VOLUME XXVII JUNE, 1928 No. 4

AN INVESTIGATION OF THE BACILLI OF THE *CAPSULATUS-MUCOSUS* GROUP.

BY J. BAMFORTH, M.D., M.R.C.P.

(*Assistant Director of Pathology, St Thomas's Hospital, S.E.* 1.)

INTRODUCTION.

THE *Bacillus capsulatus-mucosus* group may be said to have originated with the discovery of the pneumobacillus by Friedländer in 1883. This organism was isolated from the lung in a case of croupous pneumonia, and it is interesting to note, as can be ascertained from a study of the literature, that the discovery appears to have been of an accidental nature, and, as was pointed out by Weichselbaum, that Friedländer had really confused it with the pneumococcus. Subsequently, numerous other bacilli, which showed such marked similarity to the pneumobacillus in morphological, cultural and biological characteristics as to render differentiation among them a matter of extreme difficulty, were isolated by various workers from widely different sources. In a paper published in 1896 Fricke furnishes a list of the representatives of this group of organisms which had up to that time been found. Many of these were subsequently used as type strains by later workers and some are still available and frequently employed.

In addition to the *B. pneumoniae*, this list comprises the following organisms:

1. *B. pseudo-pneumonicus* Passet, isolated from the pus of an abscess.
2. *B. rhinoscleromatis*, from the nose and throat in rhinoscleroma.
3. *B. canalis capsulatus* Mori, from canal water.
4. *B. "proteus" hominis capsulatus* Bordoni-Uffreduzzi, from the blood and organs of two individuals who both died of one of the so-called rag diseases.
5. *B. capsulatus* Pfeiffer, from the peritoneal exudate of a guinea-pig which had died from natural causes.
6. *B. capsulatus* Mandry, from tracheal and bronchial secretion.
7. *B. capsulatus* Kockel, from liver and kidney cysts and pus from meningitis.
8. *B. capsulatus* Loeb, from the infiltration of the stratum corneum of the skin in keratomalacia infantum.
9. *B. capsulatus* Cohn, from pulmonary abscess.
10. *B. capsulatus-mucosus* Fasching, from nasal secretion and tuberculous sputum.
11. *B. capsulatus-mucosus* Paulsen, from nasal secretion in atrophic rhinitis and from laryngeal secretion in ozaenae.
12. *B. capsulatus-mucosus* Abel, from nasal secretion in ozaenae.

13. *B. capsulatus* von Dungern, from thrombosed umbilical arteries in a case of haemorrhagic septicaemia in the new-born.
14. *B. capsulatus* Marchand, from the exudate in croupous pneumonia.
15. *B. capsulatus* Nicolaier, from abscess of kidney in suppurative nephritis.
16. *B. capsulatus* Wicklein, from a case of chronic liver abscess and chronic purulent cholecystitis with diffuse peritonitis.
17. *B. capsulatus* Wright and Mallory, from broncho-pneumonia and from pus of liver abscess and different organs in a case of septicaemia.
18. *B. capsulatus* Chiari, from pus in otitis media and meningitis in a case of pyaemia following suppurative nephritis.
19[1]. *B. crassus sputigenes* Kreibohm, from the sputum and from the surface of the tongue.
20. *B. buccalis muciferens* Miller, from blood and organs of mice which had died after injection of sputum, and
21. B. of sputum-septicaemia Miller from a similar source.

Thus gradually arose a great group of organisms, some of which, judged by the methods of identification then in use, were characterised by minor degrees of difference, whilst others were apparently identical. Yet all showed certain general characteristics. This group of bacteria Fricke designated with the common name "*Bacillus mucosus capsulatus.*"

According to Fricke the general characteristics of this group are as follows: the bacilli are short, possess capsules and show marked pleomorphism; they are non-motile, do not form spores, and are decolorised by Gram's method; in gelatine cultures they form the so-called nail growth, but do not produce liquefaction, and most of them form a moderate amount of indol.

The organisms belonging to this group have been found to be more widely distributed in nature than would be thought, even from a perusal of the list of organisms quoted from Fricke. They have been encountered by many workers outside the body, *e.g.* in the air, in water, in dust and in soil. In healthy human beings it is stated that they have been found in the nose, in the mouth more rarely, in the saliva and in the gastro-intestinal tract. Perkins, in his investigations, was astonished at the frequency with which they were found in plate cultures made as a routine practice at autopsies. In addition, from time to time they were found in a large number of morbid conditions. In diseases of the respiratory tract they were found in rhino-scleroma, in ozaena, in inflammation of nasal passages and sinuses, in lobar and broncho-pneumonia, in abscess and gangrene, and in tuberculous affections of the lungs. In inflammation of serous cavities they have been found in pleurisy, pericarditis and peritonitis. In diseases of the digestive tract, they have been isolated from cases of stomatitis, gastro-enteritis, colitis and from cases showing dysenteric symptoms. In diseases of the urinary tract

[1] This organism should probably not have been included, because it is stated that, though it showed great similarity to *B. pneumoniae* in cultural behaviour, yet it differed in being Gram positive. It also appeared to form spores at 35° C.

they have been found in cystitis, pyelitis, pyonephrosis and pyelonephritis, in abscess and in tuberculous affections of the kidneys. In addition they have been found in otitis media, in meningitis and cerebral abscess and rarely in acute ulcerative endocarditis. Occasionally they have been isolated from cases of general infection usually of haemorrhagic type.

The above enumeration of diseased conditions from which bacilli of the *capsulatus-mucosus* group have been recovered, sometimes in pure culture, sometimes in association with other bacteria, although it comprises by far the greater number, is not to be regarded as comprehensive. From the clinical point of view, their association with pulmonary, intestinal and urinary affections and their frequent occurrence in chronic diseases of the nasal fossae renders these organisms of definite importance.

The classification of such an enormous group of bacteria obtained from so many different sources and showing smaller or greater degrees of difference from one another has always proved a matter of extreme difficulty. The great diversity in cultural, biological, and pathogenic characteristics suggesting classifications from corresponding points of view and the difficulties encountered in attempting a serological classification offer an adequate explanation. So conflicting and so indefinite are the findings of various workers upon this subject that it is quite impossible to draw any conclusions from a careful study of the literature. A few of the classifications from some of the more important references only will be given here.

Working with 14 strains, 6 of which were obtained from cases of otitis media and 6 from cases of enteritis or gastro-enteritis, Fricke was of opinion that differences in the appearance of the growth upon potato were sufficient to warrant a division into two groups: (1) a profuse moist shiny yellowish growth including *B. pneumoniae* and *B. capsulatus-mucosus* Pfeiffer; (2) a profuse viscid almost colourless growth including *B. ozaenae* Abel. Some strains were found to occupy an intermediate position. Perkins, however, although distinguishing different appearances in potato culture with different strains, judging from his own results and from the results of other workers, came to the conclusion that the potato was of no advantage in classification.

Strong attempted to divide the organisms of the *B. capsulatus-mucosus* group into two main types according to differences noted in their growth upon agar. (1) Young colonies colourless, older ones whitish; this type was represented by *B. pneumoniae*, *B. capsulatus-mucosus* Wright and Mallory, *B. ozaenae* Abel, *B. crassus sputigenes* Kreibohm, and perhaps *B. rhinoscleromatis*. (2) Colonies white from the first; this type was represented by *B. lactis-aërogenes* Escherich (isolated from the faeces of breast-fed infants), *B. capsulatus-mucosus* Pfeiffer and *B. capsulatus-mucosus* Kruse (a strain which he obtained from Králs Laboratory at Prague); capsules were harder to demonstrate in this type. This classification however did not receive support from subsequent workers who considered that the appearance of the colonies on agar were inconstant and unreliable.

Clairmont in an exhaustive study comprising observations on the cultural and biological characters and a series of agglutination experiments, which do not appear to be sufficiently comprehensive, put forward the following classification:

Type I. *B. capsulatus-mucosus:*

Species (i) *B. pneumoniae* Friedländer, *B. ozanae* Abel.
,, (ii) *B. capsulatus-mucosus* Fasching.
,, (iii) *B. rhinoscleromatis.*

Type II. *B. aërogenes:*

Species (i) *B. capsulatus-mucosus* Pfeiffer.
,, (ii) *B. lactis-aërogenes* Escherich and *B. coli immobilis* Wilde.

He considered that the *B. capsulatus-mucosus* Pfeiffer occupied an intermediate position between the *aërogenes* and the Friedländer groups.

Perkins suggested a classification based on the fermentation reactions obtained in the following media: lactose, glucose, levulose, maltose, mannite, saccharose, arabinose and glycerin. Group I comprised all the strains which fermented all these carbohydrates with gas formation and includes *B. lactis-aërogenes* Escherich, *B. capsulatus-mucosus* Pfeiffer and the bacillus of haemorrhagic septicaemia Howard. Group II fermented all carbohydrates except lactose with gas formation and includes *B. pneumoniae*, *B. capsulatus-mucosus* Fasching, *B. sputigenes crassus* Kreibohm and *B. ozaenae* Abel. Group III fermented all carbohydrates except saccharose with gas formation and includes *B. acidi lactici* (classified by many authorities in the *B. coli* group) and *B. capsulatus-mucosus* Blumer (isolated from a case of haemorrhagic septicaemia). The position of *B. rhinoscleromatis* was doubtful. Perkins found that the culture with which he worked refused to form gas in any of the media, but Strong found that another more recent culture produced acid and gas in glucose and saccharose, but not in lactose, so that probably it should have been included with *B. pneumoniae* in Group II.

Fricke, Clairmont and Perkins studied the pathogenicity of different strains in laboratory animals, but the results are so variable and inconclusive that no mention is made of them in this paper. Perkins expressed the view that animal inoculation, immunisation and agglutination showed results which are far too variable to admit of classification.

Besson in his *Technique microbiologique et sérothérapique* devotes two chapters to the bacilli of the *capsulatus-mucosus* group. In Chapter XXII under the title "Le pneumobacille de Friedländer" he included in addition to Friedländer's bacillus, *B. lactis-aërogenes* Escherich and the *B. ozaenae* Abel. He states that no valid distinction can be drawn between the *B. lactis-aërogenes* and the pneumobacillus and that the demonstration of the identity of the two organisms sketched by Denys and Martin was completed by Grimbert and Legros, whose observations were confirmed by Bertarelli, who

considered the *B. lactis-aërogenes* to be merely a variety of the pneumobacillus and cannot be separated from it. In Chapter XXXIII under the title "*Bacillus mucosus capsulatus*" he describes three organisms, namely, *B. rhinoscleromatis*, *B. mycogenes*, which was isolated by Edwards from infected wounds and which shows all the characteristics of *B. rhinoscleromatis*, and *B. capsulatus-mucosus* Fasching. He states that these three organisms appear to belong to one species, of which at the most they constitute different races.

Besson holds that the pneumobacillus ferments sugars with production of gas and reduces neutral red, whilst *B. mucosus capsulatus* ferments sugars without gas formation and does not reduce neutral red, but that microscopically and culturally they are identical. He also quotes Bertarelli to the effect that a serum prepared against the pneumobacillus agglutinates both the pneumobacillus and *B. lactis-aërogenes*, but that it is without effect upon the bacilli of Group B described in Chapter XXXIII. He mentions that active sera are difficult to prepare against the bacilli of the latter group and that agglutination tests give variable results.

Small and Julianelle investigated the biological and serological characters of 27 strains of the *B. capsulatus-mucosus* group. Of these 11 strains were obtained from cases of inguinal granuloma, 13 were of respiratory origin, 2 were stock cultures of *B. aërogenes* and 1 a stock culture of *B. rhinoscleromatis*. In order to eliminate capsules they resorted to prolonged cultivation on plain agar for several months, but were not successful in all instances. Nine agglutinating sera were prepared but 8 of the 27 strains were not agglutinated by any of them. Six strains were agglutinated, usually only in high serum concentration, by one serum. The remaining 15 strains showed a tendency to group agglutination, each by three or four of the immune sera. Further, they observed that capsule bearing strains agglutinated only in high concentration of immune sera and that the reactions more closely resembled precipitin reactions, because the volume of the clumped mass was larger than that to be expected from the turbidity of the antigens. Capsule-free strains, which were the most prone to be affected by group agglutinins, agglutinated in serum dilutions as high as 1/2500 and gave a finely granular precipitate. They found that a serum might agglutinate a heterologous antigen to a much higher titre than the homologous antigen. Heterologous strains which were agglutinated by a certain serum were inactive in removing homologous agglutinins of considerably less titre. It was noted also that common immunologic strains showed diverse carbohydrate reactions.

Such anomalous results must of necessity cause considerable difficulty in the classification of this group of organisms on a serological basis, and in his later work Julianelle sought to provide an explanation by applying the principles governing the immunological relationships of the pneumococci. Heidelberger and Avery, Avery and Morgan, and Avery and Neill showed that in the pneumococcus the bacterial cell was made up of two constituents: (1) a polysaccharide—the soluble specific substance which endows the cell

with type specificity, (2) a protein which, regardless of type derivation, exhibits immunologically only the common and undifferentiated characteristics of the species. The dissociation of the polysaccharide from the cell deprives the organism of its type specific antigenic power. They state that in general antipneumococcus serum contains both the type specific and the species specific antibody, the occurrence of the latter depending upon the extent of the dissociation of the antigenic complex which may take place both *in vivo* and *in vitro*. The presence of the protein antibody in appreciable amounts may therefore mask and sometimes obliterate the type specificity of an organism. Under unfavourable conditions the organisms are known to lose the function of elaborating the soluble specific substance—a condition resulting in loss of type specificity and capsule formation and accompanied by loss of virulence. These degraded strains lose the property of stimulating the type specific antibody and as antigen provoke only the common protein response (Stryker, Reimann and Amos). Working on these lines with 30 strains of Friedländer's bacilli, 22 of which were obtained from human sources, chiefly from cases of lobar pneumonia, Julianelle obtained three types, A, B, and C, and Group X, a heterogeneous group of bacilli which did not fall into any of the three types. The bacilli of Type B were also agglutinated by the antiserum of pneumococcus Type II, as had already been shown by Avery, Heidelberger and Goebel. This grouping was supported by precipitin reactions in which he showed that the polysaccharide of each type reacted only with the immune serum of the corresponding type and occurred at a high dilution of the polysaccharide.

Just as Arkwright had described smooth and rough types of colonies for the intestinal group of bacteria, so Julianelle in a second paper distinguished smooth and rough (S. and R.) types of Friedländer's bacillus, as had been indicated by previous workers. He stated that the change from smooth to rough is accompanied by (1) loss of capsule and mucoid characteristics, (2) loss of agglutinability in type specific sera, (3) attenuation of virulence, (4) rough and irregular appearance of the surface of the colonies. For destruction of capsules he employed two methods—(i) subculture for several days in broth containing homologous serum, (ii) hydrolysis with weak acid according to the method of Porges. He found that anti R. sera did not agglutinate S. strains, even though the organism used for immunisation was derived from the S. strain later used for agglutination, and that anti R. sera contained no precipitins for the soluble specific substances of the corresponding S. strains. An immune serum prepared against an encapsulated strain of Type A contained only a small amount of the species antibody which was equally operative against four R. strains, each of which was derived from an S. organism of a serologically different type. All R. strains were found to be reciprocally agglutinated by each of the different anti R. sera. The agglutinin titres of anti R. sera were high (1 in 2500); of anti S. sera low (1 in 40, 1 in 80). Absorption of an anti R. serum with any R. strain removed both homologous

and heterologous agglutinins. Encapsulated organisms heated in the presence of acid were not appreciably agglutinated in purely anti S. sera of types serologically different from that yielding the decapsulated cells, such agglutination depending upon the presence of species antibody. In antisera of the parent strains there was a definite precipitin reaction referable to the presence of unhydrolysed soluble specific substance.

In a third paper Julianelle showed that the polysaccharide, the soluble specific substance of Friedländer's bacillus, is non-antigenic when dissociated from the cell, but that the nucleo-protein was antigenic. Anti-protein sera did not agglutinate encapsulated organisms of either homologous or heterologous type, but agglutinated equally well and in high dilution capsule-free organisms of any of the serologically different types. In addition anti-protein sera of Friedländer's bacillus caused precipitation of protein derived from any of the different types and from *B. aërogenes*, *B. coli* and the granuloma bacillus. This phenomenon, he considered, lent considerable assistance in the interpretation of the results of former investigators who observed that anti-Friedländer sera caused agglutination of *B. rhinoscleromatis* (Sicard, von Eisler and Porges, Galli-Valerio, Fitzgerald), *B. aërogenes* (Bertarelli), *B. typhosus* (Klemperer and Scheier) and granuloma bacillus (Small and Julianelle). The explanation of such cross agglutinations was, he thought, to be found in the fact that immunisation with non-encapsulated strains stimulated the formation of agglutinins, which act not only on R. cells of Friedländer's bacillus, but also on R. cells of a closely related species.

The object of this investigation has been to study the organisms of the *B. capsulatus-mucosus* group which are found in association with disease in man, to ascertain what types of these bacteria commonly occur, to attempt some form of classification rather from a point of view of determining if a particular type is associated with a particular disease, and to enquire as to the pathogenicity or non-pathogenicity of the diseased condition.

For this purpose 81 strains of bacilli belonging to this group have been collected. Of these 17 were isolated by routine investigation from the nasal discharge of 60 patients attending the Out-patient Department of St Thomas's Hospital for diseases of the nose and throat. The remaining 64 strains have been obtained from specimens which have been sent to the Pathological Department for bacteriological examination. Of these 64 strains, 32 have been isolated from faeces, 16 from urine, 9 from sputum, 2 from nasal swabs, and 5 from miscellaneous sources which will be mentioned later. The fact that these 64 strains are all that have been collected in the laboratory during the past three years demonstrates that these organisms are not very frequently encountered.

In the course of routine bacteriological examination in the laboratory, plate cultures of litmus lactose agar are made directly from specimens of urine. For specimens of faeces and sputum small portions are dried upon

porous tiles according to Dudgeon's method, as described by Wordley, with a view to effecting elimination of water, and from the resulting powder plate cultures are made. Litmus lactose agar, blood agar and simple nutrient agar are the media usually employed. From the series of 60 patients attending the Throat Department for diseases of the nose and sinuses nasal swabs were taken, and from these plate cultures of litmus lactose agar were made directly.

The recognition of the colonies of the *capsulatus-mucosus* group upon litmus lactose agar plates is in most cases not very difficult. A short account only is given here, but a more detailed description of cultural characteristics with the differences between the various strains will be found later. After 24 hours' incubation the colonies are found to be circular in shape and raised in a markedly spherical manner from the surface of the medium. The centre of the colony often shows a greater or less degree of opacity. Viewed by transmitted light the edge is smooth and the periphery of the colony clear and transparent. As the majority of the strains investigated produce acid in media containing lactose these colonies are pink in colour. Lastly, the glistening and mucoid appearance and the sticky nature of the colonies when tested by the platinum loop, although varying in degree in different strains, are so constant as to be of very great importance. Further, incubation shows a progressive increase in size of the colonies, and although in most strains the opacity of the central portion is more intensified and in a large number the colour changes to blue, yet despite the increased size the characters described above are well preserved. Examination of stained films from such colonies show Gram negative bacilli with larger involution forms, but slight differences in morphology are met with in different strains. The capsules of these bacilli, as examined in contact with the body fluids, *e.g.* in sputum, faeces and nasal discharge, are quite easily detected. But in films made from the colonies on plate cultures or from the growth on sloped agar by various methods recommended in bacteriological text-books, I have not been successful in demonstrating them, except in a very few cases. In all the strains examined a true motility has never been observed.

At times a certain amount of difficulty is experienced in distinguishing the colonies of *B. capsulatus-mucosus* from colonies formed by certain strains of *B. coli*. Occasionally, colonies are formed by organisms of the colon group which appear to be more glistening and mucoid than usually obtains. But after further incubation for one or two days the difficulty will be found to have disappeared. The colonies have become much duller and more opaque throughout; no clear bordering zone can be distinguished, and the resemblance is no longer seen. At times one also meets with colonies similar to those of *B. capsulatus-mucosus* formed by air organisms; but the examination of stained films will frequently show both bacilli and spores which in many cases are Gram positive, and on further incubation such colonies often become dry, crusty and corrugated.

The colonies of the bacilli of the *B. capsulatus-mucosus* group as above

described were picked off the culture plates and grown separately upon agar slopes. It is important to take more than one colony, because more than one strain may be present in the same specimen.

The fermentative reactions were tested upon the following media: lactose, glucose, mannite, maltose, saccharose and dulcite; inulin, salicin and starch were also employed, but as no additional advantage was derived from their use, the reactions with the first six media only are shown in this paper. The media were made with a Lemco broth basis and contained 1 per cent. peptone and 1 per cent. carbohydrate. As indicator 1 per cent. of 0·06 per cent. phenol red was used. The reaction with milk was also tested. In all cases these cultures were incubated for 10 days and the results noted daily. No precise calculations with regard to the nature and amounts of acid and gas formed were undertaken.

The medium used for determining the production or non-production of indol was a solution containing 1 per cent. peptone (Hopkins and Williams) and 0·5 per cent. sodium chloride and made up with distilled water. It was sterilised in the autoclave at 110° C. for 20 minutes. All the organisms investigated were grown in the medium at 37° C. for 10 days before the test for indol was done. The reagent employed was Ehrlich's paradimethylamidobenzaldehyde. On addition of this reagent to the peptone water cultures by gently pouring a little down the side of the test tube so as to form a layer on the surface, and on subsequent application of gentle heat, it was found that all strains tested gave a positive indol reaction without requiring the addition of an oxidising reagent such as potassium persulphate. These results were so much opposed to the results obtained by previous workers, many of whom found that a large number of strains of *B. capsulatus-mucosus* were negative, that the above method was discontinued, and all the 81 strains were tested for indol formation by preliminary extraction with ether. The cultures were shaken vigorously with ether and then allowed to settle. A little of the Ehrlich's reagent was carefully added so as to form a layer between the ether and the culture medium, and then finally saturated potassium persulphate was carefully added. If indol was present the typical rose coloration was formed between the ether and the culture medium. The results obtained by this method were quite different, and a very large number of the strains were found to be negative.

All strains were tested for haemolysis by incubating for 24 hours in tubes containing peptone and sodium chloride in which human red cells were added according to the procedure adopted for the examination of *B. coli* by Dudgeon, Wordley and Bawtree.

In addition, the effect upon gelatin was studied by incubating at 22° C. for 14 days upon gelatin slopes. A certain number of stab-cultures were made in gelatin for the purpose of examining the so-called nail growth, but as from the point of view of this research no useful purpose appeared to be served, this procedure was not used as a routine practice.

In order to make classification among the various strains, rabbits were inoculated intravenously with the object of making agglutinating sera. In the majority of cases living cultures could be used from the commencement, but in a few killed cultures were used for the first two or three injections and live cultures subsequently. The strength of the agglutinating sera produced differed considerably with different strains. With some strains agglutinating sera with high titres were easily produced, but with others, especially for example those isolated from nasal secretion, the titres of the sera produced were low. It will be shown later that it was important to employ both formolised Dreyer and live agar emulsions as antigens in performing the agglutination tests.

For the purpose of identification of the 81 strains of *B. capsulatus-mucosus* comparison was made not only between strains isolated from the different habitats, *e.g.* faeces, urine, nasal discharge, etc., but also with certain type strains obtained from the Lister Institute of Preventive Medicine. The type strains from the Lister Institute were the following: *B. lactis-aërogenes* 124, *B. lactis-aërogenes* 243, *B. lactis-aërogenes* 418 (American Museum of Natural History), "Leather bacillus" 672 (isolated by Houston from leather washer of water tap and considered to be closely related to *B. lactis-aërogenes*), *B. pneumoniae* Friedländer, probably original Friedländer strain, *B. rhinoscleromatis* (isolated in Sumatra from case of rhinoscleroma), and *B. ozaenae* 459 (Ferry).

Bacilli of the *capsulatus-mucosus* Group isolated from Nasal Discharge.

Clinical Notes. Nineteen strains of *B. capsulatus-mucosus* were recovered from cases of disease of the nasal passage and sinuses. Of these, as mentioned above, 17 were obtained by routine investigation of 60 patients attending the Out-patient Department for nasal disease. Two additional strains, 6947 and F.A.P., were obtained from the nasal discharge of two individuals similarly affected. In the great majority of these cases the affection was of long standing, and the diagnoses include such conditions as chronic ethmoiditis with ozaena, atrophic rhinitis, chronic maxillary antritis, chronic frontal sinusitis and ethmoiditis.

Cultural Characteristics. The colonies formed upon agar plates are characterised by a greater transparency and a more gelatinous appearance as compared with colonies formed by strains isolated from other sources. In addition, the growth upon agar slopes shows little opacity and a much more sticky consistence which is retained for long periods of time and after subculturing at intervals of 3 or 4 weeks for many months shows but little impairment. Especially noteworthy in this respect were the strains N. 41 and N. 58.

Considerable variation is shown in the fermentation reactions of these 19 strains. With the exception of N. 52 all strains produced either acid or acid and gas in lactose. All produced either acid or acid and gas in glucose,

mannite and maltose. Three strains N. 4, N. 52 and N. 60 produced acid, but not gas, in these media, whilst all the remainder with the exception of strain F.A.P. produced acid and gas. The strain F.A.P. showed reactions intermediate in type, giving acid only in lactose and glucose, but acid and gas in maltose and mannite. Ten strains only attacked saccharose, and it is significant that the strain N. 8 was the only one which fermented dulcite. It is important to note that, when both acid and gas were formed, the amount of gas was quite small. The reactions obtained in milk were variable, some producing acid, others both acid and clot. All strains were found to be non-haemolytic. None produced indol or liquefied gelatin (see Appendix, Table I).

Serological Reactions. Agglutination tests were performed with two sera prepared against the strains 6947 and N. 1. Attempts to produce agglutinating sera of high titre against these two organisms were unsuccessful. The serum prepared against N. 1 after five intravenous injections of live agar emulsions ranging from 200 to 4000 millions of bacilli possessed a titre of only 1 in 400 against its homologous antigen. The experience of a few strains showed that there was no appreciable difference in agglutination if either live agar emulsions or formolised Dreyer suspensions were used as antigens. The results shown in Table I are those obtained with live agar emulsions. The sera 6947 and N. 1 agglutinated all strains except N. 8, N. 41 and N. 58. By this common agglutinative property a group of 16 strains is apparently indicated.

Further consideration will be given later to strains N. 8, N. 41 and N. 58.

Table I.

Live agar emulsions	6947	N. 1	N. 4	N. 5	N. 8	N. 23	N. 24	N. 32	N. 35	N. 41
Serum 6947	200	100	100	50	0	50	50	50	50	0
Serum N. 1	200	400	200	200	0	50	—	100	50	0

Live agar emulsions	N. 50	N. 51	N. 52	N. 53	N. 54	N. 56	N. 58	N. 60	F.A.P.
Serum 6947	100	50	50	100	50	50	0	50	100
Serum N. 1	100	50	50	50	50	—	0	50	—

BACILLI OF THE *CAPSULATUS-MUCOSUS* GROUP ISOLATED FROM SPUTUM.

Clinical Notes. Nine strains of bacilli numbered S. 1 to S. 9 were recovered from sputum. The strain S. 1 was recovered from the sputum of a patient suffering from bronchiectasis. Two strains S. 2 and S. 3 were isolated from the sputum of one individual with the same disease. The organisms S. 4 and S. 5 were obtained from two patients suffering from tonsilitis and naso-pharyngeal catarrh, and S. 6 from a patient with chronic pulmonary fibrosis. The strain S. 8 was recovered from a patient who was expectorating a large quantity of very purulent sputum containing a large amount of blood but in whom no physical signs were discovered. No tubercle bacilli were found in this sputum.

Cultural Characteristics. The strains S. 7, S. 8 and S. 9 showed cultural characteristics upon agar and fermentation reactions similar to those described for the bacilli isolated from nasal discharge. With the exception of S. 5 the

remaining strains formed colonies upon agar plates which showed a greater degree of opacity than in the case of the "nasal" strains. The growth upon agar slopes was more opaque, less gelatinous in appearance and less viscid when tested with the platinum loop. In cultures a few days old, and especially after repeated subculturing, these differences became accentuated. The strain S. 5 formed colonies upon agar plates very mucoid and gelatinous in appearance and a very sticky growth upon agar slopes. There was considerable variation in the fermentation reaction of strains S. 1 to S. 6. Strains S. 1 and S. 3 acidified without gas formation lactose, glucose, mannite, maltose and saccharose: S. 2, S. 4, S. 5 and S. 6 produced acid and gas in these media with the exception that S. 5 did not affect lactose. In the case of S. 5 and S. 6 the acidity was shortly succeeded by alkalinity, a phenomenon described by Besson by the term "caméléonage." The amount of gas formed was considerably greater than in the case of the "nasal" strains. Dulcite was unaffected by all six strains. The reaction in milk varied, some producing acid, others acid and clot. S. 4 was the only strain which produced indol. All strains were non-haemolytic, and none produced liquefaction of gelatin (see Appendix, Table II).

Serological Reactions. Strains S. 7, S. 8 and S. 9 were agglutinated in live agar emulsion by serum 6947 in dilutions of 1 in 100, 1 in 100, and 1 in 50 respectively. All the remaining strains with the exception of S. 5 were agglutinated by S. 1 serum as is shown in Table II. The importance of using both formolised Dreyer (F.D.) and live agar (L.A.) emulsions is also demonstrated. The agglutination reactions of the strain S. 5 will be shown later.

Table II.

Sera	S. 1		S. 2		S. 3		S. 4		S. 5		S. 6	
Type of emulsion	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.
S. 1	5000	1000	1000	0	2000	0	1000	500	0	0	250	250

BACILLI OF THE *CAPSULATUS-MUCOSUS* GROUP ISOLATED FROM FAECES.

Clinical Notes. Thirty-two strains numbered F. 1–32 were recovered from the faeces of 32 patients. Eleven strains were isolated from cases of ulcerative colitis, 11 from cases of gastro-enteritis, enteritis or dysentery, 5 from cases of typhoid or paratyphoid B fever in convalescence, 1 from a typhoid carrier and 1 from a case of pernicious anaemia. In the three remaining cases no clinical data were obtained. It is interesting to note that one strain (F. 25) was recovered almost in pure culture from the faeces of a girl at a boarding school in which a small outbreak of enteritis occurred. This outbreak was investigated by Dr C. F. Selous, who kindly submitted specimens of milk and cheese supplied to the school which also contained organisms of the same group.

Cultural Characteristics. The appearance of the colonies on agar plates and of the growth upon agar slopes produced by these 32 faecal strains

correspond to those described for the organisms S. 1, S. 2, S. 3, S. 4 and S. 6 isolated from sputum. Such degrees of differences as might occur in various strains were so slight as to be of no value in differentiation. The fermentation reactions of these 32 strains showed marked differences. Twelve strains produced acid and gas in all six media, lactose, glucose, mannite, maltose, saccharose and dulcite. Of the remaining 20 strains all fermented lactose, glucose, mannite and maltose; 12 did not ferment either saccharose or dulcite; 7 fermented saccharose but not dulcite, whereas 1 strain fermented dulcite but not saccharose. Acidity was always accompanied by gas formation which was again much larger than obtained in the case of strains isolated from nasal discharge. With further incubation a large proportion of the strains showed the phenomenon of "caméléonage." All strains formed acid and clot in milk. Twelve strains gave a positive indol reaction. All were found to be non-haemolytic. One strain only, F. 4, produced liquefaction of gelatin, a property which was confirmed by repetition of the experiment (see Appendix, Table III).

Serological Reactions. The results of the agglutination reactions of the 32 faecal strains are shown in Table III which also demonstrates the importance of employing as antigens both formalised Dreyer and live agar emulsions. A number of positive results would not have been obtained if only one type of antigen had been used. In order to obtain a clear representation of the relationship existing between the various strains 15 different agglutinating sera were prepared. It is shown that 9 strains, F. 1, F. 2, F. 5, F. 6, F. 8, F. 11, F. 12, F. 18 and F. 21, are very closely related if not identical in that they exhibit a common agglutination with sera prepared against F. 1 and F. 6. The strain F. 9 is agglutinated by F. 1 serum in high dilution, but fails to show any result whatever with F. 6 serum: in addition, the strain F. 9 was also agglutinated by sera prepared against F. 3, F. 9, F. 10, F. 15, F. 16, F. 17, F. 20, F. 24 and F. 25, although none of these except the serum F. 24 produced any reaction with the strain F. 1. This shows a distinct connection between the 9 strains first mentioned and a number of other strains. A further consideration of this table on similar lines shows that with the exception of F. 27 a relationship can be traced amongst all the remaining 31 strains and probably, if additional sera had been prepared and further tests done, the strain F. 27 would have been brought into line also. It would appear that the differences both in cultural and serological properties exhibited by these organisms are really rather of minor importance and that these organisms are descendants of some common ancestor.

BACILLI OF THE *CAPSULATUS-MUCOSUS* GROUP ISOLATED FROM URINE.

Clinical Notes. Sixteen strains numbered U. 1–16 were recovered from the urine of 16 patients suffering from urinary affection. In the large majority of these cases the disease was recent and the urine was acid, contained numerous pus cells and gave a pure growth of *B. capsulatus-mucosus*. In a few cases the urine at first showed a pure infection with *B. coli* (in one case

Table III.

Sera	F. 1		F. 3		F. 6		F. 9		F. 10		F. 13		F. 15		F. 16		F. 17		F. 19		F. 20		F. 22		F. 24		F. 25		F. 27	
Type of emulsion	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.
Strains																												
F. 1	500	10,000	0	0	0	10,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0
F. 2	20,000	50	—	—	10,000	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F. 3	0	0	200	100	0	0	0	0	0	0	0	1000	0	0	0	0	25	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 4	0	0	0	0	0	0	0	0	400	0	0	0	0	0	500	0	0	—	0	500	—	—	—	—	0	50	100	0	0	—
F. 5	20,000	20,000	—	—	10,000	5,000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F. 6	20,000	20,000	—	—	5,000	200	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F. 7	0	2,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	200	0	200	50	—	—	—	—	250	1000	0	0	0	0
F. 8	4,000	5,000	—	—	4,000	2,000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F. 9	2,000	5,000	400	0	0	0	2000	2000	2000	1000	0	0	250	0	500	250	25	0	0	0	100	100	0	0	250	100	0	500	0	0
F. 10	0	0	0	0	0	0	0	0	2000	1000	0	0	0	0	0	0	25	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 11	20,000	10,000	—	—	2,000	2,000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F. 12	20,000	10,000	—	—	2,000	2,000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F. 13	0	0	0	0	0	0	0	0	0	0	100	500	0	0	0	0	25	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 14	0	0	0	200	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 15	250	0	0	0	0	0	0	0	0	0	0	0	2000	250	0	0	0	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20,000	10,000	0	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	200	0	0	0	0	0	0	0	0	0	0	0	0
F. 18	20,000	20,000	—	—	10,000	5,000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F. 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	250	250	0	0	25	50	0	0	0	0	0	0
F. 20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	50	0	0	0	0	0	0	0	0
F. 21	20,000	20,000	—	—	10,000	10,000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F. 22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	200	0	0	0	0	0	0
F. 23	0	0	0	0	0	0	50	50	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—	0	0	100	100	0	0
F. 24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	400	0	4000	50	0	0	0	0
F. 25	0	0	0	0	0	0	50	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0
F. 26	100	0	0	0	0	0	250	0	250	0	0	0	0	0	50	0	0	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	400	400
F. 28	0	0	0	0	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 29	0	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0	0	0	0	0	—	—	—	—	0	0	0	0	0	0
F. 30	0	0	0	0	250	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. 31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	50	0	0	0	0	0	0	0	0	0	0
F. 32	100	0	0	0	100	0	100	0	500	0	0	0	100	0	100	0	0	0	0	0	—	—	—	—	0	0	0	0	0	0

F.D. = formol Dreyer.

L.A. = live agar.

with *B. proteus*), but specimens obtained later in the course of the disease showed a large growth of *B. capsulatus-mucosus* in addition. In some cases a condition of pyelitis was present, but in the majority the infection was probably limited to the bladder.

Cultural Characteristics. In general the cultural characteristics of the growth formed upon agar plates and slopes by the urinary strains are like those found in the case of the faecal strains. A certain number of these organisms, however, as exemplified by the strain U. 6, showed a greater translucency and viscidity of growth in this respect approaching that formed by the strains isolated from nasal secretion. The fermentation reactions of these 16 strains corresponded in general to those obtained with the faecal strains. With the exception of two, U. 5 and U. 12, all fermented lactose. A few strains showed the phenomenon of "caméléonage," and a number produced indol from peptone water. One strain, U. 13, showed definite haemolytic properties, but all failed to liquefy gelatin (see Appendix, Table IV).

Serological Reactions. Table IV shows the agglutination reactions. Ten agglutinating sera were prepared by means of which all strains were agglutinated with the exception of strain U. 15. Here again the necessity of using formolised Dreyer and live agar emulsions was experienced. In the case of the urinary strains such a close inter-relationship as was shown in the faecal strains was not demonstrated. The strain U. 9, for example, was agglutinated only by serum U. 9, which serum reacted with no other strain in the series. Nevertheless a study of this table shows that a relationship can be established amongst the majority of the members of this series.

BACILLI OF THE *CAPSULATUS-MUCOSUS* GROUP ISOLATED FROM MISCELLANEOUS SOURCES.

Clinical Notes. Six strains were obtained from miscellaneous sources. Of these one was obtained from human milk (74 milk); one from purulent discharge from a faecal fistula in a case of septicaemia (Eales pus); two from the cow's milk and from the cheese which constituted part of the food taken for examination at the time of the outbreak of enteritis in the boarding school to which reference has been previously made (p. 354); one from the cervical discharge of a patient suffering from chronic gonorrhoea (V.D. 6913); and one from the conjunctival sac prior to operation for cataract (Saville).

Cultural Characteristics. With the exception of the strain "Saville" these organisms showed cultural characteristics upon agar like those shown in the case of the faecal strains. The strain "Saville" however showed appearances like those found in strains N. 41 and N. 58 isolated from nasal discharge. The fermentation reactions show similar variations to those mentioned in the case of the strains isolated from other sources. All fermented lactose. Two strains, 74 milk and V.D. 6913, showed "caméléonage." The strains isolated from cow's milk and from cheese produced indol. All were non-haemolytic and none liquefied gelatin.

Table IV.

Sera	U. 3		U. 4		U. 5		U. 6		U. 7		U. 8		U. 9		U. 10		U. 11		U. 12	
Type of emulsion	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.
Strains																				
U. 1	0	0	0	500	0	0	0	0	0	500	0	0	0	0	0	0	0	500	100	0
U. 2	200	0	400	500	0	0	500	0	0	0	0	0	0	0	0	0	0	0	50	0
U. 3	50	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. 4	0	0	250	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. 5	0	0	0	0	100	500	0	0	0	0	0	0	0	0	0	0	0	0	0	100
U. 6	200	0	0	0	25	0	5000	2500	0	0	100	0	0	0	0	0	0	500	0	0
U. 7	0	0	0	0	0	0	0	0	20,000	20,000	0	0	0	0	0	0	0	0	0	0
U. 8	0	0	0	200	0	0	0	0	0	50	0	1000	0	0	0	25	0	200	0	0
U. 9	0	0	0	0	0	0	0	0	0	0	0	0	500	1000	50	0	0	0	0	0
U. 10	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	200	250	0	0	0
U. 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0
U. 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2500	10,000
U. 13	1000	1000	0	0	0	0	250	250	0	0	100	100	0	0	25	0	0	0	100	0
U. 14	0	0	0	0	0	1000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. 16	0	0	0	0	0	100	500	0	0	0	100	200	0	0	0	50	0	50	0	0

F.D. = formol Dreyer. L.A. = live agar.

https://doi.org/10.1017/S0022172400032071 Published online by Cambridge University Press

Serological Reactions. The agglutination reactions of these organisms with a serum prepared against the strain 74 milk are shown in Table V.

Table V.

Strains	74 milk		Eales pus		Cow's milk		Cheese		V.D. 6913		Saville	
Type of emulsion	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.
Serum 74 milk	250	500	0	0	0	0	0	0	0	10,000	—	—

F.D. = formol Dreyer. L.A. = live agar.

Serum 74 milk was found to agglutinate the strain V.D. 6913 and the homologous strain, but it failed to react with the strains "Eales pus," "Cow's milk" and "Cheese."

A COMPARISON OF THE CULTURAL CHARACTERISTICS AND SEROLOGICAL REACTIONS OF THE STRAINS OF *B. CAPSULATUS-MUCOSUS* ISOLATED FROM DIFFERENT SOURCES WITH THOSE OF THE TYPE STRAINS.

An account of the cultural characteristics, fermentation and serological reactions of 82 strains of *B. capsulatus-mucosus* arranged according to their source has been given with certain exceptions in the preceding pages. In order to describe the cultural characteristics and fermentation reactions more fully comparison was made with certain type cultures obtained from the Lister Institute.

Five of these type strains, namely, the three type strains of *B. lactis-aërogenes*, the "leather bacillus" and *B. pneumoniae*, exhibit cultural characteristics which very closely resemble one another. After 24 hours' incubation the colonies formed upon agar plates by all these strains are circular, moderately large, raised, mucoid and of a yellowish-white colour. After 48 hours the centre of the colony becomes opaque and surrounded by a translucent greyish bordering zone which is separated from the opaque centre by a slight depression. Similar colonies are produced by the 30 faecal strains, the majority of the urinary strains, the strains isolated from sputum except S. 5, S. 7, S. 8 and S. 9, and the strains from miscellaneous sources with the exception of the strain "Saville." A certain number of urinary strains, as exemplified by U. 6, showed colonies which were more mucoid and translucent in appearance, a property which was enhanced in the case of the nasal strains and strains S. 7, S. 8 and S. 9, and especially in the strains S. 5, N. 41, N. 58 and "Saville."

After 48 hours' incubation the colonies formed by *B. rhinoscleromatis* are yellowish-white but less intensely coloured. They are homogeneous raised colonies with uneven margins and the central portions are not distinguishable from the remainder. The colonies show the mucus-like characteristic in a high degree. The colonies formed by the *B. ozaenae* are flatter and of a greyish white colour. Like those of *B. rhinoscleromatis*, they are less opaque and of uniform appearance from centre to periphery. They are not mucoid in character and are easily distinguishable from any of the above.

A somewhat greater differentiation in cultural characteristics is shown by the use of litmus lactose agar. After 48 hours' incubation the colonies formed by the three type strains of *B. lactis-aërogenes* and by the faecal strains are large pink dome-shaped colonies with prominent opaque white centres and bordering pink zones of a light colour and much less opacity. They are of shiny mucoid appearance and their edges are clear and transparent. The colonies of *B. pneumoniae* and of certain strains isolated from sputum, as exemplified by S. 1, are rather smaller colonies, only slightly raised and pink in colour. The centres are not so prominent as in the case of the faecal strains. The colonies formed by some of the urinary strains are like those of the faecal strains, but those formed by the remaining urinary strains, as exemplified by U. 6, are almost homogeneous colonies, pink or blue in colour according to their action upon lactose. The colour is only slightly intensified in their centres. As on ordinary agar, the colonies formed by the nasal strains, by S. 7, S. 8 and S. 9, and also by S. 5, N. 41, N. 58 and "Saville," are characterised by a still greater translucency and gelatinous appearance. The colonies of *B. rhinoscleromatis* are slightly raised homogeneous blue colonies and are very shiny and mucoid in character. *B. ozaenae* forms blue flat homogenous colonies which are not shiny, and as before, can be easily distinguished from any of the preceding.

These differences are magnified when one studies the growth upon agar slopes. As stated previously, the strains obtained from nasal discharge show little opacity in their growth and a much more sticky consistency which is retained for a long time and is little changed even after repeated subculturing. Especially does this statement apply to the strains S. 5, N. 41, N. 58 and "Saville." In the case of the type strains of *B. lactis-aërogenes* and those resembling them the growth upon agar slopes is much more opaque, less translucent and gelatinous in appearance, and less viscid when tested with the platinum loop. As time goes on and with repeated subculturing these differences become accentuated, although replating upon litmus lactose agar even after the lapse of several months reproduces the characteristic colonies observed at the time of the first isolation.

Table VI shows the fermentation reactions of the seven type strains. The three type strains of *B. lactis-aërogenes* produced in carbohydrate media the primary acidity followed by alkalinity which has been noted in so many strains in the course of this investigation. The reactions of *B. pneumoniae* and the "leather bacillus" show only slight differences. Both produced acid and gas with lactose, glucose, mannite, maltose and saccharose, but in addition *B. pneumoniae* fermented dulcite, and the "leather bacillus" produced indol. Similar types of reaction have been encountered in many of the strains isolated from sputum, faeces, urine and miscellaneous sources. *B. rhinoscleromatis* produced no reaction with either lactose or dulcite, and formed acid without gas in glucose, mannite, maltose and saccharose. The reactions of *B. ozaenae* were peculiar in that this organism attacked slowly lactose,

Table VI.

Days of incubation ...	Lactose 1	3	5	10	Glucose 1	3	5	10	Mannite 1	3	5	10	Maltose 1	3	5	10	Saccharose 1	3	5	10	Dulcite 1	3	5	10	Milk	Indol	Haemolysis	Gelatin
B. lactis-aërogenes 124	ag	ag	ag	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ac	–	–	0
B. lactis-aërogenes 243	ag	ag	ag	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	–g	alk.g	a	alk	alk	alk	ac	–	–	0
B. lactis-aërogenes 418	ag	ag	ag	alk.g	ag	–g	alk.g	alk.g	ag	–g	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	a	alk	alk	alk	ac	–	–	0
Leather bacillus	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	a	alk	alk	alk	ac	++	–	0
B. pneumoniae Friedländer 204	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ac	–	–	0
B. rhino-scleromatis	–	–	–	–	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	–	–	alk	alk	a	–	–	0
B. ozaenae 459	–	–	alk.g	alk.g	–	a	ag	ag	–	–	alk	alk	–	–	alk.g	alk.g	–	–	alk	alk	–	–	alk	alk	alk	++	–	–

a = acid. ac = acid and clot. ag = acid and gas. alk = alkaline. alk.g = alkaline and gas. –g = neutral and gas.

Table VII.

Sera / Type of emulsion / Strains	Lactis-aërogenes 243 F.D.	L.A.	Pneumoniae Friedländer F.D.	L.A.	F. 1 F.D.	L.A.	F. 6 F.D.	L.A.	F. 13 F.D.	L.A.	F. 19 F.D.	L.A.	F. 24 F.D.	L.A.	S. 1 F.D.	L.A.	74 milk F.D.	L.A.	Rhino-scleromatis F.D.	L.A.	Ozaenae F.D.	L.A.
B. lactis-aërogenes 124	0	0	0	0	50	50	0	0	250	500	1000	500	50	0	0	0	100	50	—	—	—	—
B. lactis-aërogenes 243	500	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. lactis-aërogenes 418	100	100	0	0	0	0	0	0	0	0	0	0	250	0	0	0	0	0	—	—	—	—
Leather bacillus	0	0	0	0	2000	5000	2000	1000	—	—	—	—	—	—	250	250	250	250	—	—	—	—
B. pneumoniae Friedländer	1000	1000	10,000	10,000	0	500	500	1000	—	—	—	—	—	—	500	1000	0	5000	0	100	0	50
B. rhino-scleromatis	200	0	0	0	0	0	0	0	—	—	—	—	—	—	0	0	—	—	200	0	0	0
B. ozaenae 459	0	0	0	0	0	0	—	—	—	—	—	—	—	—	0	0	—	—	0	0	10,000	10,000

F.D. = formol Dreyer. L.A. = live agar.

glucose and maltose, and produced no reaction with mannite, saccharose or dulcite. *B. ozaenae* and the "leather bacillus" were the only type strains which produced indol.

Table VII shows the agglutination reactions of various sera upon the seven type strains. The three type strains of *B. lactis-aërogenes* showed the same peculiarities which were found in the agglutination of the faecal strains. Serum lactis-aërogenes 243 agglutinated both *B. lactis-aërogenes* 243 and 418, but had no action upon *B. lactis-aërogenes* 124, which organism was however agglutinated in high dilution by sera F. 13 and F. 19 and in low dilution by sera F. 1, F. 24 and 74 milk. Serum F. 24 also agglutinated *B. lactis-aërogenes* 418 to a titre of 1 in 250.

It is important to note that *B. pneumoniae* was agglutinated in high dilution by sera lactis-aërogenes 243, pneumoniae, F. 1, F. 6, S. 1 and 74 milk, and in low dilution by sera rhinoscleromatis and ozaenae, but that serum pneumoniae had no effect except upon the homologous strain. The "leather bacillus" was agglutinated by sera F. 1 and F. 6 in high dilution, and by sera S. 1 and 74 milk in a somewhat lower dilution. The table shows also the existence of a relationship between *B. rhinoscleromatis*, *B. pneumoniae* and *B. lactis-aërogenes* 243. *B. ozaenae* was agglutinated only by its homologous serum.

Table VIII shows the results of testing the agglutinating power of sera lactis-aërogenes 243, pneumoniae, F. 1, F. 6 and 74 milk upon the six strains S. 1–6. Serum pneumoniae gave positive results, though only in low dilution, with strains S. 2 and S. 3, but the remaining sera agglutinated all strains except S. 5. The strain S. 5 reacted with none of these sera.

Table VIII.

Type of emulsion ...	Serum lactis-aërogenes 243		Serum pneumoniae Friedländer		Serum F. 1		Serum F. 6		Serum 74 milk	
	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.
S. 1	250	500	0	0	20,000	1,000	2000	1000	5000	1,000
S. 2	50	0	0	250	20,000	50	2000	0	4000	10,000
S. 3	50	0	100	50	10,000	0	2000	0	4000	0
S. 4	500	0	0	0	20,000	10,000	5000	0	1000	250
S. 5	0	0	0	0	0	0	0	0	0	0
S. 6	50	50	0	0	2,000	1,000	1000	500	250	250

F.D. = formol Dreyer. L.A. = live agar.

Table IX shows the results of testing the agglutinating reactions of sera lactis-aërogenes 243, pneumoniae, S. 1 and 74 milk, U. 4 and U. 5 upon the strains F. 1–32. Nine strains were agglutinated by serum lactis-aërogenes 243, 9 strains by serum S. 1, and 13 strains by serum 74 milk. Only a small number of the strains were tested by sera U. 4 and U. 5, but two strains were agglutinated by each serum. It is to be noted that serum pneumoniae had no reaction upon any of the strains F. 1–32.

Table X shows the agglutination reactions of sera lactis-aërogenes 243,

https://doi.org/10.1017/S0022172400032071 Published online by Cambridge University Press

Table IX.

Type of emulsion...	Serum lactis-aërogene 243		Serum pneumoniae Friedländer		Serum S. 1		Serum 74 milk		Serum U. 4		Serum U. 5	
	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.
F. 1	0	100	0	0	0	1000	0	1000	0	0	0	0
F. 2	1000	50	0	0	2000	500	5000	500	—	—	—	—
F. 3	0	0	0	0	0	0	0	0	—	—	—	—
F. 4	100	0	0	0	0	0	0	0	—	—	—	—
F. 5	50	250	0	0	500	500	2000	1000	—	—	—	—
F. 6	50	0	0	0	200	0	1000	0	0	0	2000	0
F. 7	0	0	0	0	0	0	0	0	—	—	—	—
F. 8	250	250	0	0	500	500	4000	1000	—	—	—	—
F. 9	0	0	0	0	0	0	2000	2000	200	0	200	50
F. 10	0	0	0	0	0	0	0	0	—	—	—	—
F. 11	50	100	0	0	250	1000	1000	4000	—	—	—	—
F. 12	0	0	0	0	500	500	250	250	—	—	—	—
F. 13	0	0	0	0	0	0	0	0	—	—	—	—
F. 14	0	0	0	0	0	0	0	0	—	—	—	—
F. 15	0	0	0	0	0	0	0	0	—	—	—	—
F. 16	0	0	0	0	0	0	0	0	—	—	—	—
F. 17	0	0	0	0	0	0	0	0	0	0	0	0
F. 18	100	100	0	0	500	1000	2000	2000	—	—	—	—
F. 19	0	0	0	0	0	0	0	0	0	0	0	0
F. 20	0	0	0	0	0	0	0	0	0	0	0	0
F. 21	250	250	0	0	500	500	2000	500	—	—	—	—
F. 22	0	0	0	0	0	0	0	0	0	0	0	0
F. 23	0	0	0	0	0	0	0	0	—	—	—	—
F. 24	0	0	0	0	0	0	0	0	100	0	0	0
F. 25	0	0	0	0	0	0	0	0	—	—	—	—
F. 26	0	0	0	0	0	0	1000	0	—	—	—	—
F. 27	0	0	0	0	0	0	0	0	0	0	0	0
F. 28	0	0	0	0	0	0	250	0	—	—	—	—
F. 29	0	0	0	0	0	0	0	0	—	—	—	—
F. 30	0	0	0	0	0	0	0	0	—	—	0	0
F. 31	0	0	0	0	0	0	0	0	—	—	—	—
F. 32	0	0	0	0	0	0	100	0	—	—	—	—

F.D. = formol Dreyer. L.A. = live agar.

Table X.

Type of emulsion...	Serum lactis-aërogenes 243		Serum pneumoniae Friedländer		Serum F. 1		Serum F. 6		Serum S. 1	
	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.
U. 1	0	0	0	0	0	0	0	0	0	0
U. 2	0	0	0	0	0	0	0	0	0	0
U. 3	0	0	0	0	0	0	0	0	0	0
U. 4	0	0	0	0	0	0	0	0	0	0
U. 5	0	50	0	0	0	50	0	50	0	0
U. 6	0	0	0	100	0	0	0	0	0	0
U. 7	0	0	0	0	0	0	0	0	0	0
U. 8	0	0	0	0	0	0	0	0	0	0
U. 9	0	0	0	0	0	0	0	0	0	0
U. 10	0	0	0	0	0	0	0	0	0	0
U. 11	0	0	0	0	0	0	0	0	0	0
U. 12	0	0	0	0	0	0	0	0	0	0
U. 13	0	0	100	250	0	0	0	0	0	0
U. 14	0	0	0	0	1000	1000	0	1000	0	500
U. 15	0	0	0	0	0	0	500	100	0	0
U. 16	0	0	1000	1000	0	0	0	0	0	0

F.D. = formol Dreyer. L.A. = live agar.

https://doi.org/10.1017/S0022172400032071 Published online by Cambridge University Press

pneumoniae, F. 1, F. 6 and S. 1 against the 16 strains U. 1–16. Six strains only were agglutinated, the strain U. 5 by sera lactis-aërogenes 243, F. 1 and F. 6, the strains U. 6, U. 13 and U. 16 by serum pneumoniae, the strain U. 14 by sera F. 1 and F. 6, and the strain U. 15 by serum F. 6 only.

Table XI shows the agglutination reactions of sera lactis-aërogenes 243, pneumoniae, F. 1, F. 6, F. 19 and S. 1 upon the strains obtained from miscellaneous sources. It is noteworthy that serum S. 1 agglutinated a live agar

Table XI.

Type of emulsion...	Serum lactis-aërogenes 243		Serum pneumoniae Friedländer		Serum F. 1		Serum F. 6		Serum F. 19		Serum S. 1	
	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.	F.D.	L.A.
74 milk	0	0	0	0	0	0	0	0	0	0	0	500
Eales pus	0	0	0	0	0	0	0	0	100	0	0	0
Cow's milk	0	0	0	0	0	0	0	0	0	0	0	0
Cheese	0	0	0	0	0	0	0	0	50	50	0	0
V.D. 6913	0	100	0	100	0	20,000	0	2000	—	—	0	500
Saville	—	—	0	0	0	0	—	—	—	—	0	0

F.D. = formol Dreyer. L.A. = live agar.

antigen of the organism 74 milk to a titre of 1/500. After experiments with many different sera, serum F. 19 was found to agglutinate the organisms "Eales pus" and "cheese." The strain V.D. 6913 was agglutinated by the five sera employed, including serum pneumoniae in low dilution. The strain "Saville" was not agglutinated by any of the three sera F. 1, S. 1 and pneumoniae and the strain "cow's milk" gave negative results with all the six sera employed.

A serum prepared against the organism isolated from cow's milk agglutinated the homologous strain in a dilution of 1 in 250. It was found to agglutinate strains F. 24 and F. 9 in dilutions of 1 in 100 and 1 in 1000 respectively. This strain therefore is obviously related to the strains obtained from faeces.

In order to give a comprehensive view of the serological relationship existing between the type strains and the strains isolated in the course of this investigation, the cross-agglutination reactions of four type strains, *B. rhinoscleromatis*, *B. lactis-aërogenes* 243, *B. pneumoniae* and *B. ozaenae*, and four strains B. 6947, F. 1, S. 1 and U. 6, which may be considered as representative of the latter, are given in Table XII. The completely isolated position of B. 6947 is clearly shown. It is true that the serum prepared against *B. ozaenae* agglutinated *B. pneumoniae* and S. 1, but compared with the end point of agglutination with the homologous organism, the titre is so low as to be disregarded. A relationship between the remaining six strains is clearly indicated.

Table XII.

Type of emulsion ...	Serum *B. rhino-scleromatis* F.D.	Serum *B. rhino-scleromatis* L.A.	Serum *B. lactis-aërogenes* 243 F.D.	Serum *B. lactis-aërogenes* 243 L.A.	Serum pneumoniae Friedländer F.D.	Serum pneumoniae Friedländer L.A.	Serum *B. ozaenae* F.D.	Serum *B. ozaenae* L.A.
B. rhinoscleromatis ...	200	0	200	0	0	0	0	0
B. lactis-aërogenes 243 ...	0	0	500	200	0	0	0	0
B. pneumoniae Friedländer	0	100	1000	1000	10,000	20,000	0	50
B. ozaenae	0	0	0	0	0	0	10,000	10,000
B. 6947	0	0	0	—	0	0	0	0
F. 1	0	0	0	100	0	0	0	0
S. 1	0	0	250	500	0	0	50	0
U. 6	0	100	0	0	0	100	0	0

Type of emulsion ...	Serum B. 6947 F.D.	Serum B. 6947 L.A.	Serum F. 1 F.D.	Serum F. 1 L.A.	Serum S. 1 F.D.	Serum S. 1 L.A.	Serum U. 6 F.D.	Serum U. 6 L.A.
B. rhinoscleromatis ...	0	0	0	0	0	0	0	0
B. lactis-aërogenes 243 ...	0	0	0	0	0	0	500	0
B. pneumoniae Friedländer	0	0	0	500	500	1000	0	500
B. ozaenae	0	0	0	0	0	0	0	0
B. 6947	200	200	0	0	0	0	0	0
F. 1	0	0	500	10,000	0	1000	0	0
S. 1	0	0	20,000	1,000	5000	1000	0	0
U. 6	0	0	0	0	0	0	5000	0

F.D. = formol Dreyer. L.A. = live agar.

CROSS-AGGLUTINATION REACTIONS BETWEEN STRAINS N. 8, N. 41, N. 58, S. 5, "SAVILLE" AND THE TYPE STRAINS *B. PNEUMONIAE* AND *B. RHINOSCLEROMATIS*.

The position of the strains N. 8, N. 41, N. 58, S. 5 and "Saville" will now be considered. It was found that serum pneumoniae agglutinated a formol Dreyer antigen of N. 8 in a dilution of 1 in 10,000 and that a serum prepared against N. 8 agglutinated a similar antigen of *B. pneumoniae* in a dilution of 1 in 1000, and both formol Dreyer and live agar antigens of *B. pneumoniae* of 1 in 4000 and 1 in 2000 respectively. Serum N. 8 also produced a definite agglutination of a formol Dreyer antigen of S. 5 in a dilution of 1 in 50. Serum S. 5 agglutinated all three organisms N. 8, S. 5 and *B. pneumoniae* in high dilution. It would appear therefore that the organism N. 8 is practically identical with *B. pneumoniae* and that the organism S. 5 is closely related. A serum prepared against the strain "Saville" agglutinated not only the homologous strain but also strains N. 41 and N. 58, which strains were not affected by serum pneumoniae. These three strains therefore, which as mentioned above showed similar cultural characteristics, also showed common agglutination reactions and are presumably identical organisms. All these results are shown in Table XIII, which also demonstrates the relationship of *B. rhinoscleromatis* to the five strains under consideration. The comparison with *B. rhinoscleromatis* was made because a relationship between it and *B. pneumoniae* has been asserted by previous workers. It was found that a formol Dreyer antigen of *B. rhinoscleromatis* was agglutinated by serum N. 8 in a dilution of 1 in 2000 and by sera pneumoniae, S. 5 and "Saville" in dilutions appreciably comparable to that found in the agglu-

https://doi.org/10.1017/S0022172400032071 Published online by Cambridge University Press

Table XIII.

Type of emulsion ...	Serum pneumoniae Friedländer F.D.	Serum pneumoniae Friedländer L.A.	Serum N. 8 F.D.	Serum N. 8 L.A.	Serum S. 5 F.D.	Serum S. 5 L.A.	Serum Saville F.D.	Serum Saville L.A.	Serum rhino-scleromatis F.D.	Serum rhino-scleromatis L.A.
N. 8	10,000	0	1000	0	2000	0	0	0	0	0
N. 41	0	0	0	0	0	0	200	50	0	0
N. 58	0	0	0	0	0	0	100	100	0	0
S. 5	0	0	50	0	1000	0	0	0	0	0
Saville	0	0	0	0	0	0	100	200	0	0
Pneumoniae Friedländer	10,000	20,000	4000	2000	5000	2000	0	0	0	100
Rhinoscleromatis ...	100	0	2000	0	100	0	200	0	200	0

F.D. = formol Dreyer. L.A. = live agar.

tination obtained with homologous serum. A serum prepared against *B. rhinoscleromatis* agglutinated *B. pneumoniae* and *B. rhinoscleromatis*.

Saturation Experiments.

With the object of throwing additional light upon the relationship existing amongst the various strains as demonstrated by agglutination reactions, a number of saturation experiments was done. The number of these was, of necessity, limited and representative strains were chiefly employed.

Heavy suspensions of the bacilli from agar cultures were made in saline. To the immune serum to be tested and already diluted 1 in 5 with saline an equal amount of the required bacillary emulsion was added and the resultant mixture placed in the ice-safe for several days. It was shaken at intervals. The treated serum was separated off in the centrifuge and tested for agglutinins along with the control serum which had been diluted 10 times with saline and kept in the ice-safe under precisely similar conditions.

The first three experiments were done with pneumoniae serum, S. 1 serum and F. 6 serum, and the following organisms—*B. pneumoniae*, *B. lactis-aërogenes* 243, S. 1, F. 1, F. 6 and *B. rhinoscleromatis*—were used for saturation. The results are shown in the following tables:

Experiment No. 1.

Serum	End-point of agglutination with live agar antigen of *B. pneumoniae*
Control serum pneumoniae	10,000
Pneumoniae serum saturated with *B. pneumoniae* ...	100
,, ,, *B. lactis-aërogenes* 243	5,000
,, ,, S. 1	10,000
,, ,, F. 1	10,000
,, ,, *B. rhinoscleromatis* ...	10,000

Experiment No. 2.

Serum	Agglutination results after saturation of S. 1 serum on: S. 1	F. 1	F. 6	*B. pneumoniae*
Control serum S. 1	2000	1000	500	1000
Serum S. 1 saturated with F. 1	1000	0	0	1000
,, ,, *B. lactis-aërogenes* 243	1000	0	0	50
,, , *B. pneumoniae* ...	2000	200	200	0

https://doi.org/10.1017/S0022172400032071 Published online by Cambridge University Press

Experiment No. 3.

Serum	Agglutination results after saturation of F. 6 serum on			
	F. 1	F. 6	S. 1	*B. pneumoniae*
Control serum F. 6	1000	1000	2000	2000
Serum F. 6 saturated with F. 6	0	0	250	250
,, ,, S. 1	1000	500	0	2000
,, ,, *B. pneumoniae* ...	1000	1000	2000	0

These experiments show that *B. lactis-aërogenes* 243 occupies an intermediate position between F. 1 and *B. pneumoniae*. It would also appear that, although F. 1 and F. 6 are very closely related, F. 6 is more allied to *B. pneumoniae* than F. 1. The relationship of S. 1 to these strains is also shown.

Exps. 4 and 5 are concerned with the relationships of the strains N. 8, S. 5, *B. pneumoniae* and *B. rhinoscleromatis*.

Experiment No. 4.

Serum	Agglutination results after saturation of S. 5 serum on	
	S. 5	*B. pneumoniae*
Control serum S. 5	500	1000
Serum S. 5 saturated with S. 5	0	1000
,, ,, *B. pneumoniae*	500	0

Experiment No. 5.

Serum	Agglutination results after saturation of N. 8 serum on			
	N. 8	S. 5	*B. pneumoniae*	*B. rhinoscleromatis*
Control serum N. 8	2000	50	1000	2000
Serum N. 8 saturated with *B. pneumoniae* ...	0	0	0	250

The results show that the organisms N. 8 and *B. pneumoniae* are probably identical and both are related to S. 5 and *B. rhinoscleromatis*.

FURTHER EXPERIMENTS WITH REFERENCE TO THE OBSERVATIONS OF L. A. JULIANELLE.

Reference was made in the introduction to this paper to the work of Small and Julianelle on the biological and serological characters of 27 strains of the *B. capsulatus-mucosus* group. These authors observed that capsule-bearing strains agglutinated only in high concentration of immune sera and that the reactions more closely resembled precipitin reactions, but that capsule-free strains, which are the most prone to be affected by group agglutinins, agglutinated in high dilutions of sera and gave a finely granular precipitate. Julianelle later sought to provide an explanation of these anomalous results by applying the principles governing the immunological relationships of the pneumococci as demonstrated by Heidelberger, Avery and others. By this means, working with 30 strains of Friedländer's bacilli he obtained three types, A, B and C and group X, a heterogeneous group of bacilli which did

not fall into any of the three types. He distinguished smooth and rough (S. and R.) types of colonies, and stated that the change from smooth to rough was accompanied by loss of capsule and mucoid characteristics and loss of agglutinability in type specific sera. He found that smooth encapsulated (S.) strains only showed type specificity and that S. strains were not agglutinated by sera prepared by immunisation with capsule-free (R.) strains even though the organism employed for immunisation was derived from the S. strain later used for agglutination. He found that anti S. sera reacted irregularly with capsule-free strains, and that anti R. sera reacted with all R. forms regardless of type derivation. The agglutinin titres of anti R. sera were high (1 in 2500) and of anti S. sera low (1 in 40, 1 in 80). Further, he found that a closely related organism, *Encapsulatus granulomatis*, when decapsulated was also agglutinated by anti R. sera, and thought that this explained in a measure the confusion experienced with allied encapsulated Gram-negative organisms. Later he immunised rabbits with the nucleoprotein isolated from representative strains of types A, B and group X, and found that the anti-protein sera obtained did not react with the encapsulated cell of either homologous or heterologous type. Capsule-free cells derived from any of the serologically different types agglutinated equally well in all the anti-protein sera. Anti-protein sera of Friedländer's bacillus reacted with protein derived from *B. aërogenes*, *B. coli* and granuloma bacillus. He considered that these findings lent considerable assistance in the interpretation of the results of former investigators who found that anti-Friedländer sera caused agglutination of *B. rhinoscleromatis* (Sicard, von Eisler and Porges, Galli-Valerio, Fitzgerald), *B. aërogenes* (Bertarelli), *B. typhosus* (Klemperer and Scheier) and granuloma bacillus (Small and Julianelle).

It is obvious that due consideration should be given to these observations with reference to the results obtained in this investigation. A reference to Table III shows that each of the sera prepared against F. 1 and F. 16 agglutinated a live agar suspension of the homologous strain in a dilution of 1 in 10,000. In addition in each case a finely granular precipitate was obtained. Both sera could therefore be justifiably regarded as anti R. sera produced by non-encapsulated strains, but no trace of cross-agglutination with those same strains was obtained. Yet there is no reason to doubt that F. 1 and F. 16 are allied organisms.

Julianelle employed two methods of effecting capsule destruction. The first method was to grow the organism in broth containing 10 per cent. of homologous immune serum and to transplant daily into the same medium. Plate cultures were made daily and examined after 18–24 hours' incubation. With 6 to 10 transplants cultures were obtained in which rough colonies were observed. Cultures in plain broth from single typical R. colonies were made and the roughness of the colonies confirmed by animal inoculation, as loss of virulence also was found to accompany the change from rough to smooth. The second method was the method employed by Porges—the destruction of

the capsule by chemical means. Smooth strains were grown on agar. Saline suspensions were made and acidified with one-quarter volume of $N/4$ HCl and heated for 15 minutes at 80° C. The suspensions were then cooled immediately under tap water and neutralised with an equivalent quantity of $N/4$ NaOH.

With a view to confirming these observations in so far as they concern the organisms examined in this investigation, two bacilli 6947 and U. 4, which retained for long periods of time a marked mucoid appearance in agar culture and with which it was only possible to produce agglutinating sera of low titre, were treated according to the first method by growing in broth containing homologous immune serum. Plate cultures were made daily and examined after 18 to 24 hours, but no differentiation into rough and smooth colonies was observed.

The results of a number of agglutination tests performed with antigens prepared according to the method of Porges are shown in Table XIV.

Table XIV. *Experiments with certain sera of high titre and antigens prepared according to the method of Porges.*

	Antigens							
Sera	U. 6 agar	U. 6 agar (Porges)	F. 27 agar	F. 27 agar (Porges)	F. 1 agar	F. 1 agar (Porges)	*B. pneumoniae* agar	*B. pneumoniae* agar (Porges)
F. 1	0	0	0	0	10,000	100	500	—
B. pneumoniae	100	0	0	0	0	0	10,000	2000
U. 9	0	0	0	0	—	—	—	—
F. 27	—	—	200	200	0	—	—	—
U. 6	2500	1000	—	—	—	—	—	—

These results show that a live agar antigen of F. 27 is inagglutinable by sera F. 1, *B. pneumoniae* and U. 9, and that even when treated by Porges' method it is still inagglutinable. The slight degree of agglutination given by *B. pneumoniae* serum with the strain U. 6 entirely disappears when the antigen is treated by the same method. *B. pneumoniae* serum failed to agglutinate F. 1 agar antigen either in the untreated or treated state, although the fact that F. 1 serum agglutinated *B. pneumoniae* shows that they are allied organisms.

These experiments, although limited in scope, serve to show, I think, that the various organisms of the *B. capsulatus-mucosus* group when decapsulated do not contain an agglutinable substance which is common to all.

DISCUSSION.

It is difficult to draw definite conclusions with regard to the classification of bacteria which give such diverse and confusing experimental results as have been obtained in the 81 strains of *B. capsulatus-mucosus* which are included in this paper. Experience with other groups such as the group *B. dysenteriae* has shown that little stress can be laid upon differences in

fermentation properties, and this certainly holds good for the *B. capsulatus-mucosus* group in which, as pointed out by Small and Julianelle and other authors, common immunologic strains may show diverse carbohydrate reactions. The chief data for providing classification depend upon differences in serological reactions, which are notoriously difficult in the case of this group of organisms.

It seems clear, however, that a primary division into two main groups A and B is justifiable. Group A comprises 19 strains, 16 obtained from nasal discharge (*i.e.* all except N. 8, N. 41 and N. 58), and three strains S. 7, S. 8 and S. 9 from sputum. It is represented by the strain B. 6947. There appears to be little doubt that the organism of the *B. capsulatus-mucosus* group most frequently encountered in association with chronic nasal disease is a bacillus of the B. 6947 type. In spite of variations in cultural characteristics as shown in the various carbohydrate media these bacilli are classified in one group by their common agglutinative properties as tested by sera B. 6947 and N. 1. So far as could be ascertained, this type of *B. capsulatus-mucosus* shows no serological relationship to any other type encountered in this research or to any of the type strains obtained from the Lister Institute. It is interesting to note that it shows no resemblance either in cultural or serological properties to *B. ozaenae* 459 (Lister Institute). To some extent it resembles the type strain of *B. pneumoniae*, but its fermentative power as tested by various carbohydrates is much smaller, and serologically no relationship could be established.

The sera of several patients from whom this bacillus was recovered were tested for agglutinins, but although in almost all cases the disease was of long standing none were found. Four patients were treated with autogenous vaccines of B. 6947 type with doses commencing at 10 million rising to 500 million organisms, but in no case was any improvement observed, and in addition no agglutinins were demonstrated in the patients' sera after this procedure.

The remaining 62 strains, which include F. 1–32, S. 1–6, U. 1–16, N. 8, N. 41 and N. 58 and the five strains from miscellaneous sources, belong to group B, as do also all the type strains from the Lister Institute with the exception of *B. ozaenae*. A further sub-division of group B into sub-groups is, I think, undesirable. There is no doubt that there are many types of organisms included in this group, but connecting links between them are easily established. Table XII illustrates these points. In this table the results of cross-agglutination experiments among the representative strains *B. rhinoscleromatis*, *B. lactis-aërogenes* 243, *B. pneumoniae*, *B. ozaenae*, B. 6947, F. 1, S. 1 and U. 6 are shown. The table clearly shows that the strain B. 6947 is quite unconnected with any of the remaining strains, and for this reason has been placed in group A. The organism *B. ozaenae* is not agglutinated by any other than its homologous serum. The cultural characters of this organism as given by the Lister strain are so unlike those of the *B. capsulatus-mucosus* group in

general that there is no reason for including it. It is true that serum *B. ozaenae* produced positive agglutination with *B. pneumoniae* and S. 1 in a dilution of 1 in 50, but this titre is so small as compared with the end-point of the serum that, in our opinion, it should be disregarded. The relationship existing among the remaining six strains is quite clearly shown in the table. The view held by Denys and Martin, Grimbert and Legros, Bertarelli and others that a close relationship exists between the *B. lactis-aërogenes* and the pneumobacillus is certainly supported by the results obtained in this investigation. In addition, both are serologically related to *B. rhinoscleromatis*. On the other hand, connections between *B. lactis-aërogenes* and the pneumobacillus and the three strains F. 1, S. 1 and U. 6 are clearly shown. *B. lactis-aërogenes* 243 appears to occupy an intermediate position between F. 1 and the pneumobacillus. The organism U. 6 more closely resembles the pneumobacillus.

The *B. pneumoniae* as represented by the type strain from the Lister Institute fermented all the six carbohydrates employed with the formation of gas. The reactions of this organism have been found to be different by various observers. For example, Fricke and Clairmont state that it forms both acid and gas in lactose, but Perkins states that it forms acid but not gas. Fricke found that it produced both acid and clot in milk, but Clairmont and Perkins found no coagulation. The strains found most resembling *B. pneumoniae* are N. 8 (which gives precisely similar carbohydrate reactions and is probably identical), U. 6, U. 13, U. 16 and to a less extent S. 5 (see Tables X and XII). The strains "Saville," N. 41 and N. 58, which are probably identical with one another, have been shown to be serologically related to *B. rhinoscleromatis*, and consequently to *B. pneumoniae*. It is remarkable that *B. pneumoniae* should be agglutinable in so many different antisera prepared against organisms of this group.

Although definite serological relationship between *B. rhinoscleromatis* and several organisms included in this research have been established no organism exactly comparable to *B. rhinoscleromatis* has been isolated.

The organism *B. lactis-aërogenes* is the type of *B. capsulatus-mucosus* which has most frequently been found in the course of this research. A reference to Table VI shows that in the case of all three Lister strains of this organism the preliminary acidity produced in the various carbohydrates was shortly succeeded by alkalinity. Many bacilli giving similar carbohydrate reactions and showing serological relationship have been isolated from faeces, sputum, urine and miscellaneous sources. *B. lactis-aërogenes* 243, as mentioned above, appears to occupy an intermediate position between F. 1 and the pneumobacillus. At the same time it shows a definite serological relationship with *B. pneumoniae* and *B. rhinoscleromatis*.

Group B therefore comprises a number of organisms showing definite degrees of difference but distinctly related to one another and represented by various types, such as *B. rhinoscleromatis*, *B. pneumoniae*, *B. lactis-aërogenes*, etc.

It is stated by various authors that *B. capsulatus-mucosus* is quite frequently encountered in the examination of normal human faeces. This statement however is not supported by the experience of this work. The 32 organisms (F. 1–32) represent all the strains of *B. capsulatus-mucosus* recovered from 976 specimens of faeces examined over a period of 3 years. As mentioned above, 11 of the 32 strains were isolated from cases of ulcerative colitis, 11 from cases of gastro-enteritis, enteritis or dysentery, five from cases of convalescent typhoid or paratyphoid B fever, one from a typhoid carrier and one from a case of pernicious anaemia. In three cases no history was obtained. In 29 cases at least therefore there was evidence that an abnormal or pathological condition of the intestine was present. Dudgeon, in a study of the intestinal flora under normal and abnormal conditions, found that *B. capsulatus-mucosus* occurred in 5·5 per cent. of cases and usually in patients suffering from an abnormal condition of the intestinal tract.

The type of *B. capsulatus-mucosus* most commonly found in the faeces is *B. lactis-aërogenes*. Its occurrence in the faeces in cases of gastro-enteritis was noted many years ago by Fricke who, amongst several other cases, described two occurring in a man and his wife after partaking of raw milk. Vomiting and diarrhoea were present in both cases, and cultures made from the faeces shortly after commencement of the illness showed an almost pure growth of *B. capsulatus-mucosus*. In a few days the patients recovered, and *B. capsulatus-mucosus* disappeared from the faeces. The small outbreak of enteritis occurring at a girls' school is interesting in this connection, but the bacilli isolated from the milk and cheese, although belonging to the *capsulatus-mucosus* group, were not serologically identical with the strain (F. 25) isolated from the faeces of one of the patients or with one another. The sera of 10 patients suffering from gastro-enteritis, enteritis or ulcerative colitis were examined for agglutinins at different periods, but the results were in every case negative. Four cases of ulcerative colitis, in which condition *B. capsulatus-mucosus* is often persistently present in the faeces, were treated by autogenous vaccines. In two cases definite improvement took place, but even after a course of several injections agglutinins could still not be demonstrated in the serum, and there is no evidence to show that benefit had resulted from the treatment. Fricke believes that in cases of gastro-enteritis, owing perhaps to some catarrhal condition of the intestinal wall, the composition of the intestinal secretion is changed. This change permits of the rapid growth and multiplication of bacilli of the *capsulatus-mucosus* group which, having been ingested with the food, are passing through the alimentary tract, but owing to their extreme scarcity are under normal circumstances unrecognisable by the usual laboratory examination. The normal composition of the intestinal secretion does not permit of any multiplication of these chance inhabitants. This explanation would also account for the presence of large numbers of these organisms in cases of typhoid and paratyphoid fevers, ulcerative colitis, pernicious anaemia and other conditions.

The bacteria of the *B. capsulatus-mucosus* group found in association with urinary affections, so far as the results of this investigation show, apparently belong also to group B. Three strains U. 6, U. 13 and U. 16 show serological relationship to *B. pneumoniae*, and three strains U. 5, U. 14 and U. 15 to the organism F. 6. In addition, cross-agglutination experiments show many connections between these six strains and other of the 16 urinary strains isolated. In six cases the sera of the patients were examined for agglutinins, and the organisms U. 1 and U. 2 were agglutinated in dilutions of 1 in 200 and 1 in 1000 of the sera of the respective patients. In both instances the organisms were grown from the urine in pure culture. In the remaining four cases the results were negative.

Of the nine strains of *B. capsulatus-mucosus* recovered from sputum three strains S. 7, S. 8 and S. 9 belong to group A. They show similar cultural and serological characteristics to the strains obtained from nasal secretion and may have been derived from that source. The remaining six strains belong to group B. Of these the strain S. 5 shows a marked relationship to *B. pneumoniae*. The remaining five strains although differing in cultural characteristics show definite affinities with one another, with the strains F. 1 and F. 6 and with the type strains *B. lactis-aërogenes* 243 and *B. pneumoniae*. The strains S. 1, S. 2 and S. 3 were tested with the sera of the patients from the sputum of whom they were obtained, but were not agglutinated.

The six strains isolated from miscellaneous sources also should be regarded as belonging to group B. The strain "Saville" showed precisely similar cultural characters to the strains N. 41 and N. 58, and serological examination showed that these three strains were probably identical. The remaining five strains have been shown to be related to other organisms belonging to group B isolated from other localities. No examination for agglutinins in the sera of the patients concerned was done.

CONCLUSIONS.

1. Eighty-one strains of bacilli belonging to the *capsulatus-mucosus* group have been isolated from nasal secretion, sputum, faeces, urine and miscellaneous sources. They are divisible into two groups—group A, represented by the strain B. 6947, and group B, a heterogeneous collection of organisms represented by various types, *B. lactis-aërogenes*, *B. pneumoniae*, *B. rhinoscleromatis*, F. 1, U. 6, etc.

2. The bacillus found most commonly in association with chronic nasal disease is a bacillus of the 6947 type. No connection was found between this organism and any of those belonging to group B. No evidence was found that this bacillus was pathogenic. It shows no relationship either to *B. pneumoniae*, *B. rhinoscleromatis* or *B. ozaenae*.

3. The cultural characteristics of the organism *B. ozaenae* as represented by the Lister Institute strain are so different that this organism should not be placed in the *capsulatus-mucosus* group.

4. With regard to group B, the various types of organisms as represented by *B. lactis-aërogenes*, *B. pneumoniae*, *B. rhinoscleromatis*, F. 1, U. 6 and others, although they show marked differences in cultural reactions, can be shown to be serologically related to one another. Intermediate types are common. These facts render a further division into sub-groups difficult and unjustifiable.

5. The anomalous serological reactions found amongst these organisms cannot be wholly explained on the basis of encapsulation and non-encapsulation of the strains.

6. The presence of *B. capsulatus-mucosus* in the faeces should be regarded as indicative of an abnormal condition of the intestine although no direct evidence of pathogenicity was obtained. The common type found in the faeces is *B. lactis-aërogenes*.

7. The bacilli of the *capsulatus-mucosus* group found in the sputum may belong to either group A or group B. The latter show obvious serological relationships to those obtained from other sources.

8. The bacilli obtained from urine and from miscellaneous sources also belong to group B. In two patients suffering from urinary affection there was evidence that the organism was causal.

I am indebted to Prof. L. S. Dudgeon and Dr John F. Taylor for pathological material and for the many strains which they have given to me. This investigation has been made possible by my tenure of the Louis Jenner Research Scholarship.

APPENDIX.

Table I.

Days of incubation...	Lactose 1	3	5	10	Glucose 1	3	5	10	Mannite 1	3	5	10	Maltose 1	3	5	10	Saccharose 1	3	5	10	Dulcite 1	3	5	10	Milk	Indol	Haemo-lysis	Gelatin
B. 6947	–	–	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	alk	alk	alk	a	alk	alk	alk	a	–	–	–
N. 1	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	–	alk	alk	–	–	alk	alk	a	–	–	–
N. 4	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	–	–	alk	alk	–	alk	alk	alk	a	–	–	–
N. 5	–	–	–	ag	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	–	–	alk	–	–	alk	alk	a	–	–	–
N. 8	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	a-ac	–	–	–
N. 23	–	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	–	–	alk	–	–	alk	alk	a	–	–	–
N. 24	–	–	–	ag	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	–	alk	ag	–	–	alk	alk	a	–	–	–
N. 32	a	ag	ag	ag	a	ag	ag	ag	a	ag	ag	ag	a	ag	ag	ag	–	alk	alk	alk	–	alk	alk	alk	a-ac	–	–	–
N. 35	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	–	ag	ag	–	alk	alk	alk	a-ac	–	–	–
N. 41	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	alk	alk	alk	a	–	–	–
N. 50	a	ag	ag	ag	a	a	ag	ag	a	ag	ag	ag	a	a	ag	ag	–	–	a	ag	–	–	alk	alk	a-ac	–	–	–
N. 51	a	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	–	alk	alk	–	–	–	alk	a-ac	–	–	–
N. 52	–	–	alk	alk	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	–	–	alk	alk	a	–	–	–
N. 53	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	–	ag	ag	–	–	alk	alk	a-ac	–	–	–
N. 54	a	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	–	ag	ag	–	–	–	alk	a	–	–	–
N. 56	ag	ag	ag	ag	a	a	a	a	a	ag	ag	ag	a	a	a	a	ag	ag	ag	ag	–	–	–	–	a-ac	–	–	–
N. 58	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	alk	alk	alk	a-ac	–	–	–
N. 60	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	–	–	alk	–	–	–	alk	a-ac	–	–	–
F.A.P.	–	–	–	a	a	a	a	a	–	a	ag	ag	a	a	ag	ag	–	alk	alk	alk	–	alk	alk	alk	a	–	–	–

a = acid. ag = acid and gas. alk = alkaline.

Table II.

Days of incubation...	Lactose 1	3	5	10	Glucose 1	3	5	10	Mannite 1	3	5	10	Maltose 1	3	5	10	Saccharose 1	3	5	10	Dulcite 1	3	5	10	Milk	Indol	Haemo-lysis	Gelatin
S. 1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	–	alk	alk	alk	a	–	–	–
S. 2	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	alk	alk	alk	ac	–	–	–
S. 3	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	–	–	alk	alk	a	–	–	–
S. 4	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	alk	alk	alk	ac	+	+	–
S. 5	–	alk	alk	alk	ag	alk.g	alk.g	alk.g	ag	ag	ag	alk.g	ag	ag	ag	alk.g	ag	ag	ag	ag	–	alk	alk	alk	a	–	–	–
S. 6	ag	ag	–g	alk.g	ag	–g	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	ag	alk.g	ag	ag	ag	ag	–	alk	alk	alk	ac	–	–	–
S. 7	–	–	a	a	a	a	a	a	a	a	a	a	a	ag	ag	ag	–	–	ag	ag	–	alk	alk	alk	a	Tr	–	–
S. 8	a	ag	ag	ag	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	–	a	ag	ag	–	–	alk	alk	ac	–	–	–
S. 9	a	a	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	a	a	a	a	–	a	ag	ag	–	alk	alk	alk	a	–	–	–

a = acid. ac = acid and clot. ag = acid and gas. alk = alkaline. alk.g = alkaline and gas. –g = neutral and gas. Tr = trace.

Journ. of Hyg. xxvii

Table III.

Days of incubation...	Lactose				Glucose				Mannite				Maltose				Saccharose				Dulcite				Milk	Indol	Haemolysis	Gelatin
	1	3	5	10	1	3	5	10	1	3	5	10	1	3	5	10	1	3	5	10	1	3	5	10				
F. 1	ag	ag	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	−g	alk.g	alk.g	ac	−	−	0
F. 2	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	−	alk	alk	alk	ac	−	−	0
F. 3	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	−	ag	ag	−	alk	alk	alk	ac	−	−	0
F. 4	−	−	ag	alk.g	ag	alk.g	alk.g	alk.g	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	−	alk	alk	alk	ac	−	−	++
F. 5	ag	ag	ag	ag	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	ag	ag	alk.g	ag	−g	alk.g	alk.g	ac	−	−	0
F. 6	a	ag	ag	alk.g	ag	alk.g	alk.g	alk.g	ag	−g	alk.g	alk.g	ag	−g	alk.g	alk.g	ag	ag	ag	alk.g	a	alk.g	alk.g	alk.g	ac	−	−	0
F. 7	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	−	alk	alk	ag	ag	ag	alk.g	ac	−	−	0
F. 8	ag	ag	ag	alk.g	ag	ag	alk.g	alk.g	ag	ag	ag	alk.g	ag	ag	−g	alk.g	ag	ag	−g	alk.g	−	alk	alk.g	alk.g	ac	−	−	0
F. 9	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	ac	+	−	0
F. 10	ag	ag	ag	ag	ag	alk.g	alk.g	alk.g	ag	ag	ag	alk.g	ag	alk.g	alk.g	alk.g	ag	ag	ag	ag	ag	ag	−g	alk.g	ac	−	−	0
F. 11	ag	ag	−g	alk.g	ag	−g	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	−g	alk.g	alk.g	ag	ag	ag	alk.g	−	alk.g	alk.g	alk.g	ac	−	−	0
F. 12	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	alk	alk	alk	ac	−	−	0
F. 13	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	−	alk	alk	alk	−	alk	alk	alk	ac	+	−	0
F. 14	ag	ag	ag	ag	ag	ag	alk.g	alk.g	ag	−g	alk.g	alk.g	ag	ag	ag	ag	−	alk	alk	alk	−	alk	alk	alk	ac	+	−	0
F. 15	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	−	alk	alk	ag	−	alk	alk	alk	ac	−	−	0
F. 16	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	alk	alk	alk	alk	−	alk	alk	alk	ac	+	−	0
F. 17	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	alk	alk.g	alk.g	ag	ag	alk.g	alk.g	ac	+	−	0
F. 18	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	ag	ag	ag	alk.g	alk.g	alk.g	ac	−	−	0
F. 19	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	a	a	ag	ag	−	alk	alk	alk	ac	+	−	0
F. 20	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	ag	ag	ag	ag	ag	−g	alk.g	ac	+	−	0
F. 21	ag	ag	ag	alk.g	ag	alk.g	alk.g	alk.g	ag	−g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	ag	ag	ag	ag	ag	alk.g	alk.g	ac	−	−	0
F. 22	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	alk	alk	alk	−	alk	alk	alk	ac	−	−	0
F. 23	ag	ag	ag	ag	ag	ag	−g	alk.g	ag	ag	ag	ag	ag	ag	alk.g	alk.g	−	alk	alk	alk	−	alk	alk	alk	ac	+	−	0
F. 24	ag	ag	−g	alk.g	ag	ag	−g	alk.g	ag	ag	−g	alk.g	ag	−g	−g	alk.g	ag	ag	ag	ag	ag	ag	−g	alk.g	ac	−	−	0
F. 25	ag	ag	−g	alk.g	ag	ag	−g	alk.g	ag	ag	−g	alk.g	ag	ag	−g	alk.g	−	alk	alk	alk	−	alk	alk	alk	ac	+	−	0
F. 26	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	alk.g	−	alk	alk	alk	−	alk	alk	alk	ac	+	−	0
F. 27	ag	ag	ag	ag	ag	ag	ag	alk.g	ag	ag	ag	alk.g	ag	ag	ag	ag	alk	alk	alk	alk	alk	alk	alk	alk	ac	−	−	0
F. 28	ag	ag	ag	ag	ag	ag	ag	ag	ag	−g	alk.g	alk.g	ag	ag	ag	ag	−	alk	alk	alk	−	alk	alk	alk	ac	−	−	0
F. 29	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	alk	alk	alk	−	alk	alk	alk	ac	Tr	−	0
F. 30	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	−	alk	alk	alk	ac	−	−	0
F. 31	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk	alk	alk	alk	alk	alk	alk	alk	ac	+	−	0
F. 32	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	alk	alk	alk	−	alk	alk	alk	ac	−	−	0

a = acid. ac = acid and clot. ag = acid and gas. alk = alkaline. alk.g = alkaline and gas. −g = neutral and gas. Tr = trace.

Table IV.

Days of incubation...	Lactose				Glucose				Mannite				Maltose				Saccharose				Dulcite				Milk	Indol	Haemolysis	Gelatin
	1	3	5	10	1	3	5	10	1	3	5	10	1	3	5	10	1	3	5	10	1	3	5	10				
U. 1	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	ag	ag	alk.g	alk.g	ac	−	−	−
U. 2	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	ag	ag	ag	−	−	alk	alk	ac	−	−	−
U. 3	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	alk	alk	alk	−	alk	alk	alk	ac	+ + +	−	−
U. 4	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	alk	alk	alk	−	alk	alk	alk	ac	+ +	−	−
U. 5	alk	alk	alk	alk	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	alk	alk	alk	alk	ac	−	−	−
U. 6	ag	ag	ag	ag	ag	ag	ag	alk.g	ag	ag	ag	alk.g	ag	ag	−g	alk.g	ag	ag	ag	−g	ag	alk.g	alk.g	alk.g	ac	+ +	−	−
U. 7	ag	ag	alk.g	alk.g	ag	−g	alk.g	alk.g	ag	−g	alk.g	alk.g	ag	ag	alk.g	alk.g	ag	ag	alk.g	alk.g	−	alk	alk	alk	ac	−	−	−
U. 8	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	−	−	−	ac	+ +	−	−
U. 9	ag	ag	ag	ag	ag	ag	ag	alk.g	ag	ag	alk.g	alk.g	ag	ag	ag	ag	−	alk	alk	alk	ag	ag	alk.g	alk.g	ac	−	−	−
U. 10	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−g	−g	ag	−	alk	alk	alk	−	alk	alk	alk	ac	+	−	−
U. 11	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	alk.g	alk	alk	alk	alk	−	alk	alk	alk	ac	−	−	−
U. 12	−	alk	alk	alk	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	ag	alk.g	alk.g	alk.g	−	alk	alk	alk	ac	−	−	−
U. 13	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	ag	ag	ag	ag	ag	ag	ag	ag	−	ag	alk.g	alk.g	ac	+	+	−
U. 14	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk.g	ac	−	−	0
U. 15	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	−	alk	alk	alk	ac	−	−	0
U. 16	ag	ag	ag	ag	ag	alk.g	alk.g	alk.g	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	ag	alk.g	alk	alk	alk	alk	ac	−	−	0

a = acid. ac = acid and clot. ag = acid and gas. alk = alkaline. alk.g = alkaline and gas. −g = neutral and gas.

REFERENCES.

ABEL, R. (1893). *Centralbl. f. Bakt.* **13**, 161 (1896); *ibid.* **21**, 89.
AMOS, H. L. (1925). *J. Exp. Med.* **41**, 649.
ARKWRIGHT, J. A. (1921). *J. Path. and Bact.* **24**, 49.
AVERY, O. T. and MORGAN, H. J. (1925). *J. Exp. Med.* **42**, 347.
AVERY, O. T. and NEILL, J. M. (1925). *Ibid.* **42**, 355.
BERTARELLI, E. (1906). *Centralbl. f. Bakt.* 1, Abt. Ref. **37**, 343.
BESSON, A. (1924). *Technique microbiologique et sérothérapique*, p. 784.
BLUMER, G. and LAIRD, A. T. (1901). *Bull. Johns Hopkins Hosp.* **12**, 45.
BORDONI-UFFREDUZZI, G. (1888). *Zeitschr. f. Hyg. u. Infectionskr.* **3**, 333.
CHIARI, H. (1895). *Prager med. Wochenschr.* 251.
CLAIRMONT, P. (1902). *Zeitschr. f. Hyg. u. Infectionskr.* **39**, 1.
COHN, T. (1893). *Deutsche med. Wochenschr.* **19**, 804.
DENYS, J. and MARTIN, J. (1893). *La Cellule*, **9**, 261.
DUDGEON, L. S. (1926). *J. Hygiene*, **25**, 120.
DUDGEON, L. S., WORDLEY, E. and BAWTREE, F. (1921). *Ibid.* **20**, 137.
v. DUNGERN, E. (1893). *Centralbl. f. Bakt.* **14**, 541.
EDWARDS [quoted from Besson, *v. supra*, p. 793].
v. EISLER, M. and PORGES, O. (1906). *Centralbl. f. Bakt.* 1 Abt. Orig. **42**, 660.
ESCHERICH, T. (1886). *Die Darmbakterien des Säuglings.* Stuttgart.
FASCHING, M. (1892). *Centralbl. f. Bakt.* **12**, 304.
FITZGERALD, J. G. (1912–13). *Proc. Soc. Exp. Biol. and Med.* **10**, 52.
FRICKE, C. (1896). *Zeitschr. f. Hyg. u. Infectionskr.* **23**, 380.
FRIEDLÄNDER, C. (1883). *Fortschr. d. Med.* **1**, 715.
GALLI-VALERIO, B. (1910). *Centralbl. f. Bakt.* 1 Abt. Orig. **53**, 477.
—— (1911). *Ibid.* **57**, 481.
GRIMBERT, L. and LEGROS, G. (1900). *Compt. rend. Soc. de Biol.* **52**, 491.
HEIDELBERGER, M. and AVERY, O. T. (1923). *J. Exp. Med.* **38**, 73.
—— —— (1924). *Ibid.* **40**, 301.
HEIDELBERGER, M., GOEBEL, W. F. and AVERY, O. T. (1925). *Ibid.* **40**, 701.
HOWARD, W. T. (1899). *Ibid.* **4**, 149.
JULIANELLE, L. A. (1926). *Ibid.* **44**, 113, 683 and 735.
KLEMPERER, F. and SCHEIER, M. (1902). *Zeitschr. klin. Med.* **14**, 133.
KOCKEL (1891). *Fortschr. d. Med.* **9**, 331.
KREIBOHM, R. (1889). *Ueber das Vorkommen pathogener Mikroorganismen im Mundsecret.* Dissert. Göttingen.
KRUSE (1899). Review in *Centralbl. f. Bakt.* **25**, 49.
LOEB (1891). *Ibid.* **10**, 369.
MANDRY (1890). *Fortschr. d. Med.* **8**, 205.
MARCHAND (1894). Review in *Centralbl. f. Bakt.* **15**, 428.
MILLER, W. D. (1892). *Die Mikroorganismen der Mundhöhle*, 320–322.
MORI, R. (1888). *Zeitschr. f. Hyg. u. Infectionskr.* **4**, 47.
NICOLAIER, A. (1894). *Centralbl. f. Bakt.* **16**, 601.
PASSET, J. (1885). *Untersuchungen über die Aetiologie der eitrigen Phlegmone des Menschen.* Berlin.
PAULSEN (1890). *Centralbl. f. Bakt.* **8**, 344.
PERKINS, R. G. (1900–01). *J. Exp. Med.* **5**, 389.
PFEIFFER, R. (1899). *Zeitschr. f. Hyg. u. Infectionskr.* **6**, 145.
PORGES, O. (1905). *Wien. klin. Wochenschr.* **18**, 691.
REIMANN, H. A. (1925). *J. Exp. Med.* **41**, 587.
SICARD, A. (1899). *Compt. rend. Soc. Biol.* **51**, 813.
SMALL, C. and JULIANELLE, L. A. (1922). *J. Infect. Dis.* **32**, 456.
STRONG, L. W. (1899). *Centralbl. f. Bakt.* **25**, 49.
STRYKER, L. M. (1916). *J. Exp. Med.* **24**, 49.
WEICHSELBAUM, A. (1886). *Wien. med. Jahrb.* p. 483.
WICKLEIN, E. (1895). *Centralbl. f. Bakt.* **18**, 425.
WILDE, M. (1896). *Ueber den Bacillus pneumoniae Friedländer's und verwandte Bakterien.* Bonn.
WORDLEY, E. (1921). *J. Hygiene*, **20**, 60.
WRIGHT, J. H. and MALLORY, F. B. (1895). *Zeitschr. f. Hyg. u. Infectionskr.* **20**, 220.

(*MS. received for publication* 11. II. 1928.—Ed.)