TYPES OF DYSENTERY BACILLI ISOLATED AT No. 3 AUSTRALIAN GENERAL HOSPITAL, CAIRO, MARCH—AUGUST, 1916, WITH OBSERVATIONS ON THE VARIABILITY OF THE MANNITE FERMENTING GROUP.

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The results of attempts to isolate Dysentery bacilli from 217 cases in which the stools contained muco-pus with or without blood are tabulated below. In many cases the amount of mucus in the stool was very small. All these cases occurred amongst Australian troops of the E.E.F.

Table I.

Amoeba histolytica or cysts present		63
B. dysenteriae Shiga isolated		47
Mannite fermenting dysentery bacilli	Agglutinated at time of isolation by Y serum*	64
	Not agglutinated at time of isolation by Y serum	12
No causative organism recovered		36
	Total	222

* Serum made from Hiss and Russell's Y bacillus at the Lister Institute, original titre 1/8000.

In five instances dysentery bacilli were isolated from patients with amoebic infections.

The method employed was to wash the mucus, break it up in sterile broth and plate off some drops on the surface of a MacConkey plate. Next day likely colonies were picked off, sown into warm broth and incubated for a few hours. They were examined for motility and if motile discarded. The non-motile broth cultures were sown into glucose, lactose and mannite peptone water and on to an agar slope. If glucose or both glucose and mannite were fermented with the formation of acid only and lactose unchanged an emulsion was made from the agar

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slope and tested as regards agglutinability against a Shiga or Y serum respectively. Macroscopical methods were employed.

In forty-nine instances the further biochemical characteristics of the strains were investigated immediately.

The Shiga types of dysentery bacilli isolated were all true to type and agglutinated as a rule well at a dilution of 1/800 with a serum made

TABLE II.

Variation in the Fermentations, etc., of organisms agglutinated by Flexner-Y Serum.

	Maltose	Saccharose	Dextrin	Raffinose	Arabinose	Isodulcite	Sorbite	Glycerin	Indol
1	A	O	A	A	O	O	O	O	+ +
2	O	O	A[4]	A	A[6]	O	O	O	+ +
3	A	O	O	A	O	O	O	O	O
4	O	O	O	A	A[4]	O	O	O	O
5	O	O	O	O	A[4]	O	A	O	+ + +
6	O	O	O	A	A[6]	O	O	O	O
7	A	O	O	O	O	O	A	O	+ + +
8	A	O	O	O	A[4]	O	A	O	+ + +
9	O	O	O	A	O	O	O	O	+ +
10	O	A[7]	O	A	O	O	O	O	O
11	O	O	O	O	O	O	O	O	O
12	O	O	O	A	O	O	A	O	+
13	O	O	O	A	O	O	O	O	O
14	A	A[6]	A	A	O	O	O	O	+
15	O	O	O	A	O	O	O	O	+
16	O	O	O	A	O	O	O	O	+
17	O	O	O	A	O	O	O	O	+
18	O	O	—	O	—	O	A	O	+ + +
19	A	O	—	O	—	O	A	O	+ + +
20	A	O	—	O	—	O	A	O	O
21	A	O	—	O	—	A[3]	O	O	O
22	A	O	—	A	—	O	O	O	+
23	O	O	—	O	—	O	O	O	O
24	A	O	—	O	—	O	O	O	+
25	O	O	—	A[5]	—	O	A	O	O
26	A	O	—	O	—	O	O	O	+ + +
27	O	O	—	O	—	O	A	A[9]	+ +
28	A	O	—	O	—	O	O	O	O
29	O	O	—	O	—	O	O	O	O
30	A	O	—	A	—	O	O	O	+
31	O	O	—	O	—	—	A	O	+
32	O	O	—	O	—	—	A	O	+
33	O	O	—	A	—	—	O	O	+
34	O	O	—	A	—	—	O	O	O
35	O	A[7]	—	A	—	—	O	O	O
36	O	O	—	O	—	—	O	O	O
37	O	O	—	A	—	—	O	O	+

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at the Lister Institute. Higher dilutions were not tried as the serum, although specific, had not a high titre for the homologous organism at the time we were using it.

The organisms of the mannite fermenting group were, on the contrary, very variable, both as regards their biochemical activities and agglutinability. Forty-nine of them were tested upon a variety of carbohydrates. They all failed to form acid in lactose, dulcite, inulin and adonite. They all produced acid in glucose, mannite and galactose and turned milk first acid then alkaline. Their action upon maltose, saccharose, dextrin, raffinose, arabinose, isodulcite (rhamnose), sorbite and glycerin and their power to form indol varied with different strains and the same strain varied at different times. The details of these variable reactions are given in Tables II and III. Table II includes all those which at the time of isolation were agglutinated by our Y serum, Table III those which were not.

TABLE III.

Variable Fermentations, etc., of organisms not agglutinated by Y Serum at the time of isolation.

	Maltose	Saccharose	Dextrin	Raffinose	Arabinose	Isodulcite	Sorbite	Glycerin	Indol	Agglut. by Patient's serum	Agglut. by polyvalent curative serum	Agglut. by Y serum 6 months after isolation
38	A	O	—	A	—	O	O	A^3	+	1/400	1/250	O
39	A	O	—	A	—	A	O	A^3	+	O	—	1/1600
40	A	O	—	O	—	—	O	O	O	O	—	—
41	A	O	—	O	—	A	O	O	+	—	—	—
42	A	O	—	O	—	—	O	O	+	—	—	—
43	O	O	—	O	—	O	O	A^5	O	O	O	O
44	O	O	—	O	—	—	O	O	O	—	—	—
45	A	O	—	O	—	A	O	O	O	1/200	—	1/800
46	A	O	—	O	—	A	O	A^9	O	1/800	—	1/400
47	O	O	—	O	—	O	O	O	+	O	—	1/1600
48	A	O	O	—	A	A	A	A^4	O	1/400	1/250	O
49	O	O	—	O	—	O	A	O	+	—	O	O

A = Acid only in peptone water containing the various carbohydrates in 24 hrs.
A^3 = „ „ „ „ „ „ „ „ „ 3 days.
O = Growth but no change in reaction.
— = Not tried.
\+ = Indol formation determined by paradimethylaminobenzaldehyde and for + + and + + + strong reaction of indol with above reagent.

Thirty of the cultures in Table II were kindly re-examined for us by Miss Rhodes of the Lister Institute, about six months after isolation. Meantime they had been subcultured at intervals on agar slopes. Out of the three originally fermenting saccharose two had lost this property.

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Five had lost the power to split maltose and other five had acquired it; four had lost the power to split sorbite, and the other two had acquired it. In six cases the action upon raffinose was reversed, three ceasing to ferment this sugar and three gaining this faculty.

The proportion of indol was the most variable; the property being lost in nine cases and acquired in four.

The instability in biochemical activities manifested by these Egyptian strains, other than in the action upon lactose, dulcite, glucose and mannite, is in accordance with observations upon organisms of this group isolated in different parts of the world, and a survey of the voluminous literature on this subject convinces us that any attempt to separate the mannite fermenting dysentery bacilli into groups on the ground of their action upon carbohydrates is unsound.

Fermentation of Saccharose.

Strong (1900) isolated a bacillus from cases of dysentery in the Philippines which differed from Flexner's organism derived from the same source in not fermenting maltose but attacking saccharose. On these grounds it has been regarded as a new species of dysentery bacillus but we understand from Prof. Strong that he is not of this opinion.

The inference that saccharine fermentation differentiates Strong's from Flexner's bacillus was disturbed when Hiss (1904) found that at this time two out of three strains of the latter fermented saccharose in peptone water more readily than the former, twelve days elapsing in the case of Strong's bacillus before the development of acidity.

There are two strains of Strong's bacillus at the Lister Institute which have been propagated for several years. When one of us had occasion to examine these two strains a few years ago they would neither of them attack saccharose. We notice also that Fraser (1916) says that a culture of Strong's bacillus obtained by him from the Director of the Bureau of Science, Manila, failed to ferment saccharose. The capacity to split cane sugar is obviously a variable characteristic, with a tendency to disappear upon laboratory media. Three of our cultures fermented saccharose at the time they were isolated and two retained this power after six months. The property may more quickly disappear. Hehewerth (1916) found that three out of twenty-six strains isolated during a small epidemic fermented saccharose but all lost this faculty four months later. Fraser (1916), too, gives instances to the same effect, in which the faculty had disappeared in fourteen days.

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So far we have been principally concerned with the disappearance of the power to split cane sugar. By artificial selection strains possessing this characteristic may also be derived. Twort (1907) propagated a Flexner's bacillus, which at the time did not ferment saccharose, upon relays of peptone water containing this sugar, subculturing about every fourteen days. After some months the strain was found to possess the characteristic of readily fermenting saccharose.

The same faculty may arise spontaneously. In 1915 it was noticed that the strains of Flexner at the Lister Institute were inconstant as regards the fermentation of saccharose and maltose in peptone water and at the same time displayed a tendency to form secondary colonies[1].

Variations in carbohydrate fermentation were studied in detail by Massini (1907) and Burk (1908) and Kowalenko (1910) with Neisser's (1906) *Bacterium coli mutabile*. Lactose fermentation is, however, the stable character of this organism, acid-forming colonies breeding true and non-acid-forming colonies breeding both kinds. It seemed more likely that the nature of the character of saccharose fermentation would, in the case of dysentery, more closely resemble the isodulcite fermentation of typhoid and dysentery bacilli investigated by Müller (1908, 1911) and by Penfold (1911), and the lactose fermentation of typhoid bacilli by Penfold (1911). In these latter cases the faculty to ferment the sugar is the variation from type. The white colonies on Endo or MacConkey's media breed true and the red colonies mixtures, and on subculture on to media devoid of the particular sugar the faculty becomes undiscoverable after a few subcultures.

In the case of dysentery bacilli, however, even when propagated on broth media, the fermentation of saccharose may again, for reasons unknown, become a characteristic of the strain of sufficient prominence to be observable.

We investigated one of our strains which at the time of isolation produced acid in peptone water after a few days. Our object was to determine whether the faculty could be enhanced by selection and whether it possessed any stability. We were unfortunate in our choice for of the three strains originally fermenting saccharose this particular one soon lost this power, whereas the others have retained it during six months.

To increase the number of microbes in the culture capable of fermenting the sugar we used the method of Neisser (1906).

[1] Personal communication by Dr Harriette Chick.

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17. VI. 16.	Sown in saccharose peptone water.
24. VI. 16.	Acid reaction developed.
27. VI. 16.	The 10th day, plated on neutral red saccharose agar.
28. VI. 16.	50 pink colonies; 800 colourless colonies which remained so five days. *Subcultures from colourless colonies bred only colourless colonies, which remained colourless.*
	Two pink colonies sown in saccharose peptone water.
3. VII. 16.	One of the peptone waters developed acid in five days, other not.
	From former a plate of saccharose neutral red agar made.
4. VII. 16.	300 colonies.
6. VII. 16.	All pink.
7. VII. 16.	18 *colonies have developed daughter colonies of bright red colour.*
	Red bud sown in peptone water.
8. VII. 16.	Peptone water strongly acid. Loopful planted on saccharose agar.
9. VII. 16.	350 colonies developed.
10. VII. 16.	All colonies pink; 50 developing buds.
11. VII. 16.	Buds well grown and bright red. Bud sown in saccharose peptone water and on to another saccharose plate.
12. VII. 16.	Peptone water acid in 16 hours. About 800 colonies on plate all pink.
14. VII. 16.	45 colonies developed red buds.
15. VII. 16.	Material from a bud plated on to saccharose agar.
18. VII. 16.	Numerous colonies developed red buds but the colonies were too thick for enumeration.
	One bud sown in saccharose peptone water.
19. VII. 16.	Peptone water acid in 15 hours.
	At this stage the process of artificial selection was suspended as we were apparently not increasing the character. We had long since ceased to get any colourless colonies. All colonies turned pink, but the proportion developing secondary colonies remained about the same.
20. VII. 16.	Material from a bud was sown into broth and subcultured every few days for one month.
20. VIII. 16.	Broth culture sown in saccharose peptone water and plated on saccharose neutral red agar.
21. VIII. 16 to 31. VIII. 16	No acid formed in 10 days in the peptone water. Only permanently colourless colonies developed upon the saccharose plate.

In this strain the power to ferment cane sugar was a feeble characteristic, tending to disappear and only developed and maintained by natural or artificial selection.

During the course of this experiment an interesting and unusual change in the agglutinability of the organism occurred. On the 4. VII. 16 it was agglutinated completely by a dilution of 1 in 800 of our Y serum. On the 9. VII. 16, that is, after we had selected from daughter

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colonies, it was retested and was agglutinated equally well, but on the 21. VII. 16 it was not agglutinated by a dilution of 1 in 50. This was repeated and the serum we were using tested and there was no apparent source of error. The non-agglutinable culture was kept and propagated upon nutrient agar, being subcultured at fortnightly intervals. A few months later it was retested and found to agglutinate as well as ever.

As far as we are aware, variations, whether natural or induced, in the fermentative characters of bacilli are not usually accompanied by serious alterations in their agglutinability. Kowalenko (1910, p. 289), Penfold (1911, p. 51).

Jacobsen (1910) has, however, recorded an instance of daughter colonies possessing different agglutinability to the mother colonies in the case of his *Bacterium typhii mutabile* which was isolated from cases of typhoid fever in a lunatic asylum. The mother colonies of this organism were inagglutinable by typhoid serum, whereas the daughter colonies were agglutinated in a dilution of 1 in 10,000. Further, by propagating from single bacilli of the mother colonies in broth, agglutinability gradually developed until in four months the full sensitiveness was reached.

Fermentation of Maltose.

Maltose and saccharose fermentation were properties utilized by Lentz (1913) to differentiate mannite fermenting dysentery bacilli into groups. To what extent this is likely to be useful in the case of saccharose has been just discussed. The fermentation of maltose is a considerably less stable property of these dysentery bacilli than that of saccharose. Hiss (1904) showed that his Y bacillus fermented maltose after successive cultures on media containing this sugar. In our observation one-third of the strains examined had in six months changed in this respect. Hehewerth (1916) examined his cultures at intervals up to four months after isolation and found that in twelve out of twenty-six the action was reversed, some acquiring the faculty to ferment maltose, others losing it. Individual colonies from the same patient behaved differently.

Other Carbohydrates. With the remaining carbohydrates, sorbite, raffinose, isodulcite and dextrin, the action is uncertain and varies from time to time. Indol production is equally capricious.

The action of the organisms isolated in Egypt upon isodulcite demands a short comment. It will be noticed that but one in Table II

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fermented this sugar and that late, whereas five out of the twelve which were not, at the time isolated, agglutinated by Y serum, did so.

Isodulcite is only exceptionally fermented by any of the Flexner or Y strains, but, as shown by Müller (1908), by growing the former upon agar containing this pentose, daughter colonies may be formed which readily attack it. On the other hand Morgan (1911) found that a strain of Strong's bacillus *B. pseudo-dysenteriae* D Kruse and some of Ruffer and Willmore's (1909) El Tor strains produced acid in isodulcite with varying rapidity.

Morgan also examined the cultural characteristics of a number of strains of dysentery-like organisms fermenting mannite which Ledingham had isolated in the course of an extensive investigation upon typhoid carriers in Great Britain. In no case was there any reason to suppose that the individuals from whom they were derived were suffering or had suffered from dysentery. The majority of these strains fermented isodulcite and about half of them were agglutinated by Y serum in high dilution.

We come to the conclusion therefore that for purposes of isolation and identification of the dysentery bacilli, the only carbohydrates of service are glucose, mannite, lactose and dulcite.

The Agglutination of the Mannite fermenting dysentery bacilli.

Hiss (1904) divided these bacilli into three groups, Y, Strong and Flexner, by serological reaction. He showed that a serum made with his Y bacillus agglutinated Flexner's bacillus nearly as well as the homologous serum. On the other hand, a serum made with Flexner's bacillus did not agglutinate the Y bacillus in high dilution. The agglutinins for the Flexner bacillus in a serum made from Y bacillus were completely absorbed by an emulsion of Flexner's bacillus, leaving those for Y intact. Reciprocally Y bacilli only absorb the agglutinins for Y out of a Flexner serum, leaving the titre for the Flexner bacillus undiminished.

Kruse (1907) found at least six groupings necessary to accommodate the strains he examined, the sixth being a cave of Adullam for those which would not comfortably fit into one or other of the first five groups. The majority of organisms isolated from epidemics in Germany, up to this time, fell into either his Group A or D. Kruse suggested that his Group D is the same as that represented in America by the Y bacillus of Hiss and Russell, but had not a culture of the latter for comparison.

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Morgan (1911), however, found that although Y serum agglutinated *B. pseudo-dysenteriae* A Kruse and one specimen of *B. pseudo-dysenteriae* D Kruse to the full titre of 1/20,000 of the serum and another specimen to 1/500 only, in no case did these three bacilli remove the agglutinin for Y. *B. pseudo-dysenteriae* D Kruse must therefore be regarded as serologically distinct from Y.

The agglutinations of Egyptian "El Tor" strains of dysentery were examined by Ruffer and Willmore (1909). From experiments with an El Tor serum, a Flexner serum and a *B. pseudo-dysenteriae* D serum, Ruffer and Willmore placed the mannite fermenting dysentery bacilli into two groups. The first contains El Tor No. 1 and *B. pseudo-dysenteriae* D Kruse, and in the second they place *B. dysenteriae* Flexner and *B. pseudo-dysenteriae* A Kruse. The members of each group could, however, be differentiated by absorption experiments.

Later, Morgan (1911) studied El Tor bacilli, *two years after their isolation*, along with other well-known strains, and found that the former as well as the latter were all agglutinated in a dilution of 1/2000 of a serum he prepared from a specimen of Hiss and Russell's Y bacillus and, with one exception, by a Flexner serum but in a much lower dilution.

Winter (1912) also examined thirty-one strains of this type of dysentery bacillus; thirty were derived from German sources and one was a Flexner bacillus obtained from America. He found Castellani's absorption method unsatisfactory and confined his observations to cross agglutination experiments. On this basis eleven of his strains appear to correspond to Kruse's *B. pseudo-dysenteriae* D and seven to Kruse's A Group. Three are so poorly agglutinated by any of his sera that it is uncertain how they should be classed, but they appear to belong to Group A. Two are intermediate between Groups A and D, and the remaining twelve cannot be grouped by the sera employed.

Hutt (1913) emphasizes the futility of grouping dysentery bacilli according to their action upon carbohydrates. This had previously been pointed out by Kruse. He used the absorption method and thereby confirmed Kruse's groupings, indeed increasing their number.

The position may be summed up by saying that the mannite fermenting dysentery bacilli comprise a number of strains serologically distinguishable by the absorption method, but overlapping considerably as regards agglutination. For the diagnosis of an organism suspected to belong to this dysentery group no one serum is adequate; sera made with a member of each of Kruse's groups A, D and E, would embrace

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most of the German strains. Y serum seems to be least specific and covers the greatest range.

From Morgan's work Y serum seemed to be particularly indicated for diagnosis work in Egypt, and we used it exclusively. Nevertheless, nearly one-sixth of the bacilli we recovered in 1916 were not agglutinated by Y serum directly after isolation. The titre of our serum against the homologous organism was 1/4000 at the time of using.

Eight of these strains were kindly re-examined by Miss Rhodes six months later. Four of them were then agglutinated by the same Y serum in dilutions, varying from 1/400 to 1/1600, so that in the meantime they had acquired some sensitivity by cultivation. Two of the four strains which still failed to be agglutinated were clumped by the polyvalent curative serum of the Lister Institute[1] in a dilution of 1/250.

In eight of the cases we were able to test the organism isolated against the patient's own serum and in four of them it was agglutinated in dilutions above 1/100 (see Table III), indicating that he had been infected by the microbe recovered from his stool. Two of the strains which did not acquire agglutinability on culture are included amongst those which were agglutinated by the patient's serum.

It would be wrong to assume that a bacillus with the morphological, cultural and biochemical characters of the Flexner group of dysentery bacilli is discredited as an etiological factor because it is not agglutinated by Y or any other univalent serum. This group of organisms is clearly in an unstable condition and records frequently occur of strains which are not agglutinated by sera prepared from the common type-strains. A recent instance, which is interesting on account of the unusual toxicity of the strain for rabbits, is given by d'Herelle (1916). This organism, which possessed the usual characteristics of the Flexner group, was isolated in five cases during a small epidemic amongst a troop of Dragoons and was not agglutinated by the polyvalent Flexner Y serum of the Pasteur Institute.

Summary and Conclusions.

1. Of 123 dysentery bacilli isolated, forty-seven were *B. dysenteriae* Shiga, seventy-six were mannite fermenters.

2. The biochemical activities of forty-nine strains of the latter were investigated immediately after isolation and again six months later.

[1] In the process of immunization the horses furnishing this serum had been injected with a number of Egyptian strains received from Sir Armand Ruffer some years ago.

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They showed variability as regards the fermentation of maltose, saccharose, dextrin, raffinose, arabinose, isodulcite, sorbite and glycerin and in the formation of indol. The same strain varied at different times, some gaining, others losing one or other of the above properties.

Similar observations by other observers and the authors are discussed and the conclusion is arrived at that the separation of the mannite fermenting dysentery bacilli into groups on the ground of their action upon the above carbohydrates is unsound and that the only carbohydrates of service for their identification are glucose, mannite, lactose and dulcite.

3. An experiment conducted during two and a half months upon a particular strain of dysentery bacillus shows that the fermentation of saccharose was in this strain a "recessive character" and only maintained by artificial selection.

4. One-sixth of the mannite fermenting dysentery organisms isolated were not agglutinated by a univalent Y (rabbit's) serum at the time of isolation but half of these acquired this property by cultivation. Others were well agglutinated by the patient's serum. An experiment is described in which agglutinability to Y serum was lost under prolonged cultivation on saccharose peptone media. In this experiment successive cultures were made every few days and the material for subcultivation was taken from daughter colonies (buds), which arose in the saccharose plates. Agglutinability was rapidly regained by propagation in broth. From the author's experience and from a survey of the literature, the conclusion is arrived at that no one univalent serum will agglutinate all dysentery bacilli of the mannite fermenting type and that a bacillus with the morphological, cultural, and biochemical characters of dysentery bacilli of this type is not discredited as an etiological factor because it is not agglutinated by any particular univalent serum.

In conclusion we express our indebtedness to Miss Rhodes of the Lister Institute for retesting many of the cultures in London, in January, 1917, and convey to her our grateful appreciation of her kindness in so doing.

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