RELATIONSHIPS OF THE ENCAPSULATED BACILLI WITH SPECIAL REFERENCE TO BACT. AEROGENES[1]

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The purpose of this paper is to present the results of work on the encapsulated bacilli and to discuss the relationships between some of the members of this group. This work is the outgrowth of a study of encapsulated bacilli found in cases of genital diseases in horses. These organisms have been described by Dimock and Snyder (1925), Dimock and Edwards (1927) and Edwards (1928). While these organisms were being compared with encapsulated bacilli isolated from human sources it was noted that certain cultures of *Bact. aerogenes* were closely related to the cultures from equine sources and to some of the cultures of human origin. This relationship seemed worthy of further study; so a number of cultures of *Bact. aerogenes* were collected and compared with the human and equine cultures in our possession.

SOURCE OF CULTURES

The cultures of Friedländer and granuloma bacilli which have been used in this work were obtained from several investigators who forwarded them to us. With one exception the strains of *Bact. aerogenes* were obtained in the same way. One of the cultures of *Bact. aerogenes* and the organisms from genital diseases in horses were isolated in this laboratory. Following are given the designations of the cultures and the sources from which they were obtained.

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E1, E2, E8—isolated from uteri of infected mares F3, F5, F6, F8, F9, F10, F11, F13, F18, F20, F22, F23, F24, F25, F28, FR—Pneumonia, human
F1, F2—extirpated appendix, human
F21—extirpated tonsil, human
F26—chronic colitis, human
F27—chronic cystitis, human
F15—mitral regurgitation, human
F19—pleuritis, human
G2, G6—inguinal granuloma, human
AB—*Bact. aerogenes*, milk
AR, A9, A10, A14, AH9—*Bact. aerogenes*, feces
A3, A5, A7, A8, A11, A12, A50, A65, A70, A73, A97, A111, A112, A113, A114, A115, A153, AM8C—*Bact. aerogenes*, soil
A6, A13—*Bact. aerogenes*, water

The cultures E1, E2 and E8 represent a group of sixty-five encapsulated organisms recovered from cases of metritis in mares. Forty of these sixty-five strains have been studied and found identical in morphological, cultural, biochemical and serological properties. All are identical with type B of the Friedländer bacillus as described by Julianelle (1926). Since these cultures exhibit the same properties only three of them are included in this study.

CULTURAL CHARACTERISTICS

The cultural characteristics of the majority of the organisms included in this study are those generally attributed to the members of the *B. mucosus* group. An abundant, dirty white to slightly yellow growth which is moist, glistening and slimy occurs on agar slants. Agar colonies are large, raised, round and entire. The bacteria are Gram negative, non-sporing, non-motile rods which do not liquefy gelatin. Most of the organisms produce wide capsules on artificial media. Some of the cultures which have been grown on artificial media for long periods no longer produce the capsules typical of this group. Such strains do not exhibit a mucoid growth on agar and their virulence is reduced. This lack of virulence, loss of capsules and failure to exhibit a mucoid growth on agar occurred among all the groups studied,

the Friedländer, equine and granuloma strains as well as the cultures of *Bact. aerogenes* being affected in this way.

METHODS

The methods used in fermentation tests, immunization of rabbits, agglutination, agglutination absorption, and precipitin tests are those employed by Edwards (1928). The protein antigens used in the precipitin tests were prepared by the method of Julianelle (1926b).

TABLE 1

Biochemical reactions

	AMMONIA PRODUCTION	NITRATE PRODUCTION	INDOL PRODUCTION	METHYL RED	VOGES-PROSKAUER	GROWTH IN URIC ACID	GROWTH IN CITRATE	ACTION IN LITMUS MILK
E1, E2, E8, F6, F26, A3, F11, A5, A7, A10, A13, A50, A65, A113, A115, A150, A153 AB, AH9, G6.	+	+	−	−	+	+	+	AC
F3, F5, F9, F15, F18, F19, F23, F25, F28, A6, A11, A14, A114	+	+	−	−	+	+	+	A
F2, F8, F10, F24, F27, FSc.	+	+	−	+	−	+	+	A
F21, A97, A112	+	+	−	+	−	+	+	AC
F1	+	+	−	+	−	−	+	A
F13, FR	+	+	−	+	−	−	−	A
F20	+	+	−	−	+	−	−	A
F22	+	+	−	−	+	−	−	Al
AR, A8, A9, A111	+	+	+	−	+	+	+	AC
A73, AM8C	+	+	+	−	+	+	+	A
A70	+	+	+	+	−	+	+	A
A12	+	+	−	+	−	+	−	A
G2	+	+	−	−	−	+	+	AC

BIOCHEMICAL REACTIONS

The cultures studied have been subjected to the tests usually applied to the organisms of the colon-aerogenes group. The strains have been tested for indol production, growth in citrate and uric acid media, nitrate reduction, production of ammonia, action in litmus milk and response to the methyl red and Voges-Proskauer tests.

TABLE 2

Fermentative reactions

	GLUCOSE	LEVULOSE	GALACTOSE	LACTOSE	SUCROSE	GLYCEROL	DULCITOL	MANNITOL	SORBITOL	ARABINOSE	XYLOSE	RAFFINOSE	MALTOSE	ADONITOL	SALICIN	INULIN	DEXTRIN	SOLUBLE STARCH
E1, E2, E8, F3, F5, F8, F6, F13, F15, F19, F20, F21, F24, F25, F26, F28, AB, A3, A13, A14, A50, A112, A113, A114, G2	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F_2	AG	AG	AG	AG	O	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F23	AG	AG	AG	O	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F10, F27, A150, AH9	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
Sc	A	A	A	A	A	A	A	AG	AG	A	A	A	A	A	A	O	AG	AG
F1	A	A	A	A	O	A	O	A	A	A	A	A	A	A	A	O	A	A
F11	A	A	A	A	A	O	O	A	O	A	A	O	O	O	O	O	O	O
F18	A	A	A	A	A	A	O	A	A	A	A	A	A	A	A	O	A	A
F22	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
G6	A	AG	A	AG	A	A	O	A	A	A	A	A	A	AG	A	O	AG	AG
AR, A6, A9, A97	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
A5, A7	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	O	AG	O	AG	AG
A10	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	O	AG	AG
A11, A150, AH9	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
A12	AG	AG	AG	AG	AG	O	O	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	O
A65	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	O	AG	O	AG	AG
A115	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
A153	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	O	AG	O	AG	O
A8, A70, A73, A111	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG

From table 1 it can be seen that the organisms studied are divisible into several groups on the basis of their reactions to the tests employed. These groups do not appear to be significant however, since members of the various species are dispersed through several groups. It is evident that this combination of tests cannot be employed in the separation of the various species. If the results of each of the tests be considered separately it may be seen that not one of the tests employed can be used to differentiate the various species.

FERMENTATIVE REACTIONS

The cultures studied were tested for their ability to form acid and gas from a number of fermentable substances. An examination of table 2 shows that the cultures of the various types cannot be separated by their action upon the fermentable substances used. Certain groups are established by the fermentation tests but the various species are dispersed throughout these groups. The groups established by the fermentation reactions do not agree with those established by the biochemical reactions given in table 1, nor, as will be shown later, are they related to the groups established by the serological tests. Both the cultures of *Bact. aerogenes* and the Friedländer bacilli are rather variable in their fermentative characters.

SEROLOGICAL REACTIONS

The early literature regarding the serological properties of the Friedländer group is very confusing. Later workers have successfully classified the organisms by serological methods. Toenniessen (1914) suggested that the capsular substance was antigenic in character and that Friedländer bacilli were capable of exhibiting two antigenic complexes, one residing in the capsule and the other in the endoplasm of the bacterium. Toenniessen (1921) later demonstrated that the capsule of these organisms was a galactan. Heidelberger, Goebel and Avery (1925) derived a nitrogen-free polysaccharide from Friedländer bacilli which was capable of giving positive precipitin tests with homologous immune serum. Julianelle (1926, 1926a, 1926b) by applying

these facts to the classification of Friedländer strains has established four serological groups among the encapsulated bacilli. These he has designated as types A, B, and C, and Group X. Types A, B and C are specific while group X is composed of a number of heterologous strains.

Julianelle demonstrated that organisms which produced capsules were agglutinated by serum derived from an organism of the homologous type but were not affected by sera derived from organisms of heterologous types. The manner in which these organisms were clumped was characteristic, agglutination resulting in the formation of a compact, voluminous disc at the bottom of the tube. Organisms which no longer produced capsules, or bacilli which had been stripped of their capsule by chemical methods, did not exhibit this type specificity. They were agglutinated by the immune sera of both the homologous and heterologous types. The character of the agglutination was changed. Instead of the formation of a compact disc, agglutination resulted in a fine, powdery precipitate.

In the present investigation the Friedländer, granuloma, and equine organisms have been compared with the cultures of *Bact. aerogenes* using agglutination, precipitin, and agglutinin absorption tests in an effort to determine their relationships.

AGGLUTINATION

Cultures E1, F6, FSc, G2, G6, and A3 were used to prepare antisera. Using these sera, agglutination tests were set up in dilutions ranging from 1 to 20 to 1 to 2000. Two main types are established by the agglutination tests. The first group composed of strains E1, E2, E8, F6, F26, FR, AB, A3, A13, A113, and A114 is identical with type B of the Friedländer bacillus as described by Julianelle (1926). These organisms have the cultural characters generally attributed to the soil types of *Bact. aerogenes*. It will be noted that in this type are included five strains of *Bact. aerogenes*, three isolated from soil, one from water and one from milk.

The second large group is composed of strains F3, F5, F8, F9, F18, F19, F20, F21, F23, F24, F25, F28, FSc. These strains are identical with type A of the Julianelle.

A third group established by the agglutination test is composed of three cultures, G6, A70, and A73. G6 is a culture received from Dr. J. C. Small. It was recovered from a local lesion of inguinal granuloma. The cultures A70 and A73 are soil strains of *Bact. aerogenes* received from Dr. S. A. Koser.

In addition to the specific agglutination observed in these tests several instances of group or non-specific agglutination occurred among the aerogenes strains. This was most evident in the cases of strains A12 and A14. Julianelle (1926a) attributed group agglutination among the Friedländer bacilli to the absence of capsule formation. The same cause is probably operative in the case of *Bact. aerogenes*. The cultures which were acted upon by the group agglutinin exhibited a rather dry growth on agar and no capsules could be demonstrated in these cultures.

During the course of this work the action of hydrolysis upon agglutination of the encapsulated bacilli has been studied. This method, first used by Porges (1905), and since employed with more or less success by a number of investigators, consists of treating suspensions of encapsulated bacilli with one fourth volume of 0.25 N HCl, heating at 80°C. for fifteen to thirty minutes and neutralizing the suspension by the addition of NaOH. This treatment is designed to strip the organism of its capsule and render it easily agglutinable. Beham (1911) stated that this method often rendered the bacilli spontaneously agglutinable. The results of our work with hydrolyzed suspensions have, on the whole, been unsatisfactory. Many organisms thus treated became spontaneously agglutinable. Still other strains, after they had been so treated, were not affected by sera known to be potent in group agglutinins.

However, by varying the temperature and the time of exposure to acid we have been able to render a number of the strains susceptible to group agglutinin. We have not been able to distinguish *Bact. aerogenes* from the other types by this method. We have found that, after hydrolysis, *Bact. aerogenes* strains are agglutinated by Friedländer antisera to about the same extent as the equine organisms and Friedländer and granuloma bacilli.

PRECIPITIN TESTS

The tests performed with type specific precipitin antigen confirm the results of the agglutination tests in every case. The cultures which were agglutinated by sera derived from strains E1 and F6 gave strongly positive precipitin tests with these sera while no precipitation resulted with sera derived from strains G6 and Sc. Also, strains A70 and A73 gave strongly positive tests with serum derived from strain G6 but caused no precipitation when placed in contact with the sera of other types. The strains composing type A of Julianelle were also specific in their precipitin reactions.

In the precipitin tests using protein antigens the proteins derived from strains of *Bact. aerogenes* yielded strongly positive tests with antisera derived from cultures of Friedländer and granuloma bacilli. It was not possible to distinguish between the organisms of the various types in this way. Julianelle (1926b) has observed the precipitation occurring when Friedländer antisera are placed in contact with protein derived from *Bact. aerogenes*. This reaction indicates the close serological relationship of the proteins of these bacilli.

AGGLUTININ ABSORPTION

The results of the agglutinin absorption tests further substantiate the relationships established by the agglutination tests and precipitin tests. In every case in which type specific agglutination occurred the strain so agglutinated was able to exhaust the type serum of agglutinins completely. Thus, strains E2, E8, F6, F26, FR, AB, A3, A13, A113, and A114 effected a complete removal of agglutinins active on strain E1 from antiserum derived from that strain. They were also able completely to remove the agglutinins active on strain F6 from antiserum derived from F6. Further, these strains caused a complete removal of agglutinins from A3 antiserum. This group of organisms was inactive when used to absorb the sera of other types.

Similarly strains A70 and A73 completely exhausted antiserum G6 of agglutinins for the serum strain. They were without

effect when used to absorb antisera derived from strains E1 and FSc.

The group of Friedländer strains composing type A were also apparently of homogeneous antigenic structure since they all exhausted serum FSc of agglutinins active upon the homologous strain.

The strains which were agglutinated non-specifically were unable to completely remove agglutinins from any of the antisera.

PATHOGENICITY

Baerthlein (1918) and Toenniessen (1921) have demonstrated that the virulence of Friedländer bacilli for the laboratory animals is dependent upon capsule formation. In a previous publication, Edwards (1928), we have found that the ability of organisms of this group to cause metritis in mares is also dependent upon abundant capsule formation.

In studying the effect of cultures of *Bact. aerogenes* on laboratory animals we have found that those cultures which produced wide capsules and a mucoid growth on artificial media were uniformly virulent for the laboratory animals. Using the technique described by Dimock and Edwards (1926), it has been found possible to produce metritis in mares through the introduction of *Bact. aerogenes* into the uterus. However, only those strains which were vigorous capsule producers were able to become established.

No constant differences in pathogenicity of the various types have been observed.

DISCUSSION

It is evident from the results obtained in this study and from the results of other workers that such tests as ammonia production, nitrate reduction, indol formation, growth in uric acid and citrate media, and the methy red and Voges-Proskauer tests are of no value in the differentiation of the various types of encapsulated bacilli.

The action of the organisms when grown in milk has been employed by many writers to set apart *Bact. aerogenes* from the

other members of the group. Basing their statements on the work of Wilde (1896) and Claremont (1902) they characterize *Bact. aerogenes* as a milk coagulator, while Friedländer's bacillus is described as causing no coagulation in milk. However, the results of other workers have been contradictory. Fricke (quoted by Abel and Hallwacks (1913) found a striking variation in the ability of strains isolated from pathological sources to coagulate milk. Fitzgerald (1914) observed that certain strains of Friedländer's bacillus were able to coagulate milk while other strains caused no coagulation. Even when the utmost care was used in the tests, these differences were apparent and Fitzgerald warns against the use of coagulation of milk as a differential test. Small and Julianelle (1923) studied 13 cultures of Friedländer bacilli and found that 5 of the strains coagulated milk.

In the present study we have found that four strains of Friedländer bacilli cause a coagulation in milk. It is noteworthy that two of the Friedländer strains used in this study which did not produce coagulation in milk had been previously studied by Small and Julianelle and found to be milk coagulators. Among the aerogenes cultures studied there were seven which caused a permanent acidity in milk but produced no coagulation. Others coagulated the milk only after four to ten days incubation. In addition it must be remembered that 40 cultures of equine origin, which are identical with type B of the Friedländer bacillus, rapidly coagulate milk. In view of these facts it would seem that coagulation of milk must be accepted with reservations, if it is accepted at all, as a means of differentiating *Bact. aerogenes* from the other members of the encapsulated group.

The fermentative characters of the encapsulated bacilli have been studied by a number of workers and several classifications have been based upon the action of these organisms on fermentable substances. These studies, however, are far from being in agreement. The greatest controversy has centered about the ability of Friedländer's bacillus to ferment lactose. Many writers contend that this organism produces acid and gas from glucose and sucrose but does not attack lactose. Friedländer (1882, 1883, 1884) did not mention the action of the organisms

which he isolated on milk or lactose. Strong (1889) working with strains which were presumably lineal descendants of Friedländer's original cultures found that the organism produced no acid or gas from lactose. Grimbert (1895) stated that Friedländer's bacillus was able to produce acid from lactose. Wilde (1896) reported that the strains of Friedländer bacilli which he studied formed acid from lactose. Nicolle and Hebert (1897) found that Friedländer's bacillus formed acid and gas from lactose. Russ (quoted by Abel and Hallwachs (1913) stated that Friedländer's bacillus fermented lactose. Lehman and Neumann (1901) reported that Friedländer's bacillus fermented lactose with the production of acid and gas. Claremont (1902) found that all of the ten Friedländer strains which he studied produced acid from lactose. Nine of these strains formed perceptible gas. Perkins (1904) classified the encapsulated bacilli according to their action on glucose, lactose, and sucrose. Those strains which were able to ferment glucose and sucrose but did not attack lactose he designated as Friedländer's bacillus. A culture from Kral, supposedly a descendant of the original strain of Friedländer, did not attack lactose. MacConkey (1905) on the contrary, found that a transplant obtained indirectly from the Kral culture produced acid in lactose broth. Fitzgerald (1914) found that six of seven Friedländer strains examined attacked lactose and offered evidence that fermentation could not be relied upon in the classification of the encapsulated bacilli. Coulter (1917) studied eleven strains of Friedländer bacilli and found that none of them fermented lactose and that they formed a uniform serological group. Castellani and Chalmers (1919) in their classification of the colon-typhoid group inserted the genus Encapsulatus. The type species, *Encapsulatus pneumoniae*, Friedländer 1883, was described as forming acid and gas from glucose and sucrose and acid alone from lactose. *Encapsulatus aerogenes* was said to ferment glucose, lactose, and sucrose with the formation of acid and gas. Bergey (1921) stated that Friedländer's bacillus formed acid and gas from lactose. Small and Julianelle (1923) found that eleven of thirteen Friedländer strains which they studied produced acid from lactose. In addition five of the cultures which

they studied produced gas from this sugar. Perkins (1925) supplementing his former work, again classified the Friedländer bacillus as a non-lactose fermenter.

In this work we have studied twenty-two strains of Friedländer bacilli. With the exception of three strains, all of these organisms formed acid from lactose and, in addition, sixteen of the twenty-two cultures formed a perceptible amount of gas.

The conflicting results obtained by various workers, each believing himself to be working with the original strain of Friedländer's bacillus, are worthy of note. It seems from a review of the literature that most of the cultures of excapsulated bacilli isolated from cases of pneumonia and from the respiratory tract are able to ferment lactose. If lactose fermentation is used as a criterion for the differentiation of species it will result in the separation of organisms serologically identical into different species. Such a procedure hardly seems justified when the majority of the organisms labelled as Friedländer's bacillus are lactose fermenters.

The agglutination of aerogenes strains by Friedländer sera was noted by Claremont (1902). However, the same strains of *Bact. aerogenes* when used to prepare serum did not agglutinate any of his strains of Friedländer bacilli. It is highly probable that this agglutination was non-specific or group agglutination. Bertarelli (1906) also has observed the agglutination of aerogenes by Friedländer sera. Julianelle (1926a) calls attention to the close relationships of aerogenes and Friedländer proteins. He explains the non-specific agglutination of capsule free strains of *Bact. aerogenes* on the basis of this relationship between the proteins of the two organisms.

It is the opinion of the writer that specific agglutination occurring between Friedländer's bacillus and *Bact. aerogenes* has not been heretofore recorded. Tomcsik (1927) has isolated a protein free carbohydrate-like specific substance from strains of *Bact. aerogenes*. This substance, however, did not prove reactive with anti-Friedländer sera.

In the present work it has been found that three strains of *Bact. aerogenes* isolated from soil, one strain isolated from water

and one strain isolated from milk are identical with type B of the Friedländer bacillus as described by Julianelle (1926). Two other strains of *Bact. aerogenes* have been found to be antigentically identical with a strain of the granuloma organism. Sera for only three types of the encapsulated bacilli were used in this study. If sera were prepared for the other types of Friedländer bacillus and the strains of *Bact. aerogenes* tested with these, it is probable that still further relationships would be established. Using only the three type sera, it has been found that seven of 26 cultures of *Bact. aerogenes* are identical in their antigenic structure with certain of the Friedländer and granuloma bacilli.

It will be noted that no aerogenes cultures were found to be identical with type A of the Friedländer bacillus. This type, apparently, is most commonly found in human infections, while type B seems to be found most frequently in the lower animals.

Bergey (1921) has classified *Bact. aerogenes* under the genus Aerobacter while Friedländer's bacillus is classified under the genus Encapsulatus. Perkins (1925) includes both of these organisms in the same genus, Encapsulata, and differentiates them on the basis of lactose fermentation. Weldin (1927) places *Bact. aerogenes* in the genus Aerobacter and places Friedländer's bacillus in the genus Proteus. Castellani and Chalmers (1919) place the two organisms in the same genus, Encapsulatus, and differentiate them by the formation of gas from lactose.

From the results obtained in this study it seems that *Bact. aerogenes* is so closely related to Friedländer's bacillus and the bacilli isolated from lesions of inguinal granuloma that it should certainly be placed in the same genus as the remainder of the encapsulated bacilli. There are no constant differences between the strains which we have received labelled as Friedländer's bacilli and *Bact. aerogenes*. Furthermore we have found no distinguishing characters which might be used to separate these organisms into two or more species.

CONCLUSIONS

1. The organisms which we have received from various sources labelled Friedländer bacilli cannot be distinguished from *Bact.*

aerogenes and the other members of the encapsulated group by action on milk or fermentative characters.

2. Five cultures of *Bact. aerogenes* isolated from soil, water and milk have been found to be culturally, biochemically, and serologically identical with type B of the Friedländer bacillus as described by Julianelle. Two cultures of *Bact. aerogenes* have been found serologically identical with a strain of the granuloma bacillus.

3. *Bact. aerogenes* is so closely related to the other encapsulated forms that they should be classified in the same genus. No constant differences have been observed which could be used to separate the organisms into two or more species.

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