A FURTHER STUDY OF AN UNUSUAL BACILLUS RECOVERED FROM CASES PRESENTING SYMPTOMS OF DYSENTERY.

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Introduction.

In a previous issue of this *Journal* (1929) we published a short description of an unusual organism recovered from the faeces of a number of children who suffered from symptoms of bacillary dysentery and who showed no evidence of the presence of any of the recognised pathogenic organisms found in that disease. The bacillus was isolated without difficulty in all instances examined in the acute stage and specific agglutinins were present in the blood serum of all tested for them.

As it was isolated in a Newcastle laboratory and all the strains were derived from that city or its near neighbourhood we propose, in order to make the following report more intelligible, to name it the "Newcastle dysentery bacillus."

Since the original description was written two additional strains (A and B), possessing precisely the same biochemical characteristics and agglutinating with a specific anti-serum, have been isolated in the College of Medicine laboratory from two other children, although, in one case, only one single colony was obtained on a solitary occasion.

Strain A. J. McG., a female infant aged $2\frac{1}{2}$, was admitted to the Infectious Diseases Hospital on 5. i. 1929 with a history of having suffered for six months previously from periodic attacks of diarrhoea—sometimes seven or eight motions a day—with mucus in the stools and "abdominal spasms." During her stay in hospital her general condition was fair and she had no pyrexia or pain, but for two or three days after admission there was mucus and a little blood in the faeces. From the latter the Newcastle bacillus was recovered on five consecutive occasions at weekly intervals, but, curiously enough, her blood serum, when tested on January 18th and also on February 25th, was entirely negative to a broth culture of her own organism as well as to saline suspensions of the previously reported Gosforth and Birtley strains of the Newcastle bacillus.

Strain B. W. R., a female child aged $2\frac{1}{4}$, was admitted to the same hospital on January 16th with a history of intermittent diarrhoea with blood and mucus in the stools for twelve weeks. She had no symptoms while in hospital and the bacillus was isolated on the one occasion only.

FURTHER OBSERVATIONS UPON THE NEWCASTLE DYSENTERY BACILLUS.

The biochemical characteristics and serological relationships of the organism have now been studied more intensively than was possible at the time of our first report and the results of this more recent investigation strengthen the impression that we are dealing with an organism which has not hitherto been recognised.

BIOCHEMICAL REACTIONS.

These are given in Table I and are there compared with those of a number of other bacilli. The latter fall into two classes and include first one or two organisms whose reactions in certain important particulars bear a somewhat close resemblance and, secondly, certain bacilli which are frequently associated with dysentery, but are not known to be specifically related thereto.

Table I shows that, with Lemco broth, Dudgeon and Pulvertaft (1927), as a basis for carbohydrates, not only are the reactions of all six strains of the Newcastle bacillus remarkably free from variation but that they differ from those of the others examined. Probably its most outstanding biochemical characteristic and the one which chiefly differentiates it from most other organisms is the slow fermentation of dulcite without any action on mannite.

According to Andrewes and Neave (1921), and Savage and Bruce White (1925), a somewhat similar action on these carbohydrates, but without gas production, occurs with the Glässer-Voldagsen sub-group of Salmonella and although, unlike the Newcastle bacillus, these are motile, they were therefore included among the bacilli examined and form the first class of organisms referred to above.

One strain of *B. typhi suis* (Glässer), and the Dammann and Wegener strains of the Voldagsen bacillus were obtained from the National Collection of type cultures and in many ways the reactions of all will be seen to bear a certain general resemblance to those of the Newcastle bacillus. This resemblance is closest in the case of the Dammann strain of the Voldagsen bacillus, but it differs in its more decided action on zylose and in not producing gas with glycerine or maltose, while it also blackens lead acetate more rapidly. An interesting point is that both strains of the Voldagsen bacillus appear to produce much more gas than they are usually credited with. Whether this capacity has been acquired as the result of continued culture or whether they share with the Newcastle bacillus the peculiarity of more readily producing gas with the Lemco broth medium is a point that has not been determined.

We have heard of one or two organisms isolated elsewhere which apparently resemble the Newcastle bacillus but which produce a late fermentation of lactose. While this is quite a possibility none of the strains hitherto examined, although kept under observation for three weeks, have shown any sign of this either in peptone water or Lemco broth. The nearest approach thereto has been a slight and quite temporary yellowing of the indicator in the latter medium after about twenty-four hours in one or two instances.

Table I. *Showing the biochemical reactions (using Lemco broth as a basis for the carbohydrates) of the six strains of the Newcastle bacillus, Part* 1, *and of strains of the Glässer-Voldagsen sub-group of the Salmonella and certain bacilli associated with, but not specifically related to, dysentery (Part* 2).

Part 1.

	Original strains				New strains		
	Newcastle (1)	Gosforth	Newcastle (2)	Birtley	J. McG. (1)	W. R. (1)	J. McG. (2)
Arabinose	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)
Xylose	? *a* to –	? *a* to –	? *a* to –	? *a* to –	? *a* to –	? *a* to –	? *a* to –
Glucose	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)
Laevulose	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)
Galactose	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)	*AG* (1)
Lactose	—	—	—	—	—	—	—
Saccharose	—	—	—	—	—	—	—
Maltose	*Ag* (1–3)	*Ag* (1–3)	*Ag* (1–3)	*Ag* (1–3)	*Ag* (1–3)	*Ag* (1–3)	*Ag* (1–3)
Raffinose	—	—	—	—	—	—	—
Inulin	—	—	—	—	—	—	—
Dextrin	? *a* to –	? *a* to –	? *a* to –	? *a* to –	? *a* to –	? *a* to –	? *a* to –
Glycerin	*AG* (6)	*AG* (6)	*AG* (6)	*AG* (6)	*AG* (5)	*AG* (5)	*AG* (5)
Mannite	—	—	—	—	—	—	—
Dulcite	*AG* (3–4)	*AG* (3–4)	*AG* (3–4)	*AG* (6)	*AG* (9)	*AG* (5)	*AG* (5)
Sorbite	—	—	—	—	—	—	—
Adonite	—	—	—	—	—	—	—
Salicin	—	—	—	—	—	—	—
Inosite	—	—	—	—	—	—	—
Lead acetate broth	*B* (3–6)	*B* (3–6)	*B* (3–6)	*B* (3–6)	*B* (5–9)	*B* (3–5)	*B* (3–5)

Part 2.

	Glässer-Voldagsen sub-group			Certain bacilli associated with, but not specifically related to, dysentery				
	B. typhi suis Glässer	Voldagsen's bacilli Dammann	Voldagsen's bacilli Wegener	Morgan's bacilli No. 1	*B. alkalescens* Andrewes	*B. dispar* Andrewes	Pseudo-dysentery Kruse A	Pseudo-dysentery Kruse D
Arabinose	*A* (2)	*Ag* (3)	? *a* to –	—	*A* (1)	*A* (1)	*AG* (1)	*AG* (1)
Xylose	*A* (1)	*Ag* (2)	*AG* (1)	—	*A* (1)	*A* (1)	*AG* (1)	*AG* (1)
Glucose	*A* (1)	*Ag* (1)	*AG* (1)	*Ag* (1)	*A* (1)	*A* (1)	*AG* (1)	*AG* (1)
Laevulose	*A* (1)	*Ag* (1)	*AG* (1)	*AG* (1)	*A* (1)	*A* (1)	*AG* (1)	*AG* (1)
Galactose	*A* (1)	*Ag* (1–3)	*AG* (1)	*AG* (1)	*A* (1)	*A* (1)	*AG* (1)	*AG* (1)
Lactose	—	—	—	—	—	*A* (4)	—	—
Saccharose	—	—	—	—	—	*A* (1)	—	—
Maltose	*A* (1)	*A* (1)	*AG* (1)	—	*A* (2)	*A* (1)	*AG* (1)	*AG* (1)
Raffinose	—	—	—	—	—	No test	*A* (10)	—
Inulin	—	—	—	—	—	—	—	—
Dextrin	? *a* to –	? *a* to –	? *a* to –	—	? *a* to –	? *a* to –	? *a* to –	? *a* to –
Glycerin	*A* (5)	*A* (3)	*Ag* (3–5)	*Ag* (4)	*A* (1)	*A* (1)	*A* (1)	*Ag* (1)
Mannite	—	—	*AG* (1)	—	*A* (1)	*A* (1)	*AG* (1)	*AG* (1)
Dulcite	*A* (5)	*Ag* (5)	—	—	*A* (1) to –	—	*AG* (1)	*AG* (1)
Sorbite	*A* (10)	—	*AG* (1)	—	*A* (3)	*A* (1)	*AG* (1)	*AG* (1)
Adonite	—	—	—	—	—	—	—	—
Salicin	—	—	—	—	—	—	—	—
Inosite	—	—	—	—	—	—	—	—
Lead acetate broth	*b* (10–20)	*B* (3)	*B* (3)	No test	*B* (6)	No test	*B* (1)	*B* (1)

A = acid; *a* = slight acid; *G* = gas; *g* = slight gas; *B* = marked blackening; *b* = slight blackening; — = no change; ? *a* to – = slight temporary acidity followed by alkalinity. Figures in brackets represent days in which reaction took place.

FURTHER EXAMINATION OF SEROLOGICAL CHARACTERISTICS.

General points. Anti-sera have now been prepared from all the four strains of the Newcastle bacillus which were referred to in the former report, and only in one case has a maximum agglutination titre exceeding 1 in 2000 been

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obtained and in that case only to a slight degree. It appears therefore to be difficult to prepare from this organism a rabbit anti-serum of anything but low titre. Table III, which gives the maximum in each instance with the homologous strain, illustrates this point.

Moreover, as in the case of the majority of the bacilli associated with, but not known to be specifically related to, dysentery, the velocity of agglutination is low and the maximum does not become evident until after standing at bench temperature overnight following upon four and a half hours in the 55° C. waterbath.

Another peculiarity is that the titre is much lower with broth cultures than with saline suspensions prepared from agar slopes, even when both are standardised to a similar opacity. For some time the agglutination experiments which had been carried out had given this impression, but the parallel tests shown in Table II with the same sera, using on the one hand saline suspensions and on the other veal broth emulsions, both standardised to the opacity of the Oxford emulsions, remove any doubt about the matter and show that, with broth cultures, the titre is very much lower.

Table II. *Showing the maximum titre of agglutination with saline suspensions and veal broth emulsions respectively.*

Dilutions	125	250	500	714	1000	1250	1666	2500	Control
	Newcastle (1) anti-serum against Newcastle (1) strain.								
Saline suspension	+	+	+	+	+	+	+ fine	−	−
Broth culture	+	+	+	±	−	−	−	−	−
	Birtley anti-serum against Birtley strain.								
Saline suspension	+	+	+	+	+	+	+	? −	−
Broth culture	+	+	+	±	−	−	−	−	−

As it was thought that this difference might depend upon the relative proportion of electrolyte present in the two emulsions an experiment was carried out in which the growth from agar slopes was washed off and all the necessary dilutions made with different concentrations of saline, including 0·2, 0·4, 0·6 and 0·9 per cent., while with one slope distilled water only was used. With this last agglutination only occurred in the first tube (1 in 25), but with the others there was little variation in the actual maximum although agglutination was rather more rapid and coarser as the concentration of saline increased.

Estimations of total chlorides in a mixture of fifteen parts of broth to ten of saline and in the saline itself showed, however, no more than a slight difference.

On account of this greater agglutinability saline suspensions of the Newcastle bacillus have been utilised for the experiments detailed below.

Agglutination tests with anti-sera prepared from the Newcastle bacillus.

As an anti-serum possessing a considerably higher titre than that used in the tests recorded in the first report was now available a good many of these were repeated.

The organisms tested may be classed under the following heads:

(1) The recognised dysentery bacilli. Of these *B. dysenteriae* Shiga again showed complete absence of agglutination and the same was the case with a strain of *B. dysenteriae* Sonne (3) which had been isolated in the laboratory, but the Oxford Sonne (3) emulsion showed doubtful traces up to 1 in 50. On the other hand, four at least out of the five common races of *B. dysenteriae* Flexner were quite definitely agglutinated and will be referred to more particularly later on.

(2) Certain bacilli frequently associated with but not known to be specifically related to dysenteric conditions.

All strains of these which could be obtained were tested with completely negative results. Included under this head were strains of *B. alkalescens* and *B. dispar* of Andrewes, Morgan's bacillus No. 1, and *B. pseudo-dysenteriae* Kruse, types A and D[1].

(3) The Glässer-Voldagsen sub-group of the Salmonella whose biochemical reactions had been found in many respects to resemble those of the Newcastle bacillus. The one strain of Glässer's bacillus and the Wegener and Dammann strains of Voldagsen's bacillus, already referred to, were tested and were likewise negative.

(4) Other intestinal bacilli.

As soon as positive agglutination was obtained with a group of bacilli differing so markedly from the Newcastle bacillus in biochemical reactions as the Flexner group of dysentery bacilli do, it was felt to be essential to examine as many as possible of the Gram negative, non-lactose fermenting organisms derived from the alimentary canal in order to exclude the possibility of any similar serological affinities. Accordingly tests were put up with the Birtley anti-serum against the Oxford emulsions of *B. typhosus*, *B. paratyphosus* (A, B and C), *B. enteritidis* Gaertner, *B. aertrycke* (Mutton and Newport strains), and against a broth culture of *B. bovis morbificans* which had been isolated in the laboratory but in all cases were completely negative.

The antigenic relationship of the Newcastle bacillus to the Flexner group of dysentery bacilli.

Despite their lack of resemblance to the Newcastle bacillus in biochemical reactions it is well known that the Flexner group possess antigenic relationships with many other species, Andrewes and Inman (1919). Moreover, it

[1] The A and D types of *B. pseudo-dysenteriae* Kruse were suggested to us as possibly related to the Newcastle bacillus, but on arrival from the National Collection were found to be considered identical with *B. enteritidis* Gaertner and they agglutinated with Oxford Gaertner anti-serum.

had been found previously that a very old anti-serum prepared in the laboratory from race W appeared to produce some agglutination with the Newcastle bacillus but, in view of the negative results with Oxford sera, the observation was considered too unreliable to be recorded at that time. The converse test with Gosforth anti-serum against the Flexner emulsions was unsatisfactory owing to the presence at that time of auto-agglutination in the controls which prevented the ill-marked clumping with this very low-titred serum from being distinguished with sufficient accuracy.

When, however, more satisfactory emulsions and the much more powerful Birtley anti-serum were available for experiment the first opportunity was taken to repeat this last test and subsequently similar tests were carried out with the other three anti-sera also and the results are tabulated in Table III.

It may be noted that the Gosforth anti-serum produced no more than traces of agglutination in 1 in 50 with races V and W, in 1 in 125 with race Y, and in 1 in 25 with race Z. The Newcastle (2) anti-serum was also ill-marked in the higher dilutions but the other two produced quite definite agglutination throughout.

Table III. *Showing the maximum agglutination titre of anti-sera prepared from the four strains of the Newcastle bacillus with the homologous organism (same strain) and the five races (V, W, X, Y and Z), of* B. dysenteriae *Flexner.*

Anti-serum from strain	Newcastle (1)	Gosforth	Newcastle (2)	Birtley
With homologous organism (same strain)	1700	500	700	2250
With *B. dysenteriae* Flexner—				
Race V	500	50	250	500
„ W	125	50	50	250
„ X	Nil	Nil	Nil	Nil
„ Y	250	125	125	500
„ Z	50	25	? Nil	125

A similar test carried out with Birtley anti-serum against saline suspensions of the five races of *B. dysenteriae* Flexner prepared from strains which had either been isolated in the laboratory or procured from the National Collection, gave slightly different readings, races W, Y and Z being agglutinated to 250 and V and X to 125, but the general result confirmed the fact that, in anti-sera prepared from the Newcastle bacillus, there was a considerable amount of agglutinin for the Flexner group. The differences probably depended upon slight variation in the strains (and, in particular, the X race from which the saline suspension was prepared and which was isolated in the laboratory, contained a considerable admixture of Y), and also upon the greater agglutinability of the Oxford emulsions taken as a whole.

The strains from which these saline suspensions were prepared were made use of to provide the material for the absorption experiments which are described below.

TESTS WITH FLEXNER GROUP ANTI-SERA AGAINST THE NEWCASTLE BACILLUS.

These had previously been completely negative but were now repeated with saline suspensions of the Birtley strain instead of the broth cultures originally used. With the Oxford anti-sera prepared from races X and Y there was again complete absence of agglutination, but those prepared from races V and W produced it definitely in a dilution of 1 : 25 and traces in 1 : 50, while that from race Z produced a trace in a dilution of 1 in 25. That the difference between this and the previous tests was entirely dependent upon the use of the saline suspensions was quickly proved by the repetition of a completely negative result with a broth culture of the same organism.

TESTS WITH ANTI-SERA PREPARED FROM OTHER INTESTINAL ORGANISMS.

Saline suspensions of the Newcastle bacillus were likewise tested with the Oxford anti-sera prepared from *B. dysenteriae* Shiga and Sonne (3), *B. typhosus*, *B. paratyphosus* (A, B and C), *B. enteritidis* Gaertner, *B. aertrycke* (Mutton and Newport), but, although the commencing dilution was as low as 1 in 12, no trace of agglutination was apparent in any of them. Similar tests with anti-sera prepared by Dr W. M. Scott of the Ministry of Health, from *B. dysenteriae* Sonne (3) and from *B. bovis morbificans* were also quite negative.

ABSORPTION EXPERIMENTS.

For this purpose the anti-serum prepared from the Birtley strain of the Newcastle bacillus, which gave the highest agglutination titre of the four, 1 in 2250, was selected. Table IV shows the titre of this serum unabsorbed.

Table IV. *Showing the maximum agglutination titre before absorption of Birtley anti-serum with saline suspensions of the four strains of the Newcastle bacillus and broth emulsions of the five races of* B. dysenteriae *Flexner.*

	Organisms								
	Strains of Newcastle bacillus				Races of *B. dysenteriae* Flexner				
	Newcastle (1)	Gosforth	Newcastle (2)	Birtley	V	W	X	Y	Z
Anti-serum Birtley unabsorbed	1500	1250	2250	2250	500	250	Nil	500	125

ABSORPTION WITH THE FIVE RACES OF *B. dysenteriae* FLEXNER.

One c.c. of this Birtley anti-serum was placed in a small sterile bottle marked at the 10 c.c. level and the growth from five agar plates, each of which had been sown with a different race of *B. dysenteriae* Flexner, was washed off into it with 1 or 2 c.c. of saline. It was filled up with the latter to the 10 c.c. mark and 0·05 carbolic added. The subsequent procedure was to incubate at 37° C. for three hours, leave it at bench temperature, with occasional shaking, for two days, and finally in the ice-chest to settle. The clearing was completed by centrifugalisation.

Table V gives the maximum titre of agglutination with the same series of organisms after this process.

Table V. *Showing the maximum agglutination titre of Birtley anti-serum with saline suspensions of the four strains of the Newcastle bacillus and broth emulsions of the five races of* B. dysenteriae *Flexner, after saturation with a mixed suspension of the latter.*

	Organisms								
	Strains of Newcastle bacillus				Races of *B. dysenteriae* Flexner				
Anti-serum	Newcastle (1)	Gosforth	Newcastle (2)	Birtley	V	W	X	Y	Z
Birtley absorbed with Flexner races	1400	1000	1785	1785	Nil	Nil	Nil	Nil	Nil

The experiment was repeated and in this case the absorbed serum was tested against saline suspensions of the Flexner races used for saturation as well as of the four strains of the Newcastle bacillus, and once again showed complete removal of all agglutinins for *B. dysenteriae* Flexner with only a slight effect upon the specific agglutinins for the Newcastle bacillus.

Absorption with the Newcastle bacillus.

Similar quantities of Birtley anti-serum were then saturated in four different experiments with each of the four strains of the Newcastle bacillus. The experiments were carried out in exactly the same way as in the case just described, except that the growth from three agar plates formed the saturating suspension for each strain and that centrifugalisation was unnecessary, as the serum cleared completely in the ice-chest. Table VI gives the result of these experiments and conclusively demonstrates that each strain of the Newcastle bacillus removes practically all agglutinins for every one of its own strains and at the same time completely gets rid of the secondary agglutinins for the Flexner group of dysentery bacilli.

There can therefore be no doubt that all four strains represent one and the same organism.

Table VI. *Showing the maximum agglutination titre of Birtley anti-serum with saline suspensions of the four strains of the Newcastle bacillus and broth emulsions of the five races of* B. dysenteriae *Flexner, after saturation with each of the former.*

	Organisms								
	Strains of Newcastle bacillus				Races of *B. dysenteriae* Flexner				
Anti-serum	Newcastle (1)	Gosforth	Newcastle (2)	Birtley	V	W	X	Y	Z
Birtley absorbed with—									
Newcastle (1)	50	50	125	125	Nil	Nil	Nil	Nil	Nil
Gosforth	50	50	125	50	Nil	Nil	Nil	Nil	Nil
Newcastle (2)	Nil	Nil	? 25	Nil	Nil	Nil	Nil	Nil	Nil
Birtley	50	50	125	125	Nil	Nil	Nil	Nil	Nil

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The same result was shown when the experiment was repeated with the saline suspensions of the Flexner races as well as of the strains of the Newcastle bacillus.

Bacterial variation.

On agar plates the colonies of the Newcastle bacillus are usually of the smooth type, but in the case of the Newcastle (1) strain, which has been longest in cultivation, and to some extent also in the case of the Gosforth strain, there is a distinct tendency to roughness with irregular edges and somewhat granular surface.

On two occasions ten 18-hour colonies have been sub-cultured into broth, formalinised next morning, diluted with saline and tested for agglutination against Birtley anti-serum after this has been saturated with a mixed suspension of the Flexner group of dysentery bacilli. In each instance the maximum titre has been practically identical with all the colonies examined. So far as these experiments go, therefore, no evidence of different phases, in the sense of Andrewes (1922), has been obtained.

Summary.

(1) The unusual organism previously described (1929) has since been recovered from two additional sources, in each case children suffering from dysenteric symptoms.

(2) It is suggested that this organism be named the "Newcastle dysentery bacillus."

(3) A more extensive study of its biochemical reactions, using Lemco broth (Dudgeon and Pulvertaft, 1927), as a basis for carbohydrates, has shown that these reactions are notably free from variation and that they exhibit decided divergences from those characteristic of certain bacilli often associated with, but not specifically related to, dysentery. They are more or less closely paralleled in the case of the Glässer-Voldagsen sub-group of the Salmonella, and particularly the Dammann strain of Voldagsen's bacillus, but nevertheless show quite definite differences.

(4) Further examination of the serological peculiarities of the Newcastle bacillus has demonstrated that it is difficult to prepare from it a very active rabbit anti-serum, that its velocity of agglutination is low, and that the titre obtained with saline suspensions is higher than that obtained with broth cultures.

(5) Complete absence of agglutination has been the result in tests with *B. typhosus*, many members of the Salmonella group, including all most frequently met with and also the Glässer-Voldagsen sub-group, and with a number of the bacilli often associated with, but not specifically related to, dysentery. On the other hand, with one strain of *B. dysenteriae* Sonne (3) there was a suggestion of agglutination and with at least four out of five races (V, W, X, Y and Z) of *B. dysenteriae* Flexner this was well-marked.

(6) Saturation with a mixed suspension of these five races completely removes all trace of these secondary agglutinins without materially affecting the specific agglutinins for the Newcastle bacillus while, on the other hand, saturation with each of the four strains of the latter has, in each separate instance, practically eliminated the specific and completely removed the secondary agglutinins.

Finally we desire to acknowledge the helpful criticism we have received from Prof. H. J. Hutchens throughout our study of this organism, the facilities placed at our disposal by Dr Harold Kerr, Medical Officer of Health for Newcastle, and the great assistance rendered by the Deputy Medical Officer of Health, Dr J. A. Charles.

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