise found them morphologically and culturally identical, and was not able to differentiate them by biochemical methods or by agglutination reactions. From absorption experiments, *melitensis* appeared to be a substrain of *abortus*. Immunization of a monkey with a killed suspension of *Alc. abortus* protected it from subsequent infection with *Alc. melitensis*. Specific pathogenicity appears to be the only known means of differentiating the two organisms and recent work by Evans (1925) indicates an extremely close relationship with regard to this character. Perhaps they should be considered varieties of one species, under the name *melitensis*.

**Specific Diagnosis:** *A non-motile rod, conforming to the generic diagnosis. Coccoid cells frequent. Gelatin is not liquefied. Nitrates are not reduced. Aerobic, or slightly micro-aerophilic. Litmus milk unchanged or slightly alkaline. Distributed in milk, particularly goat's milk. The cause of Malta fever.*

4. *Alcaligenes abortus* (Bang) Bergey *et al*, 1923.

Alternative: 67. *Bacterium abortus* (Bang) Schmidt and Weis, 1902.

SYNONYMY: Bacillus of abortion (cattle) Bang, 1887; Le bacille de Bang, Nowak, 1908; Abortus bacillus, Bang, Good, 1912; *Bacillus abortus* Evans, 1915; *Bacterium abortum* (Bang) Holland, 1920; *Brucella abortus* Meyer and Shaw, 1920.

Bang (1897) described this organism as the cause of infectious abortion in cattle. It was found to be a non-motile rod growing scantily on laboratory media. Bang described it as a microaerophile. Schmidt and Weis (1902), Nowak (1908) and Good (1912) all reported partial reduction of oxygen as necessary for cultivation. Evans (1915) stated that incubation in a closed jar with *B. subtilis* favored isolation growth, but that after the strain had become accustomed to artificial cultivation, growth was abundant on all laboratory media.

Evans (1918), Meyer and Shaw (1920), Khaled (1921) and Evans (1925) compared strains of *Alcaligenes abortus* with *Alcaligenes melitensis* and found them morphologically, culturally and agglutinatively identical. (See *Alcaligenes melitensis*.) In case they were to be considered as one species, *melitensis* would be the valid specific name since it has priority over *abortus*.

**Specific Diagnosis:** *A rod morphologically, culturally and agglutinatively identical with Alcaligenes melitensis. The cause of contagious abortion in cattle. Also causes abortion in mares, sheep, rabbits and guinea pigs.*

5. *Alcaligenes viscosum* (Adametz) Comb. nov.

Alternative: 68. *Bacterium viscosum* (Adametz) Weldin and Levine, 1923.

SYNONYMY: *Bacillus lactis viscosus* Adametz, 1889; *Bacillus viscosis lactis* (Adametz) Kruse, in Flügge, 1896; *Bacterium viscosus lactis* (Adametz) Chester, 1897; *Bacterium lactis viscosum* (Adametz) Lehmann and Neumann, 1901; *Lactobacillus viscosus* (Adametz) Bergey *et al*, 1923; *Achromobacter viscosum* (Adametz) Bergey *et al*, 1925.

Adametz (1889) apparently first described this organism. He named it *Bacillus lactis viscosus.* It was isolated from slimy or ropy milk. It was a non-spore-forming, motile in young milk cultures, short rod, producing capsules in milk. Adametz considered it Gram-negative, but more resistant to decolorization than related species. Gelatin was not liquefied.

Kruse (1896) and Lehmann and Neumann (1901) described the organism as non-motile. Buchanan and Hammer (1915) did not observe motility. It should probably be considered non-motile. Investigations by various workers have shown the organism unable to attack carbohydrates either with acid or gas production.

Chester (1897) first placed the species in the genus *Bacterium.* Weldin and Levine (1923) reduced the name to binomial form and included it in their subgenus *Alcaligenes.* They used its ability to render milk slimy to differentiate it from other members of the species.

**Specific Diagnosis:** *A rod conforming to the generic diagnosis. Capsules are produced in milk, rendering the milk slimy or ropy. Gelatin is not liquefied. Indol is not produced. Found in slimy or ropy milk.*

6. *Alcaligenes bookeri* (Ford) Bergey *et al,* 1923.

Alternative: 69. *Bacterium bookeri* (Ford) Levine and Soppeland, 1926.

SYNONYMY: Bacillus A, Booker, 1887; *Bacillus bookeri,* Ford, 1903; *Alcaligenes bookeri* Bergey *et al,* 1925.

Booker (1887) designated as "Bacillus A," an organism found in cholera infantum. It was a narrow bacillus, actively motile, liquefying gelatin rapidly and blood serum slowly, and producing a definitely alkaline reaction in milk. Ford (1903) studied an organism which he had isolated from the stomach of a foundling and believed was identical with Booker's Bacillus A. His results showed an alkaline reaction in glucose broth, lactose and sucrose not attacked, indol not formed. He named the organism, *Bacillus bookeri.*

**Specific Diagnosis:** *A motile rod* conforming to the generic diagnosis. Gelatin is liquefied. Nitrates are not reduced. Indol is not formed. Litmus milk is peptonized with reduction of the litmus. Found in the intestines of man.*

## LITERATURE CITED

ACHARD, CH., AND BENSAUDE, R.
1896. Infections paratyphoidiques. Bulletin et mémoires de la societié médicale des Hopitaux de Paris, (3), 13, 820.

ANDREWES, F. W.
1918. Dysentery bacilli. The differentiation of the true dysentery bacilli from allied species. Lancet, 1, 560.

ANDREWES, F. W., AND NEAVE, SHEFFIELD.
1921. The nature and systematic position of *B. paratyphosus C.* Brit. Jour. Exper. Path., 2, 157.

* Levine and Soppeland (1926) report a non-motile variety of Alcaligenes bookeri isolated from artificial creamery wastes.

AZZI, AZZO.
1917. Ancora su alcune specie anomale di bacilli del paratifo, isolate da soldati infermi nella zona di guerra. Sperimentale. Arch. di biol., 71, 210.

BAINBRIDGE, F. A.
1909. On the paratyphoid and "food poisoning" bacilli and on the nature and efficiency of certain rat viruses. Jour. Path. & Bacteriol., 13, 443.

BANG, B.
1897. Die Aetiologie des seuchenhaften ("infectiousen" )Verwerfens. Ztschr. f. Thiermed., 1, 241.

BANTI, GUIDO.
1888. Sopra quattro nuove specie di protei o bacilli capsulati. Sperimentale, Giomale Italiano di Scienze mediche. 62, 139.

BEIJERINCK, M. W.
1900. Schwefelwasserstoffbildung in den Stadtgräben und Aufstellung der Gattung *Aerobacter*. Centralbl. f. Bakteriol., Abt. 2. 6, 193.

BERGEY, DAVID H., *et al.*
1923. Manual of Determinative Bacteriology. Williams & Wilkins Co., Baltimore.
1925. Manual of Determinative Bacteriology. 2nd ed. Williams & Wilkins Co. Baltimore.

BERGEY, D. H., AND DEEHAN, S. T.
1908. The colon-aerogenes group of bacteria. Jour. Med. Research, 19, 175.

BOOKER, WILLIAM D.
1887. A study of some of the bacteria found in the dejecta of infants afflicted with summer diarrhoea. Trans. Ninth Internat. Med. Congress, 3, 598.

BOYCOTT, A. E.
1906. Observations on the bacteriology of paratyphoid fever and on reactions of typhoid and paratyphoid sera. Jour. Hyg., 6, 33.

BREED, R. S., CONN, H. J., AND BAKER, J. C.
1918. Comments on the evolution and classification of bacteria. Jour. Bact., 3, 445.

BRIEGER.
1884. Ueber Spaltungs-producte der Bakterien. Berl. klin., Wchnschr., No. 14.

BRION, A., AND KAYSER, H.
1902. Ueber eine Erkrankung mit dem Befund eines typhusähnlichen Bacteriums im Blute. (Paratyphus.) Munchen. med. Wchnschr., 49, 611.

BRUCE, DAVID.
1893. Sur une nouvelle forme de fiévre recontrèe sur les bords de la méditerranèe. Ann. d. l'Inst. Pasteur, 7, 289.

BUCHAN, GEORGE F.
1910. The contamination of ice cream. Jour. Hyg., 10, 93.

BUCHANAN, R. E.
1918. Studies in the nomenclature and classification of the bacteria. V. Subgroups and genera of the *Bacteriaceae*. Jour. Bact., 3, 27.
1924. Bacteriology. Rev. ed. The Macmillan Co. New York.
1925. General Systematic Bacteriology, Williams & Wilkins Co., Baltimore.

BUCHANAN, R. E., AND HAMMER, B. W.
1915. Slimy and ropy milk. Ia. Agric. Exper. Sta. Research Bull. No. 22

CASTELLANI, ALDO.
1905. Meetings of the Ceylon Branch of the British Association. 1905.*
1907. Notes on cases of fever frequently confounded with typhoid and malaria in the tropics. Jour. Hyg., 7, 1.
1912. Observations on some intestinal bacteria found in man. Centralbl. f. Bakteriol., Abt. 1, 65, 262.
1913. Report of the Advisory Committee for the Tropical Diseases Research Fund for the year 1912, Lond.*

* References not seen.

1914. Notes on cases of fever due to *Bacterium columbense* (Cast. 1905). Centralbl. f. Bakteriol., Abt. 1. Orig. 74, 197.
1916. Nota sulle infezioni tifiche, paratifiche, paratifosimili e miste nella zona adriaticobalcanica. Ann. di Med. Nav. e Colon., 11, 453.
1917. Notes on tropical diseases met wtih in the Balcanic and Adriatic zones. Jour. Trop. M. and Hyg., 20, 181.

CASTELLANI, ALDO, AND CHALMERS, ALBERT J.
1910. Manual of Tropical Medicine, 1st ed. William Wood & Co. New York.
1919. A Manual of Tropical Medicine. 3rd ed. William Wood and Co., New York.
1920. Sur la classification de certains groupes de bacilles aérobes de l'intestin humain. Ann. de l'Inst. Pasteur, 34, 600.

CATHCART, E. P.
1906. The bacterial flora of "blown" tins of preserved food. Jour. Hyg., 6, 248.

CHESTER, F. D.
1897. A preliminary arrangement of the species of the genus Bacterium. Del. Coll. Agr. Exp. Sta. 9th Ann. Rept., p. 128.
1899. Systematic Bacteriology. Rep. Del. Coll. Agr. Exp. Sta. p. 53.
1901. A Manual of Determinative Bacteriology. The Macmillan Co. New York.

COLLINS, K. R.
1905. A study of the dejecta of normal children and of those suffering from acute and subacute diarrhoea with reference to *B. dysenteriae*. Jour. Infect. Dis., 2, 620.

COOPER, MERLIN L.
1925. Capsulated bacteria with special reference to *B. typhosus*. Jour. Infect. Dis., 36, 439.

COULTER, CALVIN B.
1917. The biological identity of the Friedländer bacillus. Jour. Exper. M., 26, 763.

CORNIL AND TOUPET.
1888. Sur une nouvelle maladie bactérienne du canard (cholera des canards). Compt. rend de l'Acad. des Sciences de Paris, 106, 1737.

COX, G. L., LEWIS, F. C., AND GLYNN, E. E.
1912. The number and varieties of bacteria carried by the common house-fly in sanitary and insanitary city areas. Jour. Hyg., 12, 290.

DAWSON, CHARLES F.
1898. Asthenia (going light) in fowls. U. S. Dept. of Agr., Bur. of Anim. Indus., 15th Ann. Rep., p. 330.

DEAN, ARNOLD.
1920. The isolation of an organism resembling the paratyphoid group. Med. Jour. Australia, 1, 27.

DEBAINS, E.
1917. Sur les bacilles du groupe Flexner Y. Ann. d. l'Inst. Pasteur, 31, 73.

DENOBELE, J.
1889. Ann. de la Soc. de Gand.*

DETONI, J. B., AND TREVISAN, V.
1889. *Schizomycetaceae*, Naeg. in Saccardo, P. A. Sylloge Fungorum, 8, 923.

DITTHORN, FRITZ.
1913. Ueber dos Verhalten der Typhus and typhusähnlichen Bacillen zu verschiedenen Zuckerarten und diesen nahestehenden mehratomigen Alkoholen. Centralbl. f. Bakteriol., Abt. 1, Orig. 67, 497.

DUCLAUX, E.
1882. Memoire sur le lait. Ann. Inst. Nat. Agron., 4, 23.

* References not seen.

DUDGEON, L. S., AND URQUHART, A. L.
1921. The paratyphoid "C" bacillus as a cause of paratyphoid fever. Jour. Roy. Army Med. Corps, 36, 87.

DUNCAN, J. T.
1924. A "new" Salmonella from a case of enteric fever. Jour. Hyg., 22, 402.

DURHAM, H. E.
1901. Some theoretical considerations upon the nature of agglutinins together with further observations upon *Bacillus typhi abdominalis*, *Bacillus enteritidis*, *Bacillus coli communis*, *Bacillus lactis aerogenes*, and some other bacilli of allied character. Jour. Exper. M., 5, 353.

EBERTH, C. J.
1880. Die Organismen in den Organen bei Typhus abdominalis. Virchow's Arch. f. Path. Anat. (etc.), 81, 58.

EHRENBERG, C. G.
1828. Symbolae Physicae seu Icones et Descriptiones animalium evertebratorum depositis insectis quae ex itinere per Africam Borealem et Asiam occidentalem. IV. Evertebrata.

EISENBERG, J.
1891. Bakteriologische Diagnostik, Ed. 3. Leopold Voss, Hamburg und Leipzig.

EMERSON, HAVEN, AND NORRIS, CHARLES.
1905. "Red-leg"—An infectious disease of frogs. Jour. Exper. M., 7, 32.

EMMERICH, R.
1884. Ueber die Cholera in Neapel und die in Choleraleichen und Cholerakranken gefundenen Pilze. Deutsche Med. Wchnschr. No. 50.

ENLOWS, ELLA M. A.
1920. The generic names of bacteria. Hygienic Laboratory—Bull. No. 121. Treasury Dept., U. S. P. H. Service.

ERNST.
1890. Beitrage zur pathologie Anatomie und zur allgemeinen Pathologie. 8, 204.

ESCHERICH, T.
1885. Die Darmbakterien des Neugeborenen und Säuglings. Fortschr. d. Med. 3, 515 and 507.
1886. Die Darmbakterien des Säuglings. Ferdinand Enke, Stuttgart.

EVANS, ALICE C.
1915. *Bacillus abortus* in market milk. Jour. Wash. Acad. Sc., 5, 122.
1916. The bacteria of milk freshly drawn from normal udders. Jour. Infect. Dis., 18, 437.
1918. Further studies on *Bacterium abortus* and related bacteria. III. *Bacterium abortus* and related bacteria in cow's milk. Jour. Infect. Dis., 23, 354.
1923. The nomenclature of the *melitensis-abortus* group of bacterial organisms. Pub. Health Rep. U. S. Mar. Hosp. Serv., 38, 1943.
1925. Studies on *Brucella (Alkaligenes) melitensis*. Treasury Dept., U. S. Pub. Health Service, Hygienic Lab. Bull. No. 143.

FISCHER, BERNARD.
1902. Zur Aetiologie der sogenannten Fleischvergiftungen. Ztschr. f. Hyg. u. Infektionskrankh., 39, 447.

FITCH, C. P., AND BILLINGS, W. A.
1920. A study of the action of eight strains of *Bact. abortivo-equinus* on certain of the carbohydrates. Jour. Bact., 5, 469.

FLETCHER, W.
1918. Capsulate mucoid forms of paratyphoid and dysentery bacilli. Lancet, 195, 102.

FLEXNER, S.
1900. On the etiology of tropical dysentery. Johns Hopkins Hospital Bulletins, 11, 231.

FLUGGE, C.
1886. Die mikroorganismen, F. C. W. Vogel, Leipzig.

FORD, W. W.
1901. Classification of intestinal bacteria. Jour. Med. Research, 6, 211.
1903. Classification and distribution of the intestinal bacteria in man. Studies from the Royal Victoria Hospital, 1, 487.
1905. The carbohydrate reactions of the paratyphoid or paracolon group. Med. News, 86, 1126.

VON FREUDENREICH, ED.
1890. Ueber einen neuen, in geblähten Käsen gefundenen Bacillus *(Bacillus schafferi)*, Landwirthschaftliches Jahrbuch der Schweis, 4, 17.

FRIEDLANDER, C.
1882. Ueber die Schizomyceten bei der acuten fibrösen Pneumonie. Virchow's Arch. f. Path. Anat. (etc.), 87, 319.
1883. Die Mikrokokken der Pneumonie. Fortschr. d. Med., 1, 715.

FROST, W. D.
1903. Laboratory Bacteriology, 3rd ed. The Macmillan Co., New York.

GAERTNER.
1888. Ueber die Fleischvergiftung in Frankenhausen am Kepffhäuser und den Erreger derselben. Correspondenz-Blatter des Allgemeinen ärztlichen Vereins von Thüringen, 17, 573.

GAFFKY.
1884. Zur Aetiologie des Abdominaltyphus. Mitteil. a. d. Kaiserl. Gesundheitsamte, 4, 372.

GOLDBERG, S. A.
1917. A study of the fermenting properties of *Bact. pullorum* (Rettger) and *Bact. sanguinarium* (Moore). J. Am. Vet. M. Assn., N. 54, 203.

GOOD, EDWIN S.
1912. Etiology of infectious abortion in mares. Am. Jour. Vet. Med.

GOOD, EDWIN S., AND CORBETT, LAMBERT S.
1913. Investigations of the etiology of infectious abortion of mares and jennets in Kentucky. Jour. Infect. Dis., 13, 53-68.

GOOD, EDWIN S., AND SMITH, W. V.
1916. Further investigations of the etiology and control of infectious abortion in mares. Ky. Agr. Exp. Sta. Bull. No. 204, 335.

GOOD, EDWIN S., AND CORBETT, W. V.
1916. A study of gas production by different strains of Bacillus Abortivo-equinus. Jour. Infect. Dis., 18, 586.

GRIMBERT, L.
1895. Recherches sur le pneumobacille de Friedländer, Premier mémoire, Étude des fermentations provoquée par cet organisme. Ann. d. l'Inst. Pasteur, 9, 840.
1896. Recherches sur le pneumobacille de Friedländer. Ann. d. l'Inst. Pasteur, 10, 708.

GRIMBERT, L., AND LEGROS, G.
1900. Identité du bacille lactique aerogeine et du pneumobacille de Friedländer. Compt. rend. Soc. de biol., 52, 491.

GROTENFELT, GOSTA.
1889. Studien über die Zersetzungen der Milch. II. Ueber die Virulenz einiger Milchsaurebacterien. Fortschr. d. Med., 7, 121.

HADLEY, P. B., CALDWELL, D. W., ELKINS, M. W., AND LAMBERT, D. J.
1917. Infections caused by *Bact. pullorum* in adult fowls. R. I. Agric. Exper. Sta. Bull., p. 172.

HADLEY, P. B., ELKINS, M. W., AND CALDWELL, D. W.
1918. The colon-typhoid intermediates as causative agents of disease in birds. I. The paratyphoid bacteria. R. I. Agric. Exp. Sta. Bull., p. 174.

HAMILTON, ALICE.
1898. Ueber einen aus China stammenden Kapselbacillus *(Bacillus capsulatus chinensis* nov. spec.). Centralbl. f. Bakteriol., Abt. 2, 4, 230.

HARDING, E. R., AND OSTENBERG, Z.
1912. Studies on Endo's medium with observations on the differentiation of bacilli of the paratyphoid group. Jour. Infect. Dis., 11, 109.

HAUSER, C.
1885. Ueber die Entwickelungsgeschichte und pathogenen Eigenschaften einer fäulniserregenden Bakterienart. Sitz. -ber. d. phys. -mediz. Sozietät zu Erlangen, p. 156-171. After C. Fischer. Biol. Centralbl., 5, 36.

HENRICI, H.
1894. Beitrag sur Bakterienflora des Käses. Arb. aus dem. Bact. Inst. der Techn. Hochschule zu Karlsruhe.

HIRSCHFELD, L.
1919. A new germ of paratyphoid. Lancet, 1, 296.

HOLLAND, DOROTHY F.
1920. Generic index of the commoner forms of bacteria. Jour. Bact., 5, 215.

HUEPPE, FERDINAND.
1884. Untersuchungen über die Zersetzungen der Milch durch Mikroorganismen. Mittheilungen aus dem Kaiserlichen Gesundheitsamte, 2, 337.

JACKSON, D. D.
1911. Classification of the B. coli group. Jour. Infect. Dis., 8, 241.

JORDAN, EDWIN O.
1890. A report on certain species of bacteria observed in sewage. Report on Water Supply and Sewerage, Experimental Investigations by the State Board of Health of Massachusetts. Part II, p. 821.
1903. The kinds of bacteria found in river water. Jour. Hyg., 3, 1.
1917. The differentiation of the paratyphoid-enteritidis group. I. Jour. Infect. Dis., 20, 457.

JORDAN, E. O., AND VICTORSON, R.
1917. Differentiation of the paratyphoid-enteritidis group. II. Lead acetate agar. Jour. Infect. Dis., 21, 554.

KAYSER, H.
1902. Das Wachstum der zwischen Bacterium typhi und coli stehenden Spaltpilze auf dem v. Drigalski-conradischen Agarboden. Centralbl. f. Bakteriol., Abt. 1, Orig., 31, 426.
1904. Die Bakteriologie des Paratyphus. Centralbl. f. Bakteriol. Abt. 1, Orig. 35, 154.

KHALED, Z.
1921. A comparative study of bovine abortion and undulant fever, from the bacteriological point of view. Jour. Hyg., 20, 319.

KLEBS, E.
1881. Der Bacillus des Abdominaltyphus und der typhöse Process. Arch. f. exper. Path. u. Pharmakol., 13, 381.

KLEIN, E.
1889. Ueber eine epidemische Krankheit der Hühner, verursacht durch einen Bacillus—Bacillus gallinarum. Centralbl. f. Bakteriol., 5, 689.

KLIGLER, I. J.
1914. Studies on the classification of the colon group. Jour. Infect. Dis., 15, 187.

KRAMER.
1892. Die Bakteriologie. Carl. Gerold's Sohn, Wien.

KRUMWIEDE, C., KOHN, L. A., AND VALENTINE.
1918. Studies on the paratyphoid-enteritidis group. V. The correlation of cultural and agglutination results, with special reference to B. paratyphosus B. and B. cholera suis. Jour. Med. Research, 38, 89.

KRUMWIEDE, C., PRATT, J. S., AND KOHN, L. A.
1916. Studies on the paratyphoid enteritidis group. I. Xylose fermentation for the differentiation of B. paratyphosus "A" from other members of the Paratyphoid-Enteritidis Group. Jour. Med. Research, 34, 355.
1916. Studies on the paratyphoid enteritidis group. II. Observations on the reaction in litmus milk as a method of biological differentiation. Jour. Med. Research, 35, 55.

KRUSE, W.
1896. Flügge, Die Mikroorganismen, 3rd ed. 2, F. C. W. Vogel.

KRUSE, W.
1900. Uber die Ruhr also Volkskrankheit und ihrer Erreger. Deutsch. Med. Woch., 26, 637.

LEBLAYE, R., AND GUGGENHEIM, H.
1914. Manuele Pratique de Diagnostic Bactériologique, Viget Freres.

LEHMANN, K. B.
1894. Ueber die Sauerteiggärung und die Beziehungen der Bacillus levans zum Bacillus coli communis. Centralbl. f. Bakteriol. 15, 350.

LEHMANN, K. B., AND NEUMANN, R.
1896. Atlas und Grundriss der Bakteriologie Teil II, J. F. Lehmann, München.
1901. Atlas and Principles of Bacteriology, 2nd ed. Part II. English Translation. W. B. Saunders & Co., Phila. & Lond.

LETZERICH, LUDWIG.
1881. Experimentelle Untersuchungen über Typhus abdominalis. Arch. f. exper. Path. u. Pharmakol., 14, 212.

LEVINE, MAX.
1918. A statistical classification of the Colon-Cloacae group. Jour. Bact., 3, 253.
1920. Dysentery and allied bacilli. Jour. Infect. Dis., 27, 31.
1920. Some differential characters of the group of dysentery bacilli. Proceedings American Society Bacteriologists, Dec. 1919, Abs. Bact., 4, 15.

LEVINE, MAX, AJWANI, G. A., AND WELDIN, J. C.
1925. The Morgan group of paratyphoids. Am. J. Pub. Health, 15, 17.

LEVINE, MAX, AND SOPPELAND, LULU.
1926. Bacteria in creamery wastes. Eng. Exp. Sta., Iowa State Coll., Bull. 77.

LEWIS, C. J.
1911. Bacteriology of normal and diarrheal stools of children in Birmingham, England. Loc. Gov. Board Rep. Med. Suppl., 1910-11. Appendix B., No. 2, 314.

LIGNIERES, M.
1900. Contribution a l'etude et a la classification des Septicemies hemorrhagiques. Rec. de méd. vét., Ser. 8, 7, 331.

LOGAN, W. R.
1914. The intestinal flora of infants and young children. Jour. Path. and Bacteriol., 18, 527.
1916. The bacteriology of the faeces in diarrhoea of infants. Lancet, 2, 824.

MACCONKEY, A.
1905. Lactose fermenting bacteria in feces. Jour. Hyg., 5, 333.
1906. A contribution to the bacteriology of milk. Jour. Hyg., 6, 385.
1906. Note on some cases of food-poisoning. Jour. Hyg., 6, 570.
1909. Further observations on the differentiation of lactose-fermenting bacilli with special reference to those of intestinal origin. Jour. Hyg., 9, 86.

MACKIE, T. J.
1919. The atypical dysentery bacilli. Jour. Hyg., 18, 69.

MACKIE, F. P., AND BOWEN, G. J.
1919. Note on the character of anomalous member of the Paratyphoid Group met with in Mesopotamia. J. Roy. Army Med. Corps, 33, 154.

MAGGI, LEOPOLDO.
1886. Essai d'une classification protistologique des ferments vivants. Jour. Microg., 10, 80, 173, 327.

MAGNUSSEN, H.
1919. Joint ill in foals: Etiology. Jour. Comp. Path. & Therap., 32, 143.

MAIR, W.
1906. Note on a paracolon bacillus found in the urine. Brit. M. J., 1, 438.

MARRASINI, ALBERTO.
1913. Ueber das Vorhandensein einer den Körper einiger Bakterien umgebenden Hülle und deren besondere Bedeutung. Centralbl. f. Bakteriol., Abt. 1. Orig., 71, 113.

MATZUSCHITA, T.
1902. Bakteriologisches Diagnostik. Gustav Fischer, Jena.

MEYER, K. F., AND BOERNER, FRED.
1913. Studies on the etiology of epizootic abortion in mares. Jour. Med. Research, 29, 325.

MEYER, K. F., AND SHAW, E. B.
1920. A comparison of the morphologic, cultural and biochemical characteristics of *B. abortus* and *B. melitensis*. Jour. Infect. Dis., 27, 173.

MIGULA, W.
1895. *Schizomycetes*. Engler and Prantl. Natürlichen Pflanzenfamilien, etc., W. Engelmann, Leipzig.
1900. System der Bakterien. Gustav Fischer, Jena.

MOORE.
1895. On a pathogenic bacillus of the hog cholera group associated with a fatal disease in pigeons. U. S. Dept. Agr., Bur. An. Ind. Bul. No. 8, 71.

MORGAN, H. DE R.
1905. Some observations upon the microorganisms of meat poisoning and their allies. Brit. M. J., 1, 1257.
1906. Upon the bacteriology of the summer diarrhoea of infants. Brit. M. J., 1, 908.
1907. Upon the bacteriology of the summer diarrhoea of infants. Brit. M. J., 2, 16.

MULSOW, F. W.
1919. The differentiation and distribution of the paratyphoid-enteritidis group. VI. Avian paratyphoid bacilli: A comparative study of *B. pullorum* and *B. sanguinarium*. Jour. Infect. Dis., 25, 135.

MURRAY, E. G. D.
1918. An attempt at classification of *Bacillus dysenteriae* based upon an examination of the agglutinating properties of fifty-three strains. Jour. Roy. Army Med. Corps, 31, 257.

NANKIVELL, A. T., AND STANLEY, J. M.
1920. The contamination of oysters. Jour. Hyg., 18, 465.

NICOLL, WILLIAM.
1911. On the varieties of *Bacillus coli* associated with the house-fly (Musca domestica). Jour. Hyg., 11, 381.

NICOLLE, CH. ET HEBERT, A.
1897. Note sur un enchantillon de bacille de Friedlander. Ann. d. l'Inst. Pasteur, 11, 80.

NOCARD.
1893. Conseil d. Hygiene Publique et Salubrite du Dept. du Seine, Seance, Mar. 24, 893*.

NOWAK, JULES.
1908. Le-bacille de Bang et sa biologie. Ann. d. l'Inst. Pasteur, 22, 541.

OGATA.
1892. Zur Aetiologie der Dysenterie, Centralbl. f. Bakteriol., 11, 264.

ORLA-JENSEN, S.
1909. Die Hauptlinien des natürlichen Bakterien systems. Centralbl. f. Bakteriol., Abt. 2, 22, 305.

* References not seen.

PAPASOTIRIU, J.
1901. Untersuchungen über das vorkommen des Bakterium coli in Teig und Getreide. Arch. fur Hyg., 41, 204.

PASSET.
1885. Ueber Mikroorganismen der eiterigen Zellgewebseutzündung des Menschen. Fortschr. d. Med., 3, 33.

PEREZ, FERNAND.
1899. Recherches sur la bactériologie de l'ozéne. Ann. d. l'Inst. Pasteur, 13, 937.

PERKINS, R. G.
1904. Bacillus mucosus capsulatus. Jour. Infect. Dis., 1, 241.
1925. Classification of spore-free, gram-negative, aerobic rods with special reference to fermentation and proteolysis. Jour. Infect. Dis., 37, 232.

PERRY, H. MARRIAN.
1920. The antigenic properties of B. psittacosis. Brit. J. Exp. Path., 1, 131.

PETRUSCHKY, J.
1896. Bacillus Faecalis Alcaligenes (n. sp.), Centralbl. f. Bakteriol., 19, 187.

PFEIFFER.
1889. Ueber einen neuen Kapselbacillus. Ztschr. f. Hyg. u. Infektionskrankh, 6, 145.

PIRIE, J. H. HARVEY.
1917. Observations on East African bacillary dysentery. Jour. Hyg., 15, 565.

REDMAN, T.
1922. The classification of some lactose fermenting organisms isolated from cheeses, waters and milk. J. Path. and Bacteriol., 25, 63.

REED, WALTER, AND CARROLL, JAMES.
1900. A comparative study of the biological characters and pathogenesis of Bacillus X (Sternberg), Bacillus icteroides (Sanarelli), and the Hog-cholera Bacillus (Salmon and Smith). Jour. Exp. M., 5, 215.

RETTGER, L. F.
1909. Further studies on fatal septicemia in young chickens, or "white diarrhoea". Jour. Med. Research, 21, 115.

RETTGER, LEO F., AND HARVEY, S. C.
1908. Fatal septicemia in young chickens, or "white" diarrheoa. Jour. Med. Research, 18, 277.

RETTGER, LEO F., AND KOSER, S. A.
1917. Comparative study of Bact. pullorum (Rettger) and Bact. sanguinarium (Moore). Jour. Med. Research, 35, 443.

RETTGER, LEO F., AND SCOVILLE, MARGARET M.
1919. Bacterium anatis, Nov. spec., an organism of economic importance and a member of the paratyphoid group of bacteria. Paper presented before the Society of Amer. Bact. at the annual meeting, Dec., 1918.
1920 Bacterium anatum, N. S. The etiological factor in a widespread disease of young ducklings known in some places as "keel". Jour. Infect. Dis., 26, 217.

RITCHIE,, JOHN.
1914. Note on the non-lactose fermenters in fresh milk. Jour. Hyg., 14, 393.

ROGERS, L. A., CLARK, W. M., AND DAVIS, B. J.
1914. The colon group of bacteria. Jour. Infect. Dis., 14, 411.

ROGERS, L. A., CLARK, W. M., AND LUBS, N. A.
1918. The characteristics of bacteria of the colon type occurring in human feces. Jour. Bact., 3, 231.

RUFFER, M. A., AND WILLMORE, J. G.
1909. On the etiology of dysentery. Brit. M. J., 2, 862.

SALMON, D. E.
1885. Investigations on swine plague. U. S. Dept. Agric., Bur. Anim. Indus., 2nd Ann. Rept.
1886. Investigation of swine diseases. U. S. Dept. Agric., Bur. Anim. Indus., 3rd Ann. Rept.

SANARELLI.
1891. Ueber einen neuen Mikroorganismus des Wassers, welcher für Thiere mit veränderlicher und konstanter Temperatur pathogen ist. Centralbl. f. Bakteriol., 9, 222.
1897. Étiologie et pathogènie de la fièvre jaune. Ann. d. l'Inst. Pasteur, 11, 433.

SAVAGE, W. G.
1907. The bacteriological examination of surface wells. Jour. Hyg., 7, 477.

SCHMIDT AND WEISS.
1902. Die Bakterien, 314.

SCHMITZ, K. E. F.
1917. Ein neuer Typus aus der Gruppe der Ruhrbazillen als Erreger einer grösseren Epidemie. Ztschr. f. Hyg. u. Infektionskrankh, 84, 449.

SCHOTTMÜLLER.
1900. Ueber eine das Bild des Typhus bietende Erkrankung, hervorgerufen durch typhusähnliche Bacillen. Deutsche med. Wochenschr., 26, 511.
1901. Weitere Mitteilungen über mehrere das Bild des Typhus bietende krankheitsfälle, hervorgerufen durch typhusähnliche Bazillen (Paratyphus). Ztschr. f. Hyg. u. Infektionskrankh, 36, 368.

SCHROETER, J.
1889. Die Pilze Schlesiens, J. U. Kerns, Breslau.

SHIGA, KIYOSHA.
1898. Ueber den Erreger der Dysenterie in Japan. Centralbl. f. Bakteriol., Abt. I, 23, 599.

SMITH, J.
1924 Enteritis due to *B. dysenteriae* Sonne. Jour. Hyg., 23, 94.

SMITH, THEOBALD.
1894. Additional investigations concerning infectious swine diseases. U. S. Dept. Agric., Bur. Anim. Indus., Bul. No. 6.

SMITH, TH., AND TEN BROECK, C.
1915. A note on the relation between B. pullorum (Rettger) and the fowl typhoid bacillus (Moore). Jour. Med. Research, 31, 547.

SONNE, C.
1915. On the bacteriology of the paradysentery bacilli. Centralbl. f. Bakteriol., Abt. 1, Orig., 75, 408.

STERNBERG, G. M.
1892. Manual of Bacteriology. William Wood and Company, New York.

ST. JOHN-BROOKS, RALPH, AND RHODES, MABEL.
1923. The organisms of the fowl typhoid group. Jour. Path. and Bacteriology, 26, 433.

STEWART, M. J.
1917. A study of the coliform organisms infecting wounds of war. Jour. Hyg., 16, 291.

STRONG, L. W.
1899. Ueber die Kapselbacillen, Centralbl. f. Bakteriol., Abt. I, 25, 49.

STRONG, R. P.
1917. Bacillary dysentery. Forscheimer's Therapeusis of Internal Diseases. 5, 249. D. Appleton and Co., New York.

STRONG, H. R., AND MUSGRAVE, W. E.
1900. The bacillus of Philippine dysentery. Jour. Am. M. Assoc., 35, 498.

TENBROECK, CARL.
1920. A group of paratyphoid bacilli from animals closely resembling those found in man. Journ. Exp. M., 32, 19.
1920. Bacilli of the Hog Cholera group *(Bacillus cholerae suis)* in Man. J. Exper. M., 32, 33.

THJØTTA, TH.
1919. On the bacteriology of dysentery in Norway. Jour. Bact., 4, 355.
1920. On the bacillus of Morgan No. 1—A meta-colon bacillus. Jour. Bact., 57, 77.

TRETOP, E.
1900. La maladie des cygnes coscoroba. Ann. d. l'Inst. Pasteur, 14, 224.

TREVISAN, V.
1879. Prime linee d'introduzione al lo studio dei Batterj italiane. 2nd communication. Rendiconti. Reale Institute Lombardo di Scienze e Lettere. IV. Ser. II, 12, 133.
1882. Emilio Cornalia. Atti della Accademia. Fisio-Medico-Statistica di Milano, p. 100.
1885. Caratteri di alcuni nuovi generi di Batteriacee. Atti della Accademia Fisio-Medico-Statistica in Milano. Ser. 4, 3, 92.
1887. Sul Micrococèo della rabbia e sulla possibilita di riconoscere durante il periode d'incubazione, dall' esame del sangue della persons morsicata, se la controtta l'infezione rabbica. Rendiconti. Reale Institute. Lombardo di Scienze e Lettere. Ser. II, 20, 88.
1889. I Genera e le specie delle Batteriacee. Milan.

TRIBONDEAU, L., AND FICHET, M.
1916. Note sur les dysenteries des Dardanelles. Ann. de l'Inst. Pasteur, 30, 357.

UHLENHUTH AND HUBENER.
1908. Bazillen der Typhusgruppe. Centralbl. f. Bakteriol., Ref., 41, 230.

VANLOGHEM, J. J.
1918. Bacterium proteus anindologenes. Ann. d. l'Inst. Pasteur, 32, 295.

WARD, ARCHIBALD R.
1917. Bacterium pyogenes and its relation to suppurative lesions in animals. Jour. Bact., 2, 619.

WEISS, H., AND RICE, J. L.
1917 Studies on paratyphoid-enteritidis, typhoid, dysentery and colon groups. I. Preliminary communication on inosite fermentation. Jour. Med. Research, 35, 403.

WELDIN, JOHN C., AND LEVINE, MAX.
1923. An artificial key to the species and varieties of the colon-typhoid or intestinal group of bacilli. Abs. Bact., 7, 13.

WENNER, JOHN J., AND RETTGER, LEO F.
1919. A systematic study of the proteus group of bacteria. Jour. Bact., 4, 331.

WILSON, W. J.
1908. Bacteriological observations on colon bacilli infecting the urinary tract, with special remarks on certain colon bacilli of the "anaerogenes" class. Jour. Hyg., 8, 543.

WINSLOW, C. E. A., BROADHURST, JEAN, BUCHANAN, R. E., KRUMWIEDE, CHARLES, JR., ROGERS, L. A. AND SMITH, G. H. (Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types.)
1917. Preliminary Report. The families and genera of bacteria. Jour. Bact., 2, 552.
1920. The Families and Genera of the Bacteria. Final report of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types.

WINSLOW, C. E. A., KLIGLER, I. J., AND ROTHBERG, W.
1919. Studies on the classification of the colon-typhoid group of bacteria with special reference to their fermentative reactions. Jour. Bact., 4, 429.

WOLFINN, ALEXANDER.
1894. Hygienische Studien über Mehl und Brot. 2. Die Organismen des Sauerteigs. Arch. f. Hyg., 21, 279.

ZIRONI, A., AND CAPONE, G.
1917. Recherche exiologiche Sopra vari casi di infezioni intestinali a typo diarroico nota. Sperimentale, Arch. di. biol., 71, 422.

ZOPF, W.
1883. Die Spaltpilze. Eduard Trewendt, Breslau.
1884. Die Spaltpilze. 2nd ed. Eduard Trewendt, Breslau.
1885. Die Spaltpilze. 3rd ed. Eduard Trewendt, Breslau.

# FUNGICIDAL ACTIVITY OF FURFURAL

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Furfural was first prepared by Dobereiner (2) in 1830. Although it has been utilized for the last forty years in the quantitative determination of pentoses and pentosans its chemical and physical properties were practically unknown until within the last few years. Until recent development in its manufacture, furfural remained too high in price to encourage laboratory experiments or commercial utilization. In 1920, Monroe (7) obtained furfural by the action of sulphuric acid on corn cobs and in 1921, LaForge (3) worked out a commercial process for its preparation from this same source. Soon after this, furfural was offered on the market at a low price through the independent research of the Miner Laboratories of Chicago (6). It was prepared by the digestion of oat hulls with steam and acid. These recent developments in the manufacture of furfural have stimulated investigations looking toward its utilization.

Furfural in its properties resembled formaldehyde on the one hand and benzaldehyde on the other. This suggested the possibility of its use as a fungicide. Such studies were undertaken at Iowa State College through a fellowship of the Crop Protection Institute in January, 1924, and the results of some of the laboratory toxicity studies, together with some of the substantiating field data are presented in this paper. The termination of the project after one year precludes the presentation of all of the results obtained due to lack of adequate confirmation. The studies presented include the comparative effects of furfural and formaldehyde on the germination of seeds and sclerotia, and the influence of these same aldehydes and related compounds on the germination of spores of *Puccinia coronata* Cda. *holci, Sphacelotheca sorghi* (Lk) Cl and *Ustilago hordei* (Pers) K & S.

## Laboratory Studies

### The Comparative Toxicity of Six Aldehydes.

The comparative toxicity of furfural and other aldehydes to fungus spores was studied, using the van Tieghem cell. Since all germination tests were at room temperature, little difficulty was experienced with breaking of the hanging drop due to excessive condensation of moisture on the cover slip. The glassware composing the cell was never used twice without being taken apart and washed in xylol, dried, boiled in alkali, in cleaning solution, and in several changes of distilled water. It was dipped in alcohol and wiped dry and sterilized in an oven for one hour.

Six aldehydes: formaldehyde, acetaldehyde, propylaldehyde, butylaldehyde, furfuraldehyde and benzaldehyde were used in these experiments. Stock solutions were made up of M/10 concentration of all these except benzaldehyde, for which an M/50 solution was used. These were stored in the dark in pyrex glassware and care exercised to keep the bottles stoppered.

* The writer wishes to express his appreciation for the helpful advice and criticism given during the course of these investigations by Dr. I. E. Melhus, under whose direction this work was done.

Three organisms, *Puccinia coronata holci*, *Ustilago hordei*, and *Sphacelotheca sorghi*, were used to measure toxicity. These were uniformly variable, germinated readily in distilled water in a short period of time at room temperature and were easily obtained. The influence which a variation in the number of spores in a hanging drop has on toxicity was not observed in these tests, but an effort was made to introduce about the same number of spores in each drop. Ten trials were made on different dates, using three replications in each trial of a given species.

Criteria which may be used as an indication of toxicity in a test of this kind are: (1) percent of spores germinating and (2) average length of germ tubes after allowing the culture to incubate for a definite length of time under specified conditions. The first is the most widely used. Because of the small size of the spores of *Sphacelotheca sorghi* and *Ustilago hordei*, and because they form a dense film at the base of the hanging drop an accurate count of germination was not practical. The response of these spores to the aldehydes was based on a careful comparison with check cultures in distilled water. The rapidity of germination was determined by intermittent observation for the first 24 hours. Toxicity to the uredospores of *Puccinia coronata holci* was determined by examining the cultures at regular intervals and accurately counting the percent of germination and measuring a representative number of germ tubes from several fields in each drop.

The effect of six aldehydes on the germination of spores of *Sphacelotheca sorghi*, *Ustilago hordei*, and *Puccinia coronata holci*, after a 24 hour incubation period is shown in Table I. There is a certain degree of constancy in the molar concentration of the different aldehydes which inhibited spore germination. A concentration of 0.005 M entirely prevented the germination of the spores of at least one of the three organisms tested, and noticeably retarded germination of the others. The 0.0025 M concentration usually retarded germination, while the 0.001 M solution usually had no effect.

There are some variations in the toxic concentration of the aldehydes which indicate specific action.

Taylor (10) has demonstrated the specificity of several organic acids on four different groups of wound inhabiting bacteria, but the writer is unaware of any work on the specificity of organic compounds to fungi. The only indication of specific action was the tolerance of *Ustilago hordei* to formaldehyde and its intolerance to furfural in comparison with the tolerance of *Puccinia coronata holci* to furfural and extreme sensitiveness to formaldehyde. In the case of *Ustilago hordei*, a solution of furfural was more inhibitory to germination than a solution of formaldehyde possessing two and a half times its molar concentration. With *Puccinia coronata holci* a formaldehyde solution produced as great a toxic action as a furfural solution ten times as concentrated. Except for this sensitivity of *P. coronata holci* and the slightly greater tolerance of *Ustilago hordei* to formaldehyde, the molar concentration of the aldehydes, which inhibited spore germination, was quite uniform.

Because of the specificity of action of the different aldehydes the most toxic could not be definitely determined. However, these limited experiments indicated that benzaldehyde, formaldehyde and butylaldehyde were most toxic, followed by furfuraldehyde and propylaldehyde, with acetaldehyde the least.

TABLE I. COMPARATIVE TOXICITY OF SIX ALDEHYDES TO SPORES OF *SPHACELOTHECA SORGHI, USTILAGO HORDEI* AND *PUCCINIA CORONATA HOLCI.*

| Molecular concentration of solution tested | Sphacelotheca sorghi | | | | | | Ustilago hordei | | | | | | Puccinia coronata holci | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Formaldehyde | Acetaldehyde | Propylaldehyde | Butylaldehyde | Furfuraldehyde | Benzaldehyde | Formaldehyde | Acetaldehyde | Propylaldehyde | Butylaldehyde | Furfuraldehyde | Benzaldehyde | Formaldehyde | Acetaldehyde | Propylaldehyde | Butylaldehyde | Furfuraldehyde | Benzaldehyde |
| 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.0075 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.005 | 0 | ± | tr. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ± | 0 | ± | 0 |
| 0.0025 | ± | ± | ± | tr. | ± | tr. | + | + | 0 | 0 | 0 | 0 | 0 | + | + | ± | + | 0 |
| 0.001 | + | + | + | + | + | + | + | + | + | + | + | tr. | 0 | + | + | + | + | + |
| 0.00075 | + | | | | | | + | | | | | + | ± tr. | | | | | |
| 0.0005 | + | | | | | | + | | | | | + | | | | | | |
| 0.00025 | + | | | | | | | | | | | | + | | | | | |

0 = No germination.
tr. = Trace, an occasional spore germinating by a weak promycelium.
± = Retarded germination.
+ = Normal germination as in distilled water.

TABLE II. TOXICITY OF SIX ALDEHYDES TO SPORES OF *PUCCINIA CORONATA HOLCI* AS INDICATED BY PERCENT OF GERMINATION AND GERM TUBE LENGTH AT THE END OF 2, 4 AND 8 HOUR INCUBATION PERIODS.

| Molecular concentration | Formaldehyde | | | | | | Acetaldehyde | | | | | | Propylaldehyde | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Percent Germination | | | Average length of germ tube | | | Percent Germination | | | Average length of germ tube | | | Percent Germination | | | Average length of germ tube | | |
| | Hours | | | Hours | | | Hours | | | Hours | | | Hours | | | Hours | | |
| | 2 | 4 | 8 | 2 | 4 | 8 | 2 | 4 | 8 | 2 | 4 | 8 | 2 | 4 | 8 | 2 | 4 | 8 |
| .01 | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | |
| .0075 | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | |
| .005 | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | | 0 | 11 | 11 | | 15 | 35 |
| .0025 | 0 | 0 | 0 | | | | 29 | 22 | 22 | 20 | 38 | 40 | 57 | 56 | 100 | 33 | 80 | 100 |
| .001 | 0 | 0 | 0 | | | | 71 | 100 | 100 | 100 | 100 | 100 | 71 | 100 | 100 | 75 | 100 | 100 |
| .00075 | 0 | 0 | 5 | | | | | | | | | | | | | | | |
| .0005 | 30 | 17 | 22 | 22 | 18 | 19 | | | | | | | | | | | | |
| .00025 | 100 | 100 | 100 | 55 | 60 | 53 | | | | | | | | | | | | |
| .0001 | 140 | 100 | 100 | 111 | 120 | 125 | | | | | | | | | | | | |
| Dist. water | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

TABLE II—(Continued).

| Molecular concentration | Butyaldehyde | | | | | | Benzaldehyde | | | | | | Furfuraldehyde | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Percent Germination | | | Average length of germ tube | | | Percent Germination | | | Average length of germ tube | | | Percent Germination | | | Average length of germ tube | | |
| | Hours | | | Hours | | | Hours | | | Hours | | | Hours | | | Hours | | |
| | 2 | 4 | 8 | 2 | 4 | 8 | 2 | 4 | 8 | 2 | 4 | 8 | 2 | 4 | 8 | 2 | 4 | 8 |
| .01 | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | |
| .0075 | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | |
| .005 | 0 | 0 | 0 | | | | 0 | 0 | 0 | | | | 0 | 5 | 5 | | 3 | 8 |
| .0025 | 0 | | 17 | 0 | 10 | 30 | 0 | 0 | 0 | | | | tr. | 89 | 90 | 8.3 | 60 | 60 |
| .001 | 93 | 80 | 100 | 100 | 60 | 66 | 43 | 83 | 100 | 60 | 53 | 75 | 90 | 100 | 100 | 75 | 80 | 100 |
| .00075 | | | | | | | | | | | | | | | | | | |
| .0005 | | | | | | | | | | | | | | | | | | |
| .00025 | | | | | | | | | | | | | | | | | | |
| .0001 | | | | | | | | | | | | | | | | | | |
| Dist. water | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

### Correlation of Toxic Action on Spore Germination and Its Subsequent Growth

The toxic effect of the aldehydes on the process of germination of *Puccinia coronata holci* was studied in more detail. The culture in distilled water was put under a microscope and the time required for the first indication of germination recorded. This occurred in from 70 to 80 minutes. The other cultures were examined after a lapse of an equal period of time and in only one case, the 0.0001 M formaldehyde, where the process of germination was at a stage similar to that in the check, was there any sign of germination. All of the tests could not be made simultaneously so that the percent of germination and the average length of germ tube in the check cultures of each series of tests was considered as 100 of the aldehyde cultures figured accordingly. The figures are given in Table II.

The cultures were examined after germination had first been observed in the checks, and after two, four, eight and twenty-four hour periods. The length of the germ tubes in the eight and twenty-four hour cultures, all 750$\mu$ or more, made accurate counting and measurement of the spores and germ tubes impractical. The only information of value obtained from these examinations was that no perceptible difference between the eight and twenty-four hour cultures occurred and that the spores never fully recovered from the initial retarding effects of the aldehydes. The two hour examination showed that all of the aldehydes produced marked retarding effects near the concentration inhibiting spore germination as indicated both by the percent of germination and by the average length of germ tube. Formaldehyde, as a rule, appeared to reduce the length of germ tube more than the number of spores germinating, while with the others the reverse was quite uniformly the case. In the four hour examination the retarding effect produced by aldehydes near the concentration inhibiting germination was further brought out by the starting of germination in 0.005 M furfural and propylaldehyde and in the 0.0025 M butylaldehyde. The lower figures in the 0.0005 M formaldehyde showed that germination in this solution did not keep pace with that of the checks. The eight hour examination revealed the beginning of germination during the four to eight hour period in the 0.00075 M formaldehyde solution. It also showed a general tendency for recovery as indicated by a narrowing of the difference in germ tube length between the check cultures and those of the solutions.

### Toxicity of Metallic Furoate Salts

The furfural molecule is peculiarly constructed in that besides having the aldehyde group it has in the furyl radical an unsaturated oxygen atom. It is possible that some of the chemical reactions of furfural take place by means of this unsaturated oxygen. It appeared desirable, therefore, in a study of the toxic properties of furfural to determine the role played by the furyl radical.

The means used to determine this was a comparison of the toxicity of several metallic furoate salts. These were prepared expressly for this purpose through the kindness of Dr. J. P. Trickey of the Miner Laboratories, Chicago, Illinois.

In Table III the results obtained in testing the toxicity of five metallic furoate salts to spores of *Sphacelotheca sorghi* are given. The van Tieghem cell method described by Clark (1) was used. Apparently the unsaturated oxygen in the furyl radical was not an important factor in the toxicity

of furfural, as the calcium and ferric furoates were only slightly toxic. The calcium furoate molecule contains two furyl radicals, while furfural contains only one, consequently in equimolar solutions of calcium furoate and furfural there are twice as many furyl radicals in the former as there are in the latter. The retarding effects produced by the 0.004 M calcium furoate and the 0.0025 M furfural solutions were approximately the same. That is, a solution of furfural was as toxic as one of calcium furoate containing three times as many furyl radicals. This suggests that the aldehyde group was a more important factor in toxicity than the furyl radical.

The order of toxicity of the metallic salts corresponds with the toxicity of the metals they contain. Mercuric furoate was the most toxic. Cupric and nickle furoates were of intermediate toxicity and calcium and ferric furoates were the least toxic.

TABLE III. TOXICITY OF METALLIC FUROATE SALTS TO SPORES OF *SPHACELOTHECA SORGHI*.

| Material tested | Percent concentration | Molar concentration | Germination |
|---|---|---|---|
| Calcium furoate | 1.0 | .04 | None |
| " " | 0.1 | .004 | Abundant but retarded |
| " " | 0.01 | .0004 | Normal |
| Ferric furoate | 0.25 | .007 | None |
| " " | .025 | .0007 | Normal |
| Nickle furoate | 0.1 | .004 | None |
| " " | 0.01 | .0004 | Abundant but retarded |
| " " | 0.001 | .00004 | Normal |
| Cupric furoate | 0.01 | .004 | None |
| " " | 0.001 | .0004 | Abundant but retarded |
| " " | 0.0001 | .00005 | Normal |
| Mercuric furoate | 0.05 | .001 | None |
| " " | 0.005 | .0001 | Greatly retarded |
| " " | 0.0005 | .00001 | Normal |
| Check water | | | Normal |
| Furfural | .05 | .005 | None |
| " | .025 | .0025 | Retarded |
| " | .01 | .001 | Normal |

**The Toxic Action of Furfural on the Sclerotia of Rhizoctonia solani on Potatoes**

The comparative effectiveness of furfural and formaldehyde solutions in the control of the Rhizoctonia disease of potatoes was studied in the laboratory following the methods used by Melhus, Gilman and Kendrick (5). Potato tubers infected with sclerotia of *Rhizoctonia solani* were dipped into the solution to be tested. The sclerotia were removed from them after treatment and plated on potato agar. These were incubated for 36 to 48 hours and the number of sclerotia germinating determined. Trials were made at each temperature and concentration with 20 sclerotia per plate.

Table IV shows that raising the temperature of the solution accelerated the toxic action of furfural. With furfural solutions of equal concentration the short treatments at 50° to 60° C. were invariably more toxic than the long time soaks at room temperature. Sclerotia soaked in one percent furfural for two minutes at 55° C. to 60° C. showed only two percent and one percent germination, respectively. Although the two percent furfural solution was much more toxic at room temperature than the one percent solution, raising the temperature also markedly increased its toxicity.

Sclerotia soaked at 20° C. for 15 minutes showed 85 percent, those soaked for 30 minutes, 75 percent, and those soaked for 60 minutes 10 percent germination. Only one to two percent of the sclerotia soaked in two percent furfural at 50°, 55° and 60° C. for two minutes germinated. In general these results are in accord with the data presented by Raeder, Hungerford and Chapman (8). They found that increasing the time and temperature accelerated the toxic action of furfural, but that it was not as toxic to the sclerotia of Rhizoctonia as formaldehyde. Their results suggest further that Rhizoctonia sclerotia grown in Idaho are more resistant not only to furfural, but also to other distinfectants.

TABLE IV. EFFECT OF RAISING THE TEMPERATURE OF FURFURAL SOLUTIONS ON SCLEROTIAL GERMINATION OF *RHIZOCTONIA SOLANI.*

| Treatment | Temperature Degrees C. | Length of treatment—minutes | Percent germination |
|---|---|---|---|
| Check, no treatment | | | 100 |
| Furfural 1% | 20 | 60 | 68 |
| " 2% | 20 | 15 | 85 |
| " 2% | 20 | 30 | 75 |
| " 2% | 20 | 60 | 10 |
| " 1% | 50 | 2 | 30 |
| " 1% | 55 | 2 | 2 |
| " 1% | 60 | 2 | 1 |
| " 2% | 50 | 2 | 1 |
| " 2% | 55 | 2 | 2 |
| " 2% | 60 | 2 | 1 |

Melhus, Gilman and Kendrick (5) found that no known treatment killed all the sclerotia, but considered a treatment as sufficiently toxic which would reduce the germination to two or three percent. Accordingly, the two percent furfural treatment at 50° C. for two minutes appeared to be the minimum strength which met these requirements.

The effect of covering on the toxicity of furfural was determined by treating the tubers in two percent furfural at 50° C. for two minutes and covering in various ways. Dry burlap sacks, burlap sacks wet in two percent furfural, stone crocks inverted over the potatoes on a cement floor, and large glass jars with closely fitting tops were used as covers. Preliminary trials indicated that the type of cover had little influence on the effectiveness of the treatment. Consequently the method of using burlap sacks wet in the solution used for treating the tubers was adopted as being the most practical cover.

TABLE V. EFFECT OF LENGTH OF COVERING AFTER TREATMENT OF POTATOES WITH TWO PERCENT FURFURAL AT 50° C. FOR TWO MINUTES ON THE GERMINATION OF THE SCLEROTIA OF *RHIZOCTONIA SOLANI.*

| Duration of cover | Number of sclerotia | Percent germination |
|---|---|---|
| Check, no treatment | 100 | 93 |
| Uncovered | 100 | 5 |
| 1 hour | 400 | 2.3 |
| 5 hours | 400 | 0.5 |
| 12 hours | 300 | 0.7 |

Further trials, as shown in Table V, using wet burlap as a cover, indicated that periods of cover up to five hours increased the effectiveness of the treatment, but that nothing was gained by a longer period of cover.

The effect of the different treatments, used in the 1925 field trials on sclerotia, was tested in the laboratory. Twenty tubers were selected at random from the treated lots and the sclerotia plated out for germination tests. The results of these tests are given in Table VI. The 1-120 formaldehyde and the three and two percent furfural treatments, both one and five hour cover, were quite effective. The one percent furfural treatments were more effective than the hot 1-1000 mercuric chloride treatment, but all of these were unsatisfactory. The five hour cover, in all cases, resulted in a smaller percent of sclerotia surviving than in the one hour cover treatments. This difference, however, was small.

TABLE VI. VIABILITY OF THE SCLEROTIA OF *RHIZOCTONIA SOLANI* IN THE SEED LOTS TREATED FOR FIELD PLANTING IN 1925.

| Treatment | Length of cover in hours | Number of sclerotia | Percent germination |
|---|---|---|---|
| 1. Untreated check | 0 | 200 | 98.0 |
| 2. Formaldehyde 1-120 2 min. at 50° C. | 3 | 200 | 3.0 |
| 3. Mercuric chloride 1-1000 2 min. at 50° C. | 5 | 200 | 20.0 |
| 4. Furfural 3 percent 2 min. at 50° C. | 1 | 200 | 6.0 |
| 5. Furfural 3 percent 2 min. at 50° C. | 5 | 200 | 4.5 |
| 6. Furfural 2 percent 2 min. at 50° C. | 1 | 200 | 5.0 |
| 7. Furfural 2 percent 2 min. at 50° C. | 5 | 200 | 3.0 |
| 8. Furfural 1 percent 2 min. at 50° C. | 1 | 200 | 17.5 |
| 9. Furfural 1 percent 2 min. at 50° C. | 5 | 200 | 13.0 |

### The Relation of Moisture to the Toxic Action of Furfural

Consideration was given to the effect of moisture on the toxic action of the vapor of furfural, formaldehyde and related compounds. Three museum jars were provided for each substance tested and an unstoppered bottle containing 30 c.c. of the chemical was placed in each jar. In one jar was placed 150 kernels of dry corn, in another a like number with a small beaker of water to maintain a saturated atmosphere, and in the third, kernels which had been presoaked for four hours. At the end of 24, 48, 72 and 120 hour periods 10 kernels were removed from each jar and placed in petri dishes on moist filter paper to germinate. The results are presented in Table VII.

Table VII shows that the action of furfural and formaldehyde relative to moisture is not the same. This difference in action was most pronounced where the presoaked and dry seed were subjected to fumes from the concentrated solutions. At the end of five days presoaked corn exposed to the fumes of 95 percent furfural was dead, while the germination of that subjected to the fumes of 40 percent formaldehyde was only slightly retarded. With dry corn the reverse was true. Seed subjected for five days to the vapor from 95 percent furfural germinated normally, while that exposed to vapor from 40 percent formaldehyde was dead. With a saturated atmosphere injury to dry corn was increased with 95 percent furfural, while it was decreased with 40 percent formaldehyde. The actions of the five percent solutions of furfural and formaldehyde were similar to those of the concentrated solutions, but greatly moderated.

Furfuralcohol in no case produced injurious effects on the germination of corn. This indicated that under the conditions of the experiment it was much less toxic than furfuraldehyde and that the furyl radical did not play an important role in toxicity.

TABLE VII. THE EFFECT OF ATMOSPHERIC MOISTURE ON THE TOXICITY OF DIFFERENT VAPORS TO SEED CORN GERMINATION.

| Chemical | | State of seed | Atmospheric humidity | Germination | | | |
|---|---|---|---|---|---|---|---|
| | | | | 24 hrs. | 48 hrs. | 72 hrs. | 120 hrs. |
| Furfural | 95% | Presoaked in water | Not saturated | N | R | R+ | D |
| Formaldehyde | 40% | Presoaked | " " | N | N+ | N± | N— |
| Furfural | 5% | Presoaked | " " | N | N | N+ | R |
| Formaldehyde | 5% | Presoaked | " " | N | N | N | N |
| Furylalcohol | | Presoaked | " " | N | N | N | N |
| Furylalcohol | 5% | Presoaked | " " | N | N | N | N |
| Methyl alcohol | 95% | Presoaked | " " | N | R | D | D |
| Check | | Presoaked in water | " " | N | N | N | N |
| Furfural | 95% | Dry | Saturated | N | N | N± | N± |
| Formaldehyde | 40% | " | " | N | N | N | N |
| Furfural | 5% | " | " | N | N | N | N |
| Formaldehyde | 5% | " | " | N | N | N | N |
| Furylalcohol | | " | " | N | N | N | N |
| Furylalcohol | 5% | " | " | N | N | N | N |
| Methyl alcohol | | " | " | N | N | R | R |
| Check | | " | " | N | N | N | N |
| Furfural | 95% | " | Not saturated | N | N | N | N |
| Formaldehyde | 40% | " | " " | N | R+ | R+ | D |
| Furfural | 5% | " | " " | N | N | N | N |
| Formaldehyde | 5% | " | " " | N | N | N | N |
| Furylalcohol | | " | " " | N | N | N | N |
| Furylalcohol | 5% | " | " " | N | N | N | N |
| Methyl alcohol | | " | " " | N | R+ | R+ | D |
| Check | | " | " " | N | N | N | N |

R = Retarded N = Normal D = Dead + = Excessive ± = Slightly less

**The Comparative Cumulative Injury of Furfural and Formaldehyde to the Germination of Wheat**

Miss Hurd (4) made an intensive study of the injury to the germination of wheat seed following formaldehyde disinfection. She found that no injury was produced if the treated seed was planted immediately in damp soil or if it was stored damp until planted. The injury was cumulative, i. e., increased with length of storage after treatment. Miss Hurd concluded that the injury was due to the formation of paraformaldehyde on the seed, which slowly volatilized, thus subjecting the seed to a prolonged formaldehyde treatment.

The comparative initial and cumulative injury of furfural and formaldehyde was determined by soaking wheat seed in solutions of 95 percent furfural and 40 percent formaldehyde for varying lengths of time and planting periodically. Two hundred seeds of each treatment were planted in clean sand on a greenhouse bench immediately after treatment before the seed had dried, on the same day of treatment after the seed had dried, and after the treated seed had been stored 4, 22 and 45 days. The percent germinating seed was counted after eight days as shown in Table VIII.

The data in Table VIII show that solutions of formaldehyde were more injurious to wheat germination than were those of furfural. The only treatments which undoutedly injured germination when the seed was planted immediately after treatment, while wet, were the one, two and six

hour soaks in 0.5 percent formaldehdye, and the six hour soak in 0.33 percent formaldehyde. The only effect produced by drying the treated seed, or in storing it four days before planting was an increase in the injurious effects of those treatments, which were harmful to the seed when planted wet. Storage of the treated seed for 22 and 45 days only served to aggravate the injury caused by all of the 0.33 and 0.5 percent formaldehyde treatments. The 0.1 percent formaldehyde treatments as well as all of the furfural treatments apparently were not detrimental to the germination of wheat seed even when stored for 45 days after treatment. Injury by the stronger formaldehyde solutions was cumulative, while furfural injury was not.

## Field Experiments

### The Control of Cereal Smuts

Field experiments for the control of cereal smuts covered a period of two years. During the first year efforts were chiefly directed toward determining the possibility of substituting solutions of furfural for those of formaldehyde. The second year's trials were chiefly concerned in verifying the outstanding results of the previous year and in ascertaining the toxic properties of various furfural dust derivatives.

*Source of Seed.* Only naturally infected seed was used in these experiments. Lots of wheat, variety Prelude; oats, variety White Tartar, and barley, variety Manchuria, were otained from Mr. H. A. Rodenhiser, in charge of the Crop Protection Institute's Cereal treatment project, University Farm, St. Paul, Minnesota, and winter wheat, variety Kanred, from Mr. J. J. Wilson, Muscatine, Iowa.

*Methods Used.* The seed was treated by using methods approximating as closely as possible those used in commercial practice. The dust treat-

TABLE VIII. CUMULATIVE INJURY OF FURFURAL AND FORMALDEHYDE ON WHEAT SEED GERMINATION.

| Treatment | Duration of dip | Percent germination of seed after | | | | |
|---|---|---|---|---|---|---|
| | | 0 da. | | 4 da. | 22 da. | 45 da. |
| | | Wet | Dry | | | |
| Check water | | 93.0 | 90.5 | 93.0 | 89.0 | 72.0 |
| Furfural .1 % | 1 hour | 90.0 | 89.5 | 93.0 | 89.5 | 66.5 |
| Furfural .33 | 1 hour | 93.5 | 90.5 | 89.5 | 91.5 | 58.5 |
| Furfural .5 | 1 hour | 91.0 | 84.5 | 92.0 | 90.5 | 60.5 |
| Formaldehyde .1 | 1 hour | 81.5 | 86.5 | 92.5 | 90.5 | 59.5 |
| Formaldehyde .33 | 1 hour | 86.5 | 80.5 | 81.5 | 65.0 | 26.0 |
| Formaldehyde .5 | 1 hour | 79.5 | 67.0 | 67.5 | 57.5 | 22.0 |
| Furfural .1 | 2 hours | 91.0 | 90.0 | 94.0 | 91.5 | 76.0 |
| Furfural .33 | 2 hours | 88.0 | 90.0 | 89.0 | 88.0 | 66.0 |
| Furfural .5 | 2 hours | 91.0 | 88.5 | 90.0 | 88.0 | 63.5 |
| Formaldehyde .1 | 2 hours | 88.0 | 91.0 | 92.5 | 92.0 | 75.5 |
| Formaldehyde .33 | 2 hours | 88.5 | 78.0 | 87.0 | 83.0 | 44.5 |
| Formaldehyde .5 | 2 hours | 79.0 | 55.5 | 66.0 | 53.0 | 24.5 |
| Furfural .1 | 6 hours | 89.0 | 92.0 | 89.0 | 86.0 | 71.5 |
| Furfural .33 | 6 hours | 89.5 | 90.5 | 89.5 | 76.5 | 63.0 |
| Furfural .5 | 6 hours | 87.0 | 86.0 | 87.5 | 84.5 | 61.5 |
| Formaldehyde .1 | 6 hours | 87.5 | 92.0 | 91.0 | 83.0 | 66.5 |
| Formaldehyde .33 | 6 hours | 73.0 | 78.0 | 62.5 | 50.0 | 44.5 |
| Formaldehyde .5 | 6 hours | 49.5 | 56.5 | 35.5 | 18.0 | 10.5 |

ments were applied by placing the seed and dust in large manila envelopes and shaking until the dust was evenly distributed over the seed. For the soak treatments the seed was placed in muslin bags and dipped into the sclutions the required length of time. For the sprinkle treatments the seed was placed in a jar, one-tenth its volume (40 gal. to 50 bu.) of solution sprinkled over it, and stirred until evenly distributed. In those treatments demanding covering the seed was placed in small crocks covered with a pane of glass. After treatment the seed was spread out until thoroughly dry and then placed in individual packages for rod row planting. The dust treatments were applied from one to three weeks and the liquid treatments from two to three days before planting.

Disease control was the primary object of these experiments. Consequently each treatment consisted of a single rod row replicated four times.

The effect on germination was determined by planting 200 seeds of each treatment in sand on a greenhouse bench and counting the number germinating after seven days.

The determinations for the percent of bunt in 1924 were made by clipping 100 heads of each row and in 1925 by clipping all the heads in each row. The percent of loose smut of wheat was determined in 1924 by counting the number of heads affected with this disease in 100 heads taken from each row. The percent of covered smut of barley and of the oat smuts was found by counting all the heads in each row and the number of heads attacked by the smut organisms.

Table IX presents the data obtained on the effects of the various treatments on bunt and loose smut of wheat, covered smut of barley, and the smuts of oats.

### TILLETIA LAEVIS ON WHEAT

The low percent of bunt obtained in the check plots of the 1924 spring wheat series renders an interpretation of the results difficult. Two things stand out pre-eminently: first, despite the small amount of bunt in the check plots none of the furfural derivatives satisfactorily controlled the disease; second, the high percent of bunt obtained with all of the furfural liquid treatments was in contrast with low percent in the formaldehyde treatments.

Copper carbonate was the only dust treatment which gave satisfactory control of bunt. The five furfural dust derivatives used in this series gave varying degrees of reduction, but none completely eliminated it. An increase in the quantity of dust did not proportionately reduce bunt as the eight ounce furfuramide treatment had more smutted heads than either the two or four ounce treatments. The difference between the percent of smut in the two, four and eight ounce furfurine treatments was negligible. The eight ounce furoin treatment had only slightly less than the checks. The two ounce furoic and furfuracrylic acid treatments had more smut than the two ounce furfurine treatment and both of these chemicals were somewhat injurious to seed germination.

The furfural liquid treatments of wheat in 1924 yielded a surprisingly great increase in percent of bunt over the untreated check. In the sprinkle treatments there was no apparent correlation between the concentration of the solution used or length of cover and percent of bunt. The 0.75 percent furfural treatment gave the most and the 0.5 percent treatment gave

TABLE IX. A COMPARATIVE STUDY OF SMUT CONTROL ON CEREALS.

| Treatment | Treatment | | | Percent Tilletia triciti | | | Percent Ustilago triciti | Percent Ustilago hordei | Percent Ustilago levis and U. avenae | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Oz. per bu. | Percent | Time (min.) | Spring wheat | | Winter wheat | Spring wheat | Barley | Oats | |
| | | | | 1924 | 1925 | 1925 | 1924 | 1924 | 1924 | 1925 |
| 1. Copper carbonate | 2.00 | | | | 0.00 | 0.00 | | | | 8.4 |
| 2. " " | 3.00 | | | | | | | | | |
| 3. " " | 2.00 | | | 0.00 | | | 5.5 | 0.00 | 0.5 | |
| 4. Furfuramide | 1.00 | | | | 0.1 | 4.3 | | | | |
| 5. " | 2.00 | | | 1.5 | | 5.2 | 6.0 | 0.21 | 1.75 | |
| 6. " | 2.66 | | | | 0.1 | | | | | 14.0 |
| 7. " | 4.00 | | | 0.5 | | 6.8 | 6.0 | 0.16 | 1.25 | |
| 8. " | 8.00 | | | 2.0 | | 6.2 | 6.3 | 0.00 | 1.25 | |
| 9. Furfurine | 1.00 | | | | | 4.8 | | | | |
| 10. " | 2.00 | | | 1.0 | | 5.8 | 6.3 | 0.42 | 2.75 | |
| 11. " | 4.00 | | | 0.8 | | 2.0 | 6.5 | 0.21 | 1.25 | |
| 12. " | 8.00 | | | 0.8 | | | 6.5 | 0.16 | 0.5 | |
| 13. Furoin | 8.00 | | | 3.0 | | | 7.8 | 0.21 | 1.0 | |
| 14. Furoic acid | 2.00 | | | 1.3 | | | 7.0 | 0.21 | 1.0 | |
| 15. Furfuracrylic acid | 2.00 | | | 2.0 | | | 5.5 | 0.30 | 1.0 | |
| 16. Mercuric chloride | 2.00 | | | | 0.0 | | | | | 3.3 |
| 17. " " | 5.33 | | | | 0.0 | | | | | 3.2 |
| 18. No treatment | | | | 1.8 | 2.97 | 3.9 | 5.3 | 0.75 | 3.0 | 13.6 |
| 19. Copper stearate | 1.00 | | | | | 1.8 | | | | 11.6 |
| 20. " " | 2.00 | | | | | | | | | 10.4 |
| 21. Mercuric furoate | 2.00 | | | | 0.0 | | | | | 15.4 |
| 22. Ferric furoate | 2.00 | | | | 0.0 | | | | | 16.1 |
| 23. Calcium furoate | 2.00 | | | | 0.2 | | | | | 4.2 |
| 24. Nickle furoate | 2.00 | | | | 0.0 | | | | | 9.1 |
| 25. Cupric furoate | 2.00 | | | | 0.0 | | | | | 10.2 |
| 26. Mercuric furoate + calcium carbonate (1:4) | 4.00 | | | | 0.0 | | | | | 14.1 |
| 27. Ferric furoate + calcium carbonate (1:4) | 4.00 | | | | 0.0 | | | | | 14.2 |
| 28. Calcium furoate + calcium carbonate (1:4) | 4.00 | | | | 0.2 | | | | | 11.1 |
| 29. Nickle furoate + calcium carbonate (1:4) | 4.00 | | | | 0.0 | | | | | |

TABLE IX—(Continued).

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 30. | Cupric furoate + calcium carbonate (1:4) | 4.00 | | | | 0.0 | | | | | 7.6 |
| 31. | Calcium carbonate | 4.00 | | | | 0.0 | | | | | 12.8 |
| 32. | No treatment | | | | | | | | | | 13.1 |
| 33. | Water sprinkle | | | | | 6.9 | | | | | |
| 34. | Furfural sprinkle | | 2.00 | 0 | 16.5 | 2.0 | | 5.0 | 1.47 | 2.0 | |
| 35. | " " | | 1.00 | 0 | 16.5 | 5.6 | | 5.5 | 1.55 | 3.25 | |
| 36. | " " | | 0.75 | 0 | 32.0 | 7.1 | | 6.0 | 1.08 | 1.50 | |
| 37. | " " | | 0.50 | 0 | 12.3 | 8.8 | | 5.3 | 0.42 | 1.25 | |
| 38. | " " | | 0.33 | 0 | 23.5 | 6.7 | | 6.0 | 0.40 | 2.25 | |
| 39. | Water sprinkle | | | 3 | | 7.9 | 14.5 | | | | 18.0 |
| 40. | Furfural sprinkle | | 2.00 | 3 | 34.2 | 5.2 | 2.5 | 4.5 | 1.45 | 2.5 | 6.0 |
| 41. | " " | | 1.00 | 3 | 21.3 | 6.4 | 6.0 | 7.0 | 1.06 | 2.25 | 11.8 |
| 42. | " " | | 0.75 | 3 | 35.8 | 4.6 | | 7.3 | 0.85 | 2.25 | |
| 43. | " " | | 0.50 | 3 | 14.8 | 3.9 | | 4.5 | 0.57 | 1.00 | |
| 44. | " " | | 0.33 | 3 | 18.5 | 1.9 | 3.7 | 5.8 | 0.57 | 1.25 | 12.9 |
| 45. | Formaldehyde | | 0.03 | 3 | 0.0 | 0.0 | 0.0 | 7.3 | 0.00 | 0.00 | 0.05 |
| 46. | Water sprinkle | | | 12 | | 4.6 | | | | | |
| 47. | Furfural sprinkle | | 2.00 | 12 | 20.0 | 4.1 | | 5.0 | 1.55 | 1.00 | |
| 48. | " " | | 1.00 | 12 | 23.8 | 10.2 | | 4.8 | 1.03 | 1.00 | |
| 49. | " " | | 0.75 | 12 | 32.8 | 5.7 | | 5.0 | 0.53 | 2.25 | |
| 50. | " " | | 0.50 | 12 | 18.8 | 0.8 | | 5.8 | 0.68 | 1.00 | |
| 51. | " " | | 0.33 | 12 | 32.3 | 2.0 | | 7.8 | 0.82 | 0.75 | |
| 52. | No treatment | | | | 6.0 | 1.3 | | 7.3 | 0.63 | 2.50 | |
| 53. | Furfural soak | | 0.50 | 2 | 11.8 | | | 4.5 | 0.97 | 2.50 | |
| 54. | " " | | 0.10 | 2 | 6.3 | | | 5.0 | 0.42 | 1.75 | |
| 55. | " " | | 0.05 | 2 | 11.5 | | | 6.3 | 0.68 | 1.00 | |
| 56. | Formaldehyde soak | | 0.50 | 2 | 0.0 | | | 6.0 | 0.00 | 0.00 | |
| 57. | " " | | 0.10 | 2 | 0.0 | | | 6.3 | 0.54 | 0.00 | |
| 58. | " " | | 0.05 | 2 | 0.8 | | | 8.5 | 0.48 | 1.00 | |
| 59. | Water soak | | | 6 | | | 4.0 | | | | |
| 60. | Furfural soak | | 0.50 | 6 | 10.0 | | 0.5 | 5.5 | 1.11 | 1.00 | |
| 61. | " " | | 0.10 | 6 | 5.3 | | 7.3 | 6.8 | 0.86 | 2.75 | |
| 62. | " " | | 0.05 | 6 | 5.8 | | 10.0 | 6.8 | 1.16 | 1.50 | |
| 63. | Formaldehyde soak | | 0.50 | 6 | 0.0 | | | 5.5 | 0.00 | 0.00 | |
| 64. | " " | | 0.10 | 6 | 0.0 | | | 4.5 | 0.14 | 0.00 | |
| 65. | " " | | 0.05 | 6 | 0.0 | | | 7.5 | 0.54 | 0.00 | |
| 66. | Formaldehyde spray, 1 qt. to 50 bu. | | | 5 | | 0.0 | | | | | 0.00 |
| 67. | Furfural spray, 1 qt. to 50 bu. | | | 5 | | 0.2 | | | | | 16.2 |

the least smut, 33.5 percent and 15.3 percent, respectively. The strongest concentration averaged 23.6 percent bunt, while the weakest averaged 24.8 percent. Although the five furfural treatments, dried immediately after treatment, averaged 20.1 percent bunt, while those covered for three and twelve hours averaged 24.9 and 25.5 percent, respectively, these differences are not significant as the individual variations were large.

The formaldehyde 1-320 or 0.03 percent treatment ordinarily recommended gave complete control of bunt.

Seed soaked in furfural solutions did not show as great an increase in bunt as the sprinkle treatments. There was a marked increase, however, which, as with the sprinkle treatments, possessed little correlation with either strength of treatment or duration of soak. In all cases the two hour soak treatments had more bunt than the corresponding six hour treatments, but in two of the three instances this difference was negligible. In the two hour soak the strongest (0.5 percent) and the weakest (0.05 percent) furfural solutions gave approximately the same amount of smut, and in the six hour soak the greatest amount of smut was present in the 0.5 percent solution.

The increase in percent of bunt produced by the furfural solutions was difficult to explain. No evidence of stimulation by furfural had ever been observed in the numerous spore germination tests conducted in the laboratory using *Tilletia laevis*. However, no water treatments had been included in the spring wheat series of 1924. In all subsequent tests these were incorporated.

The check untreated plots of the 1925 spring wheat series produced even less bunt than did the 1924 series. Consequently all the positive control results were insignificant. The dust treatments which did not give perfect control may be regarded as unsatisfactory and include the one ounce furfuramide, the two ounce calcium furoate, and the four ounce calcium furoate plus lime treatments.

The sprinkle treatments gave a marked increase in percent of bunt over the checks as in the previous year. Here again there was no correlation between concentration of solution or length of cover and percent of smut. However, the water sprinkle checks showed this same increase. The average percent of smut in the 15 furfural treatments was 5.0, while in the three water treatments it was 6.4. In consideration of the great variation of bunt in the furfural treatments, from 0.8 to 10.2 percent, this difference is insignificant.

The 1924-25 winter wheat series differed in some respects from the spring wheat series. The standard treatments, formaldehyde (1-320) sprinkle, and the two ounce copper carbonate, were the only ones which gave satisfactory control. The furfural derivatives, furfuramide and furfurine, did not reduce smut as in the two spring wheat series. Except in one instance, seed treated with these substances had slightly more smut than the untreated. The furfural sprinkle treatments did not increase the percent of smut as in the spring wheat treatments. The three furfural sprinkle treatments averaged 4.0 percent smut while the check untreated had 3.9 percent. The water sprinkle check had 14.5 percent bunt, which was higher than any other treatment. In contrast with the irregular results obtained in the furfural soak treatments with spring wheat was the steady reduction in smut obtained with an increase in concentration of the

solution in the winter wheat. The 0.5 percent furfural soak for six hours, which quite severely injured the stand, gave only 0.5 percent smut, while the 0.1 percent furfural gave 7.3 percent smut and the 0.05 percent furfural gave 10.0 percent smut. The water soak treatment had only 4.0 percent smut.

### USTILAGO TRITICI ON WHEAT

The spring wheat of the 1924 series contained a considerable quantity of loose smut. Since the collection of data on the effect of the various treatments on loose smut required little additional labor, notes were taken on it. As was probably to be expected, treatments with furfural and formaldehyde, although strong enough to severely injure the seed, produced no appreciable effect on the percent of loose smut.

### USTILAGO HORDEI ON BARLEY

The effect of furfural on covered smut of barley was tried only in 1924 because of the difficulty in getting seed which would produce a heavily smutted crop. In 1924 the untreated checks averaged 0.69 percent smut. Because of this low percent in the checks, too great significance should not be attached to the results obtained. All of the dust treatments reduced the percent of smut and two of them, the eight ounce copper carbonate and furfuramide treatments, completely eliminated it. In the furfural sprinkle treatments there was a consistent increase in percent of smut from 0.6 percent in the 0.33 percent treatment to 1.5 percent in the 2.0 percent treatment. In the furfural soak treatments no consistency existed between concentration and percent of smut. The formaldehyde sprinkle and both the 0.5 percent formaldehyde soak treatments gave complete control of covered smut of barley.

### USTILAGO AVENAE AND USTILAGO LAEVIS ON OATS

The effect of furfural seed treatments on oat smut was tried in 1924 and in 1925. There was a low percentage of smut in the check plots which made the first year's trials less conclusive. These trials showed, however, that furfural dust derivatives and the furfural sprinkle and soak treatments would not control oat smut. The dust derivatives, while in all cases reducing the percent of smut, in no case gave satisfactory control. The furfural sprinkle and soak treatments gave no evidence of any effect on the amount of smut. All of the formaldehyde soak treatments completely controlled oat smut except the 0.05 percent for two hours.

The 1925 untreated plots, oat smut series, averaged 13.3 percent smut. Those furfural dust derivatives which were tried in the 1924 series and gave no evidence of control as well as the furfural soak treatments were not repeated. Some sprinkle treatments were repeated in order to determine if conditions influencing the increase of bunt in wheat were not also common to oats. A series of metallic furoate salts were tested. Also, an attempt was made to verify the results obtained by Thomas (11) with mercuric chloride dust.

Five sprinkle treatments were used. Formaldehyde (1-320) gave almost complete control of oat smut. The water sprinkle treatment had 18 percent smut, the highest in the whole series. The two percent furfural sprinkle treatment with three hour cover reduced smut to six percent, while the

one and 0.33 percent treatments had nearly the same amount as the untreated checks.

The metallic furoate salts differed greatly in their efficiency. Nickle furoate was the most toxic and cupric furoate a close second. Mercuric furoate is quite insoluble in water and probably for this reason was not as toxic as the nickle and cupric salts. Calcium and ferric furoates and calcium carbonate did not reduce the amount of oat smut. Three ounces of copper carbonate reduced oat smut, but not significantly, while two ounces of copper stearate was even less efficient.

A series of treatments with mercuric chloride as the base was used. Finely ground mercuric chloride was tested alone at the rate of two and 5.33 ounce quantities. A three ounce treatment of a mixture consisting of two parts mercuric chloride and one part copper carbonate as recommended by Thomas (11) was also used, as was one consisting of two parts mercuric chloride and one part Sil O'Cil[1] Also a mixture of one part furfuramide and two parts mercuric chloride was used in varying quantities. All of the mercuric chloride treatments gave a considerable reduction in the amount of smut, but none controlled it as did the formaldehyde treatments. The two and 5.66 ounce mercuric chloride treatments and the three ounce mixtures of mercuric chloride and copper carbonate and of mercuric chloride and Sil O'Cil gave almost the same degree of smut control, ranging from 2.9 percent to 3.3 percent. A mixture of furfuramide and mercuric chloride react to form a resin. This apparently interfered to some extent with the toxic properties as the percent of smut was higher in all cases than where the mercuric chloride alone was used. There was not a proportionate decrease for an increase in quantity of material used as the nine ounce treatment was little better than the three ounce treatment.

### THE TOXIC ACTION OF SEED DISINFECTANTS

Table X presents the results obtained in germination tests following the treatment of the seed planted in 1924 and 1925.

The dust treatments with the exception of furfuracrylic acid and those in which mercuric chloride was an ingredient were not injurious to germination. In the 1924 spring wheat series where the seed germinated poorly the dust treatments apparently were beneficial. The untreated seed averaged 71.0 percent germination, while the nine dust treatments averaged 84.9 percent. The germination of the oats and barley in 1924 and of spring wheat in 1925 was comparatively good so that any beneficial results of treatment were not apparent.

All of the mercuric chloride treatments were injurious to germination. Injury was more severe to wheat than to oats. While the mercuric chloride treatments, in most cases, caused reduction in germination, injury was much more apparent through a retardation in germination and a decrease in vigor. Plants from seed treated with mercuric chloride would emerge two to three days late and would be distorted. This retardation and distortion was proportional to the amount of mercuric chloride used. Mixing the mercuric chloride with a filler as copper carbonate, Sil O'Cil or furfuramide served to ameliorate this injurious action to some extent.

The furfural and formaldehyde liquid treatments had no stimulatory effect on germination. The weaker treatments had apparently no effect,

[1] Sil O'Cil is an inert, light weight silicious substance, a diatomaceous earth.

while the stronger ones injured germination in all cases. Injury was proportional to the concentration of the solution, the duration of soak, and the duration of cover. Formaldehyde was more injurious to germination than an equal concentration of furfural used under similar conditions. Formaldehyde was more harmful to wheat than to oats or barley and was more harmful to oats than to barley. Wheat and oats were about equally susceptible to furfural injury, while barley was more resistant.

#### The Control of Black Scurf, Rhizoctonia solani, of Potatoes in 1924 and 1925

The effectiveness of furfural solutions for the control of the black scurf disease of potatoes was tested in the field. In 1924, two early varieties, Early Ohio and Irish Cobbler, and one late variety, Rural New Yorker, were used. In 1925 only one variety, Early Ohio, was tested. The land on which the potatoes were grown in 1924 was a sandy loam, low in organic matter, and had grown three successive crops of potatoes. The land used in 1925 was a sandy loam, well supplied with organic matter, and had not grown potatoes for at least ten years.

The treatments used in the field were based on the information obtained from the laboratory experiments. In 1924 four check treatments were used: (1) clean seed treated with 1-120 formaldehyde for two minutes at 50° C.; (2) clean seed untreated; (3) infected seed treated with 1-120 formaldehyde for two minutes at 50° C.; (4) infected seed untreated. Clean seed means merely tubers that proved to be free from sclrotia on careful examination after washing. It is not impossible that small sclerotia were sometimes overlooked, but every effort was made to select only tubers free from sclerotia. The same treatment that was used for the formaldehyde checks, two minutes at 50° C., was used for the furfural treatments. Furfural solutions of 1-200, 1-100 and 1-50 and 1-33 with a one and five hour cover for each concentration were used in 1924. Covering consisted in placing burlap sacks, which had been wet with the solution used in treating potatoes, around the treated potatoes for the required time. A furfuramide dust treatment was also used in the first year's tests. The treatments in the 1925 tests differed from those of the preceding year in that another check treatment was included, infected seed treated with 1-1000 mercuric chloride for two minutes at 50° C., and in that the 1-200 furfural and the furfuramide treatments were omitted.

Field plot technique as outlined by Melhus, Gilman and Kendrick (5) was followed in these tests. Four replications of each treatment were used for each variety tested. Each replication consisted of a single row 200 feet long and in which were planted 200 seed pieces. Data were taken on total yield and percent of tubers infected with black scurf. Each row was harvested separately by hand. The tubers were then placed on a screen and washed and carefully sorted into two lots; Rhizoctonia infected, and apparently clean.

The data of the 1924 potato seed treatments are given in Table XI. Student (9) method was used for determining significance of results. As there appeared to be little difference in varietal response to the different treatments, the three varieties were merged and 12 replications used in computing the results. Odds of more than 30:1 were regarded as significant. The only treatments significantly better than the infected formaldehyde check were the two for which clean seed was used. The only

TABLE X. TOXIC ACTION OF SEED DISINFECTANTS ON SEED VIABILITY IN TERMS OF PERCENT OF GERMINATION.

| Treatment | Treatment | | | Tilletia tritici | | | Ustilago levis and U. avenae | | Ustilago hordei |
|---|---|---|---|---|---|---|---|---|---|
| | Oz. per bu. | Percent | Time cover hrs. | Spring wheat | | Winter wheat | Oats | | Barley |
| | | | | 1924 | 1925 | 1924 1925 | 1924 | 1925 | 1924 |
| 1. Copper carbonate | 2.00 | | | | 95.0 | 69.0 | | | |
| 2. " " | 3.00 | | | | | | | 86.0 | |
| 3. " " | 8.00 | | | 84.0 | | | 92.0 | | 93.5 |
| 4. Furfuramide | 1.00 | | | | | | | | |
| 5. " | 2.00 | | | 88.0 | | | 85.5 | | 96.0 |
| 6. " | 2.66 | | | | 96.5 | | | 91.0 | |
| 7. " | 4.00 | | | 83.5 | | 75.0 | 99.0 | | 95.0 |
| 8. " | 8.00 | | | 84.5 | | | 95.5 | | 94.0 |
| 9. Furfurine | 1.00 | | | | | | | | |
| 10. " | 2.00 | | | 84.5 | | 63.0 | 91.5 | | 94.5 |
| 11. " | 4.00 | | | 88.0 | | 71.0 | 95.5 | | |
| 12. " | 8.00 | | | 84.0 | | 76.0 | 91.5 | | 95.0 |
| 13. Furoin | 8.00 | | | 83.5 | | | 97.5 | | 91.5 |
| 14. Furoic acid | 2.00 | | | 84.5 | | | 88.0 | | 94.0 |
| 15. Furfuracrylic acid | 2.00 | | | 72.5 | | | 88.5 | | 69.0 |
| 16. Mercuric chloride | 2.00 | | | | 64.5 | | | 89.0 | |
| 17. " " | 5.33 | | | | 14.5 | | | 83.5 | |
| 18. Mercuric chloride and copper carbonate (2:1) | 3.00 | | | | | | | 81.0 | |
| 19. Mercuric chloride and Sil o'cil (2:1) | 3.00 | | | | | | | 88.5 | |
| 20. Mercuric chloride and Furfuramide (2:1) | 3.00 | | | | 89.0 | | | 93.5 | |
| 21. Mercuric chloride and Furfuramide (2:1) | 6.00 | | | | | | | 88.5 | |
| 22. Mercuric chloride and Furfuramide (2:1) | 8.00 | | | | 71.5 | | | | |
| 23. Mercuric chloride and Furfuramide (2:1) | 9.00 | | | | | | | 77.0 | |
| 24. No treatment | | | | 65.5 | 94.5 | 63.0 | 97.0 | 90.5 | 97.5 |
| 25. Copper stearate | 1.00 | | | | 92.0 | 71.0 | | | |

TABLE X—(Continued).

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 26. | Copper stearate | 2.00 | | | | 89.5 | 77.0 | | | |
| 27. | Mercuric furoate | 2.00 | | | | 93.0 | 87.5 | | 93.0 | |
| 28. | Ferric furoate | 2.00 | | | | 91.5 | 74.0 | | 89.5 | |
| 29. | Calcium furoate | 2.00 | | | | 91.0 | 87.5 | | 91.0 | |
| 30. | Nickle furoate | 2.00 | | | | 91.0 | 87.0 | | 87.0 | |
| 31. | Cupric furoate | 2.00 | | | | 91.5 | 86.5 | | 86.5 | |
| 32. | Mercuric furoate and calcium carbonate (1:4) | 4.00 | | | | 94.5 | 78.0 | | 85.5 | |
| 33. | Ferric furoate and calcium carbonate (1:4) | 4.00 | | | | 96.0 | 80.5 | | 90.0 | |
| 34. | Calcium furoate and calcium carbonate (1:4) | 4.00 | | | | 91.0 | 80.0 | | 85.0 | |
| 35. | Nickle furoate and calcium carbonate (1:4) | 4.00 | | | | 88.5 | 78.0 | | 90.0 | |
| 36. | Cupric furoate and calcium carbonate (1:4) | 4.00 | | | | 91.5 | 80.0 | | | |
| 37. | Calcium carbonate | 4.00 | | | | 91.5 | 76.0 | | 89.5 | |
| 38. | No treatment | | | | | | | | | |
| 39. | Water sprinkle | | | 0 | | 92.5 | | | | |
| 40. | Furfural sprinkle | | 2.0 | 0 | 64.5 | 88.5 | | 85.5 | | |
| 41. | " " | | 1.0 | 0 | 73.0 | 90.5 | | 92.5 | | 79.0 |
| 42. | " " | | 0.75 | 0 | 75.5 | 91.0 | | 94.0 | | 91.5 |
| 43. | " " | | 0.50 | 0 | 77.5 | 93.5 | | 98.0 | | 94.5 |
| 44. | " " | | 0.33 | 0 | 72.5 | 90.5 | | 96.0 | | 94.5 |
| 45. | Water sprinkle | | | 3 | | 92.0 | | | 90.5 | 89.0 |
| 46. | Furfural sprinkle | | 2.00 | 3 | 66.0 | 81.5 | | 73.0 | 76.5 | |
| 47. | " " | | 1.00 | 3 | 74.5 | 85.0 | | 94.0 | 83.5 | 80.0 |
| 48. | " " | | 0.75 | 3 | 74.5 | 89.0 | | 91.0 | | 91.5 |
| 49. | " " | | 0.50 | 3 | 76.5 | 92.0 | | 93.0 | | 94.5 |
| 50. | " " | | 0.33 | 3 | 69.5 | 87.0 | | 95.0 | 88.0 | 94.5 |
| 51. | Formaldehyde | | 0.03 | 3 | 55.0 | 85.0 | | 90.0 | 83.5 | 92.0 |
| 52. | Water sprinkle | | | 12 | | 94.0 | | | | 93.5 |
| 53. | Furfural sprinkle | | 2.00 | 12 | 37.5 | 32.5 | | 28.0 | | |
| 54. | " " | | 1.00 | 12 | 54.0 | 82.0 | | 86.0 | | 60.0 |
| 55. | " " | | 0.75 | 12 | 70.0 | 86.5 | | 84.0 | | 88.5 |
| 56. | " " | | 0.50 | 12 | 68.0 | 88.0 | | 87.5 | | 88.5 |
| 57. | " " | | 0.33 | 12 | 74.5 | 94.0 | | 85.5 | | 90.5 |
| 58. | No treatment | | | | 76.5 | 93.5 | | 94.5 | 92.5 | 92.0 |
| 59. | Furfural soak | | 0.50 | 2 | 58.0 | | 82.5 | 82.5 | | 89.5 |

TABLE X—(Continued).

| Treatment | Treatment | | | Tilletia tritici | | | Ustilago levis and U. avenae | | Ustilago hordei |
|---|---|---|---|---|---|---|---|---|---|
| | Oz. per bu. | Percent | Time cover hrs. | Spring wheat | | Winter wheat | Oats | | Barley |
| | | | | 1924 | 1925 | 1924 1925 | 1924 | 1925 | 1924 |
| 60. " " | | 0.10 | 2 | 63.5 | | 80.5 | 94.0 | | 88.0 |
| 61. " " | | 0.05 | 2 | 67.0 | | 81.0 | 95.5 | | 87.0 |
| 62. Formaldehyde soak | | 0.50 | 2 | 30.5 | | | 79.5 | | 90.5 |
| 63. " " | | 0.10 | 2 | 66.5 | | 82.0 | 93.0 | | 88.0 |
| 64. Formaldehyde dip | | 0.05 | 2 | 63.0 | | 86.0 | | 96.5 | 87.5 |
| 65. Water dip | | | 6 | | | | | | 92.0 |
| 66. Furfural dip | | 0.50 | 6 | 43.0 | | 75.0 | | 59.0 | |
| 67. " " | | 0.10 | 6 | 64.5 | | 84.5 | | 88.5 | 94.5 |
| 68. " " | | 0.05 | 6 | 58.0 | | 87.5 | | 97.0 | 95.0 |
| 69. Formaldehyde dip | | 0.50 | 6 | 20.5 | | | | 53.5 | 95.5 |
| 70. " " | | 0.10 | 6 | 53.5 | | 83.5 | | 86.5 | 84.5 |
| 71. " " | | 0.05 | 6 | 55.0 | | 74.0 | | 94.0 | 95.5 |
| 72. Formaldehyde spray, 1 qt. 50 bu. | | 50.0 | 5 | | 90.5 | | | 94.0 | 98.0 |
| 73. Formaldehyde spray, 1 qt. to 50 bu. | | 95.0 | 5 | | 90.0 | | | 94.5 | |

TABLE XI. EFFECT OF THE 1924 POTATO SEED TREATMENTS ON PREVALENCE OF RHIZOCTONIA AND THE SIGNIFICANCE OF THE VARIOUS TREATMENTS.

| Condition of Seed | Fungicide | Dilution | Temperature degree | Time | | Ave. Pct. infected in 12 replications | Mean variation from infected formaldehyde treated check | Standard duration | Factor Z | Odds in favor of infected formaldehyde treated check |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Dipped minutes | Covered hours | | | | | |
| Infected | None | | | | | 61.4 | 22.8 | 23.02 | 0.99 | 269:1 |
| " | Formaldehyde | 1-120 | 50 | 2 | 3 | 37.8 | | | | |
| " | Furfural | 1-33.3 | 50 | 2 | 1 | 37.9 | 0.1 | 20.7 | 0.005 | 1:1 |
| " | " | 1-33.3 | 50 | 2 | 5 | 35.5 | —2.3 | 22.3 | 0.10 | 1:1.7 |
| " | " | 1-50 | 50 | 2 | 1 | 44.4 | 6.6 | 24.4 | 0.27 | 4.1:1 |
| " | " | 1-50 | 50 | 2 | 5 | 35.2 | —2.6 | 22.4 | 0.11 | 1.8:1 |
| " | " | 1-100 | 50 | 2 | 1 | 39.1 | 1.3 | 23.5 | 0.05 | 1:1 |
| " | " | 1-100 | 50 | 2 | 5 | 44.2 | 6.4 | 21.9 | 0.29 | 4.5:1 |
| " | " | 1-200 | 50 | 2 | 1 | 48.8 | 11.0 | 27.0 | 0.41 | 8.8:1 |
| " | " | 1-200 | 50 | 2 | 5 | 52.0 | 14.2 | 23.2 | 0.61 | 28.1:1 |
| " | Furfuramide dust | | | | | 59.6 | 21.8 | 16.0 | 1.36 | 2380:1 |
| Apparently clean | None | | | | | 25.4 | 12.4 | 17.0 | 0.73 | 1:56 |
| Apparently clean | Formaldehyde | 1-120 | 50 | 2 | 3 | 21.9 | 15.9 | 18.0 | 0.88 | 1:138 |

TABLE XII. EFFECT OF THE 1925 POTATO SEED TREATMENTS ON PREVALENCE OF RHIZOCTONIA AND THE SIGNIFICANCE OF THE VARIOUS TREATMENTS.

| Condition of seed | Fungicide | Dilution | Temperature degree cent. | Time | | Ave. Pct. infected in 4 replications | Mean variation from infected formaldehyde treated check | Standard deviation | Factor Z | Odds in favor of infected formaldehyde treated check |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Dipped minutes | Covered hours | | | | | |
| Infected | None | | | | | 49.0 | 37.4 | 24.5 | 1.53 | 24.9:1 |
| " | Formaldehyde | 1-120 | 50 | 2 | 3 | 11.6 | | | | |
| " | Mercuric chloride | 1-1000 | 50 | 2 | 5 | 29.1 | 17.5 | 7.95 | 2.20 | 61.9:1 |
| " | Furfural | 1-33.3 | 50 | 2 | 1 | 24.3 | 12.7 | 4.69 | 2.71 | 108.9:1 |
| " | " | 1-33.3 | 50 | 2 | 5 | 26.9 | 15.3 | 6.28 | 2.44 | 80.9:1 |
| " | " | 1-50 | 50 | 2 | 1 | 31.1 | 19.5 | 5.0 | 3.9 | 302:0 |
| " | " | 1-50 | 50 | 2 | 5 | 28.9 | 17.3 | 8.33 | 2.09 | 54.3:1 |
| " | " | 1-100 | 50 | 2 | 1 | 43.3 | 31.7 | 6.91 | 4.58 | 543:1 |
| " | " | 1-100 | 50 | 2 | 5 | 36.2 | 24.6 | 1.29 | 19.0 | Infinite |
| Clean | None | | | | | 17.4 | 5.8 | 12.28 | 0.47 | 3.9:1 |
| Clean | Formaldehyde | 1-120 | 50 | 2 | 3 | 7.2 | —4.4 | 6.67 | 0.66 | 1:4.9 |

The significance is computed in the "Students Method" pairing each treatment with infected formaldehyde 1-120 for two minutes at 50° to 52° C., followed by covering for three hours.

treatments significantly inferior to the infected formaldehyde check were the infected untreated and infected treated with furfuramide dust. The 1-200 furfural treatments, one and five hour cover, were markedly inferior to the formaldehyde check. The other furfural treatments controlled potato Rhizoctonia practically as well as did formaldehyde. The most effective treatment, where infected seed was used, was the 1-50 furfural, two minutes at 50° to 52° C. with a five hour cover which had 35.2 percent Rhizoctonia, 2.7 percent less than the formaldehyde treatment.

The results of the field trials in 1925 for the effect of seed treatment on the prevalence of Rhizoctonia of potatoes are given in Table XII. The results differ greatly from those of the preceding year in that the formaldehyde treatments were comparatively much more effective. The formaldehyde treatment of infected seed reduced the amount of Rhizoctonia 76 percent over the infected not treated, whereas in 1924 it caused a reduction of 38 percent. In 1925, 5.8 percent less Rhizoctonia developed from the formaldehyde treatment of infected seed than from the clean untreated check and only 4.4 percent more than from the clean seed treated with formaldehyde. In 1924 both of these treatments were significantly superior to the infected formaldehyde check treatment. The reduction in the amount of Rhizoctonia produced by the 1-33 and 1-50 furfural treatments was approximately the same for the two years. In 1924 these treatments caused reductions of 28 to 43 percent and in 1925 of 37 to 50 percent over the infected no treatment. Apparently the greater control in the 1925 treatments was due to a difference in the amount of soil infection. The land used in 1924 had been in potatoes for three successive years, while that used in 1925 had grown no crop of potatoes for at least ten years.

A statistical examination of the 1925 results using the Student (9) method of computing probable error, shows that the hot formaldehyde treatment is significantly better than any of the furfural or the hot mercuric chloride treatments. The small odds in favor of the formaldehyde treatment in comparison with the untreated infected check are probably due to the limited number of replications (four) and to the great variation in the percent of Rhizoctonia in the individual rows (22.5 to 75.4 percent of the infected no treatment check rows.) The differences between the infected formaldehyde treatment and the clean untreated and clean formaldehyde treatments were not significant. These trials coming through two seasons, although somewhat variable, indicate clearly that furfural and formaldehyde in equal concentrations are not equally effective for potato seed disinfection. Raeder et al (8) have also suggested that furfural under Idaho conditions is less effective than formaldehyde in equal concentrations. It required in their trials a 1-60 furfural solution for ten minutes at 50° C. to compare favorably with 1-120 formaldehyde for five minutes at 55° C. Higher concentrations of furfural as 1-33 or 1-50, should they prove as effective as formaldehyde, nevertheless introduce the item of cost, which at present is in favor of formaldehyde.

## *SUMMARY*

1. Toxicity studies were made using the following organisms: *Puccinia coronata holci, Ustilago hordei,* and *Sphacelotheca sorghi,* and dilute solutions of six aldehydes as follows: formaldehyde, butylaldehyde, benzaldehyde, furfuraldehyde, propylaldehyde and acetaldehyde. The aldehydes differed in their toxic action on *Puccinia coronata holci, Ustilago hordei* and *Sphacelotheca sorghi.* Benzaldehyde, formaldehyde and butylaldehyde appeared to be most toxic to the organisms studied, followed by furfuraldehyde, propylaldehyde and acetaldehyde.

2. Apparently the furan nucleus was a minor factor in the toxicity of furfural. Metallic furoate salts were markedly toxic and their toxicity was in accord with the toxicity of the metal carried. Furfuralcohol did not display the toxicity exhibited by the aldehyde, suggesting that the furyl radical is not the toxic portion of the aldehyde or alcohol.

3. Raising the temperature of the solution accelerated the toxicity of furfural to the sclerotia of *Rhizoctonia solani.* Laboratory tests indicated that covering increased the effectiveness of the furfural treatments.

4. The toxic effect of furfural and formaldehyde on germinating wheat seed is different. Injury with furfural vapor in the presence of water was greatly increased, while with formaldehyde vapor the reverse was true.

5. Seeds treated with formaldehyde solutions, dried and stored for various lengths of time before planting showed cumulative injury, while those treated with furfural did not.

6. Soaking the seed with water or furfural solutions ranging from 0.05 to 0.5 percent or sprinkling with water or furfural solutions ranging from 0.33 to 2.0 percent greatly increased the amount of bunt in wheat. Similar treatments on oats and barley had only slight effects on the percent of smut. Soaking or sprinkling with formaldehyde solutions either reduced or eliminated these smuts.

7. Dust derivatives of furfural, while in most cases reducing the percent of smut, did not give evidence of possessing sufficient toxicity to control these diseases.

8. Mercuric chloride dust or a mixture of two parts mercuric chloride dust to one part copper carbonate, Sil O'Cil, or furfuramide reduced oat smut considerably, but did not entirely eliminate it. Two ounces of mercuric chloride dust gave as good control as 5.66 ounces. Two ounces of mercuric chloride plus one ounce of copper carbonate was no better than two ounces of mercuric chloride alone. Two ounces of mercuric chloride plus one ounce of Sil O'Cil, as a spreader, gave fully as good results as the copper carbonate mixture. The addition of furfuramide to mercuric chloride apparently reduces the efficiency of the latter.

9. Mercuric chloride dust proved injurious to wheat and oat germinations in all cases.

10. Dust derivatives of furfural and copper carbonate were beneficial to germination of wheat when seed of poor quality was used.

11. When used under similar conditions, formaldehyde was more injurious to seed germination than equal concentrations of furfural.

12. Treatment of seed potatoes infected with Rhizoctonia with solutions of 1-100, 1-50 and 1-33 furfural for two minutes at 50° C. controlled Rhizoctonia as well as the 1-120 hot formaldehyde treatment in 1924. The 1-200 fufural and the furfuramide dust treatments were unsatisfactory. In the 1925 field trials the 1-120 hot formaldehyde treatment controlled Rhizoctonia better than did any of the other treatments. The 1-33 and 1-50 furfural and the 1-1000 mercuric chloride treatments for two minutes at 50° C. although materially reducing the amount of infection were significantly inferior to the formaldehyde treatment.

13. The length of cover after treatment had little effect on the amount of Rhizoctonia, which developed in the field experiments in either 1924 and 1925.

## LITERATURE CITED

1. CLARK, J. F.
1899. On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. 28: 289-327, 378-404.

2. DOBEREINER, J. W.
1832. Ueber die medizinische und chemische Anwendung und die Vortielhafte der Ameisensaure. Liebigs Ann. Chem. 3: 141-157.

3. LAFORGE, F. B.
1923. Furfural from corncobs. Jour. Indus. Engin. Chem. 15: 499-502.

4. HURD, ANNIE MAY
1920. Injury to seed wheat resulting from drying after disinfection with formaldehyde. Jour. Agr. Res. 20: 209-244.

5. MELHUS, I. E., GILMAN, J. C., AND KENDRICK, J. B.
1920. Fungicidal action of formaldehyde. Iowa Agr. Exp. Sta. Res. Bul. 50: 355-397.

6. MINER, CARL S., TRICKEY, JOHN P., AND BROWNLEE, HAROLD J.
1922. Commercial furfural—its properties and uses. I. Metall. Chem. Eng. 27: 299-303.

7. MONROE, K. P.
1921. Preparation and technical uses of furfural. Jour. Indus. Engin. Chem. 13: 133-135.

8. RAEDER, J .M., HUNGERFORD, C. W., AND CHAPMAN, NAOMI.
1925. Seed treatment control of Rhizoctonia. Idaho Agr. Exp. Sta. Res. Bul. 4: 137.

9. STUDENT.
1908. The probable error of a mean. Biometrica. 6: 1-25.

10. TAYLOR, K.
1917. Specificity in antiseptics. Lancet. 192: 294-297.

11. THOMAS, ROY C.
1925. Effective dust treatments for the control of smuts of oats. Science n. s. 61: 47-48.

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