AN UNUSUAL BACILLUS RECOVERED FROM CASES PRESENTING SYMPTOMS OF DYSENTERY.

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In a fairly comprehensive search through the available literature upon dysentery we have been unable to recognise an organism which exhibits the precise cultural and biochemical characteristics of the bacillus to which we refer below, but we do not pretend to have been able to consult all the publications on the subject and it is possible that it may previously have been described.

In peptone water media its action upon carbohydrates bears a considerable resemblance to that of *B. dysenteriae* Shiga, and it was at first thought to be related to that organism, but was eventually found to differ culturally and in its serological reactions. It likewise presents some points of similarity to No. (1) and No. (5) of the bacilli described by Morgan (1907), and to Bowman's Bacillus S (1908), said to be responsible for much of the infantile dysentery in the Philippines, but it also shows marked divergences from all. It most nearly resembles the organisms recovered by Ross (1910–11) from the stools of normal infants and those suffering from diarrhoea and classed by her as "Group G (2) and (3)" but even these do not exactly correspond.

Great caution is always necessary when attempting to connect acute intestinal conditions with organisms hitherto unrecognised, but we believe that, in this case, we can produce a considerable amount of evidence suggestive of such a connection.

Sources of the Bacillus in Question.

Source I. It was isolated originally in September 1925 from the faeces of a girl whose whole family had suffered from diarrhoea, the outbreak starting six weeks previously, and this strain will be referred to as "*Newcastle* (1)." The stool in this instance consisted of clear fluid and slightly bile-stained mucus and microscopically showed numerous pus cells and some red blood and epithelial cells but no amoebae or cysts or other Protozoa.

From direct plates, a number of non-lactose fermenting colonies, inhibited by brilliant green, were recovered. Cultures in peptone water media showed reactions persisting for three weeks, which were akin to those of *B. dysenteriae* Shiga, but the organism repeatedly proved entirely inagglutinable with the Oxford Shiga serum.

An anti-serum, prepared in the usual way, agglutinated the homologous organism to 1 in 1000, but failed to produce the slightest trace of reaction with a known Shiga emulsion. In subculture the same characteristics were observed over a period of three years, during which cultures were examined at intervals by different persons. Only on one or two occasions a minute bubble of gas seemed to be produced in glucose.

Eventually a subculture was sent to Prof. Dudgeon, in response to his request for possible Para-Shiga bacilli and when this was tested for purity before sending it away, transient acidity and a large bubble of gas was noted in dulcite.

Source II. What appears to be a strain of the same organism was isolated for the second time about the middle of May 1928 when specimens of faeces were sent in from a group of cases occurring in a Cripples' Home at Gosforth. For purposes of reference this strain is labelled "*Gosforth.*" Clinical notes show that twelve children in all were affected in the outbreak and that the majority were the more weakly members of the community. The illness lasted from one to five or six days but averaged four and diarrhoea was an invariable symptom. Most of the patients complained of abdominal pain, six had nausea and vomiting, usually at the commencement, and seven had slight pyrexia, sometimes attended by initial headache, and rising in one or two instances as high as 103° or 104° F. From four of the more severe cases six specimens of faeces were sent to the laboratory and all consisted chiefly of muco-pus, visible blood being present also in three, while microscopically red blood and pus cells and macrophages were always demonstrated. In all instances the specimens were sent in during the acute phase of the disease and from all, without exception, the same organism was isolated, usually in large numbers and always without difficulty.

Out of fourteen colonies whose reactions were tested (in peptone water media), ten retained after many days the main characteristics of *B. dysenteriae* Shiga, while four showed transient acidity and a bubble of gas in dulcite. The reaction with maltose was not at this time included but no gas was noted in glucose.

As the Home was situated in the Northumberland County area subsequent bacteriological examinations were carried out in the County laboratory. It is understood that a second group of four cases has since occurred, that the same organism has been independently isolated there, and that one strain, in subculture, has shown a minute bubble of gas in glucose.

Agglutination with patients' blood. The blood serum of five of the patients affected in the primary outbreak was tested against the Oxford Shiga emulsion and also against an emulsion prepared from the "Gosforth" bacillus. The tests in the case of the former were uniformly negative but the "Gosforth" organism was definitely agglutinated to the extent shown in Table I.

All of the first four patients, in the early stages, had visible blood and mucus in the stools and from all the organism was isolated, but M. W. was

a healthy girl who merely complained of slight abdominal pain and headache on one day and her case was not included among the series of twelve. At a later date the same organism was recovered from her faeces in the County laboratory.

Table I. *Agglutination with patients' serum.*

Name	Duration and character of illness	Date of onset	Date of withdrawal of blood	Maximum titre of agglutination
T. R.	Severe. Four days. Marked pyrexia. Pain. Vomiting	16. v. 1928	22. v. 1928	1 in 500
L. D.	Severe. Four days. Slight pyrexia	15. v. 1928	22. v. 1928	1 in 50+ 1 in 125±
M. H.	Severe. Four days. Marked pyrexia	16. v. 1928	24. v. 1928	1 in 50
D. H.	Severe. Three days. Slight pyrexia	16. v. 1928	24. v. 1928	1 in 125
M. W.	Very mild and not included in series	—	24. v. 1928	1 in 25??

Source III. In addition to the "Newcastle (1)" and "Gosforth" strains, what is apparently the same bacillus was isolated from a third source at the end of June 1928 and is labelled for purposes of reference as "*Newcastle* (2)."

In this instance the outbreak of illness involved a family of five persons of whom three were under the age of twelve and who lived in two rooms.

The first to be affected was a boy, aged three, who became ill on June 22nd with vomiting, drowsiness, and the passage of frequent grey motions containing blood. His illness was followed on the 26th by that of his brother, aged five, and this proved fatal in twenty-four hours. On the 29th a third case occurred in the person of the sister, aged eight, whose symptoms were the vomiting of greenish material and the passage of frequent loose stools containing mucus and blood. She was removed to the Royal Infirmary where she died shortly after admission and an autopsy, held the next day, revealed marked inflammation of the alimentary canal. The spleen and portions of ligatured small intestine and colon were at once sent to the laboratory and were examined in the usual manner for organisms of the typhoid-colon group. No pathogenic organisms were found except in the colon contents from which the already-mentioned bacillus was isolated in considerable numbers.

The first fatal case was not examined until five days after death and consequently too late to make bacteriological investigation of much value, but the macroscopic appearances were very similar to those found in the case of the sister.

The third child, the original case, was sent after his sister's death to the Infectious Disease Hospital where he eventually recovered. His faeces were examined after his admission there, and consequently about ten days after the onset, with entirely negative results despite the continued presence of mucus, but his blood, collected at the same time, agglutinated in a dilution of 1 in 25 the bacillus isolated from his sister although this repeatedly failed to show a trace of clumping with normal human serum. Unfortunately none of the sister's blood was available.

In her case the organism was easily recovered from the colon contents by

direct plating, but, like the others, was inhibited by brilliant green; it produced acid in glucose rather slowly and gave reactions, in a peptone water medium, akin to those of *B. dysenteriae* Shiga, except that one colony produced transient acidity and a bubble of gas in dulcite. The blood of the father of the family was tested against all the available dysentery emulsions and likewise against the bacillus isolated but with invariably negative results.

There was some slight suspicion that the sister's illness might have been connected with the Gosforth outbreak as her recent history revealed the fact that some little time before its onset she had been lost on the Town Moor and had eventually arrived at the Gosforth police station. She might have filled the rôle of "carrier" and conveyed the infection to her brothers but there was no evidence that she had come into contact with any of the Gosforth victims and the history of the outbreak suggests that she was secondarily infected.

Source IV. Quite recently a fourth strain of a similar bacillus has been isolated from the colon contents of another fatal case in a child. It will be referred to as "*Birtley.*"

This child, a boy aged five, returned from school on the night of October 17th complaining of malaise and headache and during the night suffered from diarrhoea. Next morning he was dazed and at 11 a.m. had a fit from which he never regained consciousness. On admission to the Royal Infirmary the same day, respiration was embarrassed and he died an hour and a half after admission. An autopsy was held the day after death and portions of the ileum and the colon were sent at once to the laboratory.

A pathological report by Prof. Stuart McDonald and Dr G. E. Stephenson states that there was no doubt of the existence of an acute infective enteritis, both small and large intestines being affected. In the former the changes were best marked towards the lower end and the special feature was marked hyperplasia of the lymphoid tissue, both solitary glands and Peyer's patches being involved. There was also definite ulceration in connection with the solitary glands. The mesenteric glands, particularly in the ileo-caecal region, were definitely swollen and hyperaemic. In the large intestine the changes were even more marked. The mucosa was swollen, oedematous, and distinctly hyperaemic and the follicular ulceration was more prominent. The changes were best seen in the descending colon and upper part of the pelvic colon. The other organs merely gave evidence of a general acute toxaemia.

Bacteriological examination. Plates were sown from bile-stained mucus contained in the small intestine and from definite muco-pus in the colon but from the former no pathogenic organisms were obtained. The colon contents, however, yielded numerous non-lactose fermenting colonies in direct plates and one or two from plates sown after brilliant green reinforcement.

These gave similar reactions in peptone water media to those usually obtained in the case of the "Newcastle (1) and (2)" and "Gosforth" strains but both of the two colonies examined showed transient acidity and a bubble of gas in dulcite.

This case occurred at Birtley, some distance from Newcastle, and no connection with either of the previous outbreaks could be traced. The remaining members of the family, of whom four were under and three over the age of twelve, were unaffected.

DESCRIPTION OF THE ORGANISM ISOLATED.

(1) *Morphology and cultural characteristics.*

A gram-negative non-motile bacillus which in its growth on ordinary media such as agar and broth does not differ in any noticeable way from the other organisms in the gram-negative bacillary group. It has, however, been noted that it grows rather slowly and feebly in peptone water.

(2) *Biochemical reactions.* (*See* Table II.)

One per cent. peptone water was used as a basis for the carbohydrates. With this medium the reactions bear a fairly close resemblance to those of *B. dysenteriae* Shiga, except that there is an infrequent production of a slight amount of gas in glucose and dulcite. This is inconstant even with the same strain, occurring irregularly, sometimes when the bacillus is first isolated, sometimes after prolonged subculture. A very similar irregular production of gas has been noted by Nabarro (1927) in case of an organism which he recovered from the alimentary canal, while Rajchman and Western (1916) say that the same sometimes occurs with typical Shiga.

Table II. *Biochemical reactions of the organisms isolated, using* 1 *per cent. peptone water as a basis for the carbohydrates and for indol.*

Organism	Lactose	Glucose	Mannite	Dulcite	Saccharose	Maltose	Litmus	Milk	Indol	Liquefaction of gelatin
"Newcastle (1)"	—	A. or rarely A.G. v.sl.	—	— or occasionally A.G. sl.	—	A. sl.	A. sl. then N. or Alk. sl.		—	—
"Gosforth"	—	Same as above	—	Same as above	—	Same as above	Same as above		—	—
"Newcastle (2)"	—	A.*	—	Same as above	—	Same as above	Same as above		—	—
"Birtley"	—	A.	—	A.G. sl.†	—	Same as above	Same as above		—	—

A. = acid; A.G. = acid and gas; N. = neutral; Alk. = alkaline; sl. = slight; v.sl. = very slight.

* No gas yet noted in glucose.

† Both colonies examined showed transient acidity and a bubble of gas in dulcite.

As already stated the original culture, "Newcastle (1)," was sent to Prof. Dudgeon and was found by him to give the reactions specified in Table III. As we have never been able when using peptone water media to obtain other than the results given in Table II, enquiry was made as to the medium used in his tests and it was found to be the Lemco broth described by him in this *Journal* (1927). By making use of this medium we have had no difficulty in obtaining consistently the results recorded in Table III.

Table III. *Carbohydrate reactions when using Lemco broth, Dudgeon and Pulvertaft* (1927), *as a basis.*

Organism	Lactose	Glucose	Mannite	Dulcite	Saccharose	Maltose
"Newcastle (1)"	—	A.G. 24 hours	—	A.G. 3–4 days	—	A.G. Slow and slight gas
"Gosforth"	—	Same as above	—	Same as above	—	Same as above
"Newcastle (2)"	—	Same as above	—	Same as above	—	Same as above
"Birtley"	—	Same as above	—	A.G. 6 days	—	Same as above

We have obtained these discordant reactions with the two different media only in the case of the organism we are describing and a number of known organisms, including two strains of B. Morgan No. (1), two of *B. dysenteriae* Para-Shiga +, and *B. alkalescens* Andrewes, have given typical and identical reactions with both.

(3) *Serological reactions.*

(*a*) *Agglutination tests made with normal sera and with anti-sera prepared from known organisms.* With the "Newcastle (1)" strain, Dudgeon was unable to obtain the slightest trace of agglutination when tested with anti-sera prepared from non-indol producing strains of *B. dysenteriae* Para-Shiga.

In all the agglutination tests carried out in this laboratory the Oxford standard sera were used in the case of anti-sera prepared from known organisms and the tubes were incubated for four hours at 55° C.; the results being read at once and confirmed after standing on the bench overnight. For the organisms under test, twenty-four-hour broth cultures or occasionally peptone water cultures were used and except in the case of the recently isolated "Birtley" strain, the tests have been repeated on several occasions.

The first three strains, "Newcastle (1) and (2)" and "Gosforth" have been tested against anti-sera prepared from *B. dysenteriae* Shiga and Flexner (polyvalent and also the five separate races *V*, *W*, *X*, *Y*, and *Z*), and against *B. paratyphosus* A serum, in all cases with negative results. Similar results were likewise obtained in tests with normal human and normal rabbit serum. The "Birtley" strain has not been tested with normal serum but no agglutination was noted with Shiga, polyvalent *B. dysenteriae* Flexner, or *B. paratyphosus* A anti-sera.

(*b*) *Agglutination tests made with an anti-serum prepared from the "Gosforth" strain of the organism isolated from cases with symptoms suggestive of dysentery.* These are shown in Table IV. Although agglutinins seemed to be readily

Table IV. *Agglutination tests with an anti-serum prepared from "Gosforth."*

Cultures tested	Dilution of serum					
	1 in 25	1 in 50	1 in 125	1 in 250	1 in 500	1 in 1000
"Newcastle (1)"	+	+	+	±	–	–
"Gosforth"	+	+	+	+	±	–
"Newcastle (2)"	+	+	+	+	±	–
"Birtley"	+	+	+	±	–	–
B. dysenteriae Shiga	–	–	–	–	–	–
B. Morgan No. (1), two strains	–	–	–	–	–	–
B. "schmitz," two strains ...	–	–	–	–	–	–

All control tubes were quite negative

produced in the human patients affected, the titre of the anti-serum, prepared in the usual manner from the rabbit, in this particular instance gave no higher titre than 1 in 500 for the homologous organism.

Toxicity for Rabbits.

One-tenth of an agar slope of the "Gosforth" strain was inoculated intravenously into one young rabbit and another was fed on three successive days with a whole slope mixed with its bran and oats. Both were slightly ill but recovered perfectly. The killed cultures of the same strain used in the preparation of the anti-serum also had a slight temporary effect on the animal's appetite.

The recently isolated "Birtley" strain has been inoculated subcutaneously into a third rabbit without at present, so far as can be judged, producing the slightest effect whatever.

Summary.

Organisms which appear to be morphologically, culturally, and serologically identical have been recovered in four separate outbreaks of illness suggestive of dysentery.

They have been readily isolated in considerable numbers from all cases examined in the early phase of the attack.

When 1 per cent. peptone water is used as a basis for carbohydrates these organisms usually leave lactose, mannite, dulcite, and saccharose unaffected but ferment glucose and maltose with the production of acid. Rarely, a minute amount of gas is also produced from glucose and occasionally slight acid and gas from dulcite. On the other hand, when Lemco broth (Dudgeon and Pulvertaft, 1927) is used as a basis, glucose, dulcite, and maltose are consistently fermented with the production of acid and gas.

Agglutination tests against normal sera and against anti-sera prepared from the dysentery and paradysentery bacilli and from *B. paratyphosus* A are uniformly negative but strains of the organism derived from all four sources are agglutinated to approximately the same titre by an anti-serum prepared from one of them although this anti-serum has no effect on three organisms whose reactions present some degree of similarity.

The organisms do not appear to be toxic for rabbits.

With all patients affected in these outbreaks, whose blood was available for examination, the serum has shown the presence of specific agglutinins which could not be demonstrated in normal serum.

On the whole, therefore, the evidence suggests that these organisms may be responsible for the diseased condition.

Our thanks are due to Prof. Dudgeon for permission to publish his findings, to Prof. Stuart McDonald and Dr Stephenson for their pathological report, to Dr Mackenzie of Gosforth for kindly providing the clinical histories of the patients under his care, and to Dr Messer of the Northumberland County

Laboratory for the information with which he has been good enough to furnish us.

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(*MS. received for publication* 7. XI. 1928.—Ed.)