STUDIES ON THE KLEBSIELLA-AEROBACTER GROUP OF BACTERIA

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In spite of numerous publications on the Klebsiella-Aerobacter group within recent years, no general agreement has been reached regarding their classification. A number of alternative proposals has been made, all of which are open to certain criticisms. While the writers do not feel that they have solved satisfactorily the problems of classification within this group, they wish to present the results of work on the bacteria and to make certain suggestions in regard to the separation of the group into biotypes or species. Further, the results of capsule typing of the nonmotile, capsulated members of the group are presented and several new capsular types are characterized. No attempt will be made to review the literature systematically, since reviews recently have been published by Kauffmann (1949), Henriksen (1949, 1952, 1954) and Brooke (1951, 1951*a*, 1951*b*, 1953).

MATERIALS AND METHODS

The cultures investigated included those studied by Edwards and Fife (1952) as well as 364 Klebsiella strains received for typing since that report was published. Also included were six new capsular types of Edmunds (1954). The sources of these cultures, as well as the capsular types to which they were assigned, are presented later in tabular form. In addition, 128 motile cultures of the Aerobacter group isolated largely from stools and urines were studied. A few were derived from the upper respiratory tract, from localized infections and from the organs of fowls. The motile strains were included in the study in order that their biochemical properties could be compared with those of the nonmotile cultures which the writers considered members of the genus *Klebsiella*. During the period covered by the study many motile cultures which belonged to the Aerobacter group were examined, as well as a large number of strains of intermediate character, but only a group of representative strains was included. However, it is felt that these cultures constituted a representative sample of all the motile cultures examined which possessed the primary characteristics of the Aerobacter group.

The methods used in the biochemical and serological examination of the cultures were the same as those employed by Edwards and Fife (1952).

RESULTS

Biochemical reactions. The results of the biochemical reactions are summarized in table 1, in which the organisms are divided strictly on the basis of motility. The fallacies inherent in such an arbitrary division are realized and are discussed later. It is unfortunate that a number of cultures were not subjected to each of the tests employed and are recorded in the table simply as "no test." This situation resulted largely from the fact that, from time to time, additional tests were added to those ordinarily performed and in the meantime some of the cultures had been discarded or lost.

Of the 626 nonmotile, capsulated cultures, 26 produced indol. These were included in the study since 9 of them were type strains of Klebsiella described by Brooke (1951) and the remainder of these cultures displayed IMVIC patterns which, aside from positive indol reactions, were typical of the Klebsiella group (− − + +). Also, the organisms possessed capsular antigens similar to those of typical Klebsiella cultures.

There was some degree of correlation between indol production and gelatin liquefaction. In all, there were 35 nonmotile cultures which either liquefied gelatin or produced indol. Of these, 19 were both indol and gelatin positive, seven were indol positive and gelatin negative, and nine were indol negative and gelatin positive. Also, there was a correlation between motility and gelatin liquefaction in this group, since 85.5 per cent of the motile strains liquefied gelatin but

TABLE 1

Biochemical reactions

	Motile	Nonmotile, Capsulated
No. of cultures	128	626
Indol	126−, 2 NT	26+, 599−, 1 NT
Methyl red	1+, 125−, 2 NT	110+, 511−, 5 NT
Voges-Proskauer	124+, 1−, 3 NT	510+, 112−, 4 NT
Simmons' citrate	117+, 3−, 8 NT	606+, 19−, 1 NT
Nitrate	122+, 6 NT	621+, 5−
Urea	36+, 92−	578+, 48−
H_2S	23+, 103−, 2 NT	8+, 618−
Gelatin	99+, 17−, 12 NT	28+, 595−, 3 NT
D-Tartrate	93+, 35−	600+, 26−
Glucose (gas)	117+, 11−	598+, 28−
Lactose	69+, 41 X, 15−, 3 NT	540+, 84 X, 2−
Sucrose	111+, 7 X, 9−, 1 NT	575+, 19 X, 32−
Dulcitol	26+, 97−, 5 NT	193+, 432−, 1 NT
Salicin	90+, 36 X, 1−, 1 NT	616+, 7 X, 3−
Inositol	49+, 26 X, 47−, 6 NT	586+, 28 X, 12−
Adonitol	57+, 6 X, 60−, 5 NT	569+, 7 X, 50−
Soluble starch	20+, 43 X, 40−, 25 NT	563+, 3−, 60 NT
Soluble starch (gas)	2+, 16 X, 85−, 25 NT	468+, 64 X, 34−, 60 NT
Glycerol	101+, 4−, 23 NT	566+, 3−, 57 NT
Glycerol (gas)	30+, 30 X, 42−, 26 NT	495+, 39 X, 35−, 57 NT

Definition of symbols used in fermentation tests: +, promptly positive (24 hr); X delayed positive; −, negative after 30 days' incubation; NT, no test. In gas production from soluble starch, + indicates gas production within 4 days.

only 4.6 per cent of the nonmotile strains were gelatin positive.

Only one of the motile cultures was methyl red positive, while 110 of the nonmotile cultures were positive. Likewise, among the motile cultures there was only one Voges-Proskauer negative form, while 112 were found among the nonmotile cultures. The inverse relationship usually noted between these two tests was especially marked in the group under study. Methyl red positive, Voges-Proskauer negative forms were found in Klebsiella capsule types 1, 2, 3, 4, 5, 6, 7, 8, 14, 15, 19, 21, 25, 27, 29, 31, 32, 35, 39, 44, 54, and 65. They were especially prominent in types 1 (5 of 52), 2 (15 of 48), 3 (9 of 14), 4 (45 of 51), and 5 (7 of 8). It is obvious that members of these well-known respiratory types (A, B, C, D, and E) must be included in the genus *Klebsiella* in spite of their aberrant biochemical reactions.

There was no marked difference in the citrate reactions of the motile and nonmotile strains—approximately 3 per cent of each group failed to utilize Simmons' citrate agar. It is worthy of note that 12 of the 19 citrate negative nonmotile cultures were classified in capsule types 3 and 4. Urea was hydrolyzed by only 28 per cent of the mobile cultures while 92 per cent of the nonmotile cultures attacked the substance. Again it should be noted that 38 of the 48 nonmotile urea negative cultures belonged to capsule types 3 and 4, and 36 of them belonged to type 4 alone. Although nitrate reduction is supposed to be a constant characteristic of Enterobacteriaceae, five of the nonmotile cultures failed to reduce nitrate in repeated tests. Again, all of these aberrant cultures were members of capsular type 4.

The percentage of cultures which failed to ferment lactose was somewhat higher among the motile cultures than among the nonmotile strains, while differences in sucrose fermentation were not so pronounced. The majority of cultures of both groups failed to ferment dulcitol. Salicin was fermented by practically all of the cultures, but delayed fermentation was much more frequent among the motile cultures than among the nonmotile strains. The percentage of cultures

which fermented inositol and adonitol was higher among the nonmotile cultures (98 and 92 per cent respectively) than among the motile cultures (61 and 51 per cent respectively). However, the correlations observed by Brooke (1951*a*, 1951*b*, 1953) among strains classified as Klebsiella and *Aerobacter cloacae* in their reactions upon salicin and inositol were not evident in this series of cultures. It is true that Brooke did not divide his cultures as the present series is divided, but even when allowances are made for this difference in division of the cultures, the results obtained here do not confirm those reported by Brooke, who found that only 9 per cent of *A. cloacae* fermented salicin promptly and less than 1 per cent fermented inositol.

Soluble starch was fermented by 61 per cent of the motile cultures and by 99 per cent of the nonmotile cultures. The writers are indebted to Dr. E. Hormaeche for the suggestion that cultures be observed for their ability to produce gas from starch within four days. When this criterion was applied, less than 2 per cent of the motile cultures were positive, whereas 82.6 per cent of the nonmotile strains were positive.

Of the motile strains, 8.5 per cent failed to produce visible gas from any of the fermentable substances. Among the nonmotile cultures 4.4 per cent were anaerogenic. The majority of the anaerogenic cultures were tested also for their ability to produce gas in mannitol but the results were the same as those obtained with glucose.

Brooke (1953) suggested that the *A. cloacae* and Klebsiella groups be separated by their action on glycerol. Klebsiella cultures, it was reported, produced gas from glycerol within 24 hours, whereas *A. cloacae* cultures did not. In the present work it was found that 32 per cent of the motile cultures tested produced gas promptly from glycerol, whereas 6 per cent of the nonmotile cultures did not produce gas from glycerol at any time. The nonmotile cultures which failed to produce gas from glycerol were made up in large part of the completely anaerogenic cultures although certain aerogenic cultures also were negative. Among the cultures which failed to produce gas from glycerol were members of capsular types 1, 2, 3, and 4, which obviously must be considered as klebsiellae. In addition, 39 nonmotile cultures failed to produce gas within 24 hours but did so upon prolonged incubation. In all, 74 cultures of 569 tested, or 13 per cent, failed to produce gas from glycerol within 24 hours. Among these 74 cultures were 15 of type 1, 5 of type 2, 2 of type 3, 22 of type 4, and 4 of type 5.

In addition to the nonmotile, capsulated cultures listed in table 1, 12 cultures were encountered which were nonmotile and noncapsulated. These were omitted from the table since they could not be typed serologically. Their biochemical behavior was in no way exceptional and they resembled the capsulated cultures in their action on urea, gelatin, and the fermentable substances.

TABLE 2

Reactions of newly described capsule types

Antigens	Reacting with Antiserums	Antiserums	Reacting with Antigens
58	6(1), 7(1), 58(32)	58	7(1), 58(32)
59	59(64)	59	59(64)
60	31(2), 60(64)	60	31(2), 60(64)
61	7(1), 10(8), 18(1), 41(1), 58(1), 61(128)	61	7(4), 10(4), 18(4), 41(16), 61(128)
62	62(128)	62	62(128)
63	63(32)	63	49(4), 63(32)
64	14(16), 64(128)	64	14(32), 31(2), 36(2), 47(1), 49(1), 56(2), 59(1), 64(128), 68(1)
65	39(16), 65(32)	65	27(1), 28(2), 29(2), 39(1), 40(2), 42(1), 65(32)
66	66(64)	66	66(64)
67	67(64)	67	67(64)
68	3(16), 68(32)	68	3(4), 14(2), 36(2), 68(32)
69	2(2), 21(2), 69(32)	69	2(2), 11(1), 13(1), 21(2), 69(32)

Figures in parentheses indicate titers of capsular (quellung) reactions.

Serologic Reactions

Edwards and Fife (1952) recognized 57 capsular types among 256 Klebsiella cultures. Since that time the writers have recognized and characterized six previously unrecognized capsular antigens (types 58 to 63 inclusive) and Edmunds (1954) described six additional capsular antigens (types 64 to 69 inclusive). The reactions of these new capsular antigens with the serums of the

TABLE 3

Source and type distribution of capsulated Klebsiella cultures

Capsule Types	Lungs and Pleura	Sputum and Bronchial Exudate	Nasopharynx	Urine and Genitourinary Tract	Stools and Intestinal Materials	Pus and Abscesses	Blood	Bone and Joints	Animals and Animal Food Products	Miscellaneous	Total
1	6	27	1	3	1	7	1	3	2	1	52
2	3	29		6		4	3	1	1	1	48
3		7	2	3		1	1				14
4	1	31	11	3	1	1			2	1	51
5		6	2								8
6		6	2			1					9
7		4		23	8	2	2		1	1	41
8	1	5		9	4	1	1			1	22
9	2			6	1		2				11
10		1		6	1		2				10
14	1	8		3							12
15		3		5	5	1	1				15
16		1		5	3	1	3		1		14
17		4		9	3	1	1		6		24
18		4		2	3	1				1	11
19		6		20	1	2	1		1		31
20	1	3		4	9	2	2				21
21	2			8	1	2	2			1	16
22	3	1		4					1	1	10
25		6		11							17
35	1	2	1	4	1	1				3	13
54	1	2		5	4	1	2			2	17
Other	7	43	1	64	20	9	5		9	1	159
Total.....	29	199	20	203	66	38	29	4	24	14	626

various types as well as the reactions of the new type serums with the various antigens are summarized in table 2. While the results obtained with types 64 to 69 inclusive were not identical with those obtained by Edmunds, the results were in essential agreement. The problem of the difference in behavior of different diagnostic serums will be discussed later.

The sources of the 626 capsulated nonmotile cultures are summarized in table 3. Except for the long-recognized "respiratory types," all types represented by less than 10 cultures were arbitrarily grouped together as "additional types." As would be expected, the majority of cultures of types 1 to 6 inclusive were derived from the respiratory tract. However, their presence was by no means confined to the respiratory organs; they appeared in localized infections, urinary infections, stools and intestinal contents, blood, and infections of lower animals. The remainder of the types occurred frequently in urinary infections and in stools, but they were by no means rare in respiratory infections. In fact, types 7 to 69 were found as frequently in respiratory diseases as were types 1 to 6, and were almost three times as numerous as the latter among cultures from blood and localized infections. Thus, too great emphasis probably has been placed by various writers upon the more recently described types as incitants of urinary infections. It must be recognized that a great variety of capsular types appears in respiratory infections, in various localized infections, and in the blood stream as well as in urinary infections.

Among the cultures recorded under miscellaneous sources one culture each of types 1 and 35 were isolated from gall bladders. One culture of type 2 was isolated from ascitic fluid. One culture each of types 4, 8, 18, and 35, and two cultures of type 54 were isolated from spinal fluid. One culture of type 21 was isolated from

sinusitis and one culture of type 35 was isolated from a bottle of tonic. One culture each of types 7, 22, and 53 was isolated from water.

Among the cultures from animals and animal food products, one culture of type 1 was isolated from the spleen of a monkey and another from the lung of a dog affected with pneumonia. It should be noted that type 1 rarely has been found in animals. One culture of type 2 was isolated from the cervix of a mare. This type has been found repeatedly in genital diseases of horses (Edwards, 1929). Two cultures of type 4, one culture of type 16, six cultures of type 17, and one culture each of types 19, 22, 41, and 47 were isolated from bovine mastitis. One culture each of types 7 and 42 occurred in milk. One culture of type 50 was isolated from the liver of a turkey.

Among the 128 motile cultures were 38 strains with distinct capsules. Motile, single-colony cultures of these were tested with the Klebsiella capsular sera. Distinct capsular (quellung) reactions were observed in 19 cultures. These cultures in large part were typical strains of the motile group, all were urea negative and all but two liquefied gelatin. Of these cultures, seven reacted with type 3 serum, five with type 21 serum, one with types 11 and 21 sera, two with type 26 serum, one with type 8 serum, one with types 8, 11, and 33 sera, one with type 4 serum, and one with types 3 and 68 sera. These observations indicated that the capsular antigens of encapsulated cultures of *A. cloacae* possess many relationships to the capsular antigens of klebsiellae and emphasize the need for biochemical examination of all cultures subjected to serologic tests. It is of some interest that of the 19 motile cultures which possessed capsules related to recognized Klebsiella types, 12 produced gas promptly from glycerol, four failed to produce gas upon prolonged incubation, and three were not tested.

DISCUSSION

While the results obtained in the biochemical studies are in general agreement with those obtained by other workers, certain discrepancies are apparent. Gas production from glycerol long has been considered significant in the differentiation of organisms within this group of bacteria. The most frequently quoted studies are those of Kligler (1914) and Johnson and Levine (1917) and often one finds statements in the literature that these workers found that *A. aerogenes* produced gas from glycerol whereas *A. cloacae* did not. These statements culminated in the differentiation of the two species upon the basis of gas production from glycerol by Breed, Murray and Hitchens (1948) in the 6th edition of *Bergey's Manual*. Yet, if these early publications are scrutinized, one finds that Kligler examined only 28 cultures which fermented both sucrose and salicin and which composed the aerogenes-cloacae group in his series of cultures. Of these 28 strains, nine were classified in the cloacae division of the group. Kligler, himself, was careful to point out that his study indicated only tendencies of the bacteria studied, that the separation of the cultures was by no means absolute, and that larger numbers of cultures should be studied. When Johnson and Levine studied larger numbers of cultures the absolute correlation of characters no longer was found. Of 88 strains which they classified in the cloacae group, 9.1 per cent produced gas from glycerol. Of 44 cultures classified in the aerogenes group, 97.7 per cent produced gas from glycerol. Gelatin liquefaction was found by Kligler to be inversely correlated with production of gas from glycerol, but Griffin and Stuart (1940) found that the correlation between the two characters was by no means absolute. Thus, for many years it has been apparent that these two differential tests serve to delineate groups, but that intermediate strains and aberrant cultures must be expected. In Brooke's (1951*b*, 1953) series of cultures less than 10 per cent of cultures called *A. cloacae* fermented salicin promptly and less than 1 per cent fermented inositol, results which are in marked contrast to those reported here. While the cultures of Brooke were not divided in the same manner as the present series, the *A. cloacae* group contained all of his motile cultures and thus it is apparent that the results of Brooke and of the present writers are not in agreement. All of the motile cultures described here which promptly produced gas from glycerol were replated repeatedly and the biochemical characteristics reexamined but in no case were changes in glycerol, inositol and salicin fermentation noted. It is noteworthy that none of these motile, glycerol-fermenting strains produced gas from starch within 4 days, a further indication that mixed cultures were not involved.

From an examination of Brooke's (1953) paper it is not possible to determine exactly upon what basis his cultures were divided into groups. The only characteristic listed which gives an absolute separation of the groups is production of gas from glycerol. Yet only 117 of 290 nonmotile, capsulated cultures were tested for that property. Brooke (1951*a*, 1951*b*) reported series of cloacae strains and acapsular klebsiellae which were identical with those reported by Brooke (1953). Yet Brooke (1951*a*) reported on 311 capsulated klebsiellae while only 290 such cultures were included in the 1953 report. Among the cultures studied by Brooke (1951*a*) were eight anaerogenic cultures which apparently were not considered by Brooke (1953) when he stated that all *A. aerogenes* (*Klebsiella*) tested promptly produced gas from glycerol. As stated previously, in the present series of cultures there were many nonmotile capsulated strains in addition to the completely anaerogenic forms which failed to produce gas promptly from glycerol. Almost 30 per cent of type 1 cultures, 10 per cent of type 2, and 45 per cent of type 4 were in this group. This illustrates the futility of attempting to separate *A. cloacae* from klebsiellae on the basis of glycerol fermentation alone.

The writers may be criticized for separating the cultures strictly on the basis of motility. It is recognized that nonmotile variants occur in motile cultures and that an occasional culture included in the nonmotile series may have been derived from a motile culture. It must be remembered that the genus *Klebsiella* traditionally has been defined as nonmotile and it obviously would be erroneous to group typical Friedländer strains with motile cultures. Further, it must be remembered that *Bacillus lactis-aerogenes* of Escherich long has been regarded as a strictly nonmotile species. MacConkey (1905) reviewed the literature on the aerogenes-cloacae group and all of the very early workers quoted by him regarded aerogenes as a nonmotile form and cloacae as a motile species. MacConkey subscribed to the view that aerogenes was nonmotile and failed to liquefy gelatin whereas cloacae was motile and liquefied gelatin. MacConkey (1909) held to this view and remarked that "... no one would accept a motile bacillus as *B. lactis-aerogenes* or *B. pneumoniae* (Friedländer)." Levine (1918), in an excellent statistical study, noted the close correlation between motility, gelatin liquefaction, and production of gas from glycerol and starch. Some aberrant results were observed but Levine was inclined to the view that these were due to mixed cultures and not to atypical strains. He too defined aerogenes as a nonmotile form which did not liquefy gelatin while cloacae was stated to be motile and a gelatin liquefier. In addition, action of the organisms on glycerol and starch was included in the definition. In view of these facts it is extremely difficult to understand how motile forms came to be included in *A. aerogenes* in the 6th edition of *Bergey's Manual*. The only difference in the definition of *Klebsiella pneumoniae* and *A. aerogenes* in the *Manual* is that the one is defined as nonmotile while the other is characterized as motile or nonmotile. This situation is clearly unsatisfactory and is complicated by the questionable practice of basing the primary separation of *A. aerogenes* and *A. cloacae* upon gas production from glycerol alone, apparently largely upon the basis of nine cultures of *A. cloacae* studied by Kligler (1914), since other workers noted a lack of absolute correlation.

As early as 1901, Grimbert and Legros (quoted by MacConkey, 1905) advanced the opinion that *B. lactis-aerogenes* of Escherich and *B. pneumoniae* of Friedländer were synonymous. Edwards (1929) noted that cultures classified as *A. aerogenes* and as klebsiellae were indistinguishable by biochemical and serological methods. Hence, it seems to the writers that, for the present, all nonmotile, capsulated cultures must be grouped together since there is no satisfactory method of dividing them. It may be argued that some of the gelatin-liquefying, nonmotile cultures actually should be classified as *A. cloacae*. This raises a difficult problem since five of these cultures were established as type strains of klebsiellae by Brooke (1951). The same situation applies to indol-positive cultures, of which nine are type strains of Brooke. In fact, type 26 was composed entirely of indol-positive, gelatin-positive cultures.

Since it is not possible to separate *A. aerogenes* from Klebsiella types, the writers are inclined to place both in the genus *Klebsiella*. This can be done without redefinition of the genus since the writers agree with MacConkey (1905) that aerogenes is a nonmotile form. The species *A. aerogenes* then disappears, and since it is the type species of the genus *Aerobacter*, the genus

is no longer valid unless it is redefined with a new type. This creates the problem of the classification of the organisms presently included in *Aerobacter cloacae*. Kauffmann (1954) solved this difficulty by reintroducing the genus *Cloaca* of Castellani and Chalmers (1920) and calling the bacteria *Cloaca cloacae*. Inasmuch as the term "Aerobacter" has been widely used and is familiar to all workers, the following redefinition of that term seems logical: Motile rods conforming to the definition of the family Enterobacteriaceae, fermenting glucose and lactose with acid and gas production. Form two or more times as much carbon dioxide as hydrogen from glucose. Methyl red test negative, Voges-Proskauer test positive. Citric acid and salts of citric acid utilized as sole source of carbon. Gelatin is liquefied. Nonmotile variants and cultures which fail to liquefy gelatin occasionally occur. The type species is *Aerobacter cloacae* (Jordan) Bergey et al. It is obvious that some change must be made in the completely unsatisfactory classification of the Klebsiella-Aerobacter group as it now exists in *Bergey's Manual*. In any event, it must be recognized that, within the group, many aberrant and intermediate forms will be encountered which cannot be pigeonholed neatly into one category or another.

Differentiation of capsular types within the genus *Klebsiella* progressed slowly for a number of years but recently Kauffmann (1949), Brooke (1951), Edwards and Fife (1952), and Edmunds (1954) have characterized a large number of types among cultures previously grouped together as *A. aerogenes*. As pointed out above, these types are indistinguishable from the classical Friedländer types by biochemical methods. This rapid extension of the genus naturally has raised questions regarding speciation within the group as well as discussion regarding the questions raised in the preceding paragraphs. Kauffmann (1949, 1954) recognized but two species, *Klebsiella pneumoniae* and *Klebsiella rhinoscleromatis*, which were distinguished by biochemical methods. This view was accepted by Edwards and Fife (1952) and still seems to the writers to be a logical one. Henriksen (1952) supported Kauffmann's classification of the Klebsiella group but suggested that the organisms be divided into rhinoscleroma, ozaena, pneumonia, and aerogenes subgroups which were differentiated partially by biochemical tests and partially by serologic reactions. The writers contemplated the establishment of a species, *Klebsiella ozaenae*, made up of the biochemically atypical strains which occur so frequently in capsular types 3, 4, 5, and 6. However, analysis of the biochemical data revealed that these cultures could not be differentiated with certainty from other capsular types, particularly type 1. Since it was felt that any species should be a distinct biochemical entity, it was decided that establishment of additional species was not justified. It is felt that one is justified in reporting a Klebsiella culture of types 3, 4, 5, or 6 as belonging to a biochemical variety usually associated with ozaena, but not justified in erecting a species.

In a later series of papers, Henriksen (1954, 1954*a*, 1954*b*, 1954*c*, 1954*d*, 1954*e*) reversed his former support of Kauffmann's classification of the Klebsiella group upon the following grounds: (1) Poor reproducibility of serologic results. (2) Rapid increase in number of types. (3) Increasing number of cross reactions, of closely related types and detection of double types. (4) Questionable stability of the antigenic constitution of some types.

Henriksen emphasized the differences in the cross reactions observed with capsular serums employed by Kauffmann, Brooke, Edwards and Fife, and in his own laboratory. It seems obvious that each set of diagnostic serums prepared, whether they are derived from salmonellae, shigellae, klebsiellae, or *Escherichia coli* serotypes, will differ somewhat in their cross reactions from those of any other set of diagnostic serums. These differences are due in part to varying titers of the serums and to methods of preparation and sensitivity of antigens. It is reasonable to assume that, before any set of diagnostic serums are put into use, their individual titers and cross reactions will be determined and specific absorbed serums prepared as necessary. Certainly, no worker with adequate knowledge of the subject would set out to type shigellae or salmonellae without absorbed serums. There is no reason to assume that a similar situation would not prevail in the Klebsiella-Aerobacter group.

The rapid increase in the number of capsular types admittedly makes the typing of Klebsiella cultures a laborious procedure and each laboratory must decide whether it is profitable to do the necessary work in order to obtain the information thus gained. Within the past two years

the writers have encountered but seven cultures which could not be assigned to one of the established capsule types, yet within that time approximately 500 cultures from various sources and widely separated localities were examined. The question of cross reactions and close relationships between types may be resolved by the use of adsorbed sera, as indicated above. It should be remembered that these close relationships extend to types 4, 5, 6, and 7, the first three of which Henriksen suggests that the average laboratory should be prepared to distinguish. The occurrence of "double types," i. e., types which react with two or more supposedly specific sera, indicates that by no means all of the varieties of capsular antigens in this large group of bacteria have been characterized. Further, it must be realized that cultures assigned to the same type in diagnostic work do not necessarily possess identical capsular antigens. Experience has shown that clearly demonstrable differences exist in the capsules of strains assigned to the same type in diagnostic work. Thus, in actuality, the capsular "types" are not types, but are made up of groups of strains with related capsular antigens. This does not detract from the epidemiological value of typing to any degree and one can always determine the exact relationships of cultures when necessary. The epidemiological value of typing is evidenced in the work of Henriksen (1954*a*) in which two hospital epidemics due to type 30 were detected, although in the same paper the author questions the value and practicability of typing. The epidemiological value of typing in nosocomial epidemics further was demonstrated by the work of Ørskov (1952).

Henriksen (1954*b*, 1954*c*) commented at length upon the relationships that existed between mucoid cultures of *E. coli* and certain Klebsiella types. It is not surprising that these relationships should occur. Ewing, Edwards and Hucks (1952) demonstrated relationships and cross capsular reactions between klebsiellae and encapsulated shigellae. The relationship which exists between mucoid salmonellae and Klebsiella type 13 is well known. All of these reactions are but expressions of the extrageneric relationships which exist among the various groups of enteric bacteria and which extend to the O and H antigens as well as to the envelope, capsular, and slime antigens. The writers are unable to agree with the interpretation toward which Henriksen seems inclined, i.e., that the presence of numerous partial antigens and the occurrence of "double types" suggest the occurrence of complex, variable antigens rather than separate stable types. It is quite possible that a variation akin to form variation which affects the O antigens of salmonellae is operative in the capsular antigens of klebsiellae but such a variation has not been demonstrated. If it does occur, the writers have not found that it interferes seriously with diagnostic typing. The writers are in accord with the views expressed by Kauffmann (1949), Brooke (1951) and Edmunds (1954) that Klebsiella typing is a practicable procedure. Whether a laboratory should expend the time and effort necessary to accomplish the task is a question which must be decided in each individual case, just as it must be decided whether a laboratory is to engage in other specialized diagnostic procedures.

SUMMARY

Biochemical studies of 756 cultures of the Klebsiella-Aerobacter group are summarized. The cultures were divided into 128 motile and 638 nonmotile strains. It was pointed out that no satisfactory method yet has been found to distinguish between *Aerobacter aerogenes* and members of the genus *Klebsiella* and that for the present they should be considered as a single group. Further, no single biochemical test or combination of tests sufficed clearly to distinguish the genus *Klebsiella* and *A. aerogenes* from cultures of *Aerobacter cloacae*, and many aberrant and intermediate strains occurred. The classification of the group was considered and motility and gelatin liquefaction were found most satisfactory in separating the bacteria. It was suggested that the nonmotile forms which do not liquefy gelatin, heretofore classified in both genera, *Klebsiella* and *Aerobacter*, be placed in the former genus and that the latter genus be redefined as a motile, liquefying group with *A. cloacae* as the type.

The 629 nonmotile, capsulated cultures which were included in the study were subjected to capsular typing and the distribution of the capsular types isolated from various sources was given. The practicability and significance of capsule typing were discussed.

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