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THE DIFFERENTIATION OF THE MANNITE-FERMENTING GROUP OF *B. DYSENTERIAE* WITH SPECIAL REFERENCE TO STRAINS ISOLATED FROM VARIOUS SOURCES IN THIS COUNTRY.

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THE present paper contains the results of an enquiry into the differentiation of certain organisms of varying source, which present characters more or less closely allied to those of the "mannite" or "Flexner" type of the dysentery bacillus.

The "non-mannite" type, first discovered by Shiga in 1898, as the cause of certain forms of bacillary dysentery in Japan, and later by Kruse in 1901, as the cause of epidemic dysentery in Rhenish Westphalia, has been shown to remain remarkably true to type, and our present cultural and serological methods amply suffice to separate it off clearly from the "mannite" or "Flexner" type. We are, in fact, justified in speaking of the Shiga bacillus of Dysentery. It is otherwise with the mannite-fermenting type. The work of the last ten years in various countries has elicited the fact that the bacillus discovered by Flexner in Manila in 1901 must be regarded merely as a type of a group comprising an ever-increasing number of strains differing in certain properties from the type-strain, and from each other.

The members, or sub-types, of the mannite-fermenting group, which have been most thoroughly investigated are Flexner's bacillus, the "Y" bacillus of Hiss and Russell discovered in 1904, and the bacillus of Strong.

I do not propose at this point to enter in detail into the differential characters of these three sub-types. It will be sufficient at present to state that the Flexner bacillus is far more closely allied on biological and serological grounds to the "Y" bacillus of Hiss and Russell than either of them is to the bacillus of Strong.

In the differentiation of the Flexner from the "Y" bacillus, Lentz (1909) lays some stress on the non-fermentation of maltose by the latter and the less marked formation of indol, but it is questionable whether this distinction is invariable (*vide infra*). Differentiation of these two bacilli by agglutination methods is only partially possible and it is only by the method of absorption that a tolerably clear distinction can be drawn between them.

The bacillus of Strong, which does not appear to have been encountered with any certainty since Strong's discovery of it in 1900, remains in a distinctly separate category not only by its ability to ferment cane sugar in addition to maltose and mannite, but also by its agglutination characteristics.

All these bacilli have been isolated from cases of dysentery in different countries, and in spite of the fact that certain differences have been found to exist between them, and that in not a few cases the bacilli of the mannite and non-mannite types have been found side by side in the same case of dysentery, there is at present no sufficient evidence in support of the suggestion that the Shiga or non-mannite type is the only sufficient cause of bacillary dysentery, and that the mannite-fermenting bacilli are merely associated organisms and not of prime aetiological importance. The prevalent notion also that the Shiga bacillus gives rise to a much severer type of dysentery than that associated with the mannite-fermenting bacilli, is probably unjustified. Very severe and fatal cases of dysentery have been found to be associated with the mannite-fermenting group also.

In this country, apart from asylums and similar institutions, bacillary dysentery has been rarely met with, and still more rarely established as such by bacteriological methods. In asylum dysentery the work of Eyre (1904) showed the presence of bacilli identical with the Shiga type. Some years previously Kruse had isolated from asylum cases his so-called pseudo-dysentery bacillus, which has since been identified more or less completely with the *Bacillus "Y."*

McWeeney (1905) also isolated from a case of asylum dysentery a bacillus probably identical with *B. pseudo-dysenteriae* of Kruse.

Aveline, Boycott and Macdonald (1908) in their investigation in

a particular asylum, found the mannite type of bacillus, which they identified, on fermentation and agglutination grounds only, with the bacillus of Flexner.

Macalister (1910) has also recently found the mannite type in a number of asylum cases.

The only sporadic case in this country definitely associated with the dysentery bacillus is that recorded by Marshall (1909). Marshall's case was that of a child two years old who died in the month of January 1909 in Lambeth, of a very acute form of dysentery lasting barely 48 hours, accompanied by the presence of blood and mucus in the stools. The organism isolated in practically pure culture from this case, was identified by cultural and agglutination methods with the bacillus of Flexner, but the method of absorption served to distinguish it therefrom.

The American type of infantile diarrhoea is ascribed by many observers to either the Flexner or the Shiga type of *B. dysenteriae.* Duval and Bassett (1904) came to this conclusion in 1902 and their opinion was afterwards confirmed by many observers at the Rockefeller Institute in 1903. During the summer of that year an investigation was made, under the direction of Flexner, into the occurrence of the dysentery bacillus in the epidemic diarrhoea of children, the result of which was to prove the *B. dysenteriae* to be the cause of certain epidemics of summer diarrhoea in America.

That the dysentery bacillus is the cause of summer diarrhoea in England is unlikely, since, as far as I can ascertain, no bacillus exactly corresponding to the true Shiga or Flexner type has ever been isolated in this country from cases of that disease. The dysentery bacillus was searched for by Scholberg at Cardiff in cases of summer diarrhoea during the summers of 1904 and 1905, and by myself and others at the Lister Institute during the summers of 1905–6–7–8 (Morgan 1906, 1907, Morgan and Ledingham 1909), but no bacilli corresponding exactly to any known type of dysentery bacilli were ever isolated. At the same time, in each of these years, in a small number of cases, bacilli of an atypical Flexner type were found. The percentage of these found in each year was too low to admit of their being considered a possible cause of the disease, so that we came to the conclusion that these atypical bacilli were either members of the normal flora of the intestine, or were accidental in occurrence.

O. Mayer (1910) has also recently described a strain "Furth" which he isolated from an epidemic of dysentery in a German regiment. He

comes to the conclusion that although it culturally resembles *B. pseudo-dysenteriae* D of Kruse, it is distinguished from it by agglutination and absorption experiments. He therefore regards it as a new strain of the dysentery bacillus.

A similar strain has also been recorded by Lösener (1909).

Marshall's strain above referred to and which has also been investigated by me (see later B 1) may also require to be placed in a group by itself in virtue of the serological relations obtained with it.

Ruffer and Willmore (1909) have found a large variety of mannite-fermenting strains which they isolated from cases of dysentery in Arabia. None of these bacilli are found to be identical with any known members of this group on the application of the absorption test, and they have therefore been regarded by their discoverers as new types of the mannite-fermenting group (El Tor group).

From the foregoing examples it will be seen that recent research appears to show that undoubted cases of dysentery may be associated with bacilli more or less akin to the Flexner type but separated from it and other known sub-types by certain differential characteristics. It seemed therefore of great importance to ascertain whether, by a more extensive series of differential tests, it might be possible to group these various bacilli in a more satisfactory way.

A number of dysentery-like strains, isolated chiefly from the excreta of typhoid convalescents, or suspected typhoid "carriers," by Dr Ledingham, were placed at my disposal, so that some comparison might be made between them and known strains isolated from actual cases of dysentery in other countries. A few strains recovered from cases of dysentery at Claybury Asylum by Dr Candler were also thoroughly examined.

Source of foreign strains. Group A (*vide* Table I).

The collection of foreign strains included two strains of the bacillus of Flexner, one of Flexner-Gray, two cultures of Duval isolated from cases of infant's diarrhoea in Baltimore and New York respectively, a culture of *Bacillus "Y"* of Hiss and Russell kindly sent to me by Professor Lentz, a culture of *Bacillus Strong* from Professor Kruse, two cultures of *B. pseudo-dysenteriae* D of Kruse and one of his *B. pseudo-dysenteriae* A. To these were added thirteen strains kindly sent to me by Dr Willmore which had been isolated by Ruffer and himself from cases of dysentery occurring amongst the Arab pilgrims to Mecca at

El Tor in Arabia. Those designated by him as "El Tor" bacilli he considers to be the cause of the disease in view of their serum reactions. Willmore also sent me three strains of bacilli isolated from infant's diarrhoea in Alexandria.

Source of British strains. Group B (*vide* Table II).

B 1. From faeces of fatal case of dysentery. Marshall's case (see above).

B 2. From faeces of Mrs W. a suspected typhoid-carrier. No history of dysentery obtainable. Strain isolated 19 October, 1909. A second examination on 1 December, 1909 yielded no dysentery-like bacilli.

B 3. From faeces of typhoid convalescent F. W. discharged from hospital on 22 February, 1909. Isolated 30 August, 1909. Four examinations previous to this date and one later gave no dysentery-like bacilli. *Said to have had dysentery four years ago.*

B 4. From faeces of Miss W. daughter of Mrs W. (B 2) suspected typhoid-carrier. Isolated 1 December, 1909. No history of dysentery obtainable.

B 5, B 6. Faeces and urine of Miss N. suspected typhoid-carrier. Isolated 21 December, 1909. Mother died three weeks previously after a confinement, with symptoms of profuse diarrhoea which had lasted a considerable time and which may possibly have been due to bacillary dysentery. There was no autopsy.

B 7. From faeces of a child suffering from severe diarrhoea.

B 8. From urine of Miss J. W. sister of F. W. (B 3), typhoid convalescent. Discharged from hospital 31 March, 1909. Isolated 30 August, 1909. Four examinations previous to this date and one later gave no dysentery-like bacilli. No history of dysentery.

B 9. From faeces of Miss P. suspected typhoid-carrier. Isolated 24 September, 1909. No history of dysentery.

B 10. From spleen of a mouse which had been fed on material suspected to have caused severe intestinal disturbance in man.

B 11. From stool containing mucus and blood from mild case of clinical asylum dysentery. (Claybury.)

B 12. Same source as B 11.

B 13. Same source as B 11.

B 14. From faeces of Miss J. W. (B 8). Isolated 7 October, 1909.

B 15. Isolated post-mortem from scraping of intestines in a case of dysentery. (Claybury.)

B 16. From stool containing blood and mucus. At post-mortem there were ulcers of small intestine with dysenteric ulceration of the lowest part of the large intestine. Patient had pulmonary tuberculosis. (Claybury.)

B 17. From faeces of suspected female typhoid-carrier in an asylum. Isolated November, 1907. This strain is probably a non-motile *B. typhosus* (*vide infra*).

B 18. From urine of Miss B. (age 7 years) typhoid convalescent. Isolated 7 February, 1910.

B 19. From urine of S. typhoid convalescent.

B 20. From faeces of I. P. typhoid convalescent. Isolated 17 August, 1909.

B 21. From an apparently wholesome "Cambridge" sausage.

B 22. From faeces of F. H. suspected typhoid-carrier. Isolated 16 October, 1908.

B 23. From urine of Miss S. (5 years) suspected typhoid-carrier. Isolated 19 October, 1909.

B 24. From urine of Miss D. Y. suspected typhoid-carrier. Isolated 25 September, 1909.

B 25. From urine of Miss M. Y. sister of above, suspected typhoid-carrier. Isolated 28 September, 1909.

With regard to the strains B 2, B 3, B 4, B 5, B 6, B 8, B 9, B 14, B 17, B 18, B 19, B 20, B 23, B 24 and B 25 isolated by Dr Ledingham from typhoid convalescents or healthy persons examined in the course of searches in various districts for typhoid-carriers, it has to be admitted that definite evidence of a past or present attack of dysentery was in the majority of cases not forthcoming. There is little doubt, however, in view of the results presently to be detailed, that some at least of these strains must be regarded as genuine examples of the mannite-fermenting dysentery-group and that the harbourers of such strains may very possibly have suffered from some mild form of diarrhoea whose dysenteric origin was unrecognised.

In order to make an exhaustive comparison of these cultures, each of them was (1) tested by its cultural reactions when grown on various media, (2) by agglutination experiments with known sera, and (3) by absorption experiments with the same sera, besides which the virulence of some of them was tested on animals.

Cultural reactions.

To test their cultural reactions these bacilli were grown on solutions in peptone water of all the sugars, alcohols and glucosides obtainable, also on litmus-milk, peptone-beef-broth and gelatine. Before determining these reactions all the cultures had been plated and a separate colony picked off to ensure purity.

The gelatine cultures were grown at room temperature, liquefaction being looked for at the end of two months. The growth on the various sugars &c. took place at 37° C. and their reactions were noted at the end of two, four and seven days.

The litmus-milk tubes were examined at the end of one, three and fifteen days whilst motility was determined in broth cultures grown for five hours. Indol was tested for at the end of five days' growth on broth.

TABLE I. (*Foreign Strains.*)

Designation	No.	Morphology	Glucose	Mannite	Dulcite	Lactose	Cane-sugar	Litmus milk 1 day	Litmus milk 3 days	Litmus milk 15 days	Indol	Sorbite	Gelatine	Galactose	Maltose	Dextrin	Inulin	Salacin	Arabinose	Raffinose	Erythrite	Amygdalin	Isodulcite	Adonite	Glycerine
B. Flexner "L. I. P. M."	A 1	NMB	a	a	–	–	–	as	as	alks	++	–	–	a	a	as	–	–	a	as	–	–	–	–	–
B. Flexner "Elstree"	A 2	NMB	a	a	–	–	–	a	alks	alks	+	–	–	a	a	a	–	–	a	as	–	–	–	–	–
B. Flexner Gray	A 3	NMB	a	a	–	–	–	a	alks	alks	+	–	–	a	a	a	–	–	a	as	–	–	–	–	–
B. Duval infant's diarrhoea Baltimore	A 4	NMB	a	a	–	–	–	a	alks	alks	+	–	–	a	a	a	–	–	a	as	–	–	–	–	–
B. Duval infant's diarrhoea New York	A 5	NMB	a	a	–	–	–	a	alks	alks	+	–	–	a	a	a	–	–	a	as	–	–	–	–	–
B. "*Y*" Hiss and Russell (Lentz) ...	A 6	NMB	a	a	–	–	–	as	as	alks	+	–	–	a	a	a	–	–	a	a	–	–	–	–	–
B. Strong (Kruse) ...	A 7	NMB	a	a	a	–	a	o	a	AC	++	a	–	a	a	–	–	–	a	a	–	–	a	–	–
B. pseudo-dysenteriae A Kruse ...	A 8	NMB	a	a	–	–	–	as	as	A	++	–	–	a	–	–	–	a	a	a	–	–	–	–	–
B. pseudo-dysenteriae D Kruse ...	A 9	NMB	a	a	–	–	–	as	as	alks	++	–	–	a	–	as	–	as	a	a	–	–	a	–	–
B. pseudo-dysenteriae D Kruse ...	A 10	NMB	a	a	–	–	–	as	as	alks	++	–	–	a	–	–	–	–	a	a	–	as	–	–	–
B. Flexner Willmore (El Tor) ...	A 11	NMB	a	a	–	–	–	as	as	alks	+	–	–	a	–	as	a	–	a	as	–	–	– 4=a	–	–
B. pseudo-dysenteriae Willmore ...	A 12	NMB	a	a	–	–	–	as	o	alks	++	–	–	a	–	–	–	–	a	as	–	–	–	–	–
B. pseudo-dysenteriae Willmore ...	A 13	NMB	a	a	–	–	–	as	as	alks	++	a	–	a	a	a	–	–	a	a	–	–	–	–	–
B. pseudo-dysenteriae Willmore ...	A 14	NMB	a	a	–	–	–	as	o	alks	++	a	–	a	a	a	as	–	a	a	–	–	–	–	–
B. Tor 22 Willmore	A 15	NMB	a	a	–	–	–	as	as	alk	+	–	–	a	a	–	–	– 4=as	a	a	–	–	a	–	–
B. Tor R.H.B. Willmore	A 16	NMB	a	a	–	–	–	a	as	alks	+	–	–	a	–	a	–	–	a	a	–	–	–	–	–
B. Tor R.E.T. Willmore	A 17	NMB	a	a	–	–	–	as	as	alks	++	–	–	a	–	– 4=a	a	–	a	as	–	as	–	–	–
B. Tor 44 Willmore	A 18	NMB	a	a	–	–	–	as	as	alks	++	– 4=as	–	a	– 4=a	– 4=a	–	a	a	a	–	–	a	–	as
B. Tor 12/10 Willmore	A 19	NMB	a	a	–	–	–	as	as	alks	–	–	–	a	–	a	–	–	a	a	–	–	–	–	–
B. Tor 26/7 Willmore	A 20	NMB	a	a	–	–	–	as	as	alks	–	–	–	a	–	–	a	–	a	a	–	–	–	–	– 7=as
B. Tor 167 Willmore	A 21	NMB	a	a	–	–	–	as	as	alks	++	a	–	a	–	–	a	– 4=as	a	a	–	–	– 4=a	–	–
B. infant's diarrhoea Willmore ...	A 22	NMB	a	a	–	–	–	as	as	alks	++	–	–	a	–	–	–	–	–	a	–	–	–	–	–
B. infant's diarrhoea Willmore ...	A 23	NMB	a	a	–	–	–	as	as	alks	++	–	–	a	–	–	–	–	a	a	–	–	–	–	–
B. infant's diarrhoea Willmore ...	A 24	NMB	a	a	–	–	–	as	as	alks	+	–	–	a	–	a	–	– 4=a	a	a	–	–	–	–	–
B. 162 U.P. Willmore	A 25	NMB	a	a	–	–	–	a	a	alks	+	– 4=a	–	a	– 4=as	as	–	–	a	as	–	–	–	–	–
B. 165 U.P. Willmore	A 26	NMB	a	a	–	–	–	as	as	alks	++	a	–	a	–	–	a	–	a	as	–	–	–	–	– 7=as

N.B.—NMB = Non-motile bacillus.
a = acid.
as = slight acid.
AC = acid and clot.
o = neutral.
alk = alkaline.
alks = slightly alkaline.
4=as = 4th day slightly acid.
7=as = 7th day slightly acid.

TABLE II. (*British Strains.*)

	No.	Morphology	Glucose	Mannite	Dulcite	Lactose	Cane-sugar	Litmus milk: 1 day	Litmus milk: 3 days	Litmus milk: 15 days	Indol	Sorbite	Gelatine	Galactose	Maltose	Dextrin	Inulin	Salacin	Arabinose	Raffinose	Erythrite	Amygdalin	Isodulcite	Adonite	Glycerine
Group I	B 1	NMB	a	a	–	–	–	A	o	o	+ +	–	–	a	a	a	–	–	a	a	–	–	–	–	–
	B 2	NMB	a	a	–	–	–	A	o	o	+ +	a	–	a	a	a	–	–	a	a	–	a	– 4=a	–	–
	B 3	NMB	a	a	–	–	–	A	o	o	+ +	a	–	a	a	a	–	–	a	–	–	–	–	–	–
	B 4	NMB	a	a	–		–	as	o	o	+	a	–	a	a	a	–	–	a	–	–	–	– 4=a	–	–
	B 5	NMB	a	a	–	–	–	as	o	o	+	a	–	a	a	a	–	– 7=as	a	– 7=as	–	–	– 4=a	–	–
	B 6	NMB	a	a	–	–	–	as	o	o	+	a	–	a	a	a	–	– 4=a	a	–	–	–	a	–	–
Group II	B 7	NMB	a	a	–	–	–	A	o	o	+	a	–	a	–	–	–	–	–	as	–	–	– 4=a	–	–
	B 8	NMB	a	a	–	–	–	A	o	o	+ +	a	–	a	–	–	–	–	a	– 4=a	–	a	as	–	–
	B 9	NMB	a	a	–	–	–	A	o	o	+ +	a	–	a	–	–	a	–	a	as	–	–	– 4=a	–	–
	B 10	NMB	a	a	– 4=as	–	–	A	as	alk	+	a	–	a	–	–	–	a	–	a	– 4=a	–	a	–	– 4=as
	B 11	NMB	a	a	–	–	–	as	o	o	+	a	–	a	–	–	–	–	–	as	–	–	–	–	–
	B 12	NMB	a	a	–	–	–	as	o	o	+	a	–	a	–	–	– 4=a	–	–	a	–	a	–	–	–
	B 13	NMB	a	as	–	–	–	as	o	o	+	a	–	a	–	–	–	–	–	a	–	–	–	–	–
	B 14	NMB	a	a	– 4=a	–	–	A	o	o	–	a	–	a	–	–	–	–	a	a	–	–	–	–	as
Group III	B 15	NMB	a	a	–	–	–	as	A	AC	–	–	–	a	a	a	– 4=a	–	a	a	–	–	a	–	–
	B 16	NMB	a	a	–	–	–	as	A	AC	–	–	–	a	a	a	– 4=a	–	a	–	–	–	a	–	–
	B 17	NMB	a	a	– 4=avs	–	–	A	A	A	–	a	–	a	a	a	–	–	– 4=a	a	–	–	–	–	– 4=as
	B 18	NMB	a	as	–	–	a	as	o	o	–	a	–	a	a	a	–	a	a	–	–	as	–	–	a
	B 19	NMB	a	a	–	–	a	o	a	a	+	a	–	a	a	a	–	a	a	–	–	–	as	a	–
Group IV	B 20	MB	a	a	–	–	–	as	alks	alk	+	–	–	a	–	–	–	– 4=as	–	a	– 4=a	–	a	a	– 4=as
	B 21	MB	a	a	– 4=as	–	–	A	alks	alk	+ +	–	–	a	–	a	–	a	–	as	a	–	a	a	– 4=as
	B 22	MB	a	a	– 4=as	–	–	A	alks	alk	+ +	a	–	a	–	a	–	a	–	as	a	–	a	a	– 4=as
	B 23	MB	a	a	– 4=as	–	–	A	as	alk	+ +	–	–	a	–	a	–	a	–	as	as	–	–	– 4=a	as
	B 24	MB	a	a	–	–	–	A	alks	alk	+ +	a	–	a	–	–	–	a	–	as	as	a	a	– 4=a	– 4=as
	B 25	MB	a	a	– 4=as	–	–	A	as	alk	+ +	a	–	a	–	a	a	a	– 4=a	as	as	–	a	–	– 4=a

N.B.—NMB = Non-motile bacillus.
a = acid.
as = slight acid.
AC = acid and clot.
o = neutral.
alk = alkaline.
alks = slightly alkaline.
4 = as = 4th day slightly acid.
7 = as = 7th day slightly acid.

The results of these cultural reactions will be seen in Tables I and II.

From Table I it will be noticed that the first six cultures isolated from epidemics of dysentery and from cases of dysenteric diarrhoea in America give exactly the same reactions on all the media used, whereas the reactions of none of the others are exactly alike, nor do any of them conform in every particular to the reactions given by the members of the Flexner group. The bacillus of Strong ferments dulcite, cane sugar and sorbite, and also clots milk, thus differing from all the bacilli under investigation.

The strain of *Bacillus "Y"* received from Professor Lentz was found to ferment maltose and thus correspond completely in its fermentation characters with the Flexner strains. It is possible, however, that this strain may have had some previous training on maltose and Hiss (1904) had already shown that his bacillus though unable to ferment maltose when isolated from the body may readily acquire that power by a process of training on a medium containing the sugar. The great majority of the so-called pseudo-dysentery strains of Kruse including those isolated by Willmore at El Tor do not ferment maltose, nor do six of the seven strains of the El Tor bacillus which Willmore has placed in a separate group in view of their absorption reactions.

Certain sugars and alcohols it will be seen give little or no aid in differentiation inasmuch as they are fermented or not fermented by the majority of the strains. These are galactose, arabinose, raffinose, erythrite, adonite and possibly also glycerine and amygdalin.

Also, with five or six exceptions, all the bacilli investigated in Table I have no action on sorbite, inulin, salacin and isodulcite. That these exceptions were not due to indefinite reactions or faulty observation, however, was shown by the fact that some of these bacilli were retested after an interval of two months on the same sugars and alcohols with absolutely identical results.

Of the 25 strains in this group only seven ferment sorbite and six of these were isolated by Willmore at El Tor. They are

B. pseudo-dysenteriae (Willmore) 1.
B. pseudo-dysenteriae (Willmore) 2.
B. Tor 44 (Willmore).
B. Tor 167 (Willmore).
B. 162 U.P. (Willmore).
B. 165 U.P. (Willmore).

The remaining sorbite-fermenter was the bacillus of Strong.

The failure to ferment sorbite by the members of the A group is a most important feature of this group, and serves to separate it as a whole from the Group B which contains bacilli isolated solely from sources in this country.

On looking at Table II, it will be noticed that B 1 is the only culture which gives the same reactions as *B. Flexner*.

All the others not only differ from the Flexner type, but also with the exception of B 11 and B 12, differ from one another, and, what is equally remarkable, not one of them will be found to agree completely in its reactions with any bacillus in Table I.

The principal difference between most of the cultures and *B. Flexner* is their fermentation of sorbite.

It will be noticed that B 20 to B 25 are motile bacilli; this factor when we come to consider the agglutination reactions appears to be of very great importance as a distinguishing feature.

B 17 is a bacillus of some interest in that its cultural reactions on all the media are identical with those of the typhoid bacillus, differing from this bacillus only in its non-motility. Numerous attempts were made to render it motile, such as passage through a guinea-pig and subculturing on seventeen successive days on to peptone beef broth, but without success.

The cultures in Table II have been divided into groups according to their reactions on the various media on the list as far as and including dextrin. Thus the members of Group I differ from the Flexner group in that they ferment sorbite and the members of Group II in that they ferment sorbite while failing to ferment maltose or dextrin. The members of Group III differ from *B. Flexner* in that they clot milk and produce no indol. The members in Group IV differ from the Flexner type in many respects, one striking difference being their motility.

In contrast to the A group, only galactose, erythrite and adonite can be removed as affording no aid in differentiation. Considerable differences occur between the various bacilli of the B group in their reactions on arabinose, raffinose, glycerine and amygdalin. With regard to B 1 which is the only bacillus corresponding in all particulars to *B. Flexner*, it has to be noted that the child from whom it was isolated was in all probability infected by the father who had suffered from bacillary dysentery abroad. The attempts made by Marshall to demonstrate dysentery bacilli in the excreta of the father were not successful but little stress can be attached to the negative result in

view of the marked intermittency in the excretion of the specific bacilli in such cases.

B 5 and B 6 isolated from the faeces and urine respectively of the same case resemble each other very closely in their fermentation characters, the only discrepancy being a late reaction on raffinose by B 5.

B 2 and B 4 obtained from mother and daughter respectively differ only in their action on raffinose and amygdalin. They are otherwise identical in their fermentation characters.

B 3, B 8 and B 14 from members of the same household differ very considerably, two of them being unable to ferment maltose and dextrin.

B 11 and B 13 from different cases of dysentery in the same asylum are the only two bacilli in the whole group which give absolutely identical reactions on all the sugars tested. B 15 and B 16 also from the same asylum exactly resemble one another except on raffinose. B 12 cannot be considered identical with any of the others from this asylum.

Agglutination Reactions.

The next test applied was that of the agglutination reactions. Rabbits were immunised with *B. Flexner*, *Bacillus "Y"* of Hiss and Russell and *B. Strong* respectively, and each of the cultures in Tables I and II were tested with the sera, the results being set forth in Tables III and IV.

It will be observed that all the members of the Flexner group are agglutinated by Flexner serum to the same extent, with the exception *B. "Y,"* although conversely *B. Flexner* is agglutinated as well by "Y" serum as *B. "Y"* is itself. It will be further noticed that none of the other bacilli in Table III are agglutinated by Flexner serum in a higher dilution than 1 in 2000, with the exception of the five following, viz. A 8, or *B. pseudo-dysenteriae* A of Kruse, A 13 or *B. pseudo-dysenteriae* of Willmore, and A 22, A 23 and A 24 isolated from cases of infantile diarrhoea at Alexandria.

A 25 and A 26 are agglutinated by none of these sera. It will also be noticed that all the bacilli in this table are agglutinated in a higher dilution by "Y" serum than they are by Flexner serum, although these two sera are of the same titre.

The "Strong" serum, as might be expected from the cultural reactions of the bacillus, seems to have very little affinity for any of the bacteria in this table, with the exception of A 13 which was

TABLE III. (*Foreign Strains.*)

Designation	*B. Flexner* serum (titre 1 in 20,000)													*B. "Y"* Hiss and Russell serum (titre 1 in 20,000)												*B. Strong* serum (titre 1 in 20,000)											
	No.	1 in 20	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 1000	1 in 2000	1 in 4000	1 in 5000	1 in 20000		No.	1 in 20	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 1000	1 in 2000	1 in 4000	1 in 5000	1 in 20000		No.	1 in 20	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 1000	1 in 2000	1 in 4000	1 in 5000	1 in 20000
B. Flexner "T. J. P. M."	A 1	3	3	3	3	3	3	3	3	3	3	3		A 1	3	3	3	3	3	3	3	3	3	3	3		A 1	3	3	3	3	2	1	0	0	0	0	0
B. Flexner "Elstree"	A 2	3	3	3	3	3	3	3	3	3	3	3		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
B. Flexner Gray	A 3	3	3	3	3	3	3	3	3	3	3	3		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
B. Duval Baltimore	A 4	3	3	3	3	3	3	3	3	3	3	3		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
B. "Y" Hiss and Russell (Lentz) ...	A 6	3	3	3	3	3	3	3	2	0	0	0		A 6	3	3	3	3	3	3	3	3	3	3	3		A 6	3	3	2	1	0	0	0	0	0	0	0
B. Strong (Kruse)	A 7	0	0	0	0	0	0	0	0	0	0	0		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		A 7	3	3	3	3	3	3	3	3	3	3	3
B. pseudo-dysenteriae A Kruse ...	A 8	3	3	3	3	3	3	3	3	3	3	3		A 8	3	3	3	3	3	3	3	3	3	3	3		A 8	3	3	3	2	0	0	0	0	0	0	0
B. pseudo-dysenteriae D Kruse ...	A 9	3	3	2	0	0	0	0	0	0	0	0		A 9	3	3	3	3	3	3	3	3	3	2	0		A 9	0	0	0	0	0	0	0	0	0	0	0
B. pseudo-dysenteriae D Kruse ...	A 10	3	3	1	0	0	0	0	0	0	0	0		A 10	3	3	3	3	3	3	3	3	3	3	3		A 10	0	0	0	0	0	0	0	0	0	0	0
B. Flexner Willmore (El Tor) ...	A 11	3	3	3	3	3	0	0	0	0	0	0		A 11	3	3	3	3	3	3	3	3	3	3	3		A 11	2	2	0	0	0	0	0	0	0	0	0
B. pseudo-dysenteriae Willmore (El Tor)	A 12	3	3	0	0	0	0	0	0	0	0	0		A 12	3	3	3	3	3	3	3	3	3	3	0		A 12	0	0	0	0	0	0	0	0	0	0	0
B. pseudo-dysenteriae ,, ,,	A 13	3	3	3	3	3	3	3	3	3	3	3		A 13	3	3	3	3	3	3	3	3	3	3	3		A 13	3	3	3	3	3	3	3	3	3	3	3
B. pseudo-dysenteriae ,, ,,	A 14	3	0	0	0	0	0	0	0	0	0	0		A 14	3	3	3	3	3	3	3	3	3	3	2		A 14	0	0	0	0	0	0	0	0	0	0	0
B. Tor 22 Willmore	A 15	3	3	3	0	0	0	0	0	0	0	0		A 15	3	3	3	3	3	3	3	3	0	0	0		A 15	3	3	3	3	0	0	0	0	0	0	0
B. Tor R.H.B. ,, ...	A 16	3	3	3	3	3	3	1	0	0	0	0		A 16	3	3	3	3	3	3	3	3	3	3	3		A 16	0	0	0	0	0	0	0	0	0	0	0
B. Tor R.E.T. ,, ...	A 17	3	3	3	2	0	0	0	0	0	0	0		A 17	3	3	3	3	3	3	3	3	0	0	0		A 17	0	0	0	0	0	0	0	0	0	0	0
B. Tor 44 ,, ...	A 18	3	3	2	0	0	0	0	0	0	0	0		A 18	3	3	3	3	3	3	3	1	0	0	0		A 18	0	0	0	0	0	0	0	0	0	0	0
B. Tor 12/10 ,, ...	A 19	3	3	3	3	1	0	0	0	0	0	0		A 19	3	3	3	3	3	3	3	3	0	0	0		A 19	0	0	0	0	0	0	0	0	0	0	0
B. Tor 26/7 ,, ...	A 20	3	3	3	3	0	0	0	0	0	0	0		A 20	3	3	3	3	3	3	3	3	3	3	1		A 20	0	0	0	0	0	0	0	0	0	0	0
B. Tor 167 ,, ...	A 21	3	3	3	3	3	3	3	3	0	0	0		A 21	3	3	3	3	3	3	3	3	3	3	3		A 21	0	0	0	0	0	0	0	0	0	0	0
B. infant's diarrhoea ,, ...	A 22	3	3	3	3	3	3	3	3	3	3	2		A 22	3	3	3	3	3	3	3	3	3	3	3		A 22	2	1	0	0	0	0	0	0	0	0	0
B. infant's diarrhoea ,, ...	A 23	3	3	3	3	3	3	3	3	3	3	3		A 23	3	3	3	3	3	3	3	3	3	3	3		A 23	2	0	0	0	0	0	0	0	0	0	0
B. infant's diarrhoea ,, ...	A 24	3	3	3	3	3	3	3	3	3	3	2		A 24	3	3	3	3	3	3	3	3	3	3	3		A 24	3	3	0	0	0	0	0	0	0	0	0
B. 162 U.P. ,, ...	A 25	0	0	0	0	0	0	0	0	0	0	0		A 25	0	0	0	0	0	0	0	0	0	0	0		A 25	0	0	0	0	0	0	0	0	0	0	0
B. 165 U.P. ,, ...	A 26	0	0	0	0	0	0	0	0	0	0	0		A 26	0	0	0	0	0	0	0	0	0	0	0		A 26	0	0	0	0	0	0	0	0	0	0	0

N.B.—1 = slight reaction, 2 = distinct reaction, 3 = complete reaction.

TABLE IV. (*British Strains.*)

Group	No.	*B. Flexner* serum (titre 1 in 20,000) 1 in 20	50	100	200	400	800	1000	2000	4000	6000	20000	*B.* "Y" Hiss and Russell serum (titre 1 in 20,000) 1 in 20	50	100	200	400	800	1000	2000	4000	6000	20000	*B. Strong* serum (titre 1 in 20,000) 1 in 20	50	100	200	400	800	1000	2000	4000	6000	20000
I	B 1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	B 2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
	B 3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
	B 4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
	B 5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
	B 6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
II	B 7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
	B 8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
	B 9	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
	B 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
III	B 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 15	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	0	0	0	0
	B 16	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0
	B 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	1	0	0	0	0	0
	B 19	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	2	1	0	0	0	0	0	0
IV	B 20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B 25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

N.B.—1 = slight reaction, 2 = distant reaction, 3 = complete reaction.

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agglutinated by all three sera. The El Tor strains are seen to be agglutinated readily by *B.* "*Y*" serum but not by *B. Flexner* serum.

I may add that all the cultures in both tables were tested with normal rabbit serum, but none of them were found to be agglutinated in a higher dilution than 1 in 20 of the serum except B 15 and B 16 which were agglutinated by normal serum in dilutions of 1 in 500 and 1 in 200 respectively.

Table IV is a record of the agglutination reactions of the bacilli in Table II. Here too, as was observed in respect of Table III, all the cultures are agglutinated in higher dilutions by "Y" than they are by Flexner serum, and few of them, and those only in low dilutions, are agglutinated by "Strong" serum.

Turning to the particular cultures in this table, B 1 is agglutinated in a low dilution only (1 in 200) by Flexner serum, in much higher dilution (1 in 5000) by "Y" serum, and as might be expected from its cultural reactions, not at all by "Strong" serum.

The next cultures under consideration are those included in Group I, whose principal cultural difference from *B. Flexner* consists in their fermentation of sorbite. These are all of them agglutinated by Flexner serum but in a low dilution only. They are agglutinated by "Y" serum in very high dilutions, and either not at all, or, as in the case of B 2 and B 3, in low dilutions only by "Strong" serum.

The cultures in Group II are, for no apparent cultural reasons, again split up into two divisions by their agglutination reactions. B 7, B 8 and B 9 are agglutinated by both Flexner and "Y" sera, also to a minor extent by "Strong" serum, whereas the remaining members of this group, B 10, B 11, B 12 and B 13 failed to be agglutinated by any of the three sera, with the exception of a complete reaction given by B 13 in a dilution of 1 in 200 of "Y" serum.

B 14, it will be observed, is agglutinated by none of these sera.

The two cultures in Group III are agglutinated by all three sera, but especially well by "Y" serum. B 17 is also agglutinated by none of the sera, but, owing to its great resemblance culturally to the typhoid bacillus, its agglutination reactions were further tested with typhoid serum of a titre of 1 in 20,000, by which it proved to be agglutinated in a dilution of 1 in 5000.

B 18 and B 19, both cane-sugar fermenters, and in this respect resembling the bacillus of Strong, gave no reaction with either Flexner or "Y" serum, but are agglutinated in a low dilution of 1 in 200 only by Strong serum.

The members of Group IV, being all motile bacilli, are none of them agglutinated by any of the three sera, nor are they agglutinated by typhoid serum in even as low a dilution as 1 in 20.

From these results it will be seen how impossible it is, judging these atypical bacilli by their cultural reactions alone, to say whether they will be agglutinated by Flexner serum or not. Motility, however, appears to be a complete bar to their being agglutinated by this serum, as is shown by the cultures in Group IV.

B 5 and B 6, which resembled each other closely on the various sugars, are also alike in their agglutination reactions with the three sera.

B 2 and B 4 are also alike in their agglutination characters.

B 3 and B 8 agree closely in their agglutination characters but B 14 is not agglutinated by any of the three sera.

B 11 and B 13 are neither of them agglutinated by Flexner serum but B 13 is agglutinated up to 1 in 200 by "Y" serum. B 11 is not agglutinated by "Y" serum.

Absorption experiments.

The next test applied to these cultures was that of completely absorbing Flexner and "Y" sera respectively, with each of these cultures in turn. For this purpose a sufficient quantity of the culture was added to 1 in 100 dilution of the serum to ensure complete absorption of all the specific agglutinins for that bacillus, so that after centrifugalising the bacilli, the clear serum no longer agglutinated that bacillus. The supernatant fluid was then tested with the homologous bacillus in 1 in 200, 1 in 500, 1 in 2000 and 1 in 5000.

In making these experiments with Willmore's cultures I confined myself to the use of "Y" serum only, since Willmore had already demonstrated by means of absorption experiments that his cultures could be distinguished from *B. Flexner*, and I thought it unnecessary to repeat his experiments. The results are set forth in Table V.

It will be seen that two of Willmore's strains of El Tor bacilli A 19 and A 20 can be differentiated from *B. "Y"* by these absorption experiments.

B. Flexner (Gray) is shown to be identical with *B. Flexner* (Philippines) from which the serum was made, but *B. Flexner* when made to absorb "Y" serum can be readily differentiated from that bacillus. A 8 and A 9, *B. pseudo-dysenteriae* A and *B. pseudo-dysenteriae* D of

Kruse, are similarly distinguished from *B.* "*Y*" as also is A 22 a bacillus isolated from infant's diarrhoea at Alexandria.

Absorption experiments with fifteen strains which were found to be agglutinated by Flexner or "Y" serum were tested by means of absorption experiments with Flexner and "Y" serum. The results are set forth in Table V. On examining this table it will be seen that none of these tests gave a definitely positive result, with the exception of that applied to B 2 which absorbed all the agglutinins both from Flexner and "Y" serum for their homologous bacilli. B 4, B 5, B 6 and B 8 only effect a partial absorption of the Flexner-agglutinins.

TABLE V. *Absorption Experiments.*

Flexner serum (titre 1 in 2000) totally absorbed by the following bacilli	Still agglutinates *B. Flexner* 1 in 200	1 in 500	1 in 2000	"Y" serum (titre 1 in 20,000) totally absorbed by the following bacilli	Still agglutinates *B.* "*Y*" 1 in 200	1 in 500	1 in 2000	1 in 5000
A 3 (*Flexner* Gray)	0	0	0	A 1 (*Flexner*)	3	3	3	3
	⋮	⋮	⋮	A 8 (*Pseudo-dys.* A Kruse)	3	3	3	3
	⋮	⋮	⋮	A 9 (*Pseudo-dys.* D Kruse)	3	3	3	3
	⋮	⋮	⋮	A 19 (*B. Tor*)	3	3	3	3
	⋮	⋮	⋮	A 20 (*B. Tor*)	3	3	3	3
	⋮	⋮	⋮	A 22 (*Infant's Diarrhoea*)	3	3	3	3
B 1	3	2	0	B 1	3	3	3	3
B 2	0	0	0	B 2	0	0	0	0
B 3	3	2	0	B 3	3	3	2	1
B 4	3	0	0	B 4	3	3	3	1
B 5	3	0	0	B 5	3	3	3	0
B 6	3	0	0	B 6	3	3	3	0
B 7	3	2	0	B 7	3	3	3	0
B 8	3	0	0	B 8	3	3	3	3
B 9	3	3	0	B 9	3	3	3	1
B 15	3	3	0	B 15	3	3	3	1
B 16	3	3	0	B 16	3	3	3	0

On referring to Table II, B 2 will be found in Group I, and it will be observed that its cultural reactions differ from *B. Flexner* and *B.* "*Y*" owing to its fermentation of sorbite, amygdalin and isodulcite, so that although this bacillus appears to be capable of producing the same agglutinins as *B. Flexner* or *B.* "*Y*," it can readily be distinguished from them by its cultural reactions.

Test of cultures giving similar agglutination reactions. (Table VI.)

From the above results the question arises as to whether all the cultures in Table IV which either are, or are not, agglutinated by Flexner serum are between themselves identical. It was found that

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12 of these cultures were agglutinated and that 8 of them were not agglutinated by Flexner serum, consequently in order to solve this problem a culture from each of these two groups was selected, and rabbits were immunised with them. B 9 was selected from amongst the former and B 11 from amongst the latter as shown in Table VI. The agglutination reactions of each member of the groups were then tested with these sera.

Of those tested against B 11 serum, *i.e.* the bacilli that are not agglutinated by Flexner serum, only two were found to be agglutinated by this serum. These were B 12 in a dilution of 1 in 200, and B 13 in a dilution of 1 in 400. On referring to Table II these two cultures

TABLE VI.

Bacilli that do not agglutinate with Flexner serum.

B 11 serum, prepared from one of their number.

Titre of serum 1 in 20,000.

No.	1 in 20	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 1000	1 in 2000	1 in 5000	1 in 20000
B 10	0	0	0	0	0	0	0	0	0	0
B 12	3	3	3	2	0	0	0	0	0	0
B 13	3	3	3	3	2	0	0	0	0	0
B 14	0	0	0	0	0	0	0	0	0	0
B 17	0	0	0	0	0	0	0	0	0	0
B 19	0	0	0	0	0	0	0	0	0	0

Bacilli that agglutinate with Flexner serum.

B 9 serum, prepared from one of their number.

Titre of serum 1 in 2000.

No.	1 in 20	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 1000	1 in 2000
B 1	3	3	3	3	3	3	2	1
B 2	2	2	0	0	0	0	0	0
B 3	3	3	3	3	3	2	1	1
B 4	0	0	0	0	0	0	0	0
B 5	0	0	0	0	0	0	0	0
B 6	0	0	0	0	0	0	0	0
B 7	0	0	0	0	0	0	0	0
B 8	3	3	3	3	3	3	3	3
B 9	3	3	3	3	3	3	3	2
B 15	3	3	3	3	2	1	0	0
B 16	3	3	3	3	1	0	0	0
B. Flexner	3	3	3	3	2	0	0	0
B. "Y"	3	3	3	3	2	0	0	0

Absorption Experiments.

B 11 serum totally absorbed by the following bacilli	Still agglutinates B 11			
	1 in 200	1 in 500	1 in 2000	1 in 5000
B 12	3	3	3	3
B 13	3	3	3	3

B 9 serum totally absorbed by the following bacilli	Still agglutinates B 9			
	1 in 50	1 in 200	1 in 500	1 in 2000
B 1	3	3	3	3
B 3	0	0	0	0
B 8	0	0	0	0
B 15	3	3	3	1
B 16	3	3	3	1
B. "Y"	3	3	2	0
B. Flexner	3	3	3	0

are seen to be somewhat alike in their cultural reactions both being members of Group II.

The next experiment was to test those bacilli which do agglutinate with Flexner serum, against B 9 serum. The result was that six of them, B 1, B 3, B 8, B 9, B 15 and B 16, were found to be agglutinated fairly well by this serum whereas on testing *B. Flexner* and *B. "Y"* with this serum, the titre of which was 1 in 2000, they were found to be agglutinated in no higher dilution than 1 in 400.

Absorption experiments were then made with these two sera in a similar manner to that previously described. By this means B 12 and B 13 were distinguished from B 11, for although they were agglutinated by B 11 serum they failed to absorb all the agglutinins from that serum for its homologous bacillus.

Similar absorption experiments were made with B 9 serum in respect of those bacilli which were found to be agglutinated by it, that is with B 1, B 3, B 8, B 15 and B 16. As a result of these experiments, B 3 and B 8 appeared to be identical with B 9, that is, as far as absorption tests go, although, on referring to Table II, it will be noticed that these two bacilli are very different culturally.

From the results of all these experiments with the cultures in Table II, it will be seen that culturally only B 1 conformed to the Flexner type, and that although this bacillus is agglutinated both by Flexner and "Y" serum, it is differentiated from these bacilli by the absorption tests. Judging by the absorption test alone B 2 is the only culture which appears to be identical with the Flexner group but its cultural reactions differ widely from those of the Flexner bacilli.

Test of Virulence.

The virulence of some of these cultures was tested by injecting 0·5 c.c. of a 48 hours' broth culture intraperitoneally into guinea-pigs. The cultures selected were B 1, B 2, B 3, B 7, B 14 and B 21. All the animals died within 36 hours with the exception of the one injected with B 21, the motile culture from a sausage; this animal remained unaffected.

Analysis of Results.

It will be convenient to discuss separately the results obtained with the strains from the foreign and the home sources. With regard to the former, out of a total of 25, 22 are agglutinated by "Y" serum. Of

the 22, 12 are also agglutinated in moderately high dilutions (higher than 1 in 1000) while 10 are agglutinated by this serum only in dilutions of 1 in 400, and under (1 in 20 to 1 in 400). Thus the "Y" serum has a far more powerful effect on the members of the mannite-fermenting dysentery group generally than Flexner serum.

The employment of "Y" serum rather than Flexner serum for agglutination purposes would therefore be of distinct advantage in settling the broad question of admission to the mannite-fermenting group.

The strains which responded neither to Flexner nor "Y" serum were *B. Strong* and two of Willmore's Arabian strains. The fact that *B. Strong* will not react to Flexner or "Y" serum is, however, well known and it is of interest that a Strong serum has little action on any of the bacilli tested except the homologous one. Certainly one strain A 13 from El Tor, identified by Willmore with the *B. pseudo-dysenteriae* of Kruse, is agglutinated in equally high dilutions by Flexner, "Y" and Strong serum, while three other strains are agglutinated in dilutions varying from 1 in 50 to 1 in 400; so that after all, a Strong serum though almost specific is not absolutely so for bacilli of the Strong type. The two strains of Willmore (A 25 and A 29) which did not react towards any of the three sera must be left out of consideration in the meantime. A satisfactory explanation for their failure to react cannot at present be suggested. That both ferment sorbite is of interest, but on the other hand A 13, A 14 and A 21 (also Arabian strains), which likewise ferment sorbite, readily react to Flexner and "Y" serum.

When we come to consider the fermentation reactions of these bacilli it has to be remembered that in most papers dealing with this subject an extensive series of sugars, alcohols and glucosides has not been tested. As a rule, only glucose, mannite, cane-sugar, maltose and dextrin have been called into requisition in the differentiation of the well-known types. With the large number employed in the above work it is seen that only the members of the Flexner group and the bacillus "Y" (at least the particular strains tested) agree in all particulars on the whole series of sugars. With these exceptions, all the organisms differ in certain particulars although it must be admitted that these differences in some cases are slight.

Even a casual glance at Table I exhibits a much greater uniformity among the strains of the "A" group than exists among the strains of the "B" group. Nevertheless the fact that only the Flexner strains remove Flexner agglutinins while the other (six) "A" strains tested

failed to remove the homologous agglutinins from "Y" serum, suggests that the fermentation variations, insignificant though they appear to be at first sight, are of real importance and are correlated in some way with the variations in the receptor mechanism as evidenced by the absorption results. Turning to the second group "B" we find that one bacillus only, viz. B 1, corresponds throughout in its fermentation characters with *B. Flexner*, whereas all the others differ in certain particulars from each other and from the members of the "A" group. In regard to the agglutination tests the strains B 20 to B 25, being frankly motile bacilli and entirely uninfluenced by either Flexner or "Y" serum, may be at once excluded from the dysentery group. That they were also uninfluenced by typhoid anti-serum has already been mentioned. Of the remaining 19 strains no fewer than 11 are agglutinated by "Y" serum in dilutions varying from 1 in 2000 to 1 in 20,000, and one is agglutinated in a dilution of 1 in 200 only.

On the strains of this group also, Flexner serum has a less powerful influence than "Y" serum. On the strength of the agglutination tests we must admit these 11 strains and possibly also B 13 to membership of the mannite-fermenting dysentery group, and on referring to the source of these strains we find that eight of them came either from apparently healthy persons or from persons in late convalescence from typhoid fever in whom either no history at all, or no satisfactory history, of clinical dysentery could be obtained. It is remarkable that B 3 and B 8 isolated from two members of the same family give somewhat different fermentation reactions but the same phenomenon is apparent in the case of the strains from the same asylum (Claybury).

Of the Claybury strains it would appear from agglutination reactions that B 15, B 16 and possibly B 13 should be admitted to the mannite-fermenting group, but a further examination of the two former, viz. B 15 and B 16, showed that they were influenced quite as highly by an anti-typhoid serum. Moreover, in addition to their ready agglutinability, the fact that, like Strong, both these strains ultimately clotted milk, will not permit us to include them with any confidence in this group. On the other hand it has to be observed that B 11 although absolutely identical with B 13 in its fermentation reactions is not agglutinated at all by Flexner or "Y" serum. Nor is B 12, which however differs considerably from the other strains from this asylum in its fermentation properties.

We cannot definitely reject B 11 and B 12 from the mannite-fermenting dysentery group, however, in view of the fact that a serum

prepared by immunising with B 11 agglutinates the two strains B 12 and B 13, the latter of which is agglutinated by a "Y" serum though admittedly in a low dilution. The question must remain at present unsettled as we may be dealing with strains which are only with difficulty agglutinable by any serum.

Of the remaining strains, B 10 isolated from a mouse must be rejected from the group in question, also B 14, B 18 and B 19. With regard to the two latter, B 18 and B 19 isolated from urine of two typhoid convalescents, it is of some interest to note that both are cane-sugar fermenters and it might naturally be expected that they would give some reaction with a "Strong" serum. As a matter of fact they do agglutinate with the serum but in no higher dilution than 1 in 200, and in view of the somewhat doubtful position of the "Strong" strain itself little stress can be laid on the coincidence.

B 17 requires little notice. It agglutinates in high dilutions with a typhoid anti-serum and must be chronicled as one of the rarely encountered non-motile forms of this bacillus. Ernst (1908) and Fischer (1909) have recently reported on two such strains.

The absorption experiments carried out with this group show that five strains only, viz. B 2, B 4, B 5, B 6 and B 8, contain agglutinin-receptors more or less closely allied to those of *B. Flexner* while one only, viz. B 2, removes entirely the "Y" agglutinins from a "Y" serum. B 12 and B 13 do not remove the homologous agglutinins from B 11 serum and therefore one would again hesitate to include these bacilli in the mannite-fermenting dysentery group. Further investigation is necessary.

Again B 3 and B 8 appear as the result of absorption tests to be identical with B 9. Now B 3 and B 8 were isolated on the same day from the faeces and urine respectively of two members of the same family, one of whom was alleged to have had dysentery four years ago, and yet these two bacilli differ in several particulars when their fermentation characters are compared.

It is clear, therefore, that fermentation tests have indicated differences in individual strains which were not brought out by the absorption method. So far as the absorption tests above are concerned, these go to show that comparatively few of the strains in question, whether of foreign or home origin, can be considered identical and that in the few cases where such identity is apparently established the fermentation results on an extended series of sugars may still exhibit small but decided differences.

From the practical point of view the absorption method is a laborious one and in my experience with this group has failed to indicate a similarity between the strain tested and the various well-known types. The fermentation properties have in most cases confirmed such dissimilarity and have further indicated differences which the absorption tests mask.

In the mannite-fermenting dysentery group (excluding the "Strong" strains) must be incorporated a large and probably ever-increasing number of strains reacting with striking uniformity to one test (*i.e.* agglutination with "Y" or Flexner serum), but differing from one another markedly when their fermentation properties and receptor mechanisms are minutely investigated.

List of strains which the results of the present research permit us to include in the *mannite-fermenting dysentery group* (Flexner-"Y" *types*):

B 1, B 2, B 3, B 4, B 5, B 6, B 7, B 8, B 9 } and possibly B 11, B 12 and B 13.

Strains which like "Strong" clot milk and are readily agglutinable by any anti-serum:

B 15, B 16.

Strains which do not clot milk but which like "Strong" ferment cane-sugar and are agglutinated slightly by a "Strong" serum:

B 18, B 19.

The exact position of B 15, B 16, B 18 and B 19 cannot at present be definitely settled.

Conclusions.

1. A large number of dysentery-like strains isolated from sources not obviously connected with clinical dysentery in this country have been thoroughly tested and a certain proportion of them has been found to be entitled to membership of the mannite-fermenting dysentery group.

2. Bacillary dysentery must be more widely distributed in this country than has hitherto been believed and thorough bacteriological investigation may throw light on the aetiology of dysenteric forms of diarrhoea which at present are not clinically recognised as such.

3. Like certain foreign strains recently isolated from definite dysenteric sources, these home strains cannot be identified completely

with any of the well-known types of the group, on application of extensive fermentation and absorption tests.

4. When a sufficiently extended series of carbohydrate media is tested the fermentation properties of the mannite-fermenting group afford an indication of differences between the members of the group, which are not brought to light by agglutination and absorption tests.

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