A NEW NON-MANNITE-FERMENTING DYSENTERY ORGANISM OF THE FLEXNER GROUP

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The ability to ferment mannite is considered a fundamental and constant property of members of the Flexner group of dysentery organisms. The only exception to this rule is the Newcastle bacillus (Clayton & Warren, 1928–9), but mannite fermenting strains of this organism have been described by Downie, Wade & Young (1933) and Boyd (1938). In the present communication a non-mannite-fermenting organism is described which possesses the group antigen of the Flexner group and also a new specific antigen of its own.

SOURCE OF THE ORGANISMS

During an outbreak of diarrhoea among children at a nursery at Boston Spa, a non-motile organism fermenting glucose but no other of the commonly used carbohydrates was isolated from the faeces of several children. The organism did not form indol. It was not agglutinated by Shiga, Schmitz or Newcastle antisera. The technician considered the organism to be a commensal and discarded all cultures but one. This organism (no. 53028) proved to have marked antigenic relationship to Andrewes & Inman's (1919) types Y and X and to Boyd's (1938) type P 119.

About 6 weeks later another organism (no. 58511) of similar antigenic and biochemical properties was isolated from a patient at Scalebor Park Mental Hospital. The patient was suffering from acute gastro-enteritis. A third organism with similar characters (no. 50) was subsequently isolated from an adult female patient, residing at Brighouse, who suffered from recurrent diarrhoea. A mannite-fermenting, gas-producing variant of similar antigenic structure was also isolated from this patient.

CULTURAL AND BIOCHEMICAL CHARACTERS

These organisms were Gram-negative, non-motile, non-acid-fast rods, morphologically indistinguishable from other members of the dysentery group. They were non-capsulated and spores were not formed. The bacilli stained evenly. On agar plates after 24 hr. incubation at 37° C. small, circular, low convex, smooth and colourless colonies appeared which were about 1 mm. in diameter. Similar colonies developed on MacConkey's lactose-agar plates. Somewhat more luxuriant growth was obtained on desoxycholate-citrate agar. The organism grew well on Dorset's egg medium and on Loeffler's serum. It did not form pigment on these media and liquefaction did not take place. Growth on gelatin was slow and there was no liquefaction after 3 weeks' incubation. On horse-blood plates there was no haemolysis. In broth and peptone water there was abundant growth with uniform turbidity. The organism did not spread on 1 % agar.

The fermentation reactions with different carbohydrates were tested in two different kinds of media. In peptone water carbohydrate media acid was produced from glucose within 24 hr. but no gas was formed. Lactose, dulcite, mannite, maltose, rhamnose, xylose, salicin and inositol were not fermented even when incubation was prolonged for 21 days. Fermentation of saccharose was weak, slow and somewhat variable. Soon after isolation slight acidity in peptone water containing saccharose was observed after 8 days. After the organism had been subcultured several times, this ability to attack sucrose increased, as on subsequent occasions it broke down sucrose after 6, 4 and finally 3 days. The fermentation reactions were also examined in Dudgeon & Pulvertaft's (1927) lemco-carbohydrate-phenol-red broth. Acid and a small amount of gas were formed from glucose after 24 hr. incubation. In saccharose slight acidity but no gas appeared after 4 days' incubation. A slightly alkaline reaction developed in the presence of dulcite and mannite after 4–6 days' incubation.

The methyl-red reaction and the Voges-Proskauer test were negative. Hydrogen sulphide was not formed. Citrate could not be utilized as the only source of carbon. Methylene blue was not reduced. Ammonia was formed. The test for catalase was strongly positive. As a rule no change could be observed in litmus milk. Sometimes litmus milk was slightly acidified with subsequent reversion to neutrality.

Strain no. 58511 differed in one respect from the other two organisms, as it produced small quantities of indol. The other two strains did not produce indol.

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SEROLOGICAL REACTIONS

All three strains gave identical serological reactions. They were not agglutinated by any of the sera against any of the known pathogens of similar biochemical reactions. A very marked and immediate slide agglutination was however obtained with antisera against *B. dysenteriae* Flexner, types X, Y and P 119. A weak and delayed slide agglutination was obtained with Flexner V and Newcastle antisera. Agglutination did not occur in antisera Flexner W, Z, 103, Sonne, Shiga and Schmitz (214). Negative agglutination results were also noted with twenty-one different antisera against organisms of the Salmonella group.

Table 1 gives the results of some of the agglutination tests. The sera employed in these experiments were diluted with phenol-saline solution to give standard agglutination with the homologous organism in a dilution of 1 in 250. The results were read after 4 hr. incubation in a water-bath at 50° C.

Table 1. *Agglutination of* B. *Wakefield by different antisera*

The antisera were diluted with saline solution to give a titre of 1 in 250 with the homologous organism

Antiserum	Titre	Titre after absorption with Y strain
Flexner V	1 : 10	—
Flexner W	—	—
Flexner X	1 : 250	1 : 150
Flexner Y	1 : 250	—
Flexner Z	—	—
Newcastle	1 : 10	—
Flexner-Boyd 103	—	—
Flexner-Boyd P 119	1 : 250	1 : 250
Wakefield	1 : 250	1 : 250

ANTIGENIC STRUCTURE OF THE ORGANISM

To determine the antigenic structure of *B.* Wakefield (the name we shall use when referring to the strains under discussion), an antiserum was made and cross-absorption experiments were carried out. An antiserum of high titre was easily obtained from both rabbits used for this purpose. Strain no. 53028 was used for immunization. The titre of the serum against the homologous strain was 1 in 5000. The other strains of *B.* Wakefield were agglutinated to titre. The agglutinability of other bacteria in antiserum Wakefield is summarized in Table 2. It is of interest to note that Flexner dysentery organisms of the X, Y and P 119 types were agglutinated only in low dilutions of antiserum Wakefield, although X, Y and P 119 antisera were capable of agglutinating *B.* Wakefield to titre. Standardized suspensions of *B. dysenteriae* Shiga, Schmitz, Sonne and nine different organisms of the Salmonella group were not agglutinated by the Wakefield antiserum.

The observations of Boyd (1938) showed that all Flexner dysentery bacilli possess a common group antigen and a type specific antigen. He considers type Y a defective strain containing only the group antigen. This view is borne out by the fact that cross-agglutination between different Flexner types can be minimized and often entirely removed by absorption of the Y agglutinins from the antisera. The results of agglutination tests with *B.* Wakefield using absorbed Flexner antisera are given in Table 1.

Cross-agglutination tests were also carried out. Antisera X, Y and P 119, after having been absorbed with *B.* Wakefield, did not loose the agglutinins against the homologous strain. Samples of Wakefield antiserum, on the other hand, after having been exhaustively absorbed with Flexner X, Y and P 119 strains retained their agglutinins against *B.* Wakefield. It may be concluded from these experiments that *B.* Wakefield contains (1) the group antigen common to the whole Flexner group, (2) a type specific antigen of its own, and (3) possibly a part of the type specific antigen of P 119.

Table 2. *Agglutination tests with an antiserum prepared from* B. *Wakefield* (*strain no.* 53028)

Suspension	Titre
B. Wakefield	1 : 5000
B. dys. Flexner V	1 : 40
B. dys. Flexner W	—
B. dys. Flexner X	1 : 40
B. dys. Flexner Y	1 : 320
B. dys. Flexner Z	—
B. Newcastle	—
B. dys. Flexner-Boyd 103	1 : 80
B. dys. Flexner-Boyd P 119	1 : 160
B. dys. Shiga	—
B. dys. Schmitz	—
B. dys. Sonne	—

It has been suggested by Boyd (1940) that 'membership of the Flexner group should be extended to, and limited to those races which in addition to possessing a distinct specific antigen are also endowed with the common group antigen. The latter character constitutes a bond of relationship which is of much greater significance than less fundamental properties such as biochemical reactions.' *B.* Wakefield fulfils the requirements of this definition and may therefore be regarded as a new member of the Flexner group of dysentery organisms.

PATHOGENICITY OF *B.* WAKEFIELD

To prove the pathogenicity of any dysentery organism is difficult, and the difficulties are increased when observations on only three cases are available. There is, however, some evidence which appears to point to the etiological role of *B.* Wakefield. Organisms of similar biochemical and serological properties were never found in the faeces of normal persons. *B.* Wakefield was isolated only from persons who were suffering from gastro-enteritis, and it was the only organism of presumably pathogenic importance which was found. The organism was present in the faeces in large numbers.

A sample of serum from the patient from whom strain no. 58511 was isolated was obtained about 10 days after the commencement of the illness. It agglutinated the homologous strain and also *B. dys.* Flexner Y up to a titre of 1 in 160. To remove the group agglutinins, the patient's serum was absorbed with a Flexner Y strain. The absorbed serum retained its power to agglutinate *B.* Wakefield to the original titre. The presence of specific agglutinins in the patient's serum strongly suggests that *B.* Wakefield was pathogenic. Twenty sera from normal persons did not contain specific agglutinins against this organism.

DISCUSSION

Fletcher & Jepps (1924), working at Kuala Lumpur in the Federated Malay States, isolated ten strains of dysentery bacilli which did not ferment mannite but were serologically related to the Flexner group, particularly to types X and Y. It appears very probable that *B.* Wakefield is identical with this organism. Unfortunately the identity could not be proved, as Fletcher & Jepps' organism is not included in the National Collection of Type Cultures.

H. Sachs (1943), when investigating new types of non-mannite-fermenting bacilli from cases of bacillary dysentery in India and Egypt, found 'eight strains serologically identical with some members of the Flexner-Boyd-Newcastle group'. Further details about these strains were not given.

B. fallax and *B. inconstans* (Ornstein, 1921) possess the same fermentation reactions as *B.* Wakefield. The serological structure of these organisms was therefore of interest. The strains which were obtained from the National Collection of Type Cultures did not show any serological relation to *B. dysenteriae* Flexner, our own strains, or any other dysentery organism.

Pathogenic organisms giving fermentation reactions similar to those given by our strains are *B. dysenteriae* Shiga, Schmitz, Para-Shiga (Dudgeon & Urquhart, 1919) and Newcastle. All these organisms are serologically distinct from *B.* Wakefield.

The differential diagnosis between *Proteus morgani* and our strains can be easily made by carrying out slide agglutination of the unknown organism in Flexner Y or Wakefield antiserum. Strong agglutination in either serum will indicate that the organism is probably *B.* Wakefield. Eighteen recently isolated strains of *P. morgani* did not possess any antigenic relationship to any of the dysentery bacilli. A further characteristic distinguishing the two organisms is the motility of *P. morgani*. Before the significance of *B.* Wakefield was recognized, organisms of similar biochemical reactions when not agglutinated by Newcastle, Schmitz or Shiga antisera were discarded as *P. morgani*. In this way the presence of *B.* Wakefield may have been overlooked in the past.

SUMMARY

From three patients suffering from gastro-enteritis, non-mannite-fermenting, dysentery-like organisms were isolated which showed marked serological relationship to X, Y and P 119 strains of *B. dysenteriae* Flexner.

In carbohydrate peptone-water media the organism fermented glucose without production of gas. In Lemco broth, however, both acid and gas were produced from glucose. The only other carbohydrate which was fermented was saccharose, but fermentation was weak and occurred only when incubation was prolonged for several days.

The organism possesses the group antigen common to the whole Flexner group and also a new type specific antigen of its own. Therefore it must be regarded as a new, non-mannite-fermenting member of the Flexner group of dysentery organism.

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Subcultures of all three strains have been sent to the National Collection of Type Cultures in London. Samples of the specific antiserum may be obtained from this laboratory.

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