A STUDY OF THE *BACILLUS MUCOSUS CAPSULATUS* GROUP.

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The group of organisms included in the term *B. mucosus capsulatus* has continued to intrigue bacteriologists for more than thirty years, mainly on account of the problems of academic interest which arise when any attempt is made to define the characters of the group as a whole or to classify its members into types. Proof that such problems still exist is to be found in current text-books in which certain discrepancies occur regarding the biochemical reactions ascribed to the various members. There is also uncertainty as to the inclusion of *B. lactis aerogenes* in the group (Topley and Wilson, 1929; Wilson, 1929). Much of the difficulty arose from the fact that Friedländer's bacillus, one of the first members of the group to be isolated (1883), was studied from a morphological standpoint, while Escherich who described *B. lactis aerogenes* in 1886 stressed its biological similarity to *B. coli*. A chasm was thus created between *B. lactis aerogenes* and Friedländer's bacillus which has not been bridged to the present day, although these organisms must always have resembled, more than they differed from, each other; *e.g.* Bergey (1929) classifies *B. lactis aerogenes* with coliform types under Bacterieae and places Friedländer's bacillus in the tribe *Klebsielleae Trevisan, i.e.* encapsulated Gram-negative organisms encountered principally in the respiratory tract, whose fermentation reactions are not given in full. Organisms morphologically similar to Friedländer's bacillus were found to be widely distributed, and in 1896 Fricke named the group *B. mucosus capsulatus*. There is still little criticism to be made of his general description to the effect that the members were Gram-negative, possessed capsules, showed marked pleomorphism and did not form spores; they grew on a variety of media in a profuse slimy layer, and in gelatin stabs they did not liquefy, but showed the so-called "nail" growth; in addition to this, most of the bacteria classed under this head formed a moderate amount of indole and fermented carbohydrates in solution with production of acid and gas. In an attempt to type members of this group Perkins (1904) admitted that the current knowledge of technique had been almost exhausted in the effort to separate one member from another, but on the basis of certain biochemical differences he was able to distinguish three classes, of which the first was the largest:

(1) *Bact. lactis aerogenes*, fermenting lactose and saccharose;
(2) *Bact. pneumonicum* (Friedländer group), fermenting saccharose but not lactose;
(3) *Bact. acidi lactici*, fermenting neither lactose nor saccharose.

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In a later publication (1907) he maintained that the prototype of the group was *Bact. aerogenes*, and that the other members were variants which had lost the power to ferment certain sugars in whole or in part through modifications in environment. MacConkey (1905) in his study of the lactose fermenters in faeces, which remains the classical work on this subject, used a considerably augmented number of biochemical tests, including the Voges-Proskauer reaction. These enabled him to differentiate species that had been formerly grouped with *B. coli*, and he formed the opinion that *B. lactis aerogenes*, which he studied in some detail, was not the same organism as *B. acidi lactici* (Hüppe), nor were these organisms simply non-motile forms of *B. coli communis*.

To discuss other studies of the *B. mucosus capsulatus* at the present juncture would tend to obscure the main issue which is to determine whether *B. lactis aerogenes* can be established as a member of this group, and to define its relationship to the group of Gram-negative encapsulated bacilli associated with infections of the respiratory tract (Friedländer's bacillus). An account of the present investigation will accordingly be given for which it is claimed that the series studied was larger than any other recorded (63 strains of *B. mucosus capsulatus*, 27 strains of *B. acidi lactici*, 13 strains of *B. cloacae*, 39 strains of *B. coli communior* and 14 strains of *B. neapolitanus*), and that serological reactions were correlated with comprehensive biochemical reactions.

COLLECTION OF SERIES AND TECHNIQUE.

The collection was commenced in 1928 when 24 strains of *B. mucosus capsulatus* were isolated during an investigation into infantile diarrhoea; 9 of these strains were derived from the faeces in cases of acute enteritis, and 4 from healthy controls; 4 were isolated from milk samples and 7 from batches of flies taken from infected houses. The faecal specimens were obtained by means of the rectal swab in the majority of cases and cultures were made within 6 hours directly on to solid medium; the milk samples were also plated directly, suitable dilutions having been made; the flies were trapped in a sterile wide-mouthed bottle, asphyxiated with ether vapour and mashed up in 5 c.c. bouillon, of which a loopful yielded an abundant growth after a few hours' incubation. After preliminary plating of specimens on MacConkey's lactose-bile-salt agar, representative colonies were subcultured on agar slopes and submitted to the biochemical tests recommended by Muir and Ritchie (1927), viz. fermentation of glucose, lactose, saccharose, dulcitol, mannitol, adonitol, inulin and inositol, the behaviour in litmus milk, indole formation in peptone water, liquefaction of gelatin, the production of acetylmethylcarbinol and the methyl-red reaction.

Peptone water containing 1 per cent. of the various carbohydrates (0·5 per cent. in the case of glucose) and Andrade's indicator was used to study fermentation, the results being read after 10 days' incubation at 37° C. Indole formation was tested by means of a strip of filter paper soaked in saturated

solution of oxalic acid, which was then dried and inserted inside the test-tube attached to the plug (Gnezda reaction); this method was found to be expeditious, reliable and delicate when compared with Ehrlich's rosindole test. The Voges-Proskauer method was used to test the presence of carbinol, *i.e.* strains were grown for 3 days in 2 per cent. glucose bouillon which was then exposed to room temperature after the addition of decinormal sodium hydroxide; a positive reaction, if present, usually appeared within 4 hours. The methyl-red reaction was tested in a 3 days' growth in 0·5 per cent. peptone water containing a similar amount of glucose and di-potassium hydrogen phosphate.

During this investigation 278 lactose-fermenting bacilli were isolated and typed, the following species occurring in descending order of frequency: *B. coli communis*, *B. coli communior*, *B. acidi lactici*, *B. mucosus capsulatus*, *B. neapolitanus*, *B. cloacae*, *B. coli anaerogenes* and *B. "paracolon"* types. As particular attention was being paid to *B. mucosus capsulatus* the above list is not intended to serve as an accurate guide to the relative frequency of coliform types of faecal origin, although an attempt was made to isolate from each plate as many different types as possible. The experience gained during this work led the writer to curtail the biochemical tests in later work in the following manner: lactose fermenters on MacConkey's agar were tested in saccharose and dulcitol, and in the search for *B. mucosus capsulatus* all saccharose fermenters, whether they fermented dulcitol or not, were then tested in adonitol, inulin and inositol; they were also tested for indole and carbinol formation. This modification was based on conclusions regarding the value of the various tests which agreed with those of MacConkey (1909), although arrived at independently.

Twenty-one more strains of *B. mucosus capsulatus* were isolated in 1930 when the occurrence of this organism in adult faeces was investigated, 6 strains being derived from cases of paratyphoid fever and ileo-colitis by plating in the usual manner; the remainder were obtained from normal stools by means of enrichment in citrate broth (Koser, 1923) or by direct plating on citrate agar recommended by Simmons (1926). The superiority of Simmons' modification was demonstrated in the following test.

Experiment. With 12 samples of faeces, direct plating on Simmons' citrate agar yielded 8 strains of *B. mucosus capsulatus*; enriching each sample in 3 successive tubes of Koser's citrate broth followed by plating on MacConkey's agar yielded 5 strains; direct plating on MacConkey's agar yielded only 1 strain.

Seven strains of *B. mucosus capsulatus* were added from the National Collection of Type Cultures, also 1 stock strain of Friedländer's bacillus, 4 strains isolated from sputum, 1 from pus, 1 from chronic nasal sinusitis and 4 from urine. The entire collection thus numbered 63 strains. It may be emphasised here that, while strains were originally isolated because of cultural appearances suggestive of the mucoid-encapsulated group, all strains which failed to ferment inositol were excluded from this group. The term *B. mucosus*

capsulatus will accordingly be reserved for strains which besides other properties have the power to ferment inositol.

DISTRIBUTION.

B. mucosus capsulatus was isolated in 5 of 38 samples (13 per cent.) of normal faeces, and in 14 of 50 samples (28 per cent.) of diarrhoeal faeces, no special enrichment method being used. Not only was the frequency of the organism doubled in diarrhoeal conditions, but its relative proportion to *B. coli* was increased to such an extent as to outnumber it in many instances. This marked change could not be attributed to the mere hurrying onwards of the contents of the small intestine or caecum, since no colonies of *B. mucosus capsulatus* appeared on the primary MacConkey agar plates subcultured from the dejecta in artificial diarrhoea, produced by large doses of castor oil in 7 instances and magnesium sulphate in 5 instances. This was in marked contrast to the ease with which *B. mucosus capsulatus* was often isolated in cases of enteritis.

An attempt was made to discover the natural habitat of *B. mucosus capsulatus* in the gut by taking 4 samples of normal stools and examining a loopful of the mucus layer covering them, then after searing across the stool, examining a loopful of the solid matter. In 3 of the samples the surface mucus yielded plentiful colonies of *B. mucosus capsulatus* and *B. coli*, whereas the solid matter yielded *B. coli* almost entirely, but in much scantier growth. This would suggest that *B. mucosus capsulatus* is associated in the large intestine with the mucus-secreting cells.

B. mucosus capsulatus was found to occur very infrequently in sputa, only 4 strains being collected in an examination of 152 specimens. The same difficulty was encountered in attempting to isolate nasal strains when only 1 strain was obtained from 42 swabs. All cultures were made on serum agar slopes.

The occurrence of *B. mucosus capsulatus* in milk samples and in flies was associated with its occurrence in the faeces of infants with enteritis in the same house, except in the case of Fl.[1] 4 and 5. In one instance the strains from milk, flies and faeces, in another the strains from milk and faeces, and in a third the strains from flies and faeces proved to be serologically identical.

No strains of *B. mucosus capsulatus* were isolated from 11 soil samples nor from 13 samples of sheep droppings, although enrichment methods were employed in the examination. The soil samples were selected widely, 4 being collected from back-courts, 2 from agricultural land and 5 from moors. The moor samples were found to be sterile, but the other samples yielded a heavy growth of coliform types which included *B. cloacae* from one of the back-court soils.

[1] The following symbols are used in the text to indicate the origin of the strains: F. = faeces, Fl. = flies, M. = milk, S. = sputum and U. = urine.

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Morphology.

All strains were non-motile bacilli which reacted negatively to Gram's stain, and the irregular manner in which they frequently took up the stain gave the appearance of a centrally situated spore. The microscopical appearance when grown for 24 hours on plain agar, or on MacConkey's agar, varied with different strains from a short cocco-bacillus of medium thickness to a much stouter, longer bacillus of uniform length; with certain strains there was a mixture of longer and shorter forms; filamentous forms were fairly common and pair formation was observed. Various strains of *B. coli* presented a similar microscopical appearance. The formation of capsules was studied in 42 strains, using Muir's method of staining smears made from 24-hour serum agar cultures. All the strains tested when newly isolated, whether of faecal or non-faecal origin, showed well-defined, close-fitting capsules; the majority of the strains tested after a year's subculture were also encapsulated, while the remainder showed vestiges of capsule formation, except a few strains in which capsules were not demonstrated. Certain type cultures still showed the vestiges of a capsule after many years subculture. The width of the capsule varied with different strains, and it was a frequent occurrence for several bacilli to be enclosed within a single capsular envelope which had degenerated to mere shreds connecting one bacillus to another in certain of the older strains. A mixture of encapsulated and non-capsulated forms was not observed.

Cultural characters.

On solid media the strains gave a luxuriant slimy growth, dense white and faintly opalescent on plain agar, greyer, moister and more viscid on serum agar. The strains showed great vitality on these media, remaining alive for several months at room temperature without subculture; non-faecal strains tended to die out more rapidly than those of faecal origin. On MacConkey's agar colonies were circular, smooth-edged, slimy, pale pink to reflected light and deep red to transmitted light. When the medium was too dry the colonies showed a rough surface at first, sliminess developing after several days' growth at room temperature. These colonial appearances were simulated closely on occasion by young cultures of *B. acidi lactici*, *B. coli communior*, *B. cloacae* and *B. neapolitanus*; *B. cloacae* bore the closest resemblance to *B. mucosus capsulatus*, but might be distinguished by a somewhat dirty greyish appearance and by a slightly urinous odour; moist colonies of the other coliform types cited were less viscid than those of *B. mucosus capsulatus*, and none were actually slimy.

Selected strains of *B. mucosus capsulatus*, including *B. Friedländer* 204, *B. lactis aerogenes* 418 and *B cloacae* 408 were plated on Simmons' citrate agar. The resulting growths did not show any features by which faecal strains might be distinguished from those of non-faecal origin, but the difference was emphasised between *B. mucosus capsulatus* and those coliform types which simulated its appearance on MacConkey's agar, for whereas all strains of the

former gave luxuriant, moist, orange-green colonies with Prussian blue discoloration of the surrounding medium, *B. cloacae* gave a more restricted growth of moist greyish or bluish green colonies and less discoloration, while *B. acidi lactici*, *B. coli communior* and *B. neapolitanus* behaved more like *B. coli communis*, in that their colonies were usually small, and if they grew to any size could be readily distinguished by a greyish colour, rougher surface and absence of surrounding discoloration.

No strain of *B. mucosus capsulatus* produced liquefaction of gelatin after 2 months' culture.

BIOCHEMICAL REACTIONS.

The biochemical tests which were performed have already been enumerated; they were not so wide in their scope as those of many other observers and were modified still further in the later work (*vide* Technique). The reactions of the entire collection of *B. mucosus capsulatus* and of 93 strains of coliform types which simulated its cultural appearances are set forth in Table I. Reference to this table will show that the critical test in the separation of *B. mucosus capsulatus* from non-capsulated *B. coli* is *inositol fermentation* and not saccharose fermentation which is a property shared with *B. cloacae*, *B. neapolitanus* and *B. coli communior*, nor adonitol fermentation which was a feature of 6 of 27 strains of *B. acidi lactici*, nor carbinol formation which is given by *B. cloacae*. Of these reactions, inositol fermentation alone is exclusive to *B. mucosus capsulatus*.

All members of the *B. mucosus capsulatus* group fermented glucose, saccharose and mannitol, while fermentation of lactose, adonitol and inositol occurred so constantly (not less than 95 per cent.) as to be considered another essential property of the group. The response to the other tests varied widely; dulcitol was fermented by 26 strains, and inulin by 15, of which 10 fermented dulcitol also. Indole was formed by 17 strains, 13 being derived directly from the faeces and the others, viz. M. 4, Fl. 1 and 6 and U. 3, probably derived indirectly from the same source; 13 indole formers fermented dulcitol and 5 fermented dulcitol and inulin. A positive carbinol reaction was given by 44 strains and was not always accompanied by a negative methyl-red reaction, which was so unstable as to be regarded of little significance in the group; of these carbinol positive strains, 17 fermented dulcitol and 11 formed indole.

O'Meara (1931) questions the accuracy of the Voges-Proskauer method of testing carbinol formation, and suggests growing strains in 5 c.c. Koser's citrate broth to which 0·5 per cent. glucose and 1 per cent. sodium fumarate have been added. After 24 hours' incubation a knife-point of creatine and 5 c.c. of 40 per cent. sodium hydroxide are added to the cultures, which are then exposed to room temperature, a positive reaction developing rapidly if present. He claims that this method is a more delicate test for carbinol, and by means of it he was able to demonstrate carbinol formation in a series of *B. mucosus capsulatus* and certain coliform types, notably *B. coli communior*. Thirteen strains from this collection and 4 strains of *B. coli* (1 strain of *B. acidi lactici*, MacConkey's bacillus No. 71 and 2 strains of *B. coli communis*) were tested for carbinol formation by O'Meara's method. It was found that while this

method was a ready means of discovering a positive reaction, 2 strains of *B. mucosus capsulatus* which were carbinol-negative by the Voges-Proskauer method, viz. F. 31 and *B. Friedländer* 204, were also negative by O'Meara's method. The coliform strains were negative by each method. The conclusion is, as before, that carbinol formation is not an essential feature of the *B. mucosus capsulatus* group.

These varying biochemical reactions appeared to be of doubtful significance in an attempt to distinguish types within the *B. mucosus capsulatus* group, as they did not bear any constant relationship to each other. Their importance was doubted still further when the serological reactions demonstrated relationships between strains possessing different biochemical reactions. It was decided therefore to classify the strains into the following sub-groups according to their source: (*a*) "miscellaneous," composed of strains from sputum, nose, pus, urine and certain type cultures of non-faecal origin, viz. Nos. 204, 243, 418 and 2926; (*b*) strains derived from normal faeces; and (*c*) "enteritis" sub-group including the faecal, milk and fly strains isolated during the investigation into infantile diarrhoea. An analysis of the varying biochemical reactions of these sub-groups is to be found in Table I. With regard to the relative significance of the reactions, dulcitol fermentation being equally distributed throughout the sub-groups, has none; inulin fermentation is not a sufficiently common feature to be considered important, although it tended to accompany dulcitol fermentation, and is not encountered among the strains from normal faeces. The Voges-Proskauer reaction and indole formation remain therefore the sole means of differentiating types, if any, within the group. Of these, the former is not a constant feature of any sub-group, nor is it a reliable test to differentiate strains of faecal from those of non-faecal origin, as a considerable proportion of each are carbinol-positive. The division of the total faecal strains into sub-groups shows, however, that a positive carbinol reaction is given by 81 per cent. of the "enteritis" strains, whereas it tends to be absent in the strains from normal faeces (47 per cent.). In this manner a resemblance is shown between the "enteritis" and sub-group and "miscellaneous" strains, 76 per cent. of which are carbinol-positive. Indole is formed by 58 per cent. of the normal faecal strains, by 19 per cent. of the "enteritis" strains, and is almost negligible as a property of the "miscellaneous" sub-group. The combination of a positive carbinol and a negative indole reaction is a pronounced feature of the "miscellaneous" sub-group (70 per cent.), not so common among the "enteritis" strains (62 per cent.), and is given by only 21 per cent. of the strains from normal faeces. The reciprocal combination is encountered only among strains from normal faeces (32 per cent.). The most noteworthy feature of this table is therefore the similarity in behaviour of the "miscellaneous" and "enteritis" sub-groups and the contrast between the latter and the strains from normal faeces. While no well-defined types emerge from the analysis, there appears to be a rather definite tendency for the "miscellaneous" and "enteritis" strains, *i.e.* strains derived from a pathogenic lesion, to be carbinol-positive and indole-negative.

Table I. *Showing biochemical reactions of* 62* *strains of* B. mucosus capsulatus *arranged in sub-groups, and of* 93 *strains of* B. coli.

Strains	Glucose	Lactose	Saccharose	Dulcitol	Mannitol	Adonitol	Inulin	Inositol	Carbinol	Indole	Carbinol+ Indole−	Carbinol− Indole+
B. mucosus capsulatus												
Sub-group. "Miscellaneous" (17 strains)	17 (100)	16 (94)	17 (100)	7 (41)	17 (100)	14 (82)	6 (35)	16 (94)	13 (76)	1 (6)	12 (70)	○
"Normal faecal" (19 strains)	—	—	19 (100)	8 (42)	—	19 (100)	○	19 (100)	9 (47)	11 (58)	4 (21)	6 (32)
"Enteritis" (26 strains)	26 (100)	26 (100)	26 (100)	11 (42)	26 (100)	26 (100)	9 (35)	26 (100)	21 (81)	5 (19)	16 (62)	○
B. coli communior (39 strains)	39 (100)	39 (100)	39 (100)	39 (100)	39 (100)	○	○	○	○	39 (100)	○	39 (100)
B. cloacae (13 strains)	13 (100)	13 (100)	13 (100)	○	13 (100)	○	○	○	13 (100)	○	13 (100)	○
B. acidi lactici (27 strains)	27 (100)	27 (100)	○	○	27 (100)	6 (22)	○	○	○	27 (100)	○	27 (100)
B. neapolitanus (14 strains)	14 (100)	14 (100)	14 (100)	○	14 (100)	○	○	○	○	14 (100)	○	14 (100)

* *B. lactis aerogenes* 124 is withheld from this table as source is not known.
Figures in parentheses are percentages.
○ = No reaction; — = No test made.

Three strains were found in the "enteritis" sub-group which fermented all the carbohydrates and produced both indole and carbinol, so-called *B. oxytocus*.

The stability of the biochemical reactions was studied by retesting the "enteritis" strains after 1 year's subculture on plain agar. It was found that the fermentation reactions did not vary, but 3 strains gave an altered Voges-Proskauer reaction, 2 becoming negative and 1 positive. The methyl-red test was quite unstable and altered to a positive result in many instances. The power of indole formation had apparently been lost by 2 strains.

No special study was made of the phenomenon known as *cameleonage* which occurred in the fermentation reactions of many strains of *B. mucosus capsulatus*, as it was not found to be a property peculiar to this group; it was also possessed by certain strains of coliform types.

When a strain of *B. mucosus capsulatus* and *B. coli* were implanted together in bouillon the latter was rapidly outgrown, but not entirely suppressed, even with the *p*H adjusted as low as 4·8 or as high as 8·6. Another manifestation of the vitality of *B. mucosus capsulatus* is furnished by its resistance to the action of brilliant green added to peptone water (Browning, Gilmour and Mackie, 1913), and the 3 strains from cases of paratyphoid fever were collected in this manner.

Serological reactions.

Antisera were prepared by Dr R. Cruickshank against 3 of the "enteritis" strains (F. 5, 10 and Fl. 2), against 3 sputum strains (S. 1, 2 and 5), against *R. lactis aerogenes* 418 and against 1 of the strains of *B. cloacae* isolated during the investigation. Heat-killed emulsions of recent subcultures were administered to rabbits intravenously and care was taken not to persist with the dosage over too long a period and thus elaborate group agglutinins which might mask the action of any type-specific agglutinins present. Before proceeding further it is necessary to refer to the work of Julianelle (1926), whose findings explain the varying titres of the antisera produced, as well as the agglutination reactions described below. Applying the findings of Avery and of Heidelberger on the immunological relationships of the pneumococci to the Friedländer group, Julianelle found that recently isolated strains were smooth (*S*), *i.e.* encapsulated and virulent and that their antisera which had a low titre only agglutinated strains of the same type. Experimenting with *S* strains he was able to distinguish three serological types (A, B and C) and a heterogeneous group *X* in his series. By means of artificial decapsulation he produced strains which were completely rough (*R*), whose antisera were of high titre, had lost type specificity and agglutinated *R* strains of the other types. *R* antisera failed to agglutinate *S* strains even of the same type, but one of the *S* antisera (Type A) agglutinated incompletely *R* strains of all types.

The strains used for the production of antisera in the present investigation were of various ages; strains S. 1 and 2 were recently isolated, strains F. 5, 10, Fl. 2 and *B. cloacae* were 1 year old. No strain was decapsulated artificially, and none of the sera produced could be regarded as purely anti-*R* sera.

Strains S. 5 and F. 5 produced no general reaction in the animals on inoculation, and their antisera reached a high titre very rapidly. Difficulty was encountered in preparing the other antisera owing to the toxicity of the strains for rabbits, particularly in the case of S. 1, whose virulence might be explained by the fact that it was a recently isolated, encapsulated strain. The persistence of the capsule in strains S. 2 and F. 10, which were selected as possibly different types from S. 1 and F. 5 respectively, would explain the low titres of their antisera. The occurrence of the same low titre in the antiserum to *B. lactis aerogenes* 418 suggests that smoothness may persist for many years, even when only the vestige of a capsule is demonstrable as was the case with this strain, although this would appear to be contrary to the findings of Julianelle. After two abortive attempts to prepare an antiserum against one of the strains of so-called *B. oxytocus* derived from a case of enteritis (F. 12), a serum was finally prepared against a similar strain (Fl. 2) which possessed less toxicity for rabbits. Second lots of antisera were prepared against F. 5 and S. 1 after a year's interval, in order to test the serological relationships of the strains isolated from faeces in adults. The titres remained the same, being high in the case of the former strain and still low in the case of the latter, although this strain was no longer virulent for rabbits.

RESULTS.

The results of the agglutination tests between these antisera and the "enteritis" and "miscellaneous" strains are set forth in Tables II and III. The method used in testing was to emulsify 24-hour agar-slope cultures in 0·4 per cent. saline, thus eliminating spontaneous agglutination as far as possible, and after making the dilution with antiserum, to incubate overnight at 37° C. It will be noted firstly that there is an entire absence of cross-agglutination between *B. mucosus capsulatus* and *B. coli*; none of three antisera to the former agglutinated non-capsulated coliform types (12 strains), nor did the antiserum to *B. cloacae* agglutinate any of 12 strains of *B. mucosus capsulatus*.

Within the *B. mucosus capsulatus* group itself, the reactions were not so definite as they might have been had the tests been carried out with encapsulated strains and anti-*S* sera on the one hand, and decapsulated strains and anti-*R* sera on the other. Complete agglutination to titre, or fractions of titre, was given by a number of strains, but the majority showed partial agglutination without complete sedimentation and clearing of the bacterial emulsion. Since none of the strains had been decapsulated, the explanation of these varying serological reactions is probably to be found in the varying amounts of the *S* and *R* components in the antigen used in the production of the antisera, and in the same complexity occurring in the bacterial emulsions tested for agglutinability. Complete agglutination was held to denote a closer relationship between strains than a partial reaction.

The antiserum to F. 5, a faecal strain, with a titre of 1 : 6400, agglutinated several of the "enteritis" strains (F. 6 and 12, M. 1, 2, 3 and 4, Fl. 3, 4 and 6)

Table II. *Showing serological reactions of strains of* B. mucosus capsulatus *and* B. coli.

Serum	Strains of *B. mucosus capsulatus in* "Enteritis" sub-group																			*B. cloacae*	*B. coli communis*	*B. neapolitanus*
	F. 5	F. 6	F. 7	F. 8	F. 9	F. 10	F. 11	F. 12	F. 13	M. 1	M. 2	M. 3	M. 4	Fl. 1	Fl. 2	Fl. 3	Fl. 4	Fl. 5	Fl. 6	Fl. 7			
F. 5 (1st lot) Titre 1 : 6400	T	T	—	—	—	—	—	T/8	—	T	T	T/2	T/8	—	—	T/2	T/2	—	T/4	—	—	—	—
F. 10 Titre 1 : 160	T+	PT	—	—	—	T	P T/2	PT	—	PT+	PT+	.	.	—	.	PT+	PT	—	.	.	.	.	.
Fl. 2 Titre 1 : 400	T+	T/2	P T/4	P T/4	—	—	P T/2	PT	P T/8	P T/2	T+	T/2	T/2	T	T	PT	T+	P T/4	PT	.	—	—	—
S. 1 Titre 1 : 320	—	—	—	—	—	—	—	—	—	—	.	—	—	—	—	—	—	—	.	.	.	.	.
S. 2 Titre 1 : 320	T+	PT+	—	—	—	—	—	PT	—	PT	PT+	T+	PT	—	—	PT	PT	—	P T/2	—	.	.	.
S. 5 Titre 1 : 6400	—	—	—	—	P T/64	—	P T/16	P T/32	—	P T/16	P T/8	P T/32	P T/32	—	—	P T/32	T/16	P T/32	—	P T/16	—	—	—
B. lactis aerogenes 418 Titre 1 : 100	—	.	—	—	—	—	.	P T/4	.	.	P T/4	.	.	—	P T/4	.	.	—	.	.	.	.	.
*B. cloacae**	—	—	—	—	—	—	.	.	.	—	—	.	.	—	.	—	—	—	.	.	T	—	—

"T" = Agglutination to titre; "P" = Partial agglutination.

* This antiserum agglutinated homologous strain only.

Table III. *Showing serological reactions of miscellaneous strains of* B. mucosus capsulatus.

Serum	S. 1	S. 2	S. 3	S. 4	S. 5	N	Pus	U. 1	U. 2	U. 3	N.C. 418	N.C. 243	N.C. 204	N.C. 124	N.C. 2926
F. 5 (1st lot) Titre 1 : 6400	.	.	.	.	T/32	.	P T/16	P T	P T/16	.	—	—	P T/16	P T/8	P T/32
F. 10 Titre 1 : 160	—	—	—	.	.	.	.	.	.	.	—	.	T+	T+	.
Fl. 2 Titre 1 : 400	.	.	.	.	P T/4	.	.	.	.	.	—	—	T/2	P T+	P T+
S. 1 (1st lot) Titre 1 : 320	T	—	T/2	—	T/4	P T+	P T/4	—	—	—	—	—	T	P T/8	.
S. 2 Titre 1 : 320	—	T	—	P T	.	P T	.	P T	P T	P T	—	—	T+	—	—
S. 5 Titre 1 : 6400	—	.	—	.	T	—	.	—	—	—	—	—	T	P T/16	P T/16
B. lactis aerogenes 418 Titre 1 : 100	—	.	—	.	T+	—	.	.	.	—	T	T	P T+	P T/2	.

Table IV. *Showing serological reactions of strains of* B. mucosus capsulatus *isolated from adult faeces.*

Serum	F. 14	F. 15	F. 16	F. 17	F. 18	F. 19	F. 20	F. 21	F. 22	F. 23	F. 24	F. 25	F. 26	F. 27	F. 28	F. 29	F. 30	F. 31	F. 32	F. 33	F. 34
F. 5 (2nd lot) Titre 1 : 3200	P T/4	P T/2	—	P T/4	—	P T	T/2	—	P T/2	P T/2	P T	P T	P T	P T/2	P T/4	—	P T	—	—	—	P T
S. 1 (2nd lot) Titre 1 : 320	P T/2	P T/4	—	—	—	P T/2	P T/4	—	P T/8	P T/8	P T/2	P T/4	P T/8	P T/4	P T/2	P T/8	P T/2	P T/8	P T/4	—	P T/4

in various titre fractions, but showed only partial agglutination with members of the "miscellaneous" group. Serum F. 10, whose titre of 1 : 160 suggested that it was smooth, agglutinated strains F. 5, *B. lactis aerogenes* 124 and *B. Friedländer* 204 completely, and showed partial agglutination with all the "enteritis" strains agglutinated by serum F. 5. The failure of serum F. 5 to agglutinate strain F. 10 is probably due to the fact that *R* elements predominated in strain F. 5, whereas *S* elements predominated in strain F. 10; this would appear to bear out the findings of Julianelle that an anti-*R* serum does not agglutinate even closely related *S* strains. The antiserum to Fl. 2 (*B. oxytocus*) possessed wider agglutinating powers for the "enteritis" strains than any other serum, and provided a closer link with the strains from the respiratory tract than serum F. 10 by agglutinating strains S. 5 as well as *B. Friedländer* 204.

Of the sera derived from strains from the respiratory tract, serum S. 1 agglutinated completely, or almost completely, a few members of the "miscellaneous" group (S. 3 and 5, nasal, pus, *B. Friedländer* 204), but failed to show any reaction with the "enteritis" strains; it exhibited a weak relationship with *B. lactis aerogenes* 124. As the titre of this serum was low (1 : 320), its complete reaction with strain S. 3 was regarded as determining type specificity between these two recently isolated strains. Serum S. 2 had a titre as low as that of serum S. 1, but showed wider relationships, agglutinating incompletely strains S. 4, nasal, *B. Friedländer* 204, U. 1, 2 and 3, and 11 of the "enteritis" strains. The existence of different serological types among strains of Friedländer's bacillus, claimed by Julianelle, would appear to be borne out by serum S. 2 agglutinating strain S. 4 and failing to agglutinate strains S. 1 and 3. Serum S. 5 with a titre of 1 : 6400 agglutinated completely *B. Friedländer* 204 and elicited a weak response from certain "enteritis" strains, some of which were not agglutinated by any other serum, again demonstrating a group relationship between strains derived from different sources. The antiserum to *B. lactis aerogenes* 418, with the low titre of 1 : 100, exhibited a marked specificity for so old a strain; it showed a close serological relationship with *B. Friedländer* 204 and *B. mucosus capsulatus* 243 (descended possibly from the same original strain as *B. lactis aerogenes* 418), and a less marked relationship with *B. lactis aerogenes* 124.

The reactions between the second lots of antisera to strains S. 1 and F. 5 and strains recently isolated from adult faeces are to be found in Table IV; they should be contrasted with the reactions of the first lots of antisera given in Tables II and III. Each strain appears to have lost type specificity on subculture *in vitro*, for whereas the first lot of S. 1 serum did not agglutinate any faecal strain, the second lot with the same low titre agglutinated the majority of the faecal strains it was tested against, although only partially. Serum F. 5 (second lot) did not show the complete reactions with faecal strains that the first lot had shown, even with strains F. 14–19 derived from cases of enteritis. It elicited slightly stronger reactions than serum S. 1 (second lot), and

although these would seem to be reactions between *S* strains and an anti-*R* serum, it was held that sufficient capsule formation persisted in strain F. 5 to confer a certain amount of *S* characteristics.

The results of the entire serological tests show that all but 4 strains were agglutinated by one or more of the comparatively few antisera employed. It is admitted that the majority of the reactions are incomplete, but their importance is enhanced by the entire absence of cross-agglutination between the *B. mucosus capsulatus* group and a series of *B. coli*. The *B. mucosus capsulatus* group is therefore established as a serological entity, and making every allowance for the interaction of *S* and *R* elements in the antisera and bacterial emulsions, there is still sufficient evidence that serological types exist within the group. A study of the complete reactions in Tables II and III shows that faecal strains are more closely related to each other serologically than to those derived from the respiratory tract and *vice versa*. The serological types which emerged were (*a*) faecal, comprising strains F. 5, 6, 12, M. 1, 2, 3, 4, Fl. 3, 4, 6, *B. lactis aerogenes* 124; (*b*) non-faecal, comprising S. 1, 3, 5, pus, nasal, *B. Friedländer* 204, *B. mucosus capsulatus* 243 and *B. lactis aerogenes* 418. Connecting links between types were supplied by strain Fl. 2, which is more closely related to the faecal strains, and by strain S. 2, which is more closely related to the non-faecal strains.

Virulence. Dr R. Cruickshank tested the virulence of a few strains of *B. mucosus capsulatus* on guinea-pigs and rabbits. The results showed that freshly isolated faecal strains were even more lethal than a strain from a case of pneumonia which killed a rabbit on the third day. The experiments showed also that virulence diminished *pari passu* with type specificity.

Feeding experiment. Four guinea-pigs were supplied for 1 week with water heavily contaminated with *B. mucosus capsulatus*. Examination of cloacal swabs showed that this organism did not appear in the faeces for 3 days and that it was still present at the end of a week. Cloacal swabs from 12 healthy guinea-pigs failed to yield any colonies of *B. mucosus capsulatus*. No diarrhoea was produced by this massive infection *per os*, although the animals refused food and did not appear to be well. This experiment was repeated on young guinea-pigs with the same result.

DISCUSSION.

In analysing the results of the present investigation in conjunction with the findings of other workers, one wishes to discuss in particular (1) the biological characters which define the *B. mucosus capsulatus* group and separate it from other coliform types, and (2) the distribution of this group in the environment and in man.

(1) *Biological characters of* B. mucosus capsulatus *group.*

(*a*) *Encapsulation.* The pleomorphism shown by various strains of *B. mucosus capsulatus* has been noted by all observers, who agree that microscopical

appearances alone are insufficient to distinguish it from *B. coli*, but there is some discrepancy in their findings concerning its encapsulation. Bamforth (1928) found it very difficult to demonstrate capsules from subcultures, but easy when strains were examined in contact with body fluids; Fitzgerald (1914), who included 6 strains of *Bact. aerogenes* (Escherich) among his 44 type strains, found that capsule formation varied greatly, but could be replaced by animal passage if absent. Small and Julianelle (1923) found all their strains to be encapsulated. The writer was able to demonstrate capsules in all recently isolated strains from the various sources; the power of capsule formation was retained longer by some strains than by others.

(*b*) *Citrate utilisation.* The colonial appearances of these encapsulated bacilli on ordinary media, *i.e.* a slimy mucoid growth, was found to be simulated fairly closely by certain non-capsulated coliform types, notably *B. cloacae* and *B. acidi lactici*. When difficulty is encountered in differentiation it is suggested that use be made of Simmons' citrate agar. It was found that *B. mucosus capsulatus* gave on this medium a luxuriant and characteristic growth, dependent on its power of utilising citrate as a source of carbon more readily than *B. cloacae* and other coliform types which also grow on this medium.

(*c*) *Inositol fermentation.* The confusion in the older text-books with regard to the biochemical characters of *B. mucosus capsulatus* is still apparent in the latest manuals and is probably related, in part at least, to the inclusion of coliform strains in studies on this group, which might account for the statement that *B. Friedländer* is an indole former. Another possible reason for discrepancies is the use of type strains which are not representative in their characters; *e.g. B. Friedländer* 204 is carbinol-negative, while the majority of strains from the respiratory tract are carbinol-positive. The present study shows that coagulation of milk and fermentation of glucose, mannitol and inulin might be dispensed with in typing lactose fermenters, and it is suggested that fermentation of saccharose, dulcitol, adonitol and inositol, the carbinol and indole reactions are the only useful reactions. Of these, inositol fermentation is the critical test for the differentiation of *B. mucosus capsulatus* from coliform types, as Mackie claimed (1913), and is a test that ought to be more widely adopted. The present study has shown that biochemical reactions do not help to differentiate clearly types within the *B. mucosus capsulatus* group, although the Voges-Proskauer and indole tests showed certain differences between strains from the various sources. Thus when strains were grouped according to their source it was found by means of these tests that faecal strains derived from cases of enteritis showed a closer resemblance to the sputum strains and to other strains of miscellaneous origin than to strains derived from normal faeces. The strains in the "miscellaneous" sub-group showed greater uniformity in their reactions to these tests than those in the other sub-groups. O'Meara's claim that all strains of *B. mucosus capsulatus* are carbinol-positive by his method was not upheld by a test made during the present investigation.

(*d*) *Serological grouping.* If any further proof were required for the ex-

clusion of coliform types from the *B. mucosus capsulatus* group, it is to be found in the results of the serological tests where no relationship was demonstrated between *B. coli* and *B. mucosus capsulatus* (inositol fermenters). Within the *B. mucosus capsulatus* group, the close relationship which was shown in the biochemical tests between strains from all sources was again apparent in their serological reactions, where numerous cross-agglutinations occurred between strains of different origin. With regard to the kind of reaction, complete agglutination elicited by antisera of low titre was assumed to indicate type specificity, in accordance with Julianelle's findings, and to be dependent on the presence of polysaccharide in the capsule; similarly complete agglutination elicited by antisera of high titre was probably a partial manifestation of group specificity (protein response). A pure protein response was not obtained in any reaction, since no strain had been decapsulated, but an incompletely developed protein response might be indicated by partial agglutination. This group relationship is less apparent, although never absent, when strains are fresh and type specific, but becomes more obvious as the strains grow older and lose type specificity. The tendency shown by the "enteritis" and "miscellaneous" strains to arrange themselves each into a distinct sub-group is interesting, and could not be attributed entirely to the smoothness and roughness of the strains. This would suggest that although the term *B. mucosus capsulatus* be retained as a satisfactory designation for strains from all sources, two types might be recognised within the group, viz. faecal (*B. lactis aerogenes*) and non-faecal (Friedländer's bacillus). *B. oxytocus* seemed to be a link between the two types, although it was more closely related to the enteritis strains; it combined the biochemical reactions of both types and showed wide serological relationships. It is admitted that, in order to establish types within the *B. mucosus capsulatus* group, serological tests would have to be performed with anti-*S* sera and smooth strains obtained either by recent isolation or by animal passage.

(2) *Distribution of the group in the environment and in man.*

Opinion still varies regarding the distribution of *B. mucosus capsulatus* in the environment and its occurrence in man, either as a saprophyte or as a pathogen. For example, Topley and Wilson quote Koser (1924) and Bardsley (1926) to support their statement that *B. lactis aerogenes* is not a normal inhabitant of the intestine, but occurs on plants, grains and in soil. When, however, a study is made of MacConkey's (1909) analysis of 497 lactose fermenters gathered from various sources, it appears that the inositol-fermenting and carbinol-positive strains, viz. No. 65 (*B. oxytocus*), No. 68 (*B. Friedländer*) and No. 103 (*B. lactis aerogenes*), were met with mainly in human faeces. A few such strains occurred in soil and cheese samples, but none were isolated from animal faeces, water samples, sewage or grains. Perkins failed to discover any strains of *B. mucosus capsulatus* in soil or water samples; Ford (1927) found *B. lactis aerogenes* occurred mainly in cow faeces, but was unwilling to

state whether the environmental *B. lactis aerogenes* was the same organism as the encapsulated one found in human faeces; Bamforth concluded that *B. mucosus capsulatus* was not normally found in human faeces. The present study supports the findings of Perkins and MacConkey that *B. mucosus capsulatus* is not primarily an environmental organism, although this view is opposed to those of Bardsley(1926) and Hicks (1927), who maintained that the preponderant type of lactose fermenter found in the environment was carbinol-positive, indole-negative and grew in citrate broth. The writer's discovery of *B. mucosus capsulatus* in normal human faeces bears out the findings of Cruickshank and Cruickshank (1931), and favours their view that the presence of carbinol-positive strains in soil and water supplies is no indication of freedom from faecal contamination.

The increased occurrence of *B. mucosus capsulatus* in inflammatory conditions of the gut appears to be due to an actual overgrowth, as in artificial diarrhoea produced by castor oil, which irritates the mucous coat of the small intestine, or by magnesium sulphate, which acts on the large intestine, but no such increase was demonstrated. The negative findings after the administration of castor oil militate against Ford's claim that *B. lactis aerogenes* is found in greatest numbers in the small intestine. The possibility of the increased occurrence in enteritis being due to an infection *per os* must also be taken into account, especially when no known pathogens are isolated from the faeces. It is significant in this respect that *B. mucosus capsulatus* showed itself capable of resisting the bactericidal action of the stomach and intestines in the feeding experiment, probably on account of its encapsulation; this might explain its discovery by Kendall (1911) in the duodenum of normal adults. It is difficult to assess the importance of *B. mucosus capsulatus* in other lesions, because various observers seem to have included non-capsulated coliform types in the group (Bamforth, 1928, Small and Julianelle, 1923). Its occurrence has probably been overestimated, but it is encountered not infrequently in cases of pyuria, and much less frequently in lesions of the respiratory tract where it occurs mainly as a secondary invader.

Conclusions.

1. The term *B. mucosus capsulatus* defines a group of organisms whose main distinguishing feature, apart from encapsulation, is the power of fermenting inositol; *B. lactis aerogenes* possesses all the essential properties of the group.

2. Certain coliform types such as *B. acidi lactici*, *B. cloacae*, *B. neapolitanus* and *B. coli communior*, which may resemble *B. mucosus capsulatus* in culture, failed to ferment inositol. These types utilise citrate, but less readily than *B. mucosus capsulatus*.

3. Strains of *B. mucosus capsulatus* from cases of enteritis give similar biochemical reactions to strains from sputum and other non-faecal sources, but show less resemblance to strains from normal faeces.

4. The serological tests suggested the existence of two types within the

B. mucosus capsulatus group, a faecal (*B. lactis aerogenes*) and a non-faecal (Friedländer's bacillus). The *B. lactis aerogenes* type is closely related serologically to the Friedländer type.

5. The discovery in the environment of carbinol-positive, indole-negative strains which grow in citrate broth is not an indication of freedom from faecal contamination. *B. mucosus capsulatus*, which fulfils all these conditions, occurs normally in human faeces, and certain coliform types give these reactions.

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