THE ATYPICAL DYSENTERY BACILLI.

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As there appears to be a considerable amount of uncertainty (as evidenced by recent writings on this subject) regarding the biological relationships and the etiological significance of the so-called "atypical *B. dysenteriae*," this short communication is intended as a brief résumé of conclusions arrived at in the course of an extended investigation of dysentery bacilli in Egypt during 1916 and 1917. It is to be noted that the results recorded here were obtained from the examination of cases of dysentery observed in the earliest stages and frequently followed into convalescence. Such experience has shown that conclusions derived solely from an investigation of the later stages of the disease, *e.g.* convalescents examined after arrival from over-seas in the United Kingdom, throw little light on the problem of the bacterial etiology of the disease as a whole. In a paper by Thomson and Mackie (1917)[1] on the clinical and laboratory study of dysentery in Egypt a number of atypical dysentery strains were described, but at the time this communication was submitted for publication my observations were not sufficiently complete to make any general statements regarding organisms of this type and no attempt was made to classify them.

When atypical and inagglutinable strains were first encountered in the course of laboratory examination of dysentery cases, the tendency was to disregard their occurrence and only the classical types were accepted for diagnostic purposes. As time went on, however, and as the proportion of cases from which classical dysentery bacilli could be isolated was unexpectedly low, attention was once more directed to these atypical varieties especially when they occurred in large numbers in the excreta during the earlier phase of an acute case in which amoebae were absent. Some of them corresponded in all their cultural reactions to the Shiga or Flexner-Y types, but failed to react to specific agglutinating sera. These came to be designated "inagglutinable *B. dysenteriae* Shiga or Flexner-Y." Only a few of these became agglutinable after repeated subcultures on artificial media, when tested two to three months after isolation, and some which in primary culture appeared to correspond to this designation rapidly underwent spontaneous mutation

[1] *Journ. Royal Army Med. Corps*, XXVIII. 403. The present author is personally responsible for the bacteriological notes in this paper.

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and displayed fermentative characters which clearly differentiated them from the classical types. The close biological similarity to the group of classical dysentery bacilli was nevertheless very striking, and from their constant occurrence in a certain proportion of all the acute dysenteries and their characteristic toxic effects on animals (*v. infra*), they came to be accepted as dysentery producing organisms.

CLINICAL TYPES OF DYSENTERIC INFECTIONS.

Dysenteric infections displayed a considerable diversity in their manifestations and varied from the typical acute attack of dysentery with the characteristic blood and mucus stools, to a simple diarrhoeal illness without obvious blood or mucus discharges. Generally speaking the latter type was characterised by the presence of more or less abundant cellular exudate evident on microscopic examination of the stools. Thus two types of infection might be distinguished: (1) an acute type with blood and mucus in the stools, (2) a milder type with fluid stools containing abundant cellular exudate but without blood or mucus. This distinction is of importance, as will be seen later, in determining the relationship of the atypical dysentery bacilli to the classical organisms as regards their pathogenic effects. The typical dysentery bacilli (Shiga and Flexner-Y types) were found to be associated with both the severe and the milder infections, but the great majority of the Shiga infections were of the acute type while, in the case of the Flexner-Y infections, the proportion of acute cases was smaller and the number of ordinary diarrhoeal cases larger as compared with the Shiga infections (see also below).

B. dysenteriae Shiga strains.

The Shiga strains isolated invariably corresponded in their cultural and biochemical reactions to the classical type and showed a specific agglutination reaction (*i.e.* to end titre) with a Shiga agglutinating serum. Only a few strains which corresponded in cultural reactions to the Shiga type and did not agglutinate in primary culture, became agglutinable after repeated subculture. No non-mannite fermenting strains, which resembled the Shiga type in most of their cultural characters, but differed as regards the fermentation of maltose or the production of indol, ever agglutinated with the Shiga serum. I originally thought that such strains and also the so-called inagglutinable *B. Shiga* might be "variants" from the classical type, and this was noted in the original paper by Thomson and myself. I ultimately classified these along with the atypical dysentery bacilli.

B. dysenteriae Flexner-Y strains.

In the ultimate identification of organisms of the Flexner-Y type, the Lister Institute Y serum[1] was used: and it may be said that the strains of

[1] Prepared with the original strain of Hiss and Russell, see Chick, *Lancet*, April 22, 1916.

this group invariably corresponded in their cultural characters to the classical types and showed a *specific* agglutination reaction with this serum to end titre. Saccharose fermenting strains which reacted to specific serum have been described by other observers, *e.g.* Martin[1], Glynn and others[2], but I invariably found that the Flexner-Y strains failed to ferment saccharose and lactose, and that mannite fermenting strains which after some days' incubation fermented saccharose or lactose were not agglutinated by the Y serum, accordingly they were classified with the atypical group.

A "Flexner Serum" (R.A.M. College) was also used in parallel series in the agglutination tests of a considerable number of mannite fermenters and only a small proportion of these reacted to it even in low titres.

I prepared a high titre agglutinating serum to a strain which reacted specifically to the Lister Y serum, but not to the Flexner serum, and found that this serum only agglutinated a small number of strains which were agglutinated to end titre by the Lister Y serum, and none of these reacted to the Flexner serum. This may be represented as follows:

	Lister Y serum	Flexner R.A.M. College serum	Serum to a strain agglutinated by Y serum
A. Majority of strains of Flexner-Y group	+	–	–
B. A small number of strains of Flexner-Y group	+	+	–
C. A small number of strains of Flexner-Y group (not the same strains as are included in group B)	+	–	+

(+) indicates agglutination up to "end titre" of the serum.

This subject requires further investigation, but it would appear that the Flexner-Y group includes perhaps a number of species and that the strain with which the Lister Y serum is prepared represents antigenic properties common to practically the whole group. A strain which was agglutinated by the Flexner serum was invariably agglutinated by the Y serum. Chick[3] has also noted the more restricted degree of specificity shown by "Flexner" sera for mannite fermenting strains as compared with the action of Y serum. It is further noteworthy that some strains of "Shiga" are agglutinated up to end titre by this "Y" serum. Only a small number of strains found to be inagglutinable in primary culture became agglutinable after subculture.

With regard to the agglutination of organisms of the dysentery group, it is to be noted, that, as contrasted with the typhoid group, sedimentation occurs comparatively slowly and the clumps are much smaller and the sediment is granular rather than flocculent. The results recorded here were obtained by incubating organisms (suspensions of 24 hour agar-cultures) and serum for two hours at 37° C. and then allowing the tubes to stand overnight at room-temperature.

[1] Martin, *Brit. Med. Journ.* I. April 14, 1917.

[2] Glynn, Berridge, Foley, Price and Robinson, *Report to Medical Research Committee*, December, 1917.

[3] *Lancet*, April 22, 1916.

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The "atypical *B. dysenteriae*."

This group may now be defined as (1) Gram-negative, non-motile bacilli, not liquefying gelatin, always fermenting glucose without gas production, (2) different strains varying as regards the fermentation of lactose, dulcite, saccharose, mannite, maltose (but never producing gas in any case) and the formation of indol from peptone, and (3) not agglutinated by a Y, Flexner, or Shiga serum. The cultural reactions when once acquired are all stable, as determined by repeated examination of strains. The reactions of some of the types met with are shown in Table I.

Table I.

Atypical Dysentery Bacilli.

Atypical *B. dysenteriae*	Motility	Glucose	Lactose	Dulcite	Saccharose	Mannite	Maltose	Indol	Gelatin	
No. 1	–	⊥	–	–	–	–	–	–	–	Corresponds to *B. dysenteriae* Shiga but not agglutinable by specific anti-Shiga serum
2	–	⊥	–	–	–	–	–	+	–	
3	–	⊥	–	–	–	–	⊥	–	–	
4	–	⊥	–	–	–	–	⊥	+	–	Correspond to *B. dysenteriae* Flexner-Y but not agglutinated by specific anti-Y serum
5	–	⊥	–	–	–	⊥	–	–	–	
6	–	⊥	–	–	–	⊥	–	+	–	
7	–	⊥	–	–	–	⊥	⊥	–	–	
8	–	⊥	–	–	–	⊥	⊥	+	–	
12	–	⊥	–	–	⊥	–	⊥	+	–	
16	–	⊥	–	–	⊥	⊥	⊥	+	–	
17	–	⊥	–	⊥	–	–	–	–	–	
22	–	⊥	–	⊥	–	⊥	–	+	–	
24	–	⊥	–	⊥	–	⊥	⊥	+	–	
32	–	⊥	–	⊥	⊥	⊥	⊥	+	–	Corresponds to *B. dysenteriae* Strong
40	–	⊥	⊥	–	–	⊥	⊥	+	–	
48	–	⊥	⊥	–	⊥	⊥	⊥	+	–	
56	–	⊥	⊥	⊥	–	⊥	⊥	+	–	
64	–	⊥	⊥	⊥	⊥	⊥	⊥	+	–	

⊥ = acid no gas.

These reactions when once acquired are all stable as determined by repeated examination of strains.

The numerical classification is based on the various possible combinations of cultural reactions.

All the various types were proved to be extremely virulent by intravenous or intraperitoneal injection of rabbits, producing a characteristic haemorrhagic enteritis, which was most marked in or limited to the small intestine. In fact, the virulence experiments demonstrated a highly selective toxic action on the mucosa of the small intestine (affecting the stomach to a less degree), which on autopsy was found to be intensely inflamed with massive haemorrhages in the tissue, and the lumen of the intestine was usually distended with blood-stained muco-purulent material loaded with cellular exudate and

masses of exfoliated epithelium. In the case of intraperitoneal injections, often there was little reaction in the peritoneum, but the characteristic effect on the intestine was distinct. Cultures from the intestinal contents usually yielded an almost pure growth of the particular organism. These results were obtained as a rule by injecting $\frac{1}{8}$ or $\frac{1}{4}$ of an agar slope culture in saline and the animals died in about 24 hours. Some strains, however, including a number of atypical varieties, exhibited a much higher virulence and the intravenous injection of $\frac{1}{40}$ of a 24 hours' agar slope culture produced the characteristic haemorrhagic enteritis. Comparative tests with a recently isolated Shiga and a No. 2 strain (see table) showed that the latter was distinctly the more virulent. This capacity for producing haemorrhagic enteritis may be regarded practically as an attribute peculiar to the dysentery group—typical and atypical. The lesion differs from the punctiform haemorrhages sometimes met with in the intestinal wall in various types of septicaemia.

From their occurrence in large numbers in early cases of acute dysentery, in the absence of the classical types, and their characteristic effects in animal experiments (which were found to be similar to those produced with the classical Shiga and Flexner-Y types), together with their close biological similarity to organisms of the typical dysentery group, it was concluded that these atypical organisms were to be regarded as true dysentery bacilli[1]. This group, of course, includes organisms which have been designated "inagglutinable Shiga and Flexner types," but it would appear more rational to avoid this designation and class all the organisms with the group characters given above as atypical or paradysentery bacilli. In this group mutations and also late fermentations were frequently noted, and organisms which would on first examination have been designated inagglutinable Flexner varieties soon developed additional fermentative characters.

CLINICAL SIGNIFICANCE OF ATYPICAL *B. DYSENTERIAE*.

As regards the type of infection due to these organisms, the majority of the cases were of the milder type and the proportion of cases with the typical acute signs, *i.e.* passing of blood and mucus, was lower. Nevertheless severe types of dysentery were not infrequently met with apparently due to these varieties. To sum up, the Shiga infections were mostly of the severe type and the atypical *B. dysenteriae* infections of the milder type, while the Flexner-Y infections occupied an intermediate position in this respect. Atypical organisms have been isolated from stools within a few minutes from the time they were passed, and were the predominating organism present. Thus there is no support for the suggestion that these are accidental contaminations in faeces kept for some time.

[1] In the case of infections with *B. Shiga* and *B. Flexner* the agglutination reaction of the patient's serum was found to be so variable and unreliable and so frequently absent, except in such low titres (1:50 or less) as to introduce the fallacy of a normal serum effect, that no investigation of this reaction was carried out in the case of the atypical infections.

Mixed infections.

In certain instances mixed infections with typical and atypical organisms were noted, but these were relatively uncommon.

Method of isolating *B. dysenteriae*.

It is not out of place here to refer briefly to the isolation of the dysentery bacilli. The medium I have generally employed and found most satisfactory has been MacConkey's agar. The use of a modified MacConkey's medium containing trypsinised heart extract did not appear to give appreciably better results. On MacConkey's medium the colonies of the Shiga bacillus were usually the smallest, and those of the atypical organisms the largest, but considerable variations were noted. The faeces must be examined as soon as possible after evacuation; repeated cultures from numerous specimens have shown that in general at room-temperature (Egypt in winter) the dysentery organisms cease to be recoverable after six to eight hours, although exceptionally they may persist much longer.

Frequency of dysentery bacilli in the faeces at different stages of the disease.

During the first few days of the illness, the dysentery bacilli were present in enormous numbers and often in almost pure culture; after this they tended to disappear and to be replaced by "concomitant organisms," see Table II, *e.g. B. Morgan* No. 1 and other similar organisms (B.C.L.A., Nos. 1, 2, 3, etc.),

Table II.

Concomitant Bacilli.

	Motility	Glucose	Lactose	Dulcite	Saccharose	Mannite	Maltose	Indol	Gelatin
B. Morgan No. 1	+	⊥ +	−	−	−	−	−	+	−
B.C.L.A. No. 1	+	⊥ +	−	−	−	−	−	−	−
„ No. 2	−	+	−	−	−	−	+	+	−
„ No. 3	−	+	−	−	−	−	−	+	−
„ No. 6	+	+	−	−	−	−	+	+	−
„ No. 7	−	+	−	−	−	−	−	−	−
B. faecalis alkaligenes	+	−	−	−	−	−	−	−	−
B. paracolon types	+ −	+	−	+ −	−	+	+	+ −	−
B. proteus types	+	⊥ +	−	−	+ −	−	+ −	+ −	+

All gram-negative bacilli.

The cocco-bacillus referred to in the text is morphologically coccal, but with only a few bacillary forms.

+ = acid and gas in the fermentation tests.

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B. faecalis alkaligenes, *B. paracolon* types, *B. proteus*, *B. pyocyaneus*, *Staphylococci*, and a Gram-negative non-motile non-carbohydrate-fermenting coccobacillus. Thus at a later stage of a dysenteric illness, plate cultures showed large numbers of colonies of these concomitant bacilli and dysentery bacilli were absent. It is, of course, difficult to determine the actual part played by these organisms, but it would appear as if the dysentery bacilli proper often only initiated the lesions and that these other organisms acted in aggravating or maintaining the disease. In a considerable number of autopsies at various stages in which cultures were made directly from the floor of intestinal ulcers and from necrotic tissue no dysentery bacilli were isolated, but cultures of these various concomitants were obtained. These results render the significance of bacteriological findings in convalescent cases of extremely dubious value so far as throwing light on the etiology of bacillary dysentery is concerned.