Original Communications.

SOME INVESTIGATIONS INTO SO-CALLED "NON-AGGLUTINABLE" DYSENTERY BACILLI.

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In the routine examination of stools from cases of bacillary dysentery (a disease which can readily be diagnosed from the clinical features of the case combined with the microscopic examination of the mucous exudate), it is a common experience to isolate organisms which, in their morphological, cultural and biochemical characters are identical with the classical strains of dysentery bacilli, but which are not agglutinated by the appropriate high titre sera. Although it is the practice in the Army in India to return these as "dysentery bacilli, non-agglutinable," the compromise has several undesirable features, and the present investigation is an attempt to classify at least a proportion of these serological outcasts.

CLASSIFICATION OF DYSENTERY BACILLI.

To define the exact scope of this inquiry, a brief descriptive classification of dysentery bacilli may be given.

(a) Morphological Characters.—Dysentery bacilli are all non-motile, non-sporing, non-capsulate, Gram-negative bacilli. They can be grown on all ordinary media, and are readily maintained in artificial culture over prolonged periods.

'B. morgan, which is motile, is not considered to be a cause of tropical bacillary dysentery.
162 So-called "Non-agglutinable" Dysentery Bacilli

(b) Biochemical Characters.—A comparatively comprehensive classification can be effected by means of their biochemical reactions, particularly the fermentation of certain carbohydrates and the production of indol from peptone.

Lactose.—All dysentery bacilli are alike in failing to ferment lactose in the early days of their culture, although one group is found to produce acid after some days of incubation.

Glucose.—All ferment glucose, with acid production.

Mannite.—The reaction of this "sugar" differentiates the two chief groups. The "Flexner" and "Sonne" groups produce acid, while B. dysenteriae Shiga and B. dysenteriae Schmitz do not.

Dulcite.—According to the classification now in vogue, dysentery bacilli do not ferment dulcite. It will be found, however, that one of the organisms about to be described is a late dulcite fermenter.

Maltose.—The action on maltose is so uncertain as to be valueless. In this investigation it was tried out in a large number of cases and found of no help.

Saccharose.—Differences of opinion exist as to the action of Flexner bacilli on saccharose. The most carefully recorded results are those of Gettings [1]. Of 285 strains tested, one gave acid, another slight acid, and the remainder produced no change in saccharose. The writer has had similar positive results in rare cases, usually occurring in the third week of incubation. Whenever such results occurred, fresh inoculations in saccharose have been put up. On no occasion was the result confirmed. It has been concluded, therefore, that the first result was due, most probably, to some contamination either chemical or bacterial. It is believed that impurity of the reagent accounts for the majority of anomalous results. Non-fermentation of saccharose is therefore regarded as an essential characteristic of true Flexner bacilli.

Of the bacilli which produce acid in glucose and mannite, a certain number will be found to produce acid in saccharose, generally after a few days' incubation. The majority of these organisms will also be found to be late fermenters of lactose. This is regarded as a definite group, a member of which is B. dysenteriae Sonne. There are, however, many others which are serologically distinct from Sonne. These are commonly encountered in stools from non-dysenteric cases, and it is doubtful if many have any pathogenic action. Their importance from the point of view of the present investigation lies in the fact that they comprise a considerable proportion of the strains loosely labelled "non-agglutinable Flexner," simply because the saccharose tube is not incubated for a sufficiently long time to detect the fermentation.

There remains the large and important group of mannite fermenting, non-saccharose fermenting organisms, which includes those named B. dysenteriae Flexner. They are in India the most common cause of bacillary dysentery. It is in this group that the greatest difficulty as
regards agglutination is encountered, and it is with these non-agglutinable strains that this work is concerned.

Table I gives the above classification in graphic form.

**Table I.**

<table>
<thead>
<tr>
<th>Motile or gas-producing in glucose, <em>Enterica</em>, <em>salmonella</em>, &amp;c.</th>
<th>Non-mobile, acid, without gas in glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannite fermenting</td>
<td>Non-mannite fermenting</td>
</tr>
<tr>
<td>Saccharose acid. <em>B. dysenteriae</em> Shiga, <em>B. dysenteriae</em> Schmitz, &amp;c.</td>
<td>Saccharose not acid</td>
</tr>
<tr>
<td><em>B. dysenteriae</em> Flexner</td>
<td>Organisms with these sugar reactions, but not agglutinated with Flexner serum</td>
</tr>
</tbody>
</table>

**Previous Serological Classification of Dyentery Bacilli.**

The problem of non-agglutinable Flexners is no new one. During the war they were constantly being encountered, and much diversity of opinion existed as to the validity of various strains. To clarify matters, Sir F. Andrews, [2] under the auspices of the Medical Research Council, collected a series of cultures which he made representative as far as possible, and proceeded to elaborate the now accepted classification of V, W, X, Y, Z, VZ and WX.

In passing, it might be of value to state one's experience in typing all strains of Flexner which have come to hand in the course of two years. All Andrews' types have been encountered from time to time. In addition to these recognized types, strains embodying almost every possible variation of antigenic complex have also been encountered. From the statements in a recent article in the *Journal of the Royal Army Medical Corps* [5] one is rather led to think that there need never be any difficulty in placing an organism in one or other of the above types. This is definitely not the writer's experience, nor has it been that of several others who have written on the subject [4 and 6]. Not only do variations occur from case to case, but if several colonies be picked off the same plate, and carefully put up against identical sera, remarkable variations in antigenic structure will sometimes be revealed. From the practical point of view, this is of no importance. A good polyvalent serum will bring them all down, and there need never be any doubt about an organism which satisfies this test. It can be placed definitely in the Flexner group.

It must be noted that of the strains used by Andrews in his investigations, only one came from India. Now in 1929, in the military laboratories
So-called "Non-agglutinable" Dysentery Bacilli

of India, 894 organisms giving Flexner biochemical reactions were isolated from cases of clinical dysentery. Of these, 282, or 31.5 per cent. did not agglutinate with standard serum. It seems only reasonable to assume, therefore, that the series from which Andrewes' classification was built up was not sufficiently exhaustive, and that other serological strains are entitled to consideration as being potential causes of dysentery.

In order to select those most likely to repay further investigation, advantage has been taken of the well-known fact that the occurrence of dysentery bacilli in the stools of cases follows a very definite course. If a perfectly fresh piece of mucus from an early case be plated, numerous colonies of the pathogenic type will be encountered. As the case progresses, and macrophages come to replace the polymorphs in the mucus exudate, the organism is then much more difficult to recover. Finally, when on careful examination of the stool of the convalescent no mucus can be found, it will be seen on culture that the organism has also disappeared.

Where an organism, biochemically Flexner but non-agglutinable, presented such an incidence in the course of a case of bacillary dysentery, the strain was earmarked for further investigation.

Similarly, the agglutinating powers of these organisms were tested against very high titre Flexner sera, so that a relatively small proportion of heterologous agglutination could be detected. Such heterologous agglutination was encountered in certain strains, and these were also regarded with suspicion.

Homologous sera were prepared for certain strains selected in these ways, cross-agglutination and absorption tests were performed, and by these means definite types were identified and rendered identifiable.

In order to allow others to test the validity of these findings, it is proposed to describe without further preamble three strains determined in this way which embrace the majority of non-agglutinables occurring in Southern India.

Each of these strains will be given its laboratory index number.

While these organisms were being collected and investigated from cases of dysentery, other work of a routine nature, which constitutes a very interesting and important control, has been carried out in the laboratory. An extensive examination of so-called food-handlers has been in progress. In the course of two years the writer has examined just over 2,000 of these, with a total of between 5,000 and 6,000 platings of faeces. In the majority of these cases the platings were carried out under circumstances ideal for the recovery of any delicate organisms present. The cooks, water-carriers, waiters, bakers, dairymen, etc., were caused to swallow a dose of salts at an unearthly hour in the morning, then made to parade and produce a specimen at the laboratory, which had a special latrine and pans for the purpose. The stools were plated within a few minutes of being passed, the same medium being used that was employed.
for the investigation of the dysentery cases. On no single occasion has an 88 (one of the two strains about to be described) been recovered from these individuals.

[Strain No. 88.]

This has been isolated as follows: Bangalore, 1929, 8 times; Bangalore, 1930 and 1931, 8 times; Secunderabad, 1928, 1929, 5 times (a collection of 35 strains from this laboratory was kindly given to me by Major W. Walker); Poona, 1931, once.

All cases from which the organism was recovered presented symptoms of dysentery. Clinically, the type of case has been similar to the average infection with Flexner. The following may be taken as a typical case:—

Lieutenant McK., I.M.S.

April 21, 1930.—The illness started suddenly shortly before midnight, with violent diarrhoea accompanied by colic and tenesmus. The patient felt distinctly ill, and found his temperature to be \(102.3^\circ\) F.

April 22.—Twenty stools in the day. About the twelfth stool blood and mucus appeared. This persisted for a few stools, and then gradually disappeared. It should be noted, however, that the patient made what proved to be a correct diagnosis of his condition shortly after its onset and started vigorous saline treatment. Considerable colic and tenesmus persisted throughout the day. A specimen was brought to the laboratory about midday, and was less than five minutes old when received and plated. The mucus showed a typical bacillary exudate. The plate gave an almost pure culture of 88.

April 23.—Eight stools, watery. No very obvious mucus (report by patient’s wife). Still on saline. Morning temperature \(99.6^\circ\) F., evening temperature \(99^\circ\) F. No stools during night of 23rd to 24th.

April 24.—Two stools, watery. One sent to the laboratory. Slight flakes of mucus still present. Microscopically still cellular. Organism again recovered on culture (two colonies on the plate). Temperature normal. Much more comfortable.

Thereafter the stools became more solid (saline treatment being gradually omitted), and the patient went on sick leave, ten days from the onset, feeling and looking distinctly below normal.

Biochemical reactions of 88.—Lactose, no change. Glucose, acid. Mannite, acid. Dulcite acid (late and inconstant); 50 per cent three to four days; 33 per cent twenty-seven to twenty-eight days; 16 per cent unchanged after six weeks. Saccharose, no change. Milk, acid at first, neutral four to seven days, alkaline (majority very faint) eleven to sixteen days. Indol, negative.

It will be noted that a distinctive factor exists, namely, the fermentation of dulcite by approximately five-sixths of the strains. This point will receive further consideration at a later stage.

Serological Reactions.—(a) In relation to high titre diagnostic sera.
So-called “Non-agglutinable” Dysentery Bacilli

This strain is a distinct and constant serological entity. It is practically inagglutinable with V, W, X, Y and Z sera, the sole exception being W, with which some strains will agglutinate up to five per cent of titre of the serum.

The agglutinogenetic properties of the organism are moderate, and a serum with a titre of from 1,000 to 2,500 can be produced fairly easily. All strains agglutinate to titre with such a serum. An interesting point is

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**Fig. 1.**

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<thead>
<tr>
<th>A.</th>
<th>B.</th>
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<tr>
<td>100%</td>
<td>50%</td>
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<tr>
<td>75%</td>
<td>75%</td>
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<td>25%</td>
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<th>C.</th>
<th>D.</th>
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<tbody>
<tr>
<td>100%</td>
<td>50%</td>
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<tr>
<td>75%</td>
<td>75%</td>
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<td>25%</td>
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<th>E.</th>
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<tr>
<td>100%</td>
<td>50%</td>
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<tr>
<td>75%</td>
<td>75%</td>
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<td>25%</td>
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</table>
that this serum contains a fair proportion of heterologous agglutinins for V, W, and Y. The accompanying histograms (fig. 1), showing the agglutinin content of a serum prepared from 88, illustrate this point clearly. Sera from other strains have been prepared, and differ from this one only in showing minor variations in heterologous agglutinins.

(b) In relation to the serum of cases of dysentery.

The negative evidence may first be stated. In thirty-three cases of infection with "classical" Flexner bacilli, samples of serum taken at varying stages of the illness (two from each case) were tested against an emulsion of 88. Of these, thirty-one were completely negative, and two showed slight agglutination in a dilution of 1 in 25.

Conversely, with one exception, agglutinins for 88 have been found in all cases of infection with this strain, which it has been possible to test.

The following Tables (II, III, IV and V) show the agglutination and absorption results in four cases. They are self-explanatory, and furnish considerable evidence of the pathogenicity of the strain:

**Table II.**

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>88</th>
<th>Horn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (7th day)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (abs. hom.)</td>
<td>25</td>
<td>125</td>
<td></td>
<td>50</td>
<td></td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

**Table III.**

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>88</th>
<th>Horn.</th>
<th>Sh.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's serum, 8th day</td>
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<td></td>
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<tr>
<td>15th day</td>
<td>25</td>
<td>10</td>
<td>35</td>
<td></td>
<td>30</td>
<td>40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table IV.**

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>88</th>
<th>Horn.</th>
<th>Sh.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's serum, 11th day</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>17th day</td>
<td>25</td>
<td>35</td>
<td></td>
<td>25</td>
<td>25</td>
<td>175</td>
<td></td>
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<tr>
<td>(abs. W.)</td>
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<tr>
<td>(abs. hom.)</td>
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</table>
So-called "Non-agglutinable" Dysentery Bacilli

<table>
<thead>
<tr>
<th>Private R.</th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Table V.</strong></td>
<td><strong>V</strong></td>
<td><strong>W</strong></td>
<td><strong>X</strong></td>
<td><strong>Y</strong></td>
<td><strong>Z</strong></td>
<td><strong>S8</strong></td>
</tr>
<tr>
<td>Patient's serum 6th day</td>
<td>50</td>
<td>250</td>
<td>—</td>
<td>25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15th day</td>
<td>50</td>
<td>150</td>
<td>—</td>
<td>25</td>
<td>15</td>
<td>250</td>
</tr>
<tr>
<td>19th day</td>
<td>50</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>&quot; (abs. W)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>&quot; (abs. S8)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abs. = absorbed. Sh. = Shiga. Hom. = homologous. The emulsion of "S8" used in these tests was prepared in the same way as standard agglutinable emulsions of the other dysentery organisms. The same batch was used throughout, and for the control tests mentioned above.

**Relationship to B. alkalescens (Andrewes).**—The fact of the organism being a dulcite fermenter immediately raises the question of its relationship to *B. alkalescens* (Andrewes), which also possesses this property [7].

Table VI shows the biochemical reactions of the two organisms.

<table>
<thead>
<tr>
<th><strong>Table VI.</strong></th>
<th><strong>Alkalescens</strong></th>
<th><strong>S8</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Glucose</td>
<td>Acid</td>
<td>Acid</td>
</tr>
<tr>
<td>Mannite</td>
<td>Acid</td>
<td>Acid</td>
</tr>
<tr>
<td>Dulcite</td>
<td>Acid 24 to 48 hours</td>
<td>Acid 3rd to 31st day, if at all</td>
</tr>
<tr>
<td>Milk</td>
<td>Alkaline 3rd day</td>
<td>Acid at first</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neutral 4th to 7th day</td>
</tr>
<tr>
<td>Indol</td>
<td>Positive</td>
<td>Alkaline (faint) 11th to 16th day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
</tr>
</tbody>
</table>

A subculture of *B. alkalescens* (Andrewes' own strain) was obtained from the National Collection of Type Cultures at the Lister Institute. It gave exactly the biochemical reactions which are shown above, but, more important still, it failed completely to agglutinate with S8 serum.

S8 is, therefore, an entirely separate strain from *alkalescens*. It is to be recognized by the more delayed fermentation of dulcite, by the absence of marked alkali formation in milk (the feature which gained for *alkalescens* its specific name), by the absence of indol formation, and, most important of all, by its different antigenic complex.

It may be said, in further evidence, that true *alkalescens* of Andrewes type has been isolated here on two occasions from the stools of cases which presented no symptoms of dysentery.

**Summary and Conclusions.**—S8 occurs in relation to cases of bacillary dysentery in the same way as do the accepted dysentery bacilli, i.e., it can readily be recovered in the early stages, and rapidly disappears when convalescence sets in. It has never been found in cases of bacillary dysentery.
J. S. K. Boyd

in conjunction with accepted Flexner bacilli. It has never been recovered
in the examination of over 2,000 non-dysenteric cases.

In its principal biochemical reactions it resembles the Flexner bacillus. It
differs in being an inconstant late dulcite fermenter.

Serologically, it is a sharply defined entity. While itself unaffected by
Flexner sera, with the exception of slight reaction to W, it produces a
serum showing a fair proportion of heterologous agglutinins to V, W,
and Y.

The sera of patients infected with 88 show, particularly about the third
week, appreciable agglutinins for this organism. Such agglutinins are
lacking in sera from cases of infection with the accepted Flexners.

88 differs from B. alkalescens (Andrewes) in the following points:
(a) Dulcite fermentation, if present, is much delayed.
(b) There is no marked alkali formation in milk.
(c) Indol is never formed (always in alkalescens according to Andrewes).
(d) The antigenic complex is quite different.

STRAIN NO. 103.

This strain has been encountered as follows:
Bangalore, 1929, 5 times; Bangalore, 1930 and 1931, 5 times; Secunderabad, 1929 and 1930, 3 times; Poona, November, 1930 to February, 1931,
4 times; Mhow, 1931, once.

Like 88, it has only been isolated from cases presenting symptoms of
bacillary dysentery. These symptoms have been mild to moderately severe,
and require no special comment. The organism in its incidence presented
the features previously detailed. It has frequently been isolated on suc-
cessive days from the same case. One case after a fortnight's apparent
convalescence relapsed, diarrhea with blood and mucus in the stools
reappearing. The organism, which had been absent on two occasions in
normal looking stools in the convalescent interval, was again recovered in
considerable numbers from the blood and mucus during the relapse.

No other dysentery organisms have been found in these cases. Neither
has 103 ever been found in the 2,000 odd normal individuals who constitute
the control.

Biochemical Reactions.—Lactose, no change; glucose, acid; mannite,
acid; dulcite, no change; saccharose, no change; milk, acid, late neutral
or faint alkaline; indol, may or may not be formed. 103 thus agrees
exactly in its biochemical reactions with the classical Flexners.

Serological Reactions.—(a) In relation to high titre sera.

Using sera with a titre of 1 in 250, this organism will frequently show
agglutination in 1 in 25 against X and Y, more rarely against V and W,
but so far never against Z. Quite commonly, however, no such agglutina-
tions occur. The slight degree of agglutination has, in fact, been found to
vary from day to day in a way that is difficult to explain.

Occasionally trouble is experienced through the fact that immediately
on isolation certain strains are somewhat glutinous and difficult to emulsify. This property usually disappears after a few subcultures. The difficulty can be overcome by emulsifying the growth from an agar slope with ten per cent saline and then adding ten times the bulk of distilled water. The culture washes off into the strong saline in heavy flakes, but as soon as the distilled water is added these flakes disperse in a remarkable fashion and a perfect emulsion results. Emulsions sometimes show a slight auto-agglutination on standing; for example, a little white curd can usually be stirred up from the bottom of the control tube in an agglutination test when it has been left to stand overnight.

The properties of the homologous serum will be more conveniently described at a later point.

(b) In relation to the patient's serum.

This part of the investigation has up to date been unsatisfactory. For a variety of reasons it has been possible to test the serum of only three cases. None have shown any agglutinins for 103 as isolated; somewhat significant results were, however, got in one case (see Table VIII). It will be noted that a rise of agglutinins for V and W occurred, and that all agglutinins were removed by absorption with 103.

*Development of an Agglutinable Variant.*—By far the most interesting character of this organism remains to be detailed.

Various workers [8] have from time to time described the occurrence of a Flexner bacillus, non-agglutinable when isolated, which after a period of culture on artificial media became agglutinable with Flexner sera.

Several explanations of this phenomenon have been offered. In general it seems to have been assumed to be a property acquired at random by one or other of the classical strains which, owing to some unexplained conditions (the medium used for isolation has frequently been blamed), loses the property of agglutination, but re-acquires it after repeated subcultures, particularly in broth.

Others suggest that the agglutinability is associated with the mutation of "smooth" to "rough." To make this clear, it will be necessary to detail briefly the current doctrines regarding this change in the dysentery organisms.

On isolation, all members of the Flexner group are "smooth" (S). By smoothness is meant that colonies on a plate of solid medium have a regular outline, are devoid of any grain or texture, emulsify readily in normal saline without auto-agglutination, and grow as a generalized turbidity in broth. After subculture on artificial media over periods which vary in different cases, certain colonies of a different type appear when the organism is plated. These are irregular in outline and contour, have distinct grain or texture, auto-agglutinate when emulsified in normal saline, and grow in broth as a deposit. Such colonies have been termed "rough" (R).

R colonies are believed to represent a degeneration on the part of the
organism. This, when it has taken place, is, as far as in vitro culture goes, to all intents irreversible, i.e., rough variants, once isolated, breed true indefinitely. From the S form both S and R colonies may be produced.

As a rule, the two types, when growing side by side on a plate, show an appreciable difference in size, R tending to be from two to three times the size of S.

The essential difference between S and R, however, lies not so much in their physical characters as in antigenic composition. Whereas smooth V, W, X, Y and Z can be differentiated by an agglutination test using monospecific sera, rough V, W, X, Y and Z cannot be so separated. They are, in fact, identical. A serum prepared for rough Z will agglutinate rough V, W, X and Y to the same degree as rough Z. In other words, the antigen of the R variant is common to the whole group. Further, S sera have no action on any R organism, and vice versa, the common R serum will not agglutinate any S strain.

Though in the transition stage gradations between S and R may, and do, occur, the change of antigen as between true S and true R is absolute.

It has been suggested that late developed agglutinability in Flexner-like organisms is due to (a) the development of roughness in the strain, and (b) the use of high titre serum prepared from a strain which had gone partially rough. Interaction between the R elements is supposed to occur. This, however, is not the explanation. In fact, as far as Flexner bacilli are concerned, the fear of "roughness" in the serum is rather a bogey. The production of a high titre serum for a known R strain is a very difficult matter, and the casual presence of a few degrees of roughness in an emulsion used for making a smooth serum would have little or no effect.

The explanation of the phenomenon does, however, lie in mutation, but in mutation of a type which as far as the writer is aware has never previously been described.

In the first place, it has been found that the property of late developed agglutinability belongs to one specific strain, viz., the one described above as 103. Of the eighteen strains of this organism isolated, fifteen have already developed agglutinability. Conversely, of thirty-three other strains which were not agglutinable on isolation, or when first tested with V, W, X, Y, Z, or 103 serum, and which have been under observation for varying periods, some as long as two years, none has ever become agglutinable.

The principles involved in this mutation are so much at variance with accepted ideas that the matter must be discussed in detail. A description of the investigations and findings in connection with 103 is probably the simplest way of approaching the subject.

History of 103.—103 was isolated on June 16, 1929, from the stool of an officer suffering from typical clinical bacillary dysentery. The biochemical reactions of the organism were as given above.
So-called “Non-agglutinable” Dysentery Bacilli

On June 19, 1929, a broth-culture failed to agglutinate with polyvalent Flexner serum.

On July 18, 1929, a broth-culture agglutinated to a titre of 1 in 50 with polyvalent Flexner serum (about five per cent).

On August 7, 1929, a broth-culture agglutinated to a high titre with V, W, X, Y, and Z serum, but the agglutination was only a partial one, i.e., the emulsion was not completely cleared. End points were in consequence very difficult to read.

On August 17, 1929, the test was repeated, using emulsions washed off agar slopes with saline. Similar results were obtained. The lack of clearing was so marked that contamination was suspected, and plates were made of some of the unused emulsion.

On August 18, 1930, the plates showed two very distinct types of colony, whose characters will be given in tabular form later. Both types proved to have the correct Flexner biochemical reactions; one was, as before, non-agglutinable with Flexner serum, while the other agglutinated with V, W, X, Y, and Z serum.

The experiment of plating from the original stock strain was tried several times, and with more or less difficulty the two types of colony could always be obtained. As time went on the proportion of agglutinable colonies increased at the expense of the others, and from July, 1930, repeated attempts to isolate a non-agglutinable colony from this strain have been unsuccessful.

The physical characters of the two types of colony, as occurring in strain 103, are as follows:—

A. The original colony.

(1) Small lenticular colonies, regular in outline and texture.

(2) Emulsifies as a rule readily in 0.9 per cent saline, but occasionally does not emulsify well. Sometimes there is a slight tendency towards auto-agglutination, but this is never complete.

(3) Grows with generalized turbidity in broth.

(4) Produces both A and B type colonies, unless an A colony is carefully selected and regularly subcultured each day.

(5) Non-agglutinable with Flexner sera.

B. The variant.

Large, more or less straggling colonies of irregular outline and contour, and showing a grain or texture. When growing side by side on the same plate, these colonies are about three times the size of the A type. Emulsifies readily, and has no tendency to auto-agglutinate.

Grows with generalized turbidity in broth.

Has bred true, producing nothing but B colonies, from the day of isolation over eighteen months ago.

Readily agglutinable with Flexner sera.

A consideration of these points will show considerable resemblance to, and some variation from, the S and R variants of the orthodox Flexner strains. In points 1, 3 and 4, A resembles S, while in points 1 and 4,
B resembles R. Variation occurs in points 2 and 5 in A, and points 2, 3 and 5 in B.

An attempt was made to test the agglutinins in the patient's serum against the A and B variants, and approximately ten weeks after recovery serum was obtained, and gave the following results:

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>103 B</th>
<th>103 A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/250</td>
<td>1/250</td>
<td>Nil</td>
<td>1/125</td>
<td>Nil</td>
<td>1/350</td>
<td>Nil</td>
<td></td>
</tr>
</tbody>
</table>

From this it appears that at this interval (ten weeks) the patient's serum contained no agglutinins for the A form which was the type isolated. Conversely the B variant is agglutinated to quite a high titre.

To study the antigenic characters of these variants emulsions were prepared and rabbits immunized. In this connection it may be said that although every effort was made, it is not considered that from this particular strain a pure A emulsion was ever obtained. Production of B variants in A cultures was occurring freely, and the agglutination results confirm the fact that B (whose agglutinogenetic properties proved to be much greater than those of A) was never successfully excluded. Hereafter the variants will be referred to as 103A and 103B.

103A.

The results of cross agglutination and absorption tests are shown in fig. 2. As has been previously noted, this strain has not been agglutinated by V, W, X, Y and Z smooth sera nor by a "rough" Flexner serum. It is very slightly agglutinated by a high titre of 103B serum. 103A serum, however, contains considerable co-agglutinins for V, W, X, Y and Z, and particularly for 103B. This latter is no doubt due to the fact that, as explained above, B variants were not excluded from the emulsion used to immunize the rabbit. Absorption results confirm the nature of the co-agglutinins.

This strain has only moderate agglutinogenetic properties, but a serum with a titre of 1 in 1,000 was obtained on two occasions without much difficulty.

103B.

Here again the results are most clearly expressed by diagrams (fig. 3). It will be noted in the first place that 103B bears little if any relationship to "rough" Flexner, being agglutinated only to 2-5 per cent of the titre of the serum used in these experiments, while 103B fails entirely to agglutinate "rough" Flexner.
Conversely 103B is agglutinated to titre (actually it was slightly beyond titre) by W, X and Y, and to a relatively high titre by V and Z. It is further agglutinated by 103A serum to about three times the titre of that serum for its homologous organism. The explanation of this has already been offered.

103B has marked agglutinogenetic properties, and a serum with a titre
of 1 in 10,000 to 1 in 20,000 can readily be produced. It will be noted that
this serum contains heterologous agglutinins for smooth V, W, X, Y and Z,
and also to a small extent for 103A. Absorption tests are particularly

illuminating. They indicate a close relationship to all the classical Flexner straains, but particularly to Y.

What is the explanation of these apparently anomalous findings? It
would clearly appear to be as follows:
So-called "Non-agglutinable" Dysentery Bacilli

When originally isolated the strain was pure A with no B in its composition, hence its non-agglutinability at that time and a month later. 103A is in fact a serological entity which is practically inagglutinable with V, W, X, Y and Z serum, although capable of producing a serum with heterologous agglutinins for these strains. Subsequently B variants were produced and came to dominate the cultures. This B variant, in contrast to the R variant of the classical Flexners, is readily agglutinated by all smooth Flexner sera. Its antigenic composition seems almost identical to Y, from which it differs only in being much more sensitive to agglutination.

Briefly, this strain in its original phase is a separate entity; the variant which develops contains an antigen embodying all those found in the smooth strains of V, W, X, Y and Z.

IDENTIFICATION OF FURTHER STRAINS.

Using 103 serum as a test, a search was made among previously isolated inagglutinable Flexners for further strains of this organism. This met with immediate success.

Already in the laboratory there were two strains, inagglutinable when isolated by a predecessor, which had become highly agglutinable. These proved to be pure type B, and were agglutinated to full titre with that serum. (N.B.—As shown in figure 3, V, W, X, Y and Z agglutinate to only a low percentage of the titre of this serum.)

Four other inagglutinable strains proved to be similar to 103A. These were plated, and on one plate (from strain D35) a single B type colony was seen. This when subcultured proved highly agglutinable, and in fact was an exact replica of 103B. Greater difficulty was experienced with the other strains, but with one exception they have now all produced B variants.

Since the identification of 103, eleven newly isolated strains have come to hand, nine isolated in the writer's laboratory, and two sent from other laboratories in the Command. Of these eleven, nine have produced agglutinable variants of the B type.

The agglutinable variants have always been true to type as far as agglutinability is concerned, but in some cases the naked eye differences between A and B colonies have been much less marked than was the case with 103. Viewing the plate against the dark background of the "comparator" used for reading agglutination tests often proved helpful. A colonies appeared white and somewhat opaque, while B colonies were more clear and translucent. This character was also inconstant, and in one case there were no appreciable differences in the physical characters of the colonies, which were selected more or less at random, and proved by agglutination.

At the risk of being tedious, a few case histories are given in illustration of the way in which this mutation occurs.
Patient was admitted with a history of one day's diarrhoea with blood and mucus.

July 9, 1930. Specimen sent to the laboratory was a liquid stool containing blood and mucus. Microscopically bacillary exudate.

July 10. Two colonies taken from the plate made yesterday, which contained numerous other similar ones. Both gave the biochemical and serological reactions of 103.

Patient still has diarrhoea with colic and tenesmus. Microscopically bacillary exudate.

July 11. The same organism isolated from yesterday's plate.

Serological Findings.—The following table (Table VIII) shows the agglutinins present in the patient's serum, and the effect of various high titre sera on the strain as isolated. This strain was at first a very difficult one to emulsify.

<table>
<thead>
<tr>
<th>Patient's serum, 14th day</th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>SS</th>
<th>Sh.</th>
</tr>
</thead>
<tbody>
<tr>
<td>19th day</td>
<td>25</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(abs. hom.)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Agglutination percentage of organism on isolation

<table>
<thead>
<tr>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>103a</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>10%</td>
<td>10%</td>
<td>-</td>
<td>100%</td>
</tr>
</tbody>
</table>

July 13. Strain cultured in broth to which a drop of 103 serum had been added.


July 15. All colonies alike. Replate.

July 16. Slight variation in size of the colonies. No apparent roughness. One large colony subcultured and gave the following agglutination:

<table>
<thead>
<tr>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent 50</td>
<td>Per cent 50</td>
<td>Per cent 20</td>
<td>Per cent 100</td>
<td>Per cent 20</td>
</tr>
</tbody>
</table>

July 17. No further specimens received till to-day, when a normal stool containing no blood and mucus was submitted. Culturally negative.

Specimens received daily with similar findings till July 20.
So-called "Non-agglutinable" Dysentery Bacilli

July 18.—Further plate of the same serum broth-culture shows no variants which physically resemble 103B. One large colony selected, subcultured and agglutinated in parallel with a subculture of the strain as isolated as control; results shown in Table X.

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>&quot;Serumed&quot; colony</td>
<td>50</td>
<td>50</td>
<td>20</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>

July 21. The above test repeated, using fresh cultures of the same strains. Identical results were obtained.

August 6. Repeated attempts have been made, by plating from old broth-cultures, old agar cultures, &c., to get an agglutinable variant from the original strain without the intervention of serum broth. All have failed. A fresh serum broth-culture has been made from which was subcultured a colony giving the following results, shown in parallel with a colony from an "unserumed" source:

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>10</td>
<td>—</td>
<td>20</td>
<td>14</td>
<td>—</td>
</tr>
<tr>
<td>&quot;Serumed&quot; colony</td>
<td>80</td>
<td>100</td>
<td>14</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

The two strains giving these results have been plated, each on a half of an agar plate. In appearance the colonies could not be distinguished. As a control a colony was selected at random from each half of the plate and agglutinated against Y serum. The "original" colony gave no agglutination, and the "serum" went to titre.

Conclusion.—This strain as isolated corresponds to 103A. In straightforward culture it has retained its characteristics.

By culturing in the presence of 103A serum a variant having the serological characters of 103B has been produced.

Unlike the state of affairs with 103, it is impossible to distinguish the variants by colony characters.


August 27, 1930. Patient complained of diarrhoea with blood and mucus of one day's duration. Specimen sent to the laboratory consisted of blood and mucus only. Microscopically bacillary exudate.
August 28. A strain proving to be similar to 103, readily isolated (a). Specimen received again blood and mucus only. Bacillary exudate.

August 29. A similar organism isolated (b). Stools still show blood and mucus and bacillary exudate.

August 30. Specimen sent was a watery stool with traces of blood and mucus.

August 31. Watery stool with a little non-cellular mucus. Plate negative.

September 1. Watery stool, no blood and mucus. Plate negative.


Agglutination Results of Isolation are shown in Table XII.

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.8.30. Colony (a)</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>5.9 30. Colony (a)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5.930. Colony (b)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>8.9.30. Colony (b)</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

The variations in agglutinability commonly encountered in this series are seen here.

As isolated, the strain was very difficult to emulsify, and 0·2 per cent saline had to be used. This characteristic was lost after a few subcultures.

September 7. Inoculated in broth to which some sterile filtrate of a broth-culture of 103B had been added.

September 8. Plated.

September 9. Colonies doubtful. One subcultured and agglutinates as follows:

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>103A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Per cent</td>
</tr>
</tbody>
</table>

September 11. Sub cultured in broth containing a drop of 103A serum.

September 12. Plated.

September 13. Some colonies on the plate have the physical characters of 103B. One of these subcultured agglutinates as follows:
So-called "Non-agglutinable" Dysentery Bacilli

TABLE XIII.

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>103a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

"Serumed" colony
Original: 16.9.30

September 16. The original strain was subcultured to ascertain if B elements were appearing in the normal course of events (see Table XIII).

Conclusion.—The agglutinable variants seem to have appeared by virtue of culture in the presence of 103a serum. No such variants are present in stock strains.

STRAIN No. P 8.

October 28. Patient admitted to hospital complaining of diarrhoea with blood and mucus in stools. Much griping. Temperature found to be 99.6°F. The specimen sent to the laboratory consisted of blood and mucus, was alkaline in reaction, and microscopically showed bacillary exudate. Three colonies, proving to be 103, isolated.

October 29. Specimen still bacillary exudate.

October 30. Still bacillary exudate. The same organism again isolated.

October 31. Watery stool. No blood and mucus. Plate negative.

November 1. No blood and mucus. Culturally negative.

The colonies isolated on October 28 and 30 gave the following results:—

TABLE XIV.

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>103a</th>
<th>103a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony of 28.10.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Per cent</td>
<td>50</td>
</tr>
<tr>
<td>Colony of 30.10.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Per cent</td>
<td>50</td>
</tr>
</tbody>
</table>

November 7. Inoculated in broth and broth plus 103a serum.


November 19. A further set of plain and serumed broth-cultures put up.

November 26. Plated. Variations in size of colony present, but not suggestive of mutation. Following results obtained:—

TABLE XV.

<table>
<thead>
<tr>
<th></th>
<th>103a</th>
<th>103a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small colonies</td>
<td></td>
<td>Per cent</td>
</tr>
<tr>
<td>Large colonies</td>
<td></td>
<td>Per cent</td>
</tr>
</tbody>
</table>

Downloaded from http://jramc.bmj.com/ on October 26, 2017 - Published by group.bmj.com
December 10. Plated from plain and serum broth cultures just over a month old. Serum broth gave colonies all alike. Ordinary broth showed some B type colonies. Proved through sugars. Agglutinated gave the following results:—

**Table XVI.**

<table>
<thead>
<tr>
<th></th>
<th>103A</th>
<th>103B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth colonies</td>
<td>Per cent</td>
<td>100</td>
</tr>
<tr>
<td>Broth and serum colonies</td>
<td>66</td>
<td>1</td>
</tr>
</tbody>
</table>

It would therefore appear that the colony from the plain broth is a B type variant.

December 13. A subculture from the original strain and from the B type colony just isolated gave the following results:—

**Table XVII.**

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>103A</th>
<th>103B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original B variant</td>
<td>Per cent</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>66</td>
<td>100</td>
</tr>
<tr>
<td>B variant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Attempts to Hasten the Production of Agglutinable Variants.**

Various attempts have been made to hasten the production of agglutinable variants from the strains as isolated. Growing the organism in broth to which a few drops of 103A serum had been added proved successful in four out of twelve cases in which it was tried. In the other eight cases repeated attempts have been unsuccessful. In two of these B type variants
have not yet appeared; in two they appeared in cultures in ordinary broth to which serum had not been added; in four they appeared during routine subculture.

On the assumption that the production of B variants might rest on the presence of an enzyme, a broth-culture of 103B was made and filtered through a Chamberland filter. Some of the filtrate was added to fresh broth and the organism grown in this and then plated. This was, however, unsuccessful, and no B variant appeared.

Prolonged incubation—up to a month—in a broth medium was successful in two cases.

Two old strains have resisted all attempts to make them produce B variants. They have, however, produced variants of an entirely different nature, approximating closely to the R variant of the classical Flexner, but only agglutinating to about twenty-five per cent of titre with a serum prepared from a R strain of Z.

In two other strains, both the agglutinable variant and this R type of variant have been isolated.

MINOR VARIATIONS IN AGGLUTINABILITY.

In the process of this investigation very many plates have been made and large numbers of colonies put through the same agglutination test time after time. It was repeatedly observed that minor variations in agglutinability occurred between colonies from the same plate and also sometimes in ordinary mass subcultures made from one agar slope to another. No satisfactory explanation of this phenomenon has been reached, and the

<p>| TABLE XVIII. |
|---|---|---|---|</p>
<table>
<thead>
<tr>
<th><strong>B variant isolated</strong></th>
<th><strong>Development of B variant hastened by growth in broth containing 103A serum</strong></th>
<th><strong>Period between isolation and development of B variant</strong></th>
<th><strong>Colony characters of B variant</strong></th>
<th><strong>R type of variant developed</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>+</td>
<td>46</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Baye</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Munuswamy</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D15</td>
<td>4</td>
<td>363</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D33</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D95</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>108</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>220</td>
<td>+</td>
<td>+</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>B172</td>
<td>+</td>
<td>+</td>
<td>36</td>
<td>+</td>
</tr>
<tr>
<td>B199</td>
<td>+</td>
<td>+</td>
<td>228</td>
<td>+</td>
</tr>
<tr>
<td>B208</td>
<td>+</td>
<td>+</td>
<td>36</td>
<td>+</td>
</tr>
<tr>
<td>B215</td>
<td>+</td>
<td>+</td>
<td>36</td>
<td>+</td>
</tr>
<tr>
<td>P8</td>
<td>+</td>
<td>+</td>
<td>35</td>
<td>+</td>
</tr>
<tr>
<td>P41</td>
<td>+</td>
<td>+</td>
<td>97</td>
<td>+</td>
</tr>
<tr>
<td>P61</td>
<td>+</td>
<td>+</td>
<td>47</td>
<td>+</td>
</tr>
<tr>
<td>P81</td>
<td>+</td>
<td>+</td>
<td>121</td>
<td>+</td>
</tr>
<tr>
<td>Mhl</td>
<td>+</td>
<td>+</td>
<td>72</td>
<td>+</td>
</tr>
<tr>
<td>Mll</td>
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causes are probably very complex. They are definitely not related to the
development of B variants. In one case these variations were noted a
year before true B variants could be made to put in an appearance. One
such variation can be seen in Table XII.

Table XVIII gives the principal findings in the eighteen strains that
have been tested.

Relationship of this Mutation to Others Already Described.

While it is not the object of this paper to discuss in detail the signi-
ficance of this variant, a brief comparison with other known mutations
may be given.

(a) It is obvious that it bears no direct relationship to H and C, as
seen in the enterica-salmonella group. The organisms under discussion
are devoid of flagella, and agglutination in all cases is of a granular type.

(b) It differs fundamentally from the B to R mutation of the Flexner
group. This has been already fully detailed.

(c) Relationship to the specific and non-specific or group phases, as
observed by Andrewes in the salmonella group, requires more considera-
tion. These, however, present the following characteristics: (1) They are present
on isolation, and are not a late development; (2) no physical differences
in specific and non-specific colonies have been observed; (3) changes from
one type to the other occur.

In all these points there is variation from 103, the most important point
being the third.

Further investigations along lines suggested by the above are in
progress.

(d) That variants bearing a fairly close relationship to true "R" have
been produced by some of these strains is a fact, the significance of which
needs to be borne in mind.

It is obvious that much remains to be done in the investigation of this
strain, which is the principal reason for the publication of this paper in its
immature condition. There is ample scope in the military laboratories of
India for such investigation.

Summary and Conclusions.

(1) 103 is a Flexner-like organism which has a claim to be considered
pathogenic because of the way in which it occurs in cases of acute bacillary
dysentery, because it has never been found in healthy controls, and because
it bears a close antigenic relationship to known Flexner organisms.

(2) It has the property of producing a variant which is highly agglu-
tinable with the usual Flexner sera. This variant at first occurs in the
cultures side by side with the original type. It more or less rapidly comes
to dominate the picture, and, finally completely "smothers" any of the
original colony types which may be present.
So-called "Non-agglutinable" Dysentery Bacilli

(3) The variant once isolated has the following characters: (a) It breeds pure; (b) it may have physical characters which, in part, resemble the R variant of the recognized Flexners; (c) it has marked agglutino-genetic properties, and in its antigenic composition closely resembles Y, but is more sensitive to agglutination. Conversely, it is practically unrelated to Flexner R.

(4) The production of the variant may be hastened in some cases by growth of the organism in the presence of its homologous serum, in others by prolonged incubation in a broth medium.

(5) A more or less typical R variant has developed from four strains of 103.

**Strain No. 170.**

This strain has been encountered as follows:—

Bangalore, 1929, five times; Bangalore, 1930, four times; Secunderabad, 1928, once; Poona, 1931, once.

It has been found only in the stools of cases presenting symptoms of bacillary dysentery. These cases have in the main been mild, and correspond to the average Flexner infection as seen in India. It must be remembered that all cases are diagnosed on the clinical features of the case plus the microscopic characters of the stools, and hence are generally placed on appropriate treatment at a very early stage, a fact which no doubt greatly reduces their severity.

The following is a typical case history:—

Capt. B.—August 6, 1930. History of diarrhoea for four days, mucus having been present for the last two days, blood tinged for one day. Temperature 100°F. The specimen sent to the laboratory reached it five minutes from the time it was passed, and was a watery stool containing blood and mucus. Microscopically the exudate was that of bacillary dysentery. Plates made from the mucus showed many suspicious colonies, four of which were selected and tested. All turned out to be 170 type.

August 7. Watery stool with streaks of cellular mucus. Plates again produced many suspicious colonies. Of five selected, three were of the 170 type, the others not being dysentery organisms.

August 9. Symptoms have largely subsided. Stool sent to the laboratory was watery with a trace of mucus, non-cellular. Plates negative.


**Biochemical reactions.**—Lactose, no change; glucose, acid; mannite, acid; dulcite, no change; saccharose, no change; milk, acid, late neutral or faint alkaline; indol, negative. 170 thus corresponds exactly with the known Flexners.

**Serological reactions.**—(a) In relation to the high titre sera.

This organism has apparently no antigenic relationship to the classical
Flexners. It fails to agglutinate with any serum other than its homologous serum, even in low dilution, and its homologous serum does not agglutinate V, W, X, Y, and Z, 88 or 103A, although it does produce a peculiar powdery agglutination with 103B, to about fifty per cent of titre.

The agglutinogenetic properties of the organism are indifferent, and the highest titre reached in its serum was 1 in 1,000.

(b) In relation to the patient's serum. Of three tested, none has ever shown any agglutinins for this organism.

Conclusions.—While there are no serological grounds for considering this organism to be a cause of dysentery, the fact that it has been encountered only in the stools of clinical dysentery cases, and that it has biochemical reactions identical with the classical Flexners, gives it at least a claim to consideration.

Further, when the poor agglutinogenetic powers of the organism are considered, it is not surprising that the patient should develop no agglutinins.

Other Strains.

Other types are under investigation. Of the thirty-seven strains received from Secunderabad, seven are serologically of one type, which differs from any that have been yet described. This type has not, however, been isolated to date in the writer's laboratory, and for want of adequate data a description is not yet published.

So far, attempts to classify the remainder of the non-agglutinable strains that have been isolated have been unsuccessful, and as far as can be ascertained the latter are rather a mixed collection with very little antigenic relationship to one another. Much work, however, remains to be done before definite conclusions can be formulated. Nor must it be forgotten that only strains from the South of India have been examined. It is highly probable that similar investigations elsewhere will lead to the typing of further strains.

Tests which have not been carried out.

To forestall criticism, reference may be made to certain tests which have not been carried out in these investigations.

(1) Liquefaction of gelatine. The climatic conditions under which most of this work was done rendered this impracticable as a routine measure.

(2) Acid agglutination. Previous experience of this test in a similar investigation thirteen years ago, and again four years ago, was very unsatisfactory, and created the impression that the results of the test varied within such wide limits as to render it practically valueless. It has therefore not been employed.

(3) Testing of virulence by animal inoculation. This has been avoided for two reasons. First, because of a strong antipathy to experiments
So-called "Non-agglutinable" Dysentery Bacilli

of the kind. Second, because a perusal of similar experiments carried out by other workers raises the conviction that, unless the experiments be conducted on a scale quite outside the resources available to the writer, the results will be equivocal and valueless. At best, the logic of the conclusions drawn from such results is open to criticism. Injected intravenously into the rabbit in somewhat indifferently controlled doses, certain known dysentery organisms produce death, presumably chiefly from bacillæmia and toxæmia, but accompanied by certain lesions of the intestine. It happens, however, that very similar results are produced by other organisms which are not known to be pathogenic to man [9]. The value of such experiments with an unknown organism is therefore very doubtful, and at best the evidence can only be regarded as minor contributory evidence to the issue in question.

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