DESCRIPTION OF A NEW KLEBSIELLA TYPE: CAPSULAR TYPE 15

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THESIS

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DESCRIPTION OF A NEW *KLEBSIELLA* TYPE: CAPSULAR TYPE 15

By

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I. INTRODUCTION

Numerous attempts have been made by various investigators to classify and to define the limits of the encapsulated organisms of the coliform group of bacteria, referred to in the older literature as Friedländer bacilli and in contemporary taxonomy as the genus Klebsiella. Despite the considerable effort that has been expended in the study of these organisms, classification appears to be in a comparatively rudimentary stage. A sound approach has been made to the problem but much remains to be done before an adequate separation is made of encapsulated Escherichia coli, Aerobacter species and Klebsiella types. It is probable that a sharp separation will not be accomplished. Instead, as Edwards and West (1950a) have noted with other groups of the Enterobacteriacae, a central "core" of stable types will be found which overlap in biochemical and antigenic characteristics with those of other genera.

The work of Kauffmann (1949) is the most recent advance in our knowledge of the genus Klebsiella, or, as he prefers to express it, the Klebsiella group. Kauffmann has described relatively sharp limits to the biochemical activity of the Klebsiella and has added to the capsular types established by Julianelle (1926a) and by Snijders and Gosling (1934).

At present 14 types exist. The work to be presented in this thesis is a description of a new type of Klebsiella, to be known as Capsular Type 15.

II. HISTORICAL REVIEW

The application of immunological procedures to the study of encapsulated gram-negative bacilli became possible only after Toenniessen (1914) laid the foundation for the serology of the Klebsiella group. He made the distinction between the body or soma and the capsule or mucous envelope of the organisms in the Friedländer group. The direct relationship between virulence of the bacteria and the carbohydrate nature of the capsule was also emphasized.

The first two described antigens of the Friedländer bacillus were called the S-form or encapsulated form and the R-form or non-encapsulated form. Julianelle (1926a), while working on the serology of this group, found that the capsular antigen was type-specific and polysaccharide in nature. This polysaccharide alone would not produce an antiserum when inoculated into a rabbit, but in combination with the whole organism produced a very potent antiserum. Antisera produced in this way were type-specific and would agglutinate all homologous type organisms and give a passive protection to white mice against infection by strains of the same type. By using type-specific antisera, Julianelle was able to divide the 33 strains of Friedländer bacteria he was

studying into three groups which he called Types A, B, and C, and another not too well defined group, Type X.

Antisera produced by non-encapsulated organisms seemed, according to Julianelle (1926a), to be species-specific. The antigen from non-encapsulated organisms was his so-called R-form or somatic antigen. The antisera produced with these organisms were devoid of specific agglutinins, precipitins, and protective antibodies for the type-specific encapsulated bacilli. The antisera produced with the R-form not only reacted with the immunizing antigen but with all other R-forms, regardless of type.

Edwards (1928), studied the encapsulated gram-negative bacilli of the Enterobacteriacae and established two distinct types which he called Type 1 and Type 2. When these types were compared with those described by Julianelle, Type 1 was found identical with Type B, and Type 2 identical with Type A. The majority of the strains examined were recovered from respiratory infections and belonged in the Type 2 group, while out of 29 strains examined of Type 1, only one was from a respiratory infection. It was observed by Edwards that "type-specificity and virulence of encapsulated bacilli are dependent upon capsule formation." He also felt that if a large number of encapsulated gram-negative bacilli were examined, more types would be found.

In attempts to enlarge the encapsulated group of bacilli, Edwards (1929) found during his examination of a group of cultures labelled Friedländer bacilli derived from

human and animal sources that he could not distinguish them from Aerobacter aerogenes. Some of the cultures isolated from soil, water and milk were found to be culturally, biochemically and serologically identical with Julianelle's Type B. This was the first suggestion that the A. aerogenes and Friedländer bacilli are intimately related. Edwards was unable to demonstrate any constant difference for the separation of A. aerogenes and Friedländer organisms into separate species. He suggested combining the two groups into one species.

Julianelle (1937a), soon made observations similar to those of Edwards and found that three strains of A. aerogenes comprised three type-specific immunological entities. One strain did not appear similar to any of the previously described Friedländer types but reacted with its own antiserum. One reacted with pneumococcus Type II serum while a third reacted with Friedländer Type B and pneumococcus Type II sera. Decapsulation changed the three strains from type-specific to species-specific. The cross-reaction mentioned here with the pneumococcus Type II, was attributed by Julianelle (1926b) to similarities of chemical constitution. The matter of grouping the encapsulated bacilli was summed up by Julianelle (1937b) in the following statement: "By agglutination of R-variants, the encapsulated, gram-negative rods may be classified into two large groups - composed - in one instance, of all Friedländer bacilli, and in the other, of the organisms of rhinoscleroma, ozena, granuloma inguinale and Bact. aerogenes.

Whether the serological distinctions indicate that Friedländer bacilli arise genetically from one source, and the remaining organisms from a second and different source, remains for further investigation to solve."

While examining a group of gram-negative encapsulated bacilli, Snijders and Goslings (1934) established four groups: I. Rhinoscleroma; II. Friedländer; III. Ozena; and a heterogenous group IV. Lactis aerogenes. They confirmed the findings of Julianelle with regard to the three capsular types in the Friedländer strains and introduced three new ozena capsular types, namely D, E, and F. It was also found that the capsular type common to all rhinoscleroma bacteria was identical with Type C in the Friedländer bacteria.

A group of 130 strains of encapsulated gram-negative bacilli were examined by Kauffmann (1949) and compared with the Escherichia group. He included in his examinations bacteria heretofore designated as Friedländer, rhinoscleroma, ozena, aerogenes and aerobacter. Because of priority he designated the group as Klebsiella instead of Aerobacter.

According to Kauffmann (1949), "The Klebsiella group is defined as non-motile, gram-negative, non-sporing rods that do not form indol and which ferment adonitol, inositol and other carbohydrates or alcohols, often breaking down urea and frequently giving a positive Voges-Proskauer reaction and negative methyl-red reaction. As a rule these bacteria grow on Simmons' citrate agar, normally they possess a capsule and most of them form mucous."

Kauffmann's (1949) serological examination showed the presence of four antigens which he designated as: M (mucous envelope antigen); K (capsular antigen); O (somatic smooth antigen); and R (somatic rough antigen). It is probable, according to Kauffmann, that the M and K antigens are serologically identical but this lacks experimental evidence. Of the two antigens, the K is of chief importance in diagnostic bacteriology because of its type-specificity.

The previously described K antigens of Julianelle, and of Snijders and Goslings were confirmed by Kauffmann, with the exception of Type E as described by the latter authors. Kauffmann (1949), with the assistance of Klieneberger-Nobel, was unable to demonstrate a capsule on the only strain of Type E which was available. Eight new capsular types of Klebsiella were described. Kauffmann felt that as further investigations are made new types will be found, and he suggested the use of Arabic numerals in preference to capital letters. Therefore, he identified Types A, B, C, D, E, and F as Types 1-6, respectively; Type 7 represents the capsule of the Lactis aerogenes strain, while 8-14 are designations for new strains isolated from urine. It is noteworthy that Type E, now Kauffmann's Type 5, is given the designation of a Klebsiella type, even though the representative strain is devoid of a specific capsule. Further reference to this fact will be made in the body of this report.

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In the following investigation of a new Klebsiella type, in which Kauffmann's type strains were necessarily

studied, the K antigens only were considered. The work was deliberately limited to a study of the capsules, since, as Kauffmann and others have pointed out, classification of Klebsiella organisms is achieved through the type-specific K antigens.

III. MATERIALS AND METHODS

During two outbreaks of gastroenteritis of newborn infants which were investigated by the Michigan Department of Health, there was isolated from the stools of diarrheal infants a total of eleven cultures which appeared similar in biochemical characteristics. The organisms were gram-negative rods and appeared as mucoid, domed colonies on blood agar and fermented various carbohydrates with production of acid and gas. These attributes, together with their indol, methyl-red, Voges-Proskauer and citrate reactions and lack of motility, indicated that they were probable members of the genus Klebsiella. An antiserum was produced in a rabbit against one culture, later designated as type strain 61 which agglutinated the homologous organism as well as the other 10 strains to a similar titer. This antiserum, moreover, produced distinct capsular swelling of all of the eleven cultures.

The significance of these organisms in the particular outbreaks of infant diarrhea in which they were isolated has not been determined. In view of the fact that Escherichia coli, Type 111, B_4, was isolated in the same outbreaks, in fact from several of the infants excreting the Klebsiella-like cultures, it is probable that the encapsulated organisms

were of secondary importance. However, it was considered important that all the aerobic flora of the stools of diarrheal infants in the outbreaks under investigation be classified.

The eleven cultures from infant sources, together with one other culture from another source, comprised the strains which are designated Klebsiella Type 15 in this study. The twelfth culture was isolated by Miss A. Stoll of Glens Falls Hospital, Glens Falls, New York, and was submitted to us for identification. The organism was obtained from a catheterized bladder urine specimen. The 12 cultures bear the following original isolation numbers:

1.	Kleb.	61	4.	Kleb.	89a	7.	Kleb.	96	10.	Kleb.	105
2.	"	62	5.	"	91	8.	"	97a	11.	"	127b
3.	"	63	6.	"	92c	9.	"	99a	12.	"	678

Klebsiella type cultures were obtained from Dr. F. Kauffmann of the State Serum Institute, Copenhagen, Denmark, and from Dr. P. R. Edwards, Communicable Disease Center, United States Public Health Service, Atlanta, Georgia. The strains received from Kauffmann were as follows:

Type 1	A5054	Type 6	F5052	Type 11	Kleb. 390
" 2	B5055	" 7	Aer. 4140	" 12	" 313
" 3	C5056	" 8	Kleb. 1015	" 13	" 1470
" 4	D5050	" 9	" 56	" 14	" 1193
		" 10	" 919		

Dr. Edwards supplied Klebsiella Type 5, strain Kleb. E5051. This culture was characterized by Dr. Edwards as a slime-producing organism which also possesses a capsule. The latter substance has been demonstrated by Edwards with only one of a considerable series of specific antisera.

A. ANTISERA

Antisera were produced in rabbits for the 14 Klebsiella type strains of Kauffmann and Edwards and for two strains from our own collection, Kleb. 61 and Kleb. 96. The following schedule of inoculation was found effective for the production of most antiserum:

1st inoculation	0.25 ml.	5th inoculation	1.0 ml.
2nd "	0.5 ml.	6th "	1.0 ml.
3rd "	0.5 ml.	7th "	2.0 ml.
4th "	1.0 ml.	8th "	2.0 ml.

The animals were injected on alternate days until the total dosage of 8.25 ml. of antigen had been injected. Forty-eight hours after the last inoculation the rabbits were bled to death from the heart and the blood was processed for separation of the sera. Sterilization of the sera was accomplished by Seitz filtration. Chloroform was added as a preservative and the sera were stored at 5°C in a refrigerator.

B. ANTIGENS

Antigens for rabbit inoculation were four-hour broth cultures which were grown in a modified Duguid medium on the day of animal injection. Formalin in 0.5 per cent concentration was added to the four-hour growth. Whether the cultures were killed at the time of injection is open to question. The animals appeared to tolerate the largest dose of antigen without ill effect.

The modified Duguid medium referred to in the preceding paragraph was developed as a substrate for maximum

capsule production of Klebsiella organisms. The original medium was formulated by Duguid (1948) during a study on media for growth of Aerobacter with large-sized capsules. Duguid found that a minimum of nutrient substance together with a high carbohydrate content was optimum for his purpose. Our modification was as follows:

Salts		Nutrient	
0.2 per cent	NaCl	2.0 per cent	glucose
0.1 " "	K_2SO_4	0.2 " "	peptone
0.025 " "	$MgSO_4$	0.2 " "	yeast extract

No buffering substance was added to the ingredients listed. The various salts and other substances, with the exception of glucose, were combined and the amount of distilled water added to make up the desired volume. Sterilization was carried out at 121°C for twenty minutes. Glucose was added as a filtered 50.0 per cent solution, after autoclaving, to make a final 2.0 per cent concentration.

This medium was used for growth of cultures for agglutination tests, animal inoculation, and for microscopic tests for quellung reaction.

C. STOCK CULTURES

All cultures were preserved by the freeze-dry process and stored in a refrigerator. For convenience, a complete set of the Kauffmann and Edwards' strains, as well as the Michigan cultures, were carried on Dorset's egg medium. Since Klebsiella cultures readily undergo loss of capsules, it was necessary to plate out the cultures in immediate use at three-day intervals and to select single, opaque colonies

for transfer to fresh veal infusion agar plates. The growth on plates constituted the material that was inoculated into modified Duguid's medium.

D. BIOCHEMICAL TESTS

The carbohydrates listed in Table 1 were employed in 1.0 per cent concentration in a liquid medium of the following composition:

Bacto proteose-peptone #3	10.0	gr.
Bacto beef extract	1.0	gr.
Sodium chloride	5.0	gr.
Brom cresol purple	0.015	gr.
Distilled water	1000	ml.

The proteose-peptone, beef extract and sodium chloride were dissolved in distilled water with gentle heating. The pH was then adjusted to 6.9 or 7.0. Brom cresol purple was dissolved in a small amount of distilled water before adding it to the broth. The original volume of the broth was then restored by addition of distilled water. Sterilization was carried out at 121°C for 15 minutes. The carbohydrates were then added aseptically as a sterile 10 per cent solution to make a 1.0 per cent concentration. All carbohydrates which were not fermented within 24 hours after inoculation were reincubated for 30 days. The carbohydrate tubes were closed with sterile rubber stoppers.

The urea medium was that of Stuart, Van Stratum, and Rustigian (1945).

Voges-Proskauer and methyl-red broth were prepared according to the formula of Coblentz (1943). The test for

acetylmethylcarbinol was carried out as advised by Coblentz. Both Voges-Proskauer and methyl-red tests were made routinely after four days' incubation.

Simmons' citrate slants were prepared according to the Difco formula (1948a). These were observed for a period of four days after inoculation.

Potassium nitrate broth was also made according to the Difco formula (1948b). The test for presence of nitrites was made on the fourth day of incubation.

Motility was determined by inoculation of a culture into the semi-solid, gelatin-agar medium of Edwards and Bruner (1942).

The test for H_2S formation was made by placing lead acetate impregnated paper strips over Bacto-peptone broth. Blackening of the paper indicated the presence of hydrogen sulfide.

Bacto-peptone broth was used as the medium for production of indol. The test was carried out by the addition of Ehrlich's reagent after extraction with ether.

E. SERUM ABSORPTION TESTS

Serum absorption tests were made as a proof of similarity or dissimilarity of antigens. Because encapsulated Klebsiellae have a specific gravity very close to that of 0.85 per cent saline solution, or to that of most broth media, centrifugation of a mass of organisms is very difficult. However, a method for carrying out absorption of a small amount of serum was devised which was found to be satisfactory.

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Organisms were grown in veal infusion broth for 16 to 18 hours at 37°C. Roccal* was then added in sufficient amount to make an approximate dilution of 1:3000. The organisms were then packed by centrifugation in an angle-head centrifuge at 4000 r.p.m. They were resuspended in 0.85 per cent saline and again packed by centrifugation. The appropriate serum in 3 to 5 ml. amounts was poured on the packed cells and a thorough mixing of bacteria and serum was made with a pipette. The serum was then freed of bacteria by centrifugation.

Tests for the completeness of absorption varied from tube agglutination to slide agglutination tests, or to observations for capsular swelling, according to the particular need.

* Winthrop Chemical Company, Inc., New York.

IV. RESULTS OF BIOCHEMICAL EXAMINATIONS

Kauffmann (1949) found that Klebsiellae ferment most of the carbohydrates that are used in the study of bacteria of the Enterobacteriacae, with the exception of a variation in the splitting of dulcitol. Strains of the same type vary in their fermentation of dulcitol and strains of different type show this variation. According to Kauffmann, the differentiation of a Klebsiellae from a member of the Escherichia group may be determined by the following criteria:

	Klebsiella	Escherichia
Adonitol	+	-
Inositol	+	-
Indol	-	+
Citrate	+	-
Voges-Proskauer	+	-
Methyl-Red	-	+
Urea	+	-
Motility	-	+

The compiled results of examination of the eleven Michigan cultures and the one New York organism are presented in Table 1. It will be seen that all 12 cultures fulfill the criteria listed above. The variation in fermentation of dulcitol was noted in that cultures 61, 62, 63 and 678 failed to ferment the carbohydrate in 30 days; the other eight cultures fermented dulcitol promptly. Strain 89a was the only one that failed to ferment mannitol.

The remainder of the reactions of the 12 cultures agree in detail with those recorded by Kauffmann (1949) for his type strains. Although biochemical tests were made of the

TABLE 1

Biochemical Examination

	Adonitol	Dulcitol	Sorbitol	Arabinose	Xylose	Rhamnose	Maltose	Salicin	Inosite	Lactose	Sucrose	Mannitol	Glucose	Trehalose	Indol	H_2S	Citrate	KNO_3	V-P	M-R	Urea	Motility
Kleb. #61	A	-30	A	A	A	A	A	A	A	A	A	A^7	AG	A	-	+	+	+	+	-	+7	-
Kleb. #62	A	-30	A	A	A	A	A	A	A	A	A	A^7	AG	A	-	+	+	+	+	-	+7	-
Kleb. #63	A	-30	A	A	A	A	A	A	A	A	A	A^7	AG	A	-	+	+	+	+	-	+7	-
Kleb. #89a	A	A	A	A	A	A	A	A	A	A	A^4	-30	AG	A	-	+	+	+	+	-	+7	-
Kleb. #91	A	A	A	A	A	A	A	A	A	A	A	A^8	AG	A	-	+	+	+	+	-	+7	-
Kleb. #92c	A	A	A	A	A	A	A	A	A	A	A	A^7	AG	A	-	+	+	+	+	-	+7	-
Kleb. #96	A	A	A	A	A	A	A	A	A	A	A	A^8	AG	A	-	+	+	+	+	-	+7	-
Kleb. #97a	A	A	A	A	A	A	A	A	A	A	A	A^8	AG	A	-	+	+	+	+	-	+7	-
Kleb. #99a	A	A	A	A	A	A	A	A	A	A	A	A^8	AG	A	-	+	+	+	+	-	+7	-
Kleb. #105	A	A	A	A	A	A	A	A	A	A	A	A^8	AG	A	-	+	+	+	+	-	+7	-
Kleb. #127b	A	A	A	A	A	A	A	A	A	A	A	A^8	AG	A	-	+	+	+	+	-	+7	-
Kleb. #678	A	-30	A	A	A	A	A	A	A	A	A	A^7	AG	A	-	+	+	+	+	-	+7	-

A = Acid
AG = Acid & Gas
7 = Days after inoculation when positive.

Kauffmann and Edwards cultures, they are not recorded here because of their complete agreement with published data.

V. RESULTS OF SEROLOGICAL EXAMINATIONS

The preparation of immune sera is always attended with uncertainty, both because of irregularities in antigens and because of uncertain response in inoculated animals. Our experience with the production of Klebsiella typing sera followed the usual pattern in that many of the sera were satisfactory and a few were poor. Several of the sera caused unusual difficulties and required repeated immunization of a considerable number of animals before satisfactory products were obtained. With the two types, Klebsiella Type 5 and Klebsiella Type 11, there was complete failure to produce acceptable sera.

Special efforts were made by us to determine if the antigens of Klebsiella Types 5 and 11 are poor immunizing agents, or if the physical nature of the cultures prevents a satisfactory reaction with antisera. The latter conjecture appears to be the probable answer. Both produce an extraordinary amount of slime or extra-cellular mucous. It was possible to observe in untreated preparations of both organisms a few cells which appeared to be encapsulated. When specific sera were applied to these same preparations, there was marked clumping, microscopically, of extra-cellular, clear material which we feel was slime material. Agglutination, visible in

the test tube, probably did not result because agglutinins, as well as capsular swelling antibodies, were bound by slime.

Our experience with Klebsiella Types 5 and 11 was shared by Edwards (1950b) who eventually succeeded in producing a single serum for each type which would produce comparatively poor capsular swelling with specific antigens. We are indebted to Dr. Edwards who examined strain Kleb. 61 and excluded the possibility that this organism could be either Type 5 or Type 11.

In Table 2 are presented the results of tube agglutination tests with Klebsiella type sera and the respective antigens. It will be observed that there are no significant cross-reactions at a dilution greater than 1:20. Type 7 serum produces an agglutination in a dilution of 1:40 with homologous antigen, and a cross-reaction of 1:10 dilution with Type 6 antigen. Kleb. 61 serum has a high titer of 1:160 and also agglutinates antigens of Types 10 and 14 in a 1:20 dilution. With the exception of these slight cross-agglutinations, the results are clear cut. The specific low titers are usual for Klebsiella sera. For example, the highest titer obtained by Kauffmann (1949) for his sera was 1:128.

The tube agglutination test is seldom used in diagnostic bacteriology for typing of Klebsiella. Instead, the capsular swelling test or quellung reaction, is the accepted procedure. In this study the method used in

TABLE 2

Tube Agglutination Examination of Klebsiella Type Cultures and Sera

Sera / Cultures	Kleb.1 FH772	Kleb.2 FH758	Kleb.3 IH445	Kleb.4 FH708	Kleb.5	Kleb.6 GI423	Kleb.7 FH766	Kleb.8 FH741	Kleb.9 IH422	Kleb.10 FH779	Kleb.11	Kleb.12 BI319	Kleb.13 IH453	Kleb.14 FH702	Kleb.61 GI927	Kleb.9 MJ81
leb. 1	20	-	-	-		-	-	-	-	-		-	-	-	-	-
leb. 2	-	80	-	-		-	-	-	-	-		-	-	-	-	-
leb. 3	-	-	80	-		-	-	-	-	-		-	-	-	-	-
leb. 4	-	-	-	40		-	-	-	-	-		-	-	-	-	-
leb. 5	-	-	-	-		-	-	-	-	-		-	-	-	-	-
leb. 6	-	-	-	-		20	10	-	-	-		-	-	-	-	-
leb. 7	-	-	-	-		-	40	-	-	-		-	-	-	-	-
leb. 8	-	-	-	-		-	-	20	-	-		-	-	-	-	-
leb. 9	-	-	-	-		-	-	-	80	-		-	-	-	-	-
leb.10	-	-	-	-		-	-	-	-	80		-	-	-	20	20
leb.11	-	-	-	-		-	-	-	-	-		-	-	-	-	-
leb.12	-	-	-	-		-	-	-	-	-		80	-	-	-	-
leb.13	-	-	-	-		-	-	-	-	-		-	20	-	-	-
leb.14	-	-	-	-		-	-	-	-	-		-	-	160	20	20
leb.61	-	-	-	-		-	-	-	-	-		-	-	-	160	160
leb.96	-	-	-	-		-	-	-	-	-		-	-	-	160	160

All sera were unabsorbed
- =less than 1:10.

pneumococcus typing was found satisfactory. Wet mounts consisting of broth-antigen mixed with antiserum and aqueous methylene blue were placed at one end of a glass microscopic slide. On the opposite end were placed broth-antigen and methylene blue, the control suspension. Cover slips were placed over both the test and control fluids. The wet mounts were examined under the oil immersion lens of the microscope after a period of approximately ten minutes had elapsed from the time of slide preparation. Observation was made for both agglutination and capsular swelling.

Because of the low titer of the sera the first tests were made with undiluted, unabsorbed sera. The results of these tests are recorded in Table 3. It will be seen that a number of cross-reactions exist which were undetected by tube agglutination tests. In most instances both agglutination and capsular swelling cross-reactions were not marked. Exceptions were the agglutination and quellung reaction with Type 14 serum and antigens 6 and 14, as well as the cross-reactions with Types 8 and 9 serums and antigens 8 and 9. Of particular interest is the fact that serums for Kleb. 61 and Kleb. 96 agglutinated and produced capsular swelling with their homologous organisms and their opposite cells, but no cross-reactions with other type antigens were effected. The other specific type sera produced excellent agglutination and quellung reactions with their antigens.

TABLE 3

Undiluted *Klebsiella* Type Sera Tested for Quellung and Agglutination Reactions
Observations Made Microscopically

Serum / Cultures	Kleb.1 FH772	Kleb.2 FH758	Kleb.3 IH445	Kleb.4 FH708	Kleb.5	Kleb.6 GI423	Kleb.7 FH766	Kleb.8 FH741	Kleb.9 IH422	Kleb.10 FH779	Kleb.11	Kleb.12 BI319	Kleb.13 IH453	Kleb.14 FH702	Kleb.61 GI927	Kleb.9 MJ818
Kleb.1	4 MQ	-	-	-		-	-	-	-	-		-	-	-	-	-
Kleb.2	-	4 MQ	-	-		-	-	2 Q	3 Q	-		-	-	-	-	-
Kleb.3	-	-	4 MQ	-		-	-	-	-	-		-	-	-	-	-
Kleb.4	-	-	-	4 MQ		-	-	-	-	-		-	-	-	-	-
Kleb.5																
Kleb.6	3 MQ	-	-	-		4 MQ	2 MQ	-	-	-		-	-	4 MQ	-	-
Kleb.7	-	-	-	-		-	4 MQ	-	-	1 Q		-	-	-	-	-
Kleb.8	-	1 Q	-	-		-	-	4 MQ	1 Q	-		-	-	-	-	-
Kleb.9	-	-	-	-		-	-	4 MQ	4 MQ	-		-	-	-	-	-
Kleb.10	-	-	-	-		-	-	-	-	4 MQ		-	-	-	-	-
Kleb.11																
Kleb.12	-	-	-	-		-	-	-	-	-		4 MQ	-	-	-	-
Kleb.13	-	-	-	-		-	-	-	-	-		-	4 MQ	-	-	-
Kleb.14	-	-	-	-		-	-	-	-	-		-	-	4 MQ	-	-
Kleb.61	-	-	-	-		-	-	-	-	-		-	-	-	4 MQ	4 MQ
Kleb.94	-	-	-	-		-	-	-	-	-		-	-	-	4 MQ	4 MQ

MQ - marked quellung
Q - quellung
1 to 4 - degree of agglutination

TABLE 3

Undiluted Klebsiella Type Sera Tested for Quellung and Agglutination Reactions Observations Made Microscopically

Serum / Cultures	Kleb.1 FH772	Kleb.2 FH758	Kleb.3 IH445	Kleb.4 FH708	Kleb.5	Kleb.6 GI423	Kleb.7 FH766	Kleb.8 FH741	Kleb.9 IH422	Kleb.10 FH779	Kleb.11	Kleb.12 BI319	Kleb.13 IH453	Kleb.14 FH702	Kleb.61 GI927	Kleb.96 MJ818
Kleb.1	4 MQ	-	-	-		-	-	-	-	-		-	-	-	-	-
Kleb.2	-	4 MQ	-	-		-	-	2 Q	3 Q	-		-	-	-	-	-
Kleb.3	-	-	4 MQ	-		-	-	-	-	-		-	-	-	-	-
Kleb.4	-	-	-	4 MQ		-	-	-	-	-		-	-	-	-	-
Kleb.5																
Kleb.6	3 MQ	-	-	-		4 MQ	2 MQ	-	-	-		-	-	4 MQ	-	-
Kleb.7	-	-	-	-		-	4 MQ	-	-	1 Q		-	-	-	-	-
Kleb.8	-	1 Q	-	-		-	-	4 MQ	1 Q	-		-	-	-	-	-
Kleb.9	-	-	-	-		-	-	4 MQ	4 MQ	-		-	-	-	-	-
Kleb.10	-	-	-	-		-	-	-	-	4 MQ		-	-	-	-	-
Kleb.11																
Kleb.12	-	-	-	-		-	-	-	-	-		4 MQ	-	-	-	-
Kleb.13	-	-	-	-		-	-	-	-	-		-	4 MQ	-	-	-
Kleb.14	-	-	-	-		-	-	-	-	-		-	-	4 MQ	-	-
Kleb.61	-	-	-	-		-	-	-	-	-		-	-	-	4 MQ	4
Kleb.96	-	-	-	-		-	-	-	-	-		-	-	-	4 MQ	

MQ - marked quellung
Q - quellung
1 to 4 - degree of agglutination

In Table 4 is shown the effect of dilution in eliminating the cross-reactions recorded in Table 3. A slight dilution of 1:3 or 1:5 was sufficient to produce a specific serum in all but one instance. It was necessary to absorb Type 7 serum with Type 6 antigen in order to eliminate the persistent antibodies for this antigen. By means of dilution and absorption a set of antisera were prepared which could be used with confidence for microscopic observation of agglutination or capsular swelling.

It appears clear from the data presented in Tables 2, 3, and 4 that strains Kleb. 61 and Kleb. 96 were serologically distinct from any of the previously described types of Klebsiella organisms. Sera for Kleb. 61 and Kleb. 96 failed to agglutinate or to produce capsular swelling with any of the 14 known types, including Types 5 and 11. Absorption of Kleb. 61 serum, or Kleb. 96 serum, with antigens of Types 5 and 11 did not reduce quantitatively the agglutinin content of either serum for its specific antigen. This is shown in Table 5. Moreover, the ability of each serum to produce capsular swelling with the appropriate antigen was not impaired by such absorption.

The antigenic similarity of strains Kleb. 61 and Kleb. 96 has been indicated by the fact that their antisera produce mutual agglutination and capsular swelling. Reciprocal absorption completely removed agglutinins and capsular swelling antibodies for the homologous strains.

TABLE 4

Diluted Klebsiella Type Sera Tested for Quellung and Agglutination
Observations Made Microscopically

era / ltures	Kleb.1 1:5 FH772	Kleb.2 1:5 FH758	Kleb.3 Undil. IH445	Kleb.4 Undil. FH708	Kleb.5	Kleb.6 Undil. GI423	Kleb.7 Absor. FH766	Kleb.8 1:5 FH741	Kleb.9 1:3 IH422	Kleb.10 1:5 FH779	Kleb.11	Kleb.12 1:10 BI319	Kleb.13 Undil. IH453	Kleb.14 1:5 FH702	Kleb.61 Undil. GI927	Kleb.9 Undil
eb.1	4 MQ	-	-	-		-	-	-	-	-		-	-	-	-	-
eb.2	-	4 MQ	-	-		-	-	-	-	-		-	-	-	-	-
eb.3	-	-	4 MQ	-		-	-	-	-	-		-	-	-	-	-
eb.4	-	-	-	4 MQ		-	-	-	-	-		-	-	-	-	-
eb.5																
eb.6	-	-	-	-		4 MQ	-	-	-	-		-	-	-	-	-
eb.7	-	-	-	-		-	4 MQ	-	-	-		-	-	-	-	-
eb.8	-	-	-	-		-	-	4 MQ	-	-		-	-	-	-	-
eb.9	-	-	-	-		-	-	-	4 MQ	-		-	-	-	-	-
eb.10	-	-	-	-		-	-	-	-	4 MQ		-	-	-	-	-
eb.11																
eb.12	-	-	-	-		-	-	-	-	-		4 MQ	-	-	-	-
eb.13	-	-	-	-		-	-	-	-	-		-	4 MQ	-	-	-
eb.14	-	-	-	-		-	-	-	-	-		-	-	4 MQ	-	-
eb.61	-	-	-	-		-	-	-	-	-		-	-	-	4 MQ	4 MQ
eb.96	-	-	-	-		-	-	-	-	-		-	-	-	4 MQ	4 MQ

MQ = marked quellung
4 = degree of agglutination

TABLE 5.

Antiserum Kleb. 61

Antigen	Unabsorbed	Absorbed with Type 5	Absorbed with Type 11
Kleb. 61	1:160	1:160	1:160

Antiserum Kleb. 96

Antigen	Unabsorbed	Absorbed with Type 5	Absorbed with Type 11
Kleb. 96	1:160	1:160	1:160

TABLE 6.

Examination for Quellung and Agglutination

Sera / Antigen	Kleb. 61 GI927	Kleb. 96 MJ818
Kleb. 61	4 MQ	4 MQ
Kleb. 62	4 MQ	4 MQ
Kleb. 63	4 MQ	4 MQ
Kleb. 89a	4 MQ	4 MQ
Kleb. 91	4 MQ	4 MQ
Kleb. 92c	4 MQ	4 MQ
Kleb. 96	4 MQ	4 MQ
Kleb. 97a	4 MQ	4 MQ
Kleb. 99a	4 MQ	4 MQ
Kleb. 105	4 MQ	4 MQ
Kleb. 127b	4 MQ	4 MQ
Kleb. 678	4 MQ	4 MQ

4 = complete agglutination.
MQ = marked quellung.

The other ten strains in the Michigan collection are obviously similar antigenically to Kleb. 61 and Kleb. 96. All are agglutinated with the two sera and all show capsular swelling in the presence of either sera. These data are shown in Table 6.

VI. DISCUSSION

Kauffmann (1949) retained strain E5051 (Type 5) in his schema of Klebsiella types regardless of the fact that it did not, in his opinion, possess a capsule. Edwards (1950b) experienced the same difficulties with E5051 that Kauffmann had reported but eventually succeeded with only one serum in showing capsular swelling in some of the cells. The same difficulty was also encountered by Edwards in preparing a capsular swelling serum for strain No. 390, one of the type strains of Type 11. Our experiences with these cultures emphasize the fact that they are useless in the preparation of diagnostic antisera.

For purposes of formal taxonomy there can be no objection to describing the organisms E5051 and 390 as members of the Klebsiella group. Both have biochemical characteristics which ally them to the members of this group. Strain E5051 has "O" antigens which are shared by encapsulated Klebsiella types; strain 390, however, has an "O" antigen which is not shared by any of the other Kauffmann types. In a diagnostic schema where the capsular antigens

determine the type, it would be advantageous to substitute new types of Klebsiellae with well defined capsules for the present representatives of Types 5 and 11.

That new capsular types of Klebsiella will be forthcoming is demonstrated by the description of Klebsiella Type 15. Other undescribed types have been studied by Edwards (1950b) who has found ten types in addition to those in Kauffmann's schema. Brooke (1951) working in Kauffmann's laboratory, is reported to have found 27 new types. Some of these are similar to those of Edwards. Klebsiella Type 15, according to Edwards (1950b) is distinct from any of these.

In the minds of many bacteriologists whose interests are in fields other than enteric bacteriology there is considerable doubt about the advantage of separating various groups of the Enterobacteriacae into serologic types. From the standpoint of academic taxonomy there can be no question that an understanding has been gained from serologic typing which has advanced classification greatly. The chaotic condition of the Shigella nomenclature before serologic typing was applied is an outstanding example.

To the medical bacteriologist and the epidemiologist the value of serologic typing of enteric bacteria is unquestioned. The organism Klebsiella Type 15 is an illustration of this contention. Since the 12 strains mentioned in this report were isolated, the same type of organism has been found in infant stools during an outbreak of infant diarrhea.

One infant excreted Type 15 over a period of approximately two weeks. During the convalescent period the serum of this infant agglutinated Type 15 organisms in a dilution of 1:256. It is possible that Klebsiella Type 15 constitutes one of the causative agents of infant diarrhea. Determination of the significance of this organism is clearly dependent upon one's ability to differentiate it beyond question from similar bacteria which are present in fecal flora.

VII. SUMMARY

During studies on infant diarrhea a series of mucoid, coliform cultures were isolated which proved, on biochemical examination, to have characteristics of Klebsiella. Since the cultures were from two outbreaks it was thought worthwhile to carry out a serological study of them. An antiserum produced against type culture Kleb. 61 produced quellung reaction with the 12 organisms in the collection. This same serum produced no capsular swelling or marked agglutination with any of the 14 capsular type cultures of Klebsiella described by Kauffmann (1949). It appeared, therefore, that a new type of Klebsiella had been found.

Antisera were developed against the Kauffmann type cultures, with the exception of Klebsiella Types 5 and 11, and a complete protocol was prepared of their cross-reactions, by tube agglutination, slide agglutination and

capsular swelling. Biochemical tests were also made of the 14 Kauffmann types.

Antiserum was available for type culture Kleb. 61, but an additional serum was prepared for a second organism, Kleb. 96, from the collection of 12 Klebsiella-like organisms. Reciprocal absorption studies have been made which show that cultures Kleb. 61 and Kleb. 96 are identical antigenically. The sera of Kleb. 61 and Kleb. 96 produce marked capsular swelling for the remaining 10 cultures. Serum Kleb. 96, like serum Kleb. 61, has no effect on the 14 Kauffmann type cultures.

Type culture Kleb. 61 has been shown to possess a different capsular antigen than any of the 14 types described by Kauffmann and will be designated as Klebsiella Type 15.

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