AN ORGANISM RESEMBLING THE NEWCASTLE TYPE OF DYSENTERY BACILLUS ASSOCIATED WITH CASES OF DYSENTERY.

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Many bacteria differing from the well-recognised bacilli of the Shiga, Flexner and Sonne groups in cultural and biochemical activities have been described in association with cases of dysentery although in many instances proof of their etiological significance is lacking. The organism described by Clayton and Warren (1928, 1929) and referred to as the Newcastle dysentery bacillus was isolated by them from several small outbreaks in which three fatal cases occurred, and there seems little doubt that their organism was the infecting agent.

The present communication deals with several strains of a bacillus which resembles the Newcastle organism very closely although differing from it in the fermentation of mannite. Five of the strains were isolated from cases presenting dysenteric symptoms in the town of Denton near Manchester, while the sixth strain was isolated from a mild case of dysentery in a laboratory worker in Nigeria. The Denton cases occurred during a fairly extensive outbreak of dysentery which has been described elsewhere (McGill and Downie, 1932) and in which Sonne's bacillus was the chief infecting organism. In the cases dealt with in this communication the symptoms were similar to those suffering from Sonne infections, but no evidence of infection with Sonne's bacillus was obtained by cultural examination of the faeces or by agglutination tests carried out with the patients' sera. None of the cases was fatal.

In one family three children were affected. In the first case, a girl (L. J. E.) 5 years old, illness commenced on December 2nd, 1931, with headache, vomiting, pyrexia and severe diarrhoea with blood and mucus in the stools. No specimen of faeces was sent to the laboratory at this time but a sample of blood was taken on December 15th. The serum agglutinated the organisms isolated from the other two cases in this family up to a dilution of 1 in 640. A sample of faeces from this case, sent to the laboratory on January 31st, was negative on culture. The second member of the family, a girl (I. E.) aged 6 years, became ill on December 15th with symptoms similar to those noted

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in her sister, except that there was no obvious blood in the stools on naked eye examination. From a sample of faeces received on December 17th culture on plates of MacConkey's medium showed numerous small non-lactose-fermenting colonies similar in appearance to those of the Flexner bacilli, and inhibited by brilliant green. This organism will be referred to as *Denton* 1 and is described below. Serum from a sample of blood taken on December 23rd agglutinated this culture in a dilution of 1 in 320. The brother of these two cases (J. H. E.) aged 13 years, became ill on December 21st with severe diarrhoea and mucus in the stools. From his faeces, examined on the first day of illness, the second strain of the bacillus—*Denton* 2—was recovered. No blood was sent from this case and re-examination of the faeces a month later was negative.

The third culture—*Denton* 3—was recovered from a boy (A. H.) aged 4 years, whose illness commenced on November 16th, 1931, with vomiting, headache and high temperature followed by severe diarrhoea with blood and mucus in the stools. Faeces from this case were not examined bacteriologically during the acute stage of the disease, but from specimens sent on December 17th, 1931, and January 1st, 1932, a few colonies were found in culture on MacConkey plates which later proved to be identical with the two previous strains. A few days after the commencement of this boy's illness his father suffered from similar symptoms, but examination of his faeces on December 17th was negative and a sample of blood obtained 3 months later failed to agglutinate suspensions of *Denton* 3 in a dilution of 1 in 40. The fourth strain—*Denton* 4—was isolated from the faeces of a male (H. B.) aged 53 years, who suffered for several days from severe diarrhoea and whose serum obtained on January 16th, 1932, 18 days after the onset of his illness, agglutinated the cultures isolated up to a dilution of 1 in 320. The last strain—*Denton* 5—was recovered on February 6th, 1932, from a female child (I. D.) aged 2 years, who had suffered for 3 days previously from severe diarrhoea with blood and mucus in the motions. Cultures made from the faeces on February 21st showed the same organism, but examination was negative on February 28th and March 6th.

In the plate cultures from the faeces of these cases the non-lactose-fermenting colonies were not always very numerous. This was probably due to the fact that the specimens were sent to the laboratory by post, so that an interval up to 24 hours elapsed between the times of collection and bacteriological examination.

The Nigerian strain was recovered from an adult male whose illness commenced on June 12th, 1931, with abdominal pain followed by the passing of blood and mucus. From the first motion culture on MacConkey's medium showed a practically pure growth of small non-lactose-fermenting colonies similar in appearance to those of *B. dysenteriae* Flexner. Of 51 colonies which grew on culture from a specimen obtained on the second day of illness, 40 were of the same type as those recovered from the first specimen. In the following

discussion this organism is referred to as *Strain E*. Clinically this case was rather mild and no agglutinins for the infecting organism or for any of the Flexner types were detected in the patient's serum 14 days after the onset of symptoms.

The agglutination reactions with the sera of patients L. J. E., I. E., and H. B. are of interest in that they furnish evidence of the pathogenicity of the bacilli isolated. These sera were tested against stock suspensions of the smooth type of Sonne's bacillus which had been isolated from cases of dysentery occurring in the same district about the same time, but gave negative results in a dilution of 1 in 40, the lowest dilution tested. Agglutination tests with Oxford suspensions of the Flexner dysentery bacilli were negative except in the case of serum L. J. E. which agglutinated "*Y*" suspension in a dilution of 1 in 80. The suspensions of the Denton strains which had given positive results with these sera were put up against ten sera from cases of infection due to Sonne's bacillus and eight sera which had been sent to the laboratory for Wassermann tests. Two of the sera from Sonne cases gave slight agglutination in a dilution of 1 in 20 while the rest were negative.

Cultural characters.

The five Denton strains as noted above resembled the Flexner dysentery bacilli in colony form, and morphologically were similar, being Gram-negative non-motile bacilli. *Strain E* showed the same type of colony but when first isolated appeared to be definitely motile. When examined several months later, however, this strain was no longer motile and has remained non-motile up to the present time. All strains have been examined at intervals during the last six months, but motility has never been detected and repeated attempts to demonstrate flagella, using Kirkpatrick's method, have failed. In view of the motility shown by *strain E* on first isolation several attempts have been made to recover motile bacilli from all six cultures by the technique of Colquhoun and Kirkpatrick (1932), but without success. Further, agglutination and cross-absorption tests with suspensions which had been steamed for $1\frac{1}{2}$ hours gave no indication of the presence of a heat labile H-antigen in *strain E* or *Denton* 1.

Litmus milk was at first rendered very slightly acid but became neutral or slightly alkaline after further incubation. The test for indole was negative with all cultures. Slight blackening of lead acetate occurred after 2–3 days. Nitrites were produced in media containing potassium nitrate, the Voges-Proskauer test was negative and the methyl-red test positive. All strains were non-haemolytic.

The fermentation reactions were tested immediately after isolation and showed acid in glucose and mannite with faint acidity in maltose after several days but no change in lactose, sucrose or salicin. In peptone water sugar media the *Denton* strains showed in some cases a tiny bubble of gas in glucose or

mannite after incubation for a week, whereas *strain E* showed gas after 24 hours. In view of the irregular and slight production of gas in the peptone water media after several days' incubation all strains were tested for their fermentation reactions in the medium recommended by Dudgeon and Pulvertaft (1927), using peptone water containing 1 per cent. meat extract and 1 per cent. of the carbohydrate to be tested. Table I shows the results obtained with an extended series of carbohydrates by this method. Three strains of the Newcastle dysentery bacillus were tested at the same time and are included for purposes of comparison. The results with these are practically identical with those recorded by Clayton and Warren (1929) in their discussion of those strains.

Table I.

	Denton 1	*Denton* 2	*Denton* 3	*Denton* 4	*Denton* 5	*Strain E*	*Newcastle II*	*Birtley*	*Gosforth*
Arabinose	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$
Xylose	—	—	—	—	—	—	—	—	—
Glucose	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$
Laevulose	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$
Galactose	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$
Lactose	—	—	—	—	—	—	—	—	—
Sucrose	—	—	—	—	—	—	—	—	—
Maltose	A^4	A^4	A^4	A^4	A^5	$A^4 G^8$	$A^4 G^4$	$A^4 G^4$	$A^4 G^4$
Raffinose	—	—	—	—	—	—	—	—	—
Inulin	—	—	—	—	—	—	—	—	—
Dextrin	$A^4 G^6$	$A^4 G^4$	$A^5 G^5$	$A^4 G^5$	$A^4 G^4$	$A^6 G^8$	$A^6 G^6$	$A^6 G^6$	$A^6 G^6$
Glycerin	$A^2 G^2$	$A^2 G^2$	$A^3 G^3$	$A^2 G^2$	$A^2 G^2$	$A^2 G^5$	$A^2 G^2$	$A^2 G^2$	$A^2 G^2$
Mannite	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	$A^1 G^1$	—	—	—
Dulcite	$A^5 G^5$	$A^6 G^8$	$A^5 G^8$	$A^4 G^5$	$A^5 G^5$	$A^4 G^4$	$A^5 G^5$	$A^2 G^2$	$A^4 G^4$
Adonite	—	—	—	—	—	—	—	—	—
Salicin	—	—	—	—	—	—	—	—	—
Inosite	—	—	—	—	—	—	—	—	—

A = acid, G = gas. The figures represent the number of days elapsing before acid or gas was noted.

Table I shows that the *Denton* strains and *strain E* give results which were practically identical with those given by the Newcastle strains except that mannite was never fermented by the latter.

The amount of gas produced by *strain E* was somewhat larger than with the other cultures. The tests have been repeated on various occasions in the peptone water media containing the various carbohydrates, and in this medium gas production has been variable both with Denton and Newcastle strains, and has never been large in amount.

Variability in the fermentation reactions of the dysentery bacilli have been noted by various observers (see Gardner, 1929). Recently Rutscho (1932) has recorded investigations with numerous cultures of dysentery organisms from which, by plating from old broth cultures, he was able to isolate strains differing from the parent cultures in fermentation reactions. It seemed possible that the difference in mannite fermentation in the Denton as compared with the Newcastle strains might be subject to variation. Consequently an attempt was made to isolate strains differing from the parent strains in this respect. Broth cultures of *Denton* 1, *strain E*, *Birtley* and *Gosforth*, sealed with vaseline after 24 hours' growth at 37° C., were left at room temperature and plated on

agar after 24 hours, 1 week, 4 weeks and 4 months. On each occasion 12 single colonies of each strain were subcultured to tubes of peptone water containing mannite, but no variation from the parent strain was ever noted, that is, no mannite-fermenting cultures were obtained from the Newcastle strains, while all the cultures of *Denton* 1 and *strain E* produced acid in mannite in 24 hours. Repeated daily subculture of the Newcastle strains in peptone water containing mannite over a period of 3 weeks failed to produce any change in the behaviour of the strains to that carbohydrate. As the Newcastle strains failed to ferment mannite when isolated by Clayton and Warren 4–8 years ago it would appear that the failure to ferment mannite is a relatively stable characteristic.

Serology.

In carrying out agglutination tests it was found that broth cultures killed by heat and diluted with 0·85 per cent. saline to contain approximately 400 million organisms per c.c. were more satisfactory than saline suspensions made from agar slope cultures. This applied also to the Newcastle strains which we have examined and is contrary to the findings of Clayton and Warren with these organisms. The medium used was hormone-broth, and the difference may have been due to differences in the medium or to alteration in the agglutinability of these strains. Stock suspensions were prepared from bottles of broth culture grown for 16 hours at 37° C. and killed by heat at 56° C. for 30 min.; 0·1 per cent. formalin was added as a preservative. Agglutinating sera were prepared by the intravenous injection of rabbits with heat-killed saline suspensions prepared from agar slope cultures. Injections were commenced with strain *Denton* 1 on 10. i. 32, 25 days after isolation and with the other Denton suspensions and *strain E* on 12. ii. 32. Clayton and Warren observed that the sera produced by them from rabbits were relatively low in titre, but with our strains titres of 3200 or 6400 were obtained after five or six intravenous injections extending over a period of 10 or 12 days. There was no indication of roughness in the cultures used to prepare suspensions, and the relative agglutinability of the suspensions tested may account for the difference in titre. Results of agglutination tests were read after 4 hours in a water-bath at 55° C.

In carrying out absorption tests the growth on 3–5 agar plates after 24 hours' incubation was emulsified in 3·0 c.c. of a 1 in 10 dilution of the serum to be examined. After standing in the incubator at 37° C. overnight the suspensions were centrifuged and the supernatant fluid used in the tests.

The results of agglutination and reciprocal absorption tests with strain *Denton* 1 and the other five strains are shown in Tables II and III. In the experiments recorded in Table II the growth from three agar plates was used for 0·3 c.c. serum in each case, while in the tests shown in Table III the growth on five agar plates was used for 0·3 c.c. of each serum tested.

Although the results in Table II indicate that the absorption of the serum was not complete, the titre in each case was reduced to approximately the

same degree for the homologous strain (*Denton* 1) as for the absorbing strain. When considered with the results in Table III these findings indicate that strain *Denton* 1 is serologically identical with the other Denton strains and *strain E*. When first isolated it seemed possible that strain *Denton* 1, because of its colony form and sugar reactions, belonged to the Flexner group of dysentery bacilli, as gas production was not detected in glucose and mannite peptone water media until after 1 week's incubation. Tests with sera prepared against the various Flexner types were positive with *V* serum in a dilution of 1 in 800 and with *W* and *X* sera in a dilution of 1 in 100. The corresponding titres of these sera for the homologous organisms, using Oxford suspensions, were 1 in 25,800, 1 in 3200 and 1 in 6400 respectively. Absorption of the

Table II. *Serum* Denton 1 *absorbed with the Denton strains and* strain E.

Absorbed with	Agglutinated with	Titre after absorption	Titre before absorption
Denton 1	*Denton* 1	200	6400
	Strain E	100	3200
,, 2	*Denton* 1	200	6400
	,, 2	200	6400
,, 3	,, 1	400	6400
	,, 3	400	6400
,, 4	,, 1	100	6400
	,, 4	100	3200
,, 5	,, 1	100	6400
	,, 5	100	6400
Strain E	,, 1	400	6400
	Strain E	200	6400

Table III. *Absorption of various sera with strain* Denton 1.

Sera	Absorbed with	Agglutinated with	Titre after absorption	Titre before absorption
Denton 2	*Denton* 1	*Denton* 1	<50	3200
		,, 2	<50	3200
,, 3	,, 1	,, 1	<50	3200
		,, 3	<50	3200
,, 4	,, 1	,, 1	50	6400
		,, 4	<50	6400
,, 5	,, 1	,, 1	<50	6400
		,, 5	<50	3200
Strain E	,, 1	,, 1	<50	3200
		Strain E	<50	3200

Flexner sera with the Denton strain, using the growth from five agar plates for 0·3 c.c. serum removed the agglutinins for the Denton strain but did not affect the titre for the homologous organisms. The serum which was later prepared against *Denton* 1 was found to agglutinate Oxford suspensions of Flexner *V* in a dilution of 1 in 400, *W* 1 in 100 and *Y* 1 in 100. Absorption of the Denton serum with Flexner *V* culture, or with a mixture of Flexner strains, removed the agglutinins for the absorbing strain but had no effect on the titre of the serum for the homologous organism. A similar relationship to the Flexner bacilli has been recorded by Clayton and Warren for the Newcastle strains, and as our strains, apart for the difference in fermentation

of mannite as shown in Table I, gave identical sugar reactions to those of the Newcastle strains the two groups of bacilli were compared serologically. Two sera were prepared against two Newcastle strains, *Birtley* and *Gosforth*. The results of cross-agglutination and reciprocal absorption tests with *Denton* 1 and *Birtley* shown in Table IV indicate the serological identity of these strains.

Table IV.

Sera	Absorbed with	Agglutinated with	Titre after absorption	Titre before absorption
Denton 1	*Birtley*	*Denton* 1	100	6400
		Birtley	50	3200
,, 1	*Gosforth*	*Denton* 1	100	6400
		Gosforth	100	3200
Birtley	*Denton* 1	*Denton* 1	<50	3200
		Birtley	<50	6400
,,	*Strain E*	*Strain E*	<50	3200
		Birtley	50	6400

In view of the motility observed in *strain E* when first isolated and the slight gas production by all strains in carbohydrate media, tests were made to determine whether there was any serological relationship between our strains and other members of pathogenic Gram-negative intestinal bacilli. *Denton* 1 serum failed to agglutinate suspensions of *B. typhosus*, *B. paratyphosus* B, *B. paratyphosus* A, *B. aertrycke*, *B. enteritidis gärtner*, *B. morbificans bovis*, *B. gallinarum*, *B. pullorum*, *B. dysenteriae* Shiga and *B. dysenteriae* Sonne, nor did any agglutination of strain *Denton* 1 occur with the agglutinating sera for these organisms.

Discussion.

It has been shown above that apart from the fermentation of mannite the *Denton* strains and *strain E* are identical with the Newcastle dysentery bacillus in cultural, fermentation and serological characteristics. The reaction with mannite is of some interest for, while the action of dysentery bacilli of the Flexner group on certain carbohydrates is admitted to be inconstant, the action on glucose, lactose and mannite are generally considered reliable (Andrewes and Inman, 1919). Nevertheless Fletcher and Jepps (quoted from Gardner, 1929) have described non-mannite-fermenting dysentery bacilli which could be classed serologically as Flexner bacilli of mixed *XY* type. Gas production by dysentery bacilli in carbohydrate-containing media has been recorded by Rajchman and Western (1918) and also by Goldzieher (1919), who in a study of 66 Flexner strains noted gas production by 47, although in 27 of these the gas production was inconstant. Three recently isolated strains of bacilli of the Flexner group (type *Z*) which were examined by us soon after isolation were found to produce a tiny bubble of gas in peptone water containing mannite. When tested 2 weeks later in the same medium gas was not produced. The production of gas from carbohydrates by the dysentery bacilli in amounts detectable by our usual methods of testing must, however, be regarded as exceptional. Nevertheless the strains here described, like those of Clayton and Warren, were evidently the infecting organisms in cases of clinical

dysentery and seem to be more nearly related to the Flexner group of dysentery bacilli than to other well-known members of the class of pathogenic Gram-negative intestinal bacilli. They may be regarded as a subgroup of the dysentery bacilli, related serologically in a minor degree to the Flexner group but differing from the usual classical strains in that they form small amounts of gas in carbohydrate media under the ordinary conditions of routine testing in the laboratory.

SUMMARY.

Six strains of an organism associated with and apparently etiologically related to clinical cases of dysentery have been described. Five of these strains were isolated from cases in a town in Lancashire and the other from a case in Nigeria.

These strains seem to be identical with the Newcastle dysentery bacillus described by Clayton and Warren except for the apparently constant difference in the action on mannite. Their relationship to the Flexner group of dysentery bacilli has been discussed.

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