THE MANNITOL-FERMENTING GROUP OF DYSENTERY BACILLI.

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CONTENTS

P R E F A C E

The investigations which are presented in this thesis were started in 1929 and have been pursued as opportunity offered in the intervening years. The findings were published between 1931 and 1946 in a series of papers in different journals. These are now reproduced in chronological order as Annexures, preceded by a brief summary which outlines the work and indicates its application on a large scale in general laboratory practice. There is considerable overlapping and repetition in these successive papers, but none of them can be omitted without destroying the continuity of the series, and it has been considered best to make no deletions or amendments, but to render them exactly as they were originally printed.

S U M M A R Y

Introduction.

The term dysentery was originally used to describe any condition having as a symptom diarrhoea with stools containing blood and mucus. The discovery of Entamoeba histolytica by Losch (1875) and its incrimination as a cause of dysentery was the first step in the more accurate classification of this group of diseases. In 1898 Shiga described a non-lactose fermenting coliform bacillus and produced convincing evidence that infection with this organism gives rise to acute inflammatory and ulcerative colitis. Closely related bacilli, differing from Shiga's organism in their biochemical reactions and antigenic pattern, were isolated from cases of dysentery by Flexner (1900), Strong (1900) and others. In 1915 Sonne found yet another type, and in 1917 Schmitz added one more to the list. A critical review by Murray in 1918 gave convincing confirmation of the existence of four main types of dysentery bacilli. Three of these, Shiga's bacillus, Sonne's bacillus, and Schmitz's bacillus, were clearly defined in their biochemical reactions and antigenic structure. The fourth type - or more correctly, group - embracing the organisms named

after Flexner, Strong, Hiss, Russell, and Lentz, had identical biochemical reactions, but, though closely related, were not wholly alike in their antigenic pattern.

This latter group was investigated by Andrewes and Inman (1919) and divided into five types, which they named V, W, X, Y and Z. According to their conception, these types shared four main antigens. Each of the strains, V, W, X and Z was believed to contain a dominant quantity of one of these antigens and minor quantities of the other three, while Y contained approximately equal amounts of all four. For purposes of identification, a polyvalent antiserum was prepared from all five strains, and it was hoped that this would enable all organisms of the Flexner "spectrum" to be recognised with certainty. This hope did not materialise. Experience in India revealed that about 30% of all strains having the general and biochemical characters of the group failed to react with this 'polyvalent' antiserum. For purposes of diagnosis these inagglutinable strains were regarded as atypical but none the less pathogenic Flexner-group bacilli, but this compromise had many disadvantages, and failed to carry conviction to many who at that time still believed in the widespread occurrence of amoebic dysentery.

Identification of new types of mannitol-fermenting dysentery bacilli.

In view of this state of affairs, an investigation was started which had as its object the differentiation by serological methods of these inagglutinable strains, and the confirmation, or otherwise, of their pathogenic action.

Annexures I and II cover the initial part of this work. In all, nine distinct strains were identified which had the biochemical characters of the Flexner group but could be recognised as separate entities by virtue of their antigenic structure. These nine strains could be divided into two groups. The first, consisting of three members, not only resembled Andrewes' types biochemically, but also exhibited a certain degree of cross-agglutination with them. There was no reasonable doubt that they were members of this series, and would have been recognised as such by Andrewes had they found their way into his collection, which contained only one strain emanating from India. The remaining six strains fell into a different category, as they had no demonstrable antigenic relationship with the Flexner group. Their claims to be considered pathogenic are discussed in detail, and at the date of publication of Annexure II, it was considered that three of them could be incriminated.

Distribution and Relative Frequency of the New Types.

Shortly after this first phase of the work was completed, an opportunity arose to organise a wide-scale investigation of the incidence of the different types of dysentery bacilli, and in particular of these new types, in garrisons in India. The findings over a period of approximately three years are analysed in Annexure III. They leave no doubt that these organisms are definite entities which have a wide distribution and are found only in association with cases having the clinical symptoms of bacillary dysentery.

On a later occasion another similar opportunity presented itself, and with the co-operation of pathologists in Middle East Force, a record was kept of all dysentery organisms isolated between August 1940 and June 1943 (Annexure VI). The number totalled 23,951. Although agglutinating sera were not available for the identification of five of the less common of the new types, only 800 mannitol-fermenting strains (3.34% of all dysentery bacilli isolated) were unidentified by the field laboratories. A cross-section of 109 of these was investigated by the author. 55 were found to be of the five rarer types mentioned above. A new Flexner type, of which there were 6 strains, was found. The majority of the remainder

were not true dysentery bacilli.

It is of interest to note that in the world-wide investigations carried out by British and American bacteriologists during the 1939-45 war, general confirmation was obtained of the occurrence and pathogenic action of these new types, and all nine first found in India are accepted as true dysentery bacilli. On the other hand, new discoveries were few. Two Flexner types (one from Middle East as mentioned above) have been described by Francis (1946). Only a few strains of each have been isolated. One other type having no antigenic affinity to Andrewes' strains, which has been provisionally named Shigella etousa, has been found in considerable numbers. (Ewing, 1946, Heller and Wilson, 1946, Lavington et al, 1946).

Antigenic structure of the mannitol-fermenting dysentery bacilli.

In the course of these investigations a previously undescribed form of variation was observed in one of the new Flexner types. When first isolated from a dysenteric stool this organism shows very restricted cross-agglutination with the antisera of Andrewes' types. After some time in artificial culture, it throws off variants which are clumped to a high titre with these same antisera.

Absorption tests showed that the variant differed from the parent strain only in having lost its type-specific antigen. With the loss of the specific antigen the group antigen had become "uncovered" and was susceptible to the action of group agglutinins. A series of experiments, detailed in Annexure IV revealed the fact that this group antigen, which is of some complexity, is present in all the Andrewes' types in varying degrees. It also became apparent that Andrewes' Y strain is itself a variant, probably of W, and is not a specific type. Similarly the existing type strains of X are apparently variants of Z, though this point is less certain, as no recently isolated strains of X were available. By suitable absorption with group variants, monospecific antisera for V, W, Z, and the three related new types were prepared with difficulty.

These findings necessitate a fundamental revision of Andrewes' conception of the antigenic structure of the Flexner group. Instead of being made up of a predominant quantity of one specific antigen and minor quantities of the other specific antigens, each in fact contains one specific antigen and a background of non-specific or group antigen which varies both in quantity and quality, and is common to all members of the group.

An interesting side-issue was the correlation of the Indian Type 88 with the Newcastle bacillus of Clayton and

Warren (1929) and the Manchester bacillus of Downie, Wade and Young (1933). Although these organisms have diverse biochemical reactions, their antigenic pattern, both specific and group, is identical, and it seems indisputable that they should be regarded as biochemical variants of one type.

Andrewes' VZ type has been shown to be a V strain in which the group antigen is rich in the component normally present in large quantity in Z. It has no specific Z antigen. His WX strain has not been encountered, but is presumably to be explained in the same way.

Classification of the mannitol-fermenting dysentery bacilli.

It is considered that possession of group antigen is a major type character, and warrants splitting the mannitol-fermenting dysentery bacilli into two groups. It is therefore proposed (Annexures IV and V) that all types with specific and group antigens should be classified together under the name of Flexner, and that Andrewes' alphabetical designations should be replaced by numerals. Under this scheme there are now eight types - Flexner I to VIII. The second group, to which the writer's name has been attached, embraces those strains which have a type-specific antigen, but no group antigen. It contains the six

Indian types, and it appears logical that "Shigella etousa" should be added as a seventh.

This classification is used by the Medical Research Council "Dysentery Reference Laboratory", and also for naming the antisera issued by the M.R.C. Standards Laboratory.

REFERENCES TO RECENT PUBLICATIONS.

EWING, W.H. (1946) J. Bact., LI, 433.

HELLER, G. & WILSON, S.G. (1946) J. Path. Bact., LVIII, 98.

LAVINGTON, R.J., et al. (1946) Ibid., LVIII, 101.

A N N E X U R E I.

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

Journal of the Royal Army Medical Corps, 1931, Volume LVII, pp. 161 to 186.

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 mucus 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 enquiry, 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.[x] They can be grown on all ordinary media, and are readily maintained in artificial culture over prolonged periods.

(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.

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x B. morgan, which is motile, is not considered to be a cause of tropical bacillary dysentery.

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.

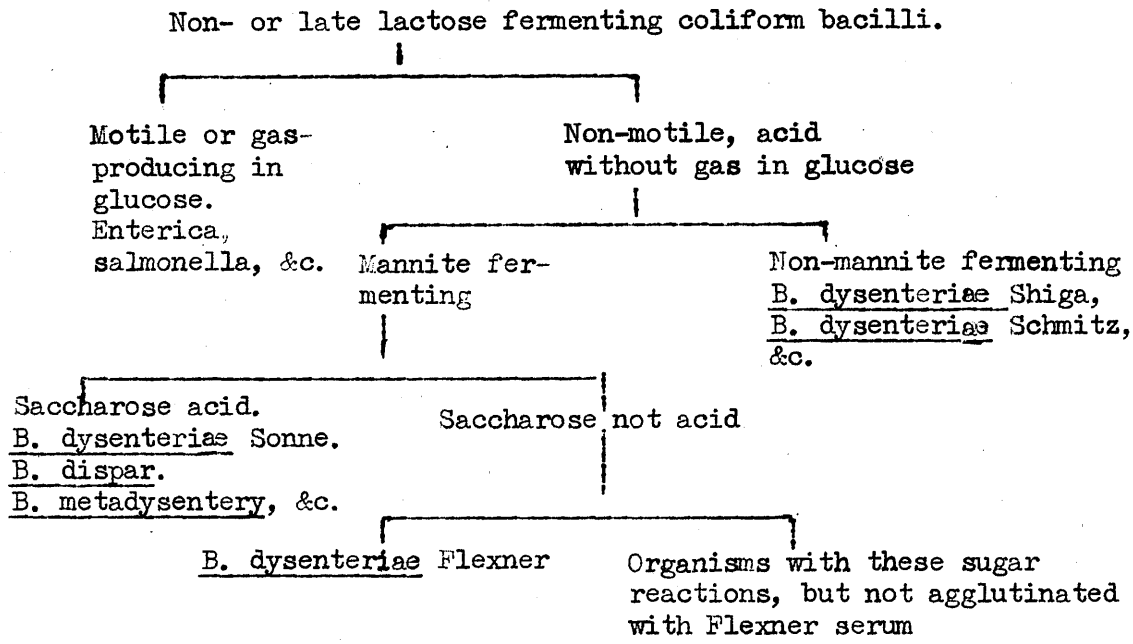
Of the bacilli which produce acid in glucose and mannite, a certain number will be found to produce acid in saccharose, generally after a few days incubation. The majority of these organisms will also be found to be late fermenters of lactose. This is regarded as a definite

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 they 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.



PREVIOUS SEROLOGICAL CLASSIFICATION OF DYSENTERY 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. Andrewes,[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 Andrewes' 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 Andrewes in his investigations, only one came from India. Now in 1929, in the military laboratories 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°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°F., evening temperature 99°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.

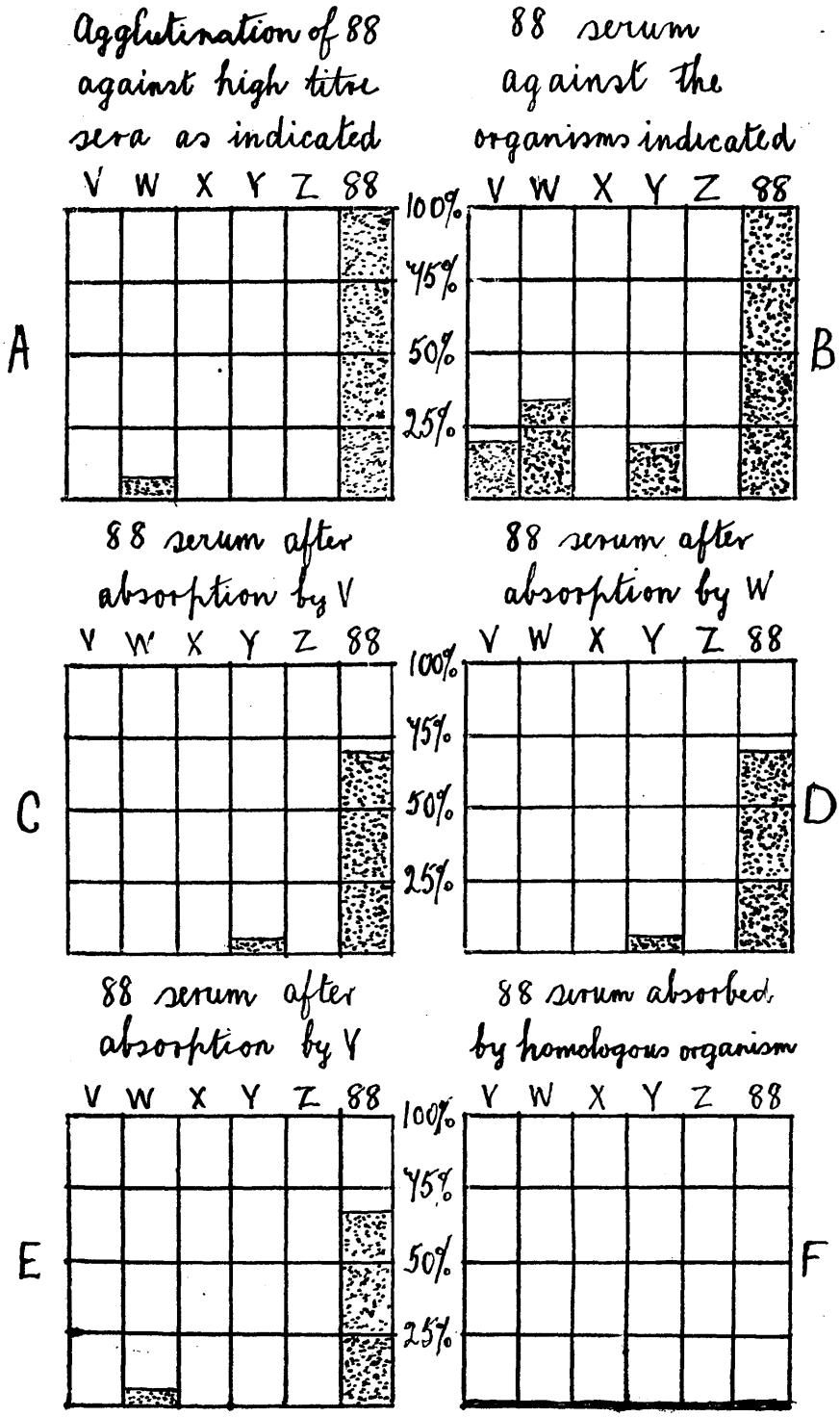
Biochemical reactions of 88. Lactose, no change. Glucose, acid. Mannite, acid. Dulcite acid (late and inconstant); 50 per cent three to four days; 33 per cent twenty-seven to twenty-eight days; 16 per cent unchanged after six weeks. Saccharose, no change. Milk, acid at first, neutral four to seven days,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

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.

Figure 1.


Agglutination of 88 against high titre sera as indicated
V W X Y Z 88
100%
75%
50%
25%
A
88 serum against the organisms indicated
V W X Y Z 88
B
88 serum after absorption by V
V W X Y Z 88
C
88 serum after absorption by W
V W X Y Z 88
D
88 serum after absorption by V
V W X Y Z 88
E
88 serum absorbed by homologous organism
V W X Y Z 88
F

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 that this serum contains a fair proportion of heterologous agglutinins for V, W, and Y. The accompanying histogram (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.

Lieutenant McK. (case described above).

	V	W	X	Y	Z	88	Hom.
Serum (7th day) ...	25	125	-	50	-	25	-
Serum (abs. hom.) ...	-	-	-	-	-	-	-

TABLE III.

Sergeant B.

	V	W	X	Y	Z	88	Hom.	Sh.
Patient's serum, 8th day	25	10	-	35	-	30	40	-
15th day	20	-	-	35	-	-	25	-

TABLE IV.

Private H.

	V	W	X	Y	Z	88	Hom.	Sh.
Patient's serum,								
11th day	25	35	-	25	25	175	-	-
17th day	50	100	-	25	-	250	25	-
" (abd. W.)	-	-	-	-	-	250	-	-
" (abs. hom.)	-	-	-	-	-	-	-	-

TABLE V.

Private R.

	V	W	X	Y	Z	88	Hom.	Sh.
Patient's serum, 6th day ...	50	250	-	25	-	-	-	-
15th day ...	50	150	-	25	15	250	-	-
19th day	50	-	-	-	-	250	-	-
" (abs. W) ...	-	-	-	-	-	100	-	-
" (abs. 88) ...	-	-	-	-	-	-	-	-

Abs. = absorbed. Sh. = Shiga. Hom. = homologous. The emulsion of "88" 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 VI.

	Alkalescens	88
Lactose ...	Nil	Nil
Glucose ...	Acid	Acid
Mannite ...	Acid	Acid
Dulcite ...	Acid 24 to 48 hours	Acid 3rd to 31st day, if at all
Milk ...	Alkaline 3rd day ...	Acid at first Neutral 4th to 7th day Alkaline (faint) 11th to 16th day
Indol ...	Positive	Negative

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 88 serum.

88 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. 88 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 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, 1928 and 1929, 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 successive days from the same case. One case after a fortnight's apparent convalescence, relapsed, diarrhoea 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 agglutinations 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 aggluntinability 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 <u>in vitro</u> 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 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.

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	B. The variant.
(1)	Small lenticular colonies, regular in outline and texture.	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.
(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.	Emulsifies readily, and has no tendency to auto-agglutinate.

	A. The original colony	B. The variant.
(3)	Grows with generalized turbidity in broth.	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.	Has bred true, producing nothing but B colonies, from the day of isolation over eighteen months ago.
(5)	Non-agglutinable with Flexner sera.	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 VII.

V	W	X	Y	Z	103B	103A
1/250	1/250	Nil	1/125	Nil	1/350	Nil

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 amd Z smooth sera nor by a "rough" Flexner serum. It is very slightly agglutinated by a high titre 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.

Figure 2.

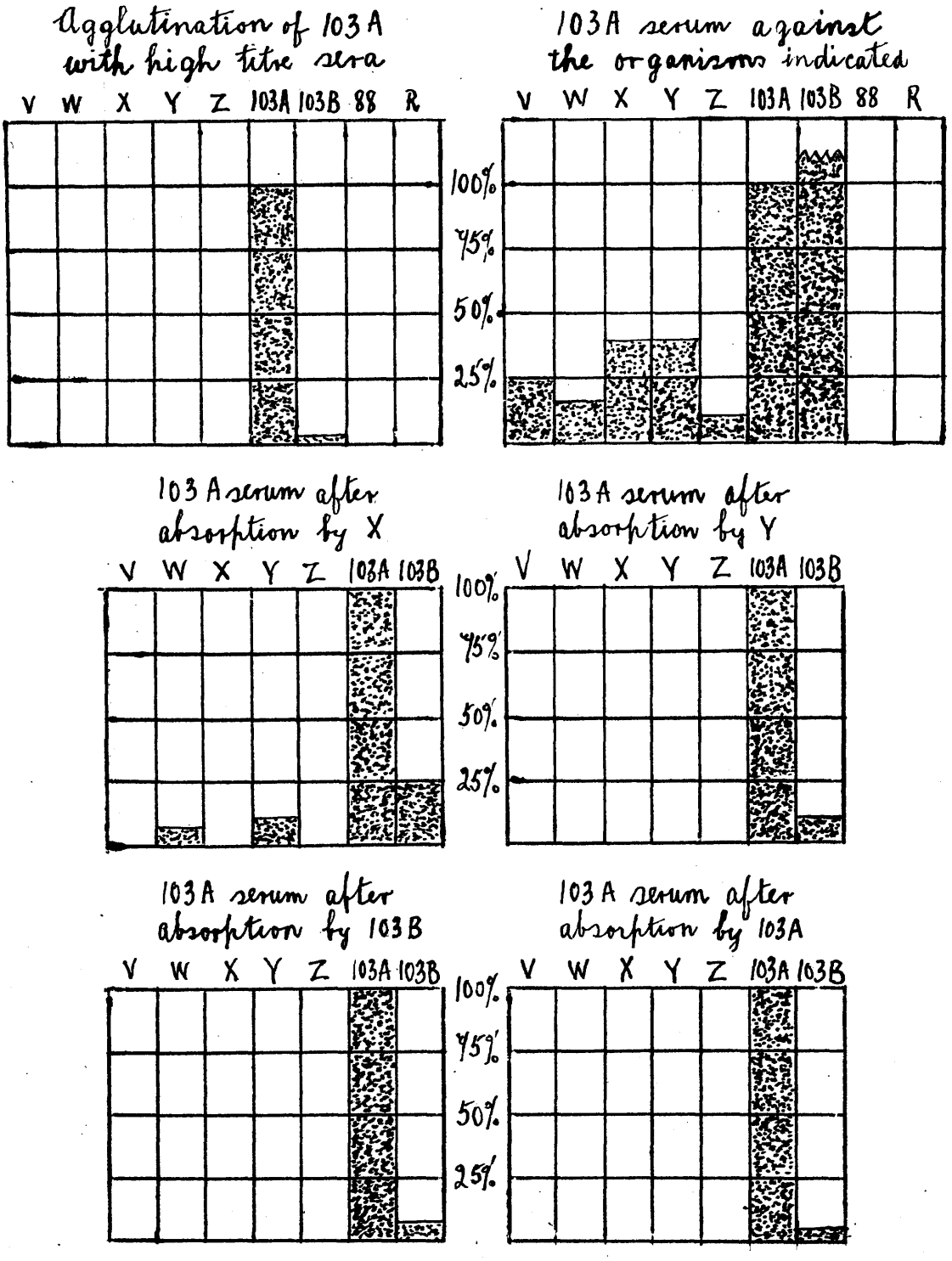
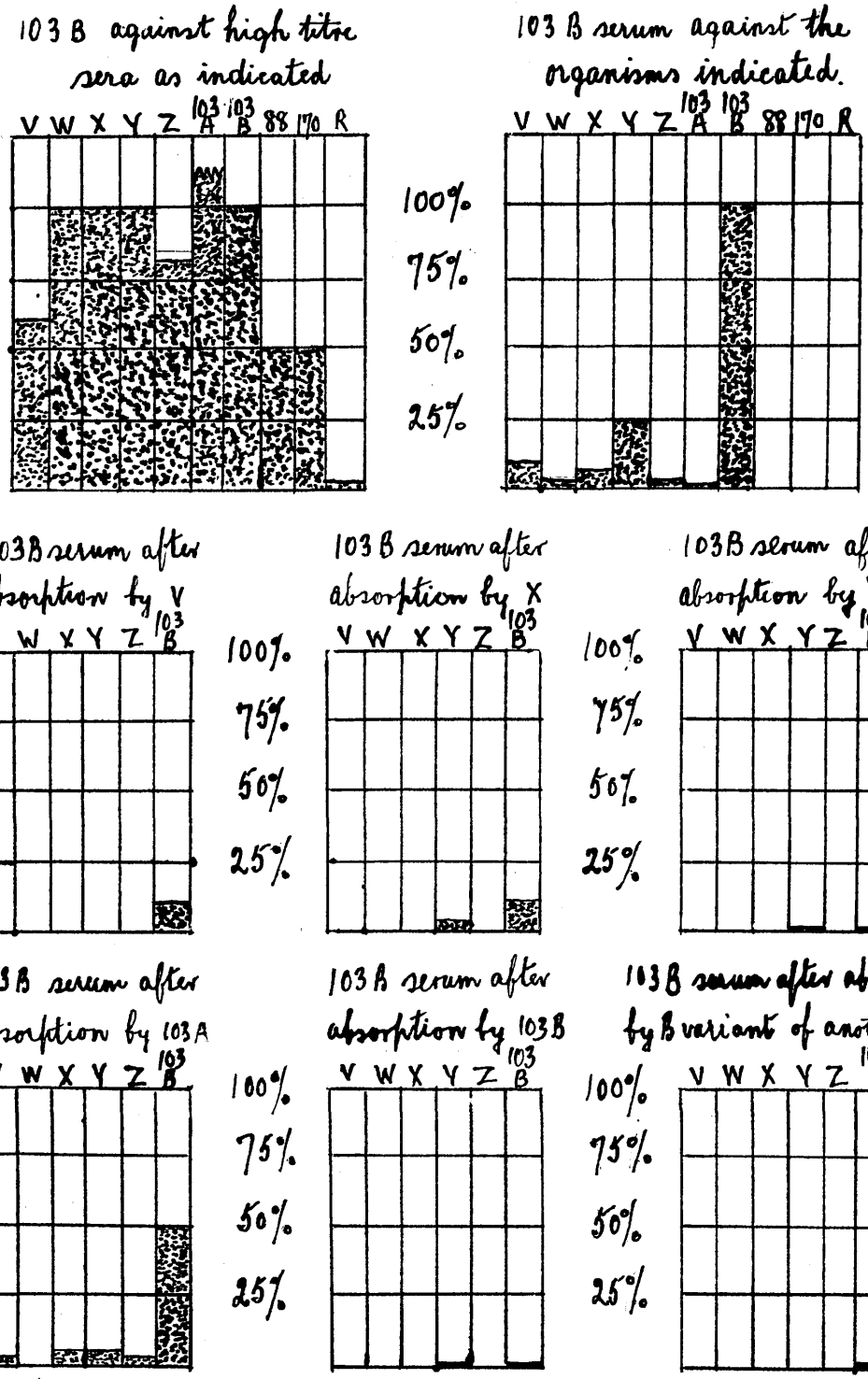

Agglutination of 103A with high titre sera
V W X Y Z 103A 103B 88 R
103A serum against the organisms indicated
V W X Y Z 103A 103B 88 R
100%
75%
50%
25%
103A serum after absorption by X
V W X Y Z 103A 103B
103A serum after absorption by Y
V W X Y Z 103A 103B
100%
75%
50%
25%
103A serum after absorption by 103B
V W X Y Z 103A 103B
103A serum after absorption by 103A
V W X Y Z 103A 103B
100%
75%
50%
25%

Figure 3.


103 B against high titre sera as indicated
V W X Y Z 103 A 103 B 88 170 R
103 B serum against the organisms indicated.
V W X Y Z 103 A 103 B 88 170 R
100%
75%
50%
25%
103B serum after absorption by V
V W X Y Z 103 B
103 B serum after absorption by X
V W X Y Z 103 B
103B serum after absorption by Y
V W X Y Z 103 B
103 B serum after absorption by 103A
V W X Y Z 103 B
103 B serum after absorption by 103B
V W X Y Z 103 B
103 B serum after absorption by B variant of another strain.
V W X Y Z 103 B

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 strains, but particularly to Y.

What is the explanation of these apparently anomalous findings? It would clearly appear to be as follows.

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 mutation occurs.

Strain No. B 172.

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 VIII

	V	W	X	Y	Z	88	Sh.	Hom.
Patient's serum, 14th day	25	-	-	-	50	-	-	-
19th day	50	125	-	-	50	-	-	-
" (abs. hom.)	-	-	-	-	-	-	-	-

	V	W	X	Y	Z	103A
Agglutination percentage of organism on isolation	-	-	10%	10%	-	100%

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

July 14. Broth-culture plated.

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 IX.

V	W	X	Y	Z
Per cent 50	Per cent 50	Per cent 20	Per cent 100	Per cent 20

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.

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 X.

	V	W	X	Y	Z
	Per cent	Per cent	Per cent	Per cent	Per cent
Original ...	-	-	20	20	-
"Serumed" colony ...	50	50	20	100	20

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 XI.

	V	W	X	Y	Z
	Per cent	Per cent	Per cent	Per cent	Per cent
Original ...	10	-	20	14	-
"Serumed" colony ...	80	100	14	100	10

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 "serumed" went to titre.

Conclusion. This strain as isolated corresponds to 103A. In straight-forward 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.

Strain No. B 215.

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.

September 3 to 9. Formed stools. A little adherent mucus on two occasions. Plates negative.

Agglutination Results of Isolation are shown in Table XII.

TABLE XII.

	V	W	X	Y	Z	103A	103B
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
30.8.30. Colony (a) ...	30	5	30	20	-	100	5
5.9.30. Colony (a) ...	-	-	6	6	-	30	-
5.9.30. Colony (b) ...	-	-	6	6	-	30	-
8.9.30. Colony (b) ...	-	-	-	-	-	100	-

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:-

V	W	X	Y	Z	103A
-	-	-	-	-	Per cent 50

September 11. Subcultured 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:-

TABLE XIII.

	V	W	X	Y	Z	103
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
"Serumed" colony	100	100	100	100	100	100
Original. 16.9.30 ...	-	-	10	10	-	100

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 on 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.

	V	W	X	Y	Z	103A	103B
						Per cent	
Colony of 28.10.30	-	-	-	-	-	50	
Colony of 30.10.30	-	-	-	-	-	50	

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

November 10. Plated from broth. No success.

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

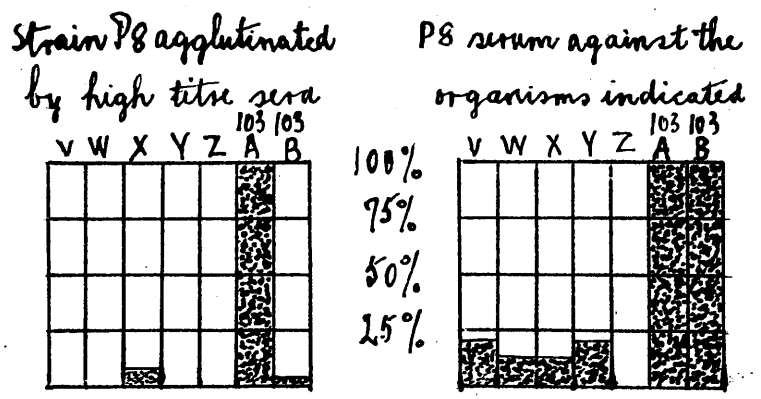
November 26. Plated. Variations in size of colony present, but not suggestive of mutation. Following results obtained:-

TABLE XV.

	103A	103B
	Per cent	Per cent
Small colonies	66	2
Large colonies	100	5

December 10. Plated from plain and serum broth cultures just over a month old. Serum broth gave colonies all alike. Ordinary broth showed

Figure 4.



some ? B type colonies. Proved through sugars. Agglutinated gave the following results:-

TABLE XVI.

	103A	103B
	Per cent	Per cent
Broth colonies	100	100
Broth and serum colonies	66	1

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.

	V	W	X	Y	Z	103A	103B
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Original	-	-	4	-	-	66	1.5
B variant	100	100	100	100	100	100	100

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 sub-culture.

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 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.

TABLE XVIII.

	B variant isolated	Development of B variant hastened by growth in broth containing 103A serum	Period between isolation and development of B variant	Colony characters of B variant			R type of variant developed
				Like 103B	Clear	Like A variant	
103 ...	+		46	+			
Baye ...	+		?	+			+
Munuswamy	+		?	+			
D15 ...		-					+
D33 ...	+	-	?		+		+
D35 ...	+		?	+			
153 ...	+	+	358	+			
220 ...							+
B172 ...	+	+	7			+	
B199 ...	+	-	228	+			
B208 ...	+	+	36		+		
B215 ...	+	+	17	+			
P8 ...	+	-	15	+			
P41 ...	+	-	97	+			
P61 ...							
P81 ...	+	-	47	+			
Mh1 ...	+	-	121	+			
Md3 ...	+	-	72	+			

RELATIONSHIP OF THIS MUTATION TO OTHERS ALREADY DESCRIBED.

While it is not the object of this paper to discuss in detail the significance 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 O, 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 S and 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 consideration. 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 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 agglutinable 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.

(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.

August 22. Watery stool with slightly cellular mucus. Plates negative.

August 25. Stools normal.

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 YET BEEN CARRIED OUT.

To forestall criticism, reference may be made to certain tests which have <u>not</u> 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 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 bacillaemia and toxaemia, 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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A N N E X U R E II.

FURTHER INVESTIGATIONS INTO THE CHARACTER AND CLASSIFICATION OF THE MANNITE-FERMENTING DYSENTERY BACILLI.

Journal of the Royal Army Medical Corps, 1932, Volume LIX, pp. 241 to 251, and 331 to 342.

A. INTRODUCTION.

The classification of the mannite-fermenting dysentery bacilli of the type generally known as B. dysenteriae Flexner, and the pathogenicity of the various races of this group, are matters which have perplexed bacteriologists and clinicians for many years.

At home it is generally accepted that the types created by the late Sir Frederick Andrewes [1] - viz., V, W, X, Y, Z, and combinations of these - embrace the majority of, if not all, the pathogenic strains, and any organism which fails to agglutinate with the serum polyvalent for these strains is regarded with considerable suspicion. This opinion is well expressed in the following sentences taken from "A System of Bacteriology," published under the auspices of the Medical Research Council[2]: "We have already seen that this (failure to

agglutinate) does not absolutely exclude the bacillus from the Flexner group, since the antigenic range of the type races is probably insufficient, by a small margin, to cover the whole group. But the great majority of such aberrant strains will be found, on thorough examination, to exclude themselves from the group by some divergent cultural or biochemical character."

In the Army in India a different state of affairs obtains. It has been the practice to report all strains which give the correct biochemical reactions as B. dysenteriae Flexner, or B. dysenteriae Flexner (inagglutinable), as the case may be. The following figures, taken from official records, indicate the extent to which these "inagglutinable" strains have been occurring.

TABLE I.

Year	Agglutinable	Inagglutinable	Per cent inagglutinable
1926	271	129	32.25
1927	342	164	32.4
1928	492	306	38.2
1929	922	286	23.7
1930	1077	360	25.4

It is obvious, therefore, either that strains in India are being too loosely classified as "Flexner", or that serological types are of common occurrence there which have not come under review at home.

The question is one of some importance. Until a few years ago it was commonly believed that most cases of dysentery occurring among the troops in India were amoebic and not bacillary in origin, but the pioneer work of Manifold[3] and others has shown that the reverse is actually the case. The frequent occurrence of these inagglutinable strains, elsewhere regarded with suspicion, has been, in the controversy which arose on the subject, a constant stumbling-block in the path of those who favour the bacillary doctrine. Further, while in certain cases these atypical strains were found to occur in such a way as to suggest strongly their pathogenicity, in others they appeared in circumstances entirely the reverse. There were, therefore, a priori grounds for suggesting that some strains were pathogenic and others were not. With a view to solving, if possible, these problems, the present investigation was undertaken.

For a time it was thought that these inagglutinable bacilli might be Andrewes' types, which for some reason or another failed to react to the agglutination test. This conception was soon found to be wrong, and it became clear that the organisms were distinct serological types which lay outside the antigenic range of the type races. As a preliminary step, it therefore became essential to define and classify these serological types. So far as strains occurring in the south of India are concerned, this has been more or less accomplished.

B. PROPOSED CLASSIFICATION.

(a) By Biochemical Reactions.

It has been found that a useful preliminary classification can be effected by the biochemical reactions of the organisms. The "sugars" used are lactose, glucose, mannite, dulcite and saccharose. Table II shows the relationship of the dysentery bacilli to other coliform organisms, as well as the inter-relationship of the three subgroups now suggested, which are differentiated by their action on dulcite, lactose and saccharose.

TABLE II.

Non-, or Late-Lactose, Fermenting Coliform Bacilli.

Motile, or gas producing in glucose. Enterica, Salmonella, etc.	Non-motile, acid without gas in glucose	
	Acid without gas in mannite	No acid or gas in mannite. *B. dysenteriae* Shiga, *B. dysenteriae* Schmitz, etc.
	Dulcite, Lactose, Saccharose: No change	Dulcite = Acid, no gas. Lactose, Saccharose: No change	Dulcite = No change. Lactose, Saccharose: (Acid, no gas, late, in either or both
	B. dysenteriae Flexner, etc.	*B. alkalescens*. Type 88, etc.	*B. dysenteriae* Sonne, *B. dispar*, etc.
	Subgroup A.	Subgroup B.	Subgroup C.

A more detailed classification by means of further "sugar" media has proved unsatisfactory, and, indeed, the reactions shown in the tables are not invariably constant, as will be shown at a later stage. Serological tests are used for the further subdivision of the strains and provide the only definite method of identifying the various types.

(b) By Serological Reactions.

High-titre sera were prepared for a large number of selected strains, and tested against every strain which had been collected, and against other strains subsequently isolated. In this way various types were identified, which were further confirmed by a series of absorption and cross-agglutination tests. These types, and the sub-groups in which they occur, are shown in Table IV. Some of these are already universally recognized; others have been previously described by the writer[4]; the remainder are now described for the first time. Each new type is given, as a means of identification, the laboratory index number of the type strain. The question of a less cumbrous nomenclature need not be considered until the work has received adequate confirmation. For convenience the

strains described by Andrewes, viz., V, W, X, Y, Z, and combinations of these, are called the "classical Flexners."

There is also embodied in the table a series of figures to show the relative frequency with which these types have occurred in one area in Southern India. With the exception of three which were accidentally lost, every strain of non-mannite fermenting, dysentery-like bacilli recovered from cases of dysentery and diarrhoea by the writer in the Madras District Laboratory, Bangalore, in 1930, and Southern Command Laboratory, Poona, in 1930 and 1931, is included in this series, which is therefore representative and entirely unselected.

Table III shows in another way the characters of the three subgroups.

TABLE III.

	Lactose	Glucose	Mannite	Dulcite	Saccharose
Subgroup A	-	A	A	-	-
" B	-	A	A	A (late)	-
" C	A (late)	A	A	-	A (late)
	+ or -				+ or -

A = Acid.

TABLE IV.

	Serological type	Number of strains	Total	Percentage	Total percentage
Subgroup A	Classical Flexners ...	138		51.69	
	Type P 119 ...	7		2.62	
	" 103 ...	24		9.00	
	" 170 ...	11		4.11	
	Unclassified ...	3		1.12	
	Total ...		183		68.54
Subgroup B	Type 88 ...	21		7.87	
	" P 288 ...	9		3.37	
	" P 274 ...	3		1.12	
	" D 1 ...	2		0.75	
	" D 19 ...	-		-	
	B. alkalescens ...	-		-	
	Total ...		35		13.11
Subgroup C	B. dysenteriae, Sonne ...	23		8.61	
	Unclassified ...	26		9.74	
	Total ...		49		18.35

It will be seen that the classical Flexners comprise slightly over half the series. Taking these together with B. dysenteriae Sonne (the only other type which is usually identified serologically) a total of approximately sixty per cent is reached. In round figures, therefore, two-fifths of all strains in this series giving in twenty-four hours correct Flexner biochemical reactions fall into the class generally labelled "inagglutinable Flexner".

C. PATHOGENICITY.

Certain tentative conclusions have been formed regarding the pathogenicity of the new types, but it is obvious that in some cases these require repeated confirmation before they can be accepted.

With dysentery bacilli it is not possible to satisfy the third of Koch's postulates, as the infection of volunteers with cultures is an impracticable proposition, and animal inoculation affords no reliable information. There are, however, data related to the first two postulates which apply to certain of the types:-

(a) The organisms have been recovered only from cases with symptoms of bacillary dysentery. This implies control investigations of the stools of healthy individuals, and such controls have been carried out on a large scale. During the three years that were occupied with these researches, over 3,000 menials were examined to ascertain if they were "carriers" of infectious bowel conditions. This involved, among other things, 8,000 odd platings of faeces, the majority of which were carried out under ideal conditions. The subject was given a saline purge, and the subsequent stool was passed in a latrine on the laboratory premises. A fresh specimen from this was at once secured and plated and examined in exactly the same way as a dysentery (or enteric) specimen. None of the strains which are considered pathogenic has ever been isolated from these controls, except in a few rare cases where, on investigation, the individual was found to be suffering from acute or chronic bacillary dysentery. In addition, by what is no doubt a coincidence, the only organisms isolated under these circumstances have been classical Flexners, Shiga or Schmitz.

(b) The organisms have been present in the stools during the same stages as the "classical" dysentery, i.e., in almost pure culture in the early acute stages and rapidly diminishing in numbers as the case progresses.

(c) Although the cases were clinically and microscopically bacillary dysentery, and were carefully investigated at the correct times, "classical" dysentery bacilli have not been isolated in association with the new types, despite the fact that several colonies were invariably selected and examined from each case. (N.B. This does not apply to the types noted as probably non-pathogenic.)

This point is mentioned because it is commonly stated that in the early stages of bacillary dysentery unusual types of organisms, which have their habitat high up in the bowel, are washed down and appear in association with the causative dysentery bacilli. This has been found by the writer in the case of B. morgan and of certain of the saccharose fermenters. It does not apply to the other strains.

(d) Certain strains have a well-marked antigenic similarity to the classical Flexners in that, when injected into a rabbit, they stimulate the production of a varying degree of heterologous agglutinins for the latter organisms.

(e) Agglutinins for the organism may develop in the patient's serum. This has been definitely found in the case of one type (88), but has not been fully investigated in the others.

D. DETAILS OF THE VARIOUS TYPES.

(a) Subgroup A.

In the 1931-32 series, 68.5 per cent of all strains isolated belong to this subgroup.

Seventy-five per cent of the subgroup is made up of classical Flexner strains. Of the remaining twenty-five per cent all but three strains fall into the new types described.

In addition to these three unclassified strains of the 1931-32 series, eight other strains with the biochemical reactions of subgroup A (collected from a variety of sources) fail to conform to any of the types. These eleven will receive separate consideration.

The serological characters of this subgroup are complex, and will be detailed in the description of each type, as will also questions of mutation and pathogenicity. With the exception of Type 170 and the unclassified series, they are closely interrelated.

(1) Classical B. dysenteriae Flexner (V, W, X, Y, Z, etc.)

There is nothing of importance to add to the knowledge which has accumulated regarding these types, and only a few general remarks need be made.

The local incidence of the types in Southern India is shown in Table V, which is an analysis of the 138 strains mentioned in Table IV. The sera used for identification were those supplied by the Enteric Laboratory, Kasauli, in the manufacture of which every care is exercised to use only smooth strains of organisms.

TABLE V.

	V	W	X	Y	Z	VZ	Others
Number	8	43	4	11	12	19	41
Percentage of 1930-31 series	3	16.1	1.5	4.12	4.5	7.1	15.35
Percentage of each type in the classical Flexner series	5.8	31.16	2.9	7.97	8.7	13.76	29.71

The only strains calling for special comment are those labelled "others". The majority were combinations such as V, W, Z; X, Y, Z, etc. A proportion, however, did not agglutinate to full titre with any of the five sera, and it is possible that such strains may possess a distinctive antigen of their own in addition to the group antigen. The next type to be described, P119, is an example of this, differing only in that its agglutination with sera of the classical strains falls far short of titre. The precise identification of strains which agglutinate approximately to titre with several sera is of little practical importance for diagnostic purposes. They are sufficiently related to the classical types to enable them to be classified along with the former without difficulty.

Mutation. Strains of the classical Flexners were not, with a few exceptions, preserved for prolonged periods, and no evidence of true mutation from S to R was ever observed.

Pathogenicity. The pathogenicity of the classical types has long been accepted. All the strains in this series were isolated from cases of dysentery, either acute,

moderate, or in some cases so mild as to be labelled merely diarrhoea.

So far as the writer's experience goes, there is no such thing as a healthy carrier of dysentery bacilli. In the few rare cases where a bacillus has been isolated from an apparently normal individual, a careful search of a large specimen of faeces, well washed in saline, invariably revealed the presence of traces of cellular mucus. Further, one would expect a true "normal" carrier to excrete the organisms on repeated occasions, as does a carrier of, say, B. typhosus. This, so far as one is aware, has never been found, other than of course in a chronic or subacute case.

Serological Diagnosis of Dysentery Caused by Organisms of this Type.

During 1930 a number of agglutination tests were carried out with the sera of patients infected with these types. No new facts emerged, and the findings of others were confirmed, viz., that the development of agglutinins is very uncertain, that it occurs so late as to be of little or no value for diagnostic purposes so far as acute cases are concerned, and that the agglutinins which develop frequently do not correspond to the antigenic type of the infecting organism. The test has been abandoned as a routine, being of no practical value.

(2) Type P119.

This type is closely allied to the classical Flexners, and particularly to X.

In all 12 strains have been isolated and investigated. 8 from Poona, 2 from Jubbulpore and 2 from Bangalore.[x]

In its biochemical reactions it corresponds exactly with the classical Flexners.

Table VI shows the agglutination reactions on isolation.

TABLE VI.

		Serum					
		V	W	X	Y	Z	P 119
Organism	P 119	10	-	10	10	-	100
	P 190	5	-	4	10	-	100
	P 272	-	-	10	10	-	100
	P 283	-	5	5	15	-	100
	P 326	5	-	10	10	-	100
	P 329	-	-	10	5	-	100
	P 493	-	-	10	10	25	100
	Dec. 6	4	-	10	10	5	100
	Dec. 7	5	-	25	10	5	100
	Md. 5	7	-	25	10	8	100
	Md. 7	5	-	25	10	5	100
	P 584	5	10	15	10	15	100

[x] A further strain has just been received from Mingaladon in Burma.

/Note - In this and all subsequent tables the figures shown are in percentages of the titre of the serum in question for its homologous organism; e.g., V serum has a titre of $\frac{1}{500}$ for its homologous organism, V; this serum agglutinates P119 in a dilution of $\frac{1}{50}$; P119 is therefore agglutinated up to 10 per cent of titre, and the figure 10 is shown in the table.

It must be clearly understood that such figures are only approximate. The apparent agglutinability of an organism varies from time to time under the influence of a variety of factors. As far as possible a uniform technique has been employed, but experience demonstrates that standard results have not been attained. The variations are, however, insufficient to upset the conclusions formed./

Table VII shows the serological interrelationship of this type with the rest of the series:-

Tables VIII and IX show absorption tests with serum prepared from Strain P119, and from Strain P493, another similar organism:-

/Note - In these and in all other absorption tests in this investigation the method of complete saturation with the absorbing organism was employed. The dose of organisms added to the serum was sufficient to ensure that

TABLE VII.

	V	W	X	Y	Z	P 119	103	170	88	P 288	P 274	D 1	D 19	B. dys. Sonne
P 119 agglutinated by various sera	10	-	10	10	-	100	5	-	-	-	-	-	-	-
P 119 serum with various organisms	12.5	2.5	17.5	25	20	100	5	-	-	-	-	-	-	-

TABLE VIII.

	V	W	X	Y	Z	103	P 119
P 119 serum control ...	12.5	2.5	17.5	25	20	2.5	100
P 119 serum absorbed "V" ...	-	1.5	5	12.5	10	-	100
" " "X" ...	-	-	-	12.5	-	-	12.5
" " "Y" ...	-	-	5	-	5	-	50
" " "Z" ...	-	-	5	5	-	-	25
" " P 119 ...	-	-	5	2.5	5	-	-

TABLE IX.

	V	W	X	Y	Z	103	P 119	P 493
P 493 serum control ...	10	2	40	25	20	2	100	100
P 493 absorbed P 493 ...	1	-	1	2	-	-	2	5
" " P 119 ...	1	-	5	-	5	-	2	10
" " X ...	-	-	2	2	-	-	10	40
" " 103 ...	2	1	10 +	10 +	10 +	-	50	100

TABLE X.

	V	W	X	Y	Z	P 119	P 493
X serum control ..	50	-	100	50	50	10	40
" absorbed P119	25	-	50	10	25	-	-
" " P493	-	-	40	29	-	-	-

after two hours at 37°C. and overnight at room termperature, the supernatant fluid still remained turbid, so that prolonged centrifuging was necessary to throw down the suspended (i.e., non-agglutinated) organism./

The capacity of X to absorb agglutinins from both the sera tested will be noted, as well as the high content of heterologous agglutinin for X in P493 serum. X serum was accordingly absorbed, with the results shown in Table X:-

From these results it will be seen that although P119 has much in common with X, it has nevertheless an antigenic structure peculiar to itself, and must therefore be regarded as a separate type.

Mutation. In view of the results obtained with Type 103 (about to be described), numerous attempts have been made to cause P119 to produce an agglutinable variant, but without success. Marked variations in colony characters were obtained, but these did not betoken any alteration in antigenic pattern.

Pathogenicity. Of the eight cases isolated in Poona, all were from typical cases of dysentery, six showing bacillary exudate, and two indefinite exudate. In no case

did a mixed infection with any other dysentery organism occur, nor was the organism isolated except during the acute phase of the disease. It was never found in the 8,000 odd platings made from non-dysenteric individuals.

No agglutination tests have been carried out with the serum of a patient harbouring this organism.

In every way except its exact antigenic complex, P119 is identical with the classical Flexners, and there seems no reason why it should not be regarded as a hitherto undescribed member of the group. It is of relatively common occurrence, as in 1931, 7 strains of this organism were isolated in Poona, as compared with 4 of V, 3 of X, and 2 of Y.

(3) Type 103.

Forty-one strains of this organism have now been isolated or identified by the writer. These have the following distribution:-

Secunderabad, 1928-29	...		3
Bangalore, 1929-30-31	...		12
Poona, 1930-31	...		19
Jubbulpore, 1931	...		1
Mhow	...	...	1
Quetta	...	...	1
Meerut	...	...	4

TABLE XI.

	V	W	X	Y	Z	P119	103	170	88	P288	P274	D1	D19	*B. alkalescens*	*B. dys.* Sonne
103 agglutinated by sera indicated	-	-	5	-	-	5	100	-	-	-	-	-	-	-	-
103 serum with the organism indicated	25	15	40	40	6	5	100	-	-	-	-	-	-	-	-

Description of Organism. As the type has already been fully described[4], only a brief résumé and a few additional facts need be given.

Its biochemical reactions are identical with those of the classical Flexners.

Its serological characters are shown in Table XI.

Mutation. This organism is unique in that, after a variable period of culture on artificial media, it produces an agglutinable variant very similar to Y, which in course of time completely overgrows and replaces the original type.

Fifteen strains have been isolated in the Southern Command Laboratory since the date of the last publication, and intermittent attempts have been made to stimulate these to form agglutinable variants. Two have done so, but the remainder still show the 103 antigenic pattern.

Strain P61, belonging to the series previously reported, has produced an agglutinable variant under circumstances worthy of record. This strain was selected because of the fact that it showed no tendency to produce agglutinable variants, and was sent to the Enteric Laboratory, Kasauli, to be used there for the production of Type 103 serum. On October 10, 1931, in the Southern Command Laboratory, a plate which was made from a twenty-four-hour

broth culture of this strain was found to contain both the original colony and the agglutinable variant. On October 15, 1931, information was received from Kasauli that their strain had become agglutinable, although no variation in colony type could be detected. A further subculture from Poona was sent to Kasauli, and the occurrence of both types of colonies in this subculture was there confirmed. The coincidence in time of the development of the agglutinable phase is striking.

Mention must be made of the fact that colonies having the physical but not the antigenic characters of the agglutinable variant have been encountered from time to time in plates from these strains. Such rough colonies agglutinated in a manner similar to newly isolated 103. Their significance is not known. They showed only a trace of agglutination with Flexner R serum.

It would, therefore, appear that 103 is liable to a variety of colony mutations.

Attention has also been drawn to variations in agglutinability which this organism shows. Certain strains fail to agglutinate beyond fifty per cent of the titre of the serum. Whether this represents a type within a type is a matter for further investigation.

Pathogenicity. All 28 strains isolated in Bangalore and Poona by the writer were from typical cases of dysentery. Twenty-seven were from cases showing bacillary exudate, and 1 from an indefinite exudate. One case - a child of 1 year progressed to a fatal termination. On the two occasions on which a suitably fresh specimen was received for examination from this case, a rich culture of 103 was obtained. It is interesting to note that therapeutic serum had no apparent action on the case. As no specific antibody for 103 is embodied in the serum this result is what might be expected.

In two cases this type was encountered in a mixed infection, the other organisms being W and 170.

It has never been found in the 8,000 controls.

No further tests with the serum of cases have been carried out. Despite the absence of confirmation by this test, no reasonable doubt is entertained that 103 is a widespread cause of bacillary dysentery.

(4) Type 170.

This organism has been isolated from 19 cases, viz., 9 from Bangalore, 7 from Poona, 1 from Secunderabad, 1 from Quetta, and 1 from Meerut.

Description of the Organism. The biochemical reactions are identical with those of classical Flexners.

Serologically the type is a distinct and separate entity. It is not agglutinated by high-titre sera of any of this series, neither does 170 serum agglutinate any of the other types.

Its agglutinogenetic properties are moderate.

No variations within the type have been found. All strains agglutinate to titre of the serum, producing somewhat heavier flocculi than is common with dysentery bacilli.

Mutation. No antigenic change has occurred in any of the strains, some of which are now over two years old.

Pathogenicity. Of the 16 cases investigated in Bangalore and Poona, 11 showed typical bacillary exudate, 4 indefinite exudate, and one was from a case of diarrhoea. In one case, 170 occurred in conjunction with 103.

170 has shown the usual cycle of incidence in acute cases, and has not been isolated from the 8,000 control platings.

Agglutination tests with the patient's serum and the homologous organism have been tried in 5 cases, all with negative results.

TABLE XII.

		Serum					
		181	197	200	B161a	P143	
Organism ..	Strain No. 73	-	-	-	-	-	Alike in not fermenting mannite for 72 hours
	Md. 2	-	-	-	-	-	
	143	-	100	-	-	-	Alike
	197	-	100	-	-	-	
	B161a	-	-	-	100	-	Alike
	P602	-	-	-	100	-	
	181	100	-	-	-	-	Serological Entity
	200	-	-	100	-	-	" "
	P143	-	-	-	-	100	" "
	Dec. 10	-	-	-	-	-	
	P373	-	-	-	-	-	

On the whole it is considered probable that 170 is a cause of dysentery, but in view of the antigenic dissimilarity from the classical Flexners, more experience is necessary before a final judgment can be given.

(5) Unclassified Strains.

There remain 11 strains of organisms giving these biochemical reactions which are serologically different from the foregoing types.

Sera have been prepared for some of these, and lead to the following further classification (vide Table XII).

Brief Notes on these Strains.

73 and Md. 2. Both were isolated from typical cases of dysentery with bacillary exudate. No other organisms isolated.

143 and 197. One from a routine examination of a food handler, the other from a case of diarrhoea with no blood and mucus. The organism has well-marked agglutinogenetic properties.

B161a and P602. Both were from cases of diarrhoea with mucus, microscopically not a typical bacillary exudate. The colonies of P602 were rough in appearance on the original plate. Moderate agglutinogenetic properties.

181. From a case of neurasthenia with occasional loose stools. No mucus. Never suggestive of dysentery. Organism isolated on one occasion only of many examinations made.

Of low agglutinogenetic power.

200. Isolated on one occasion only from a case of dysentery during convalescence. No organism isolated during the acute stages. Well-marked agglutinogenetic properties.

P143. A typical case of bacillary dysentery. Organism isolated during acute stage. Has moderate agglutinogenetic properties.

Dec. 10 and P373. Accidentally overlooked and no serum has been prepared. They may therefore be alike or distinct serologically. They have no serological relationship to any other organism studied.

(b) Subgroup B.

While the creation of a subgroup based on the fermentation of dulcite may be questioned, the arrangement in practice is a convenient one, and prevents the previous subgroup from becoming top heavy. There is evidence of inter-relationship in that two of the types in this section

produce heterologous agglutinins for the classical Flexners.

Tables XIII and XIV show the biochemical and serological characters of the various types in the subgroup.

From this it will be seen that, except in the case of P274 and B. alkalescens, the types have no common antigen.

Mutation. There is no evidence of the occurrence of true mutation in this group. On isolation the colonies are "smooth" in every way. Strains of all types which have been kept in artificial culture over long periods show superficial roughness to a greater or lesser extent. These all, however, emulsify readily, grow in broth without depositing, and agglutinate to titre with the serum made from smooth strains.

(1) Type 88.

Thirty-nine strains of this organism have been isolated or identified by us, 16 from Bangalore, 15 from Poona, 1 from Belgaum, 5 from Secunderabad, 1 from Jubbulpore, and 1 from Quetta. Strains have also been reported from Wellington and Razmak.

The biochemical reactions are as in Table XIII. In about one-third of the strains dulcite is not fermented.

TABLE XIII.

Biochemical Reactions of Subgroup B.

	Lactose	Glucose	Mannite	Dulcite	Saccharose	Milk	Indol
88	-	A	A	A 10th day	-	A Ft. alk. 15th day	-
P288 ...	-	A	A	A 5th day	-	A Alk. late	-
D1	-	A	A	A 3rd day	-	A Alk. late	-
D19	-	A	A	A 3rd day	-	A Alk. late	-
P274 ...	-	A	A	A 10th day	-	A Alk. late	-
B. alkalescens	-	A	A	A 3rd day	-	A Alk. 3rd to 5th day.	+

Note: the above times refer to cultures in media with a pH value of 7.4, and are averages.

TABLE XIV.

Serological Reactions of Subgroup B.

	Organism					
	88	P288	P274	D1	D19	B. alkalescens
Serum of 88	100	-	-	-	-	-
" " P288	-	100	-	-	-	-
" " P274	-	-	100	-	-	100
" " D1	-	-	-	100	-	-
" " D19	-	-	-	-	100	-
" " B. alkalescens	-	-	-	-	-	100

Alkali formation in milk does not occur till the second week, and is always faint.

The serological reactions are shown in Table XV. A fairly close relationship to the classical Flexners can be seen. Absorption tests recorded in a previous article[4] show that the "88" antigen is a separate entity.

Pathogenicity. Thirty cases have come under the writer's observation. Of these, 22 were typical dysentery with bacillary exudate, 5 were dysentery with indefinite exudate, and 3 were from cases with a history of dysentery which had however passed the acute stage.

No other dysentery organism has ever been recovered in association with "88" nor has this organism ever been found in the control series.

Agglutinins for "88", and also for some of the classical Flexners, particularly "W", develop in the serum of patients infected with this organism. Saturation of such a serum with "88" removes all the agglutinins, whereas the classical Flexners are capable of removing only their own (therefore heterologous) agglutinins.

Table XVI shows the findings in one case that was investigated in this way.

This type is definitely regarded as a cause of dysentery.

TABLE XV.

	V	W	X	Y	Z	P119	103	170	88	P288	P274	D1	D19	B. alkalescens	B. dys. Sonne
88 agglutinated by the sera indicated ...	-	5	-	-	-	-	-	-	100	-	-	-	-	-	-
88 serum against organism indicated ...	20	30	-	20	-	-	-	-	100	-	-	-	-	0.5 per cent	-

TABLE XVII.

	V	W	X	Y	Z	P119	103	170	88	P288	P274	D1	D19	B. alkalescens	B. dys. Sonne
P288 agglutinated by sera indicated	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-
P288 serum with organism indicated	5	-	5	5	12.5	-	-	-	-	100	-	-	-	-	-

TABLE XVI.

	V	W	X	Y	Z	88
Patient's serum, 11th day	1/25	1/35	-	1/25	1/25	1/175
" " 17th day	1/50	1/100	-	1/25	-	1/250
" " absorbed "W"	-	-	-	-	-	1/250
" " absorbed by homologous strain	-	-	-	-	-	-

(2) Type P288.

Thirteen strains of this organism have been isolated, 11 in Poona, 1 in Secunderabad, and 1 in Bombay.

Description of the Organism. The biochemical reactions of P288 are shown in Table XIII.

Serologically, as will be seen from Table XVII, a certain relationship to the classical Flexners exists. Although not agglutinated by the classical Flexner sera, a P288 serum contains a proportion of heterologous agglutinins for these types. The organism has well-marked agglutinogenetic properties.

Pathogenicity. Of the 11 Poona strains, all were from clinical bacillary dysentery, 9 being from stools showing bacillary exudate, and 2 from indefinite exudate. The Bombay strain was from a case of acute dysentery in a child.

The incidence of these cases is interesting. Nine of the Poona cases occurred between 20.6.31 and 6.8.31, and the remaining 2 cropped up simultaneously in an officer's mess in January, 1932, 5 of the 9 cases occurred in one regiment with the date of onset as follows: 16.7.31, 21.7.31, 26.7.31, 3.8.31, and 6.8.31.

No mixed infections have been discovered nor has the organism ever been found in the 8,000 controls. It has been found only during the acute stages of the cases.

In one case the patient's serum was examined for specific agglutinins with negative result.

It is considered that the evidence is in favour of pathogenicity because of (a) the fact that the organism has only been isolated from cases of clinical bacillary dysentery; (b) the definite connection between certain cases suggesting infection from a common source; and (c) the serological relationship to the classical Flexners.

(3) Type P274.

Of this type only nine strains have been encountered. Four of these occurred in the Secunderabad series, two were found in Bangalore, two in Poona, and one in Quetta.

The biochemical reactions call for no particular comment, being as shown in Table XIII. The fermentation of dulcite occurs between the eighth and sixteenth days. Indol is not formed. (Note the corresponding reactions in B. alkalescens.)

Serological Reactions. P274 is not appreciably agglutinated by high-titre sera of any other members of the series, and P274 serum has no action on any of the organisms with the exception of B. alkalescens (Andrewes) which is agglutinated to titre. Absorption tests give the results shown in Table XVIII.

TABLE XVIII.

Serum	Organisms	
	B. alkalescens	P274
B. alkalescens ...	100	0.5
P274	100	100
P274 absorbed P274 ...	1	2
P274 absorbed B. alkalescens	2	20

It will thus be seen that while P274 serum has marked heterologous agglutinins for B. alkalescens, the reverse is not true. Each possesses an antigen peculiar to itself.

P274 has good agglutinogenetic properties.

Pathogenicity. The four Secunderabad strains were all from cases of clinical dysentery with bacillary exudate.

Of four strains isolated by the writer, two were from dysentery cases with bacillary exudate, one from a case with indefinite exudate, and one from a case of intermittent diarrhoea. In one of these dysentery cases the organism was found on four successive days during the acute stage, never later. Tests of patient's serum for specific agglutinins in this case proved negative.

This type has never been encountered other than as above.

Although, therefore, there is a certain amount of evidence in favour of P274 being pathogenic, it is not yet possible to express a definite opinion on the subject.

(4) Type D1.

This is a serological type regarding which very little first-hand information can be given. Of the thirty-seven strains of "non-agglutinable Flexner" collected by Major W. Walker, R.A.M.C., in Secunderabad, and very kindly handed over to the writer, seven were found to be of this type. No similar strains were found in Bangalore or Poona till August, 1931, when two were isolated. These nine strains are all that have come to hand.

Biochemical reactions appear in Table XIII. It will be seen that, as with B. alkalescens, dulcite is fermented early, i.e., on the second or third day. Alkali formation in milk is, however, late and slight.

Serological Characters. This type is of completely different antigenic pattern from the classical Flexners. It is agglutinated by no serum in this series other than its own. Conversely D1 serum contains no heterologous agglutinins. Its agglutinogenetic properties are not of a high order.

Pathogenicity. Of the two Poona strains one was from a typical bacillary dysentery with bacillary exudate, the other from a more doubtful case with indefinite exudate. The seven Secunderabad strains were all from cases of clinical dysentery, five of which showed bacillary exudate and two indefinite exudate.

It has not been possible to test the agglutinin content of the serum of patients from whom this organism has been recovered.

While it is possible that the organism may be a cause of dysentery, there is not sufficient evidence available to admit of this being definitely asserted.

(5) Type D19.

Only two strains of this type have been found, both in the Secunderabad series. Both isolations were from cases of bacillary dysentery with typical bacillary exudate.

The biochemical and serological reactions can be seen in Tables XIII and XIV. The organism has well-marked agglutinogenetic properties.

It is impossible to say from the evidence available whether D19 is pathogenic or not.

(6) B. alkalescens (Andrewes).

This has been found five times, three times in Poona and twice in Bangalore.

The biochemical reactions at once distinguish it from other members of the group. Dulcite is rendered acid about the third day. Milk, at first acid, becomes alkaline between the third and fifth days, the degree of alkalinity ultimately being intense. Indol is formed in peptone water. B. alkalescens is the only indol-forming member of the subgroup.

The serological characters can be seen in Tables XIV and XVIII.

Pathogenicity. All five strains were recovered from the stools of normal individuals. There is, therefore, no suggestion of pathogenicity, a finding which accords with the opinion formed by Andrewes[5].

(7) Unclassified Strain.

One other dulcite-fermenting strain has been isolated which has no serological relationship to these described.

It was found in the routine examination of a normal individual.

(c) Subgroup C.

This subgroup comprises over eighteen per cent of the 1930-31 series.

It has been only somewhat superficially investigated, having been set aside for more detailed consideration when the work on the other subgroups should be completed. A transfer from practical work has rendered this impossible so far as the writer is concerned, but the work will shortly be taken up in another laboratory.

The principal member of the subgroup is, of course, B. dysenteriae Sonne, whose pathogenic properties are well known.

No attempt has been made to classify the remainder of the strains serologically. No doubt B. dispar (Andrewes and the various brands of B. metadysenteriae (Castellani) are included, but in the absence of identification by agglutination it is impossible to be certain.

The early production of colonies which are at least superficially "rough" (R) is a well-marked feature of this group. It is typified in the case of B. dysenteriae Sonne which, although usually "smooth" (S) when isolated may begin immediately to become R, and may be almost completely R within twenty-four hours. For this reason it is advisable to use for diagnostic purposes a serum capable of agglutinating both the S and the R variants, as otherwise strains which have undergone an early change to R may be missed.

Similar rapid mutation to R has been observed in all strains of this subgroup which have been studied. Many are completely R on the original plate made from the patient's stool. For this reason it is thought that such strains may be much commoner than the figures given here indicate. When typical S colonies are present on a plate, investigations, especially during the rush season, are apt to be confined to these to the exclusion of any R colonies

which may be present, which are consequently "missed". Of course there are many blue colonies with R characters which are totally unrelated to the group under discussion.

These R strains of subgroup C show well-marked differences from the R strains of subgroup A (i.e. the classical Flexners). Colonies of the latter not only appear "rough" physically, but also grow as a deposit in broth and auto-agglutinate in 0.9 per cent. saline. The subgroup C "R" strains have not these characters. Although to look at they are extremely "rough" colonies, they grow uniformly in broth, and readily emulsify in 0.9 per cent saline. B. dysenteriae Sonne nevertheless shows the same complete change of antigen in its mutation from S to R as do the classical Flexners.

In this connection there is a further point of considerable interest. The classical Flexners (V, W, X, Y, Z) have R variants with a common antigenic pattern, i.e., a serum which agglutinates V R will agglutinate equally W R, X R, etc. This is not so in subgroup C. Sonne R serum will not agglutinate any of the others, a fact which suggests the possibility that there may be a fundamental serological difference between these

biochemically similar types, and is in keeping with the suggestion that while Sonne is definitely pathogenic, these others prabably are not.

(1) B. dysenteriae Sonne.

As this is a well-known type only a few salient points regarding it will be mentioned.

Biochemical Reactions. The acid production in lactose and saccharose is generally described as taking place from the third to the fifth day, or at any rate within the first week. With the strains isolated and the media used in India this period has been more protracted, the average of twenty-six strains being fourteen days in the case of lactose, and 14.5 in the case of saccharose.

Indol is never formed.

Serological Characters. Attention is again drawn to the rapidity with which R variants may be formed. They are not infrequently seen on the original plate made from the patient's faeces. This organism is a serological entity and produces no co-agglutinins for either of the other subgroups.

Pathogenicity. Of the 31 strains isolated by the writer, 29 were from typical bacillary dysentery with bacillary exudate, and 2 from less definite cases.

In two cases Sonne occurred in conjunction with other organisms (one a "V" the other a "W"). The serum of 6 cases was tested for agglutinin production with negative result.

Infection with this organism is of common occurrence in children. Of the 31 strains, 8 were from children and 2 from enlisted boys.

(2) Unclassified Strains. In all, 39 strains have been isolated, 3 of these being sent from other laboratories.

Biochemical Reactions. The acidification of lactose and saccharose is very irregular. 26 strains produced acid in both these sugars; 9 in lactose only, and 4 in saccharose only. The period varied from one to twenty-seven days. It is possible that the non-lactose fermenters and the non-saccharose fermenters may represent different types, but the data have not been sufficiently confirmed to justify any expression of opinion.

A point worthy of note is that seventeen or nearly fifty per cent of the strains form indol, in contrast to the true Sonne, where indol production has never been found.

Serological Reactions. None of these strains are agglutinable with any of the sera prepared in this investigation against which they were tested (the earlier strains were discarded before the later sera were prepared).

An attempt to prepare a serum for one strain repeatedly isolated from a case of clinical dysentery with bacillary exudate was quite unsuccessful. In another case a weak serum with a titre of $\frac{1}{250}$ resulted.

Pathogenicity. The following list summarizes the types of case from which the thirty-six strains were recovered:-

(a)	Clinical dysentery with bacillary exudate and no known pathogenic organism isolated ...	11
(b)	Clinical dysentery with indefinite exudate and no known pathogenic organism isolated ...	1
(c)	Diarrhoea - no exudate	4
(d)	Clinical dysentery with bacillary exudate, but recognised pathogenic dysentery organisms isolated as well as this type ...	12
(e)	Convalescent dysentery	3
(f)	Cases with no intestinal symptoms	5

The biochemical reactions afford no help in the detection of pathogenic strains. Thus groups (a), (b) and (c) above include strains which ferment both lactose and saccharose; strains which ferment lactose and not saccharose, and vice versa; and strains which produce indol as well as strains which do not.

Group (d) is significant, and strongly suggests that Groups (a) and (b) represent a similar state of affairs with the causative organism missed.

In only three cases did colonies occur on the original plate in a way which was regarded as "suspicious" or "likely."

While it is possible, therefore, that some of these strains may be pathogenic, it seems fairly certain that the majority are not, and it is considered that all should be regarded as non-pathogenic till evidence to the contrary is forthcoming. There is little doubt that these are the strains which have damaged the faith of the clinician in regard to the diagnosis of "non-agglutinable" Flexner, as for the first few days they present the correct biochemical reactions and would therefore be reported as Flexner.

E. ANALYSIS OF RESULTS.

This research has been in progress for three years. During this period every non-motile coliform organism, non- or late-lactose fermenting, and acidifying without gas glucose and mannite, isolated either from dysentery cases, from routine faeces examination, or sent by other laboratories, has been kept and fully investigated. Exclusive of "classical" B. dysenteriae Flexner and B. dysenteriae Sonne, 214 strains have come to hand. Of these, 13 either died or were lost before they were properly investigated; 10 of the 13 were lost in the first year. An analysis of the remaining 201 is shown in Table XIX, which gives the stations where these organisms were isolated. With the exception of those for Bangalore, 1929-30, and Poona, 1931, the figures do not represent the toal number of isolations of these organisms, but merely the number which were sent to the writer for investigation. Every strain which reached us is included in the 214.

TABLE XIX.

Analysis of 201 Strains.

	Subgroup A				Subgroup B							Sub-group C
	103	P119	170	Un-classi-fied	88	P288	P274	D1	D19	B. alkale-scens	Un-classi-fied	Un-classi-fied
Secunderabad, 1928-29	3	-	1	-	5	1	4	7	2	-	-	1
Bangalore, 1929 ...	4	-	5	5	8	-	-	-	-	1	-	6
Bangalore and Poona, 1930	7	-	4	1	6	-	1	-	-	2	1	15
Poona, 1931 ...	17	7	7	2	15	9	2	2	-	2	-	15
Bombay, 1931 ...	-	-	-	-	-	1	-	-	-	-	-	-
Jubbulpore, 1931 ...	1	2	-	1	1	-	-	-	-	-	-	1
Bangalore, 1931 ...	3	2	-	1	2	-	1	-	-	-	-	-
Mhow, 1931 ...	1	-	-	-	-	-	-	-	-	-	-	1
Quetta, 1931 ...	1	-	1	-	1	-	1	-	-	-	-	-
Meerut, 1931 ...	4	-	1	-	-	-	-	-	-	-	-	-
Burma, 1932 ...	-	1	-	-	-	-	-	-	-	-	-	-
Poona, 1932 ...	-	1	-	1	1	2	-	-	-	-	-	-
Total ...	41	13	19	11*	39	13	9	9	2	5	1	39
Percentage (approx.)	20.5	6.5	9.5	5.5	19.5	6.5	4.5	4.5	1	2.5	0.5	19.5

* See Table XII.

F. PROPOSED FURTHER INVESTIGATIONS.

The ultimate object of this work is to provide a rapid and reliable method of differentiating between pathogenic and non-pathogenic dysentery organisms.

On the basis of the classification given above, it is proposed to continue amassing data on the various types, and a scheme has been evolved by which all these organisms isolated in the military laboratories in India will be carefully examined and records of the type of case in which they occurred tabulated. Agglutination tests will be carried out with the patient's serum where possible. It is anticipated that in a few years sufficient experience will have accumulated to enable conclusive opinions to be formed.

Special researches will be conducted by selected laboratories on the "unclassified" strains of Subgroups A and C. It may well be that certain of the strains shown in Table XII are of common occurrence in other parts of India. Also it seems probable that completely new strains may be forthcoming.

Having come to conclusions on the question of pathogenicity, there will be little difficulty in making practical application of the results.

The biochemical reactions will still be used to effect a preliminary classification, suspicious colonies from plates being inoculated into lactose, glucose and mannite, and on to an agar slope. Members of the group under discussion will reveal themselves as non-motile coliform organisms which do not ferment lactose, but produce acid without gas in glucose and mannite.

Further classification by biochemical reactions is impracticable in routine practice, as the changes are too late in occurring.

By agglutination tests, however, immediate results can be secured. The manufacture of polyvalent serum for strains classified as pathogenic is an easy matter, but in view of the number of organisms with little or no antigenic relationship, it will be necessary to split them into two or three groups, and prepare a serum polyvalent for each such group. Using these (stock) sera, and a saline emulsion of the organism from the agar slope, agglutinability can readily be determined, and strains which prove agglutinable would be reported as dysentery bacilli, all others being discarded.

G. SUMMARY.

(1) In India a large number of mannite-fermenting bacilli resembling B. dysenteriae Flexner do not agglutinate with serum polyvalent for V, W, X, Y and Z.

(2) By means of delayed biochemical reactions and serological tests a more detailed classification has been made which embraces the majority of these strains.

(3) Certain tentative opinions regarding pathogenicity are expressed. Some of the new types are believed to be definitely pathogenic, others remain doubtful, and others are probably non-pathogenic.

(4) A scheme has been elaborated by which these organisms will be investigated on a large scale.

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A N N E X U R E III.

A REVIEW OF THE DYSENTERY BACILLI OF INDIA, WITH SPECIAL REFERENCE TO CERTAIN RECENTLY DESCRIBED TYPES.

Journal of the Royal Army Medical Corps, 1936, Volume LXVI, pp. 1 to 13.

INTRODUCTORY.

In two previous papers (Boyd, 1931 and 1932) an account is given of work carried out in Bangalore and Poona with the object of differentiating the strains of dysentery bacilli previously classified in India as "inagglutinable Flexners." It is shown that the majority of these anomalous organisms can be grouped into distinct types of definite antigenic composition.

A scheme of investigation based on these findings has been drawn up along the lines indicated in the second article, and has been in operation in all military laboratories in India since July, 1932. It has proved successful in enabling all known types of dysentery bacilli to be identified quickly and with precision, and as a corollary has facilitated the detection of atypical strains, which have in their turn been made the subject of special study.

In carrying out this investigation, a comprehensive definition of the term "dysentery bacillus" has been followed. The strict definition of a dysentery bacillus must of necessity be "a bacillus capable of causing the disease known as dysentery", and with rare exceptions, such bacilli have certain morphological and cultural characters in common. They are non-motile coliform bacilli which do not liquefy gelatin, and do not produce acid from lactose in twenty-four hours, but which acidify glucose in that period. There are, however, certain organisms having these characters which do not, as far as our present knowledge goes, cause dysentery: but to avoid the possibility of missing any dysentery-producing type, all strains having the above characters have been carefully investigated.

The search for dysentery group organisms has not been confined to material from patients showing active symptoms of this disease. All strains of this kind found in the routine examination of menials and convalescents have been investigated. In the period under review, 35,126 menials were examined, and a total of 119,581 platings made from their stools. In addition, several thousands of platings were made of the stools of enteric convalescents. The results of this work consitute a massive control which has

an important bearing on the question of the pathogenic action of the various types.

The routine measures which have been followed ensure that all dysentery group organisms from every military laboratory, and from whatever source isolated, are thoroughly examined, and it is therefore considered that the figures given in this article are representative in character and make no omissions of any importance.

The present paper is a study of the results obtained in the years 1932, 1933 and 1934, during the latter two and a half years of which period the scheme has been followed. The figures and other data which form the basis of the study are taken from two sources. Those relating to organisms other than "new" types are from the Annual Reports of Command, District and Brigade Laboratories. Those concerning the "new" types are from special pro formas which have been used in connection with this investigation, and which accompany a strain through the different laboratories in which it is examined, and finally are forwarded to Army Headquarters.

The objects of the paper are twofold:-

(a) To make an analysis of all dysentery group organisms isolated in this three-year period in order to show the frequency with which the different types occur, and to emphasize the fact that the scheme embraces every type of importance which is to be found in India at present.

(b) To give a more detailed account of the cases in which the "new" types were found, and to discuss the evidence of the pathogenic action of these organisms.

ANALYSIS OF DYSENTERY GROUP BACILLI ISOLATED IN THE YEARS 1932, 1933 AND 1934.

Full details of isolations by Commands are shown in Table I.

In this table Andrewes' types (which Gardner has aptly termed the V-Z spectrum) are shown grouped together and not as individual types. While the writer's experience indicates that various criticisms of these types can be made, the fact remains that a serum which is polyvalent for the group meets all practical requirements so far as identification is concerned.

TABLE I. ANALYSIS OF DYSENTERY ORGANISMS ISOLATED IN 1932, 1933 and 1934.

	Non-Mannite Fermenters*		Mannite Fermenters														
				New Types													
	Shiga	Schmitz	V, W, X, Y, Z, etc. (Andrewes)	103	P119	170	88	P288	P274	D1	D19	Total of these "new" types	Inagglutinable strains	Strain not investigated†	Sonne	Inagglutinable lactose and saccharose fermenters	E. hystolytica
Northern Command	345	50	1,076	66	31	15	61	5	9	5	2	194	7	24	82	4	141
Eastern Command	134	72	526	4	5	21	16	3	3	2	-	54	4	32	80	37	286
Western Command	132	81	582	15	11	58	78	7	22	23	1	215	42	10	144	48	305
Southern Command	198	83	594	50	51	39	83	10	13	11	2	259	3	19	191	8	202
Burma District	11	10	110	-	2	1	2	1	-	-	-	6	1	10	11	-	31
Total	820	296	2,888	135	100	134	240	26	47	41	5	728	57	95	508	97	965
Percentage of Grand Total (Bacilli only)	14.91	5.38	52.51	2.45	1.82	2.44	4.36	0.47	0.85	0.75	0.09	13.24	1.04	1.73	9.42	1.76	

Total number of strains investigated = 5,499.

* A small number of atypical non-mannite fermenting bacilli, which are still under investigation, are not included.

† These strains were isolated and discarded, labelled "Flexner (inagglutinable)," in the first six months of 1932 before the existing scheme of classification was in operation.

NUMERICAL INCIDENCE OF THE DIFFERENT TYPES.

It will be seen that, during the three years under review, in round figures 20 per cent of all dysentery-like bacilli isolated were non-mannite fermenters (B. dysenteriae Shiga and Schmitz), 52.5 per cent were of Andrewes' types (V-Z spectrum), just under 10 per cent were B. dysenteriae Sonne, and over 13 per cent were of the "new" types.

In all, 4.53 per cent of the strains isolated do not fall within these groups.

Of these, 1.73 per cent were "atypical" strains isolated in the first six months of 1932, before the scheme of classification was adopted, and before sera for the new strains were available for general use. Under the instructions then in force they were reported as "Flexner inagglutinable" and were discarded. There is every reason to believe that the majority of them belonged to one or other of the "new" types.

A further 1.76 per cent (exclusive, of course of B. dysenteriae Sonne, which is shown separately) were late fermenters of lactose or saccharose or both. These strains have been specially investigated by Major R. A. Hepple. His work confirms the previously formed opinion, that owing to the feebleness of their agglutinogenetic properties, serological classification of this group is

impracticable. There is no evidence that they exercise any pathogenic action. The majority of them were not isolated from cases of dysentery, but were found in the normal stools of menials or others undergoing "carrier" tests. The present practice is, therefore, to regard late lactose- and saccharose-fermenting strains (other than Sonne) as a definite, if heterogenous, group having no causal relationship to dysentery. The gelatin-liquefying properties of these organisms have not been properly tested.

The remaining 1.04 per cent are the only strains which do not fall definitely into one group or another. They will be considered in detail at a later stage when it will be shown that the number of "unknowns" can be still further reduced.

The total number of strains which cannot be labelled is therefore well under 1 per cent, which would seem to show that, so far as India is concerned, the new system includes every strain of any practical importance.

GEOGRAPHICAL DISTRIBUTION OF THE "NEW" TYPES.

The distribution of the "new" types, as a whole, is not uniform throughout India. For example, isolations are proportionately much lower in the Eastern Command and

Durma District than elsewhere. There is reason to believe that this has an artificial explanation, and that in future years the disparity will be less.

Certain of the types are much more common in some districts than in others. Thus, types 103 and P119 (which are very closely related to, and indeed should form part of the V-Z spectrum) are of relatively common occurrence in the Northern and Southern Commands, but are rare in the Western Command, while members of the dulcite-fermenting group (88, P274, D1) are more frequently found in the latter Command than elsewhere.

Despite these variations, which may well be of only temporary significance, the distribution of these organisms demonstrates that they are not merely local strains of no general importance, but are organisms which have a definite and widespread association with cases showing the symptoms of bacillary dysentery.

SOURCE FROM WHICH THESE BACILLI WERE RECOVERED.

With few exceptions the recognized dysentery bacilli, and the named "new" types, were isolated from cases suffering from dysentery.

In contrast to the findings in dysentery cases, it is noteworthy that in the course of routine examination of menials for the carrier condition, dysentery bacilli have been found only on rare occasions, although all the types have at one time or another been encountered in these circumstances. Without doubt the explanation of these findings is that the individuals from whom the organisms were isolated suffered from mild chronic dysentery. In every case which came under his personal investigation the writer has been able to confirm this point by the detection of mucus exudate in the stools. In discussing the "new" types, reference will be made to all isolations from individuals not showing active symptoms.

FURTHER DETAILS REGARDING THE "NEW" TYPES.

Data which Constitute Evidence of Pathogenic Action.

Gardner (1929) cites the following four points as proof that bacilli of the Flexner group cause dysentery.

(1) The great majority of persons whose excreta contain Flexner group bacilli are suffering, or have recently suffered, from clinical dysentery. The bacilli are selectively situated in the intestinal lesions.

(2) The great majority of those who neither have, not have recently had, dysentery harbour no Flexner bacilli.

(3) The blood-serum of persons suffering from dysentery and harbouring Flexner bacilli in their intestines nearly always gives supernormal values in the agglutination and complement-fixation tests at some time during the disease.

(4) A number of instances of accidental laboratory infection of human beings with pure cultures of these bacilli are on record, the result of the infection being indistinguishable from bacillary dysentery.

To these may be added a fifth point, namely, the recovery of one suspicious type, and of no other pathogenic organism, from a series of cases of dysentery occurring as an isolated outbreak. The presumptive evidence in cases of this kind is very strong.

It may be said at once that in the case of the "new" types, evidence of the kind postulated in point (4) is, so far, lacking. While admittedly the existence of evidence of this nature would strengthen the case, its absence is of no positive significance. No fatal case resulting from infection with one or other of these organisms has occurred and hence no attempts at cultivation direct from intestinal

ulcers have been possible. Neither have complement-fixation tests been done; but in view of the agglutination results this is of little importance. The number of individuals of the category mentioned in point (2) who have been examined is, as already mentioned, approximately 35,000.

In considering the pathogenic action of these organisms, the above points will receive special attention.

103. - This type has no claim to be regarded as new, as the writer has been informed by Dr. W. M. Scott, Ministry of Health, who kindly examined certain strains sent to him, that it is identical with one of the strains previously described as B. dysenteriae Y, which, by some mischance, was not included in the series investigated by Andrewes.

It is closely allied to the V-Z series, although it is only agglutinated to a fraction of the titre of a serum polyvalent for those organisms.

As has previously been described, it undergoes a striking mutation after varying periods of life in artificial culture, and produces a highly agglutinable variant which closely resembles the Y member of the V-Z spectrum. This mutation has been observed from time to time in the

strains kept for the manufacture of high titre serum in the Enteric Laboratory, Kasauli; and it appears advisable to replace stock strains at frequent intervals by newly isolated strains, which have been carefully identified. It is highly probable that the strains which were sent three years ago to the National Collection of Type cultures have undergone this mutation and now exist in the form of variants indistinguishable from Andrewes' Y.

Of the 135 isolations shown in Table I, 134 were from cases showing the clinical picture of bacillary dysentery. No details as to severity are available regarding 5. Of the remainder 16 are recorded as severe, 43 as moderately severe, and 70 as mild. In one case the organism was isolated during the routine examination of a patient convalescent from paratyphoid A fever, who at the time showed no symptoms of active dysentery.

In 103 cases the isolation was made from blood and mucus exudate, macroscopically and microscopically typical of bacillary dysentery. In 30 cases the stools contained blood and mucus, or mucus alone, but microscopically the exudate was indefinite. In the paratyphoid case mentioned above no exudate was present. One case is unrecorded.

Mixed infections occurred as follows: With W, 1 case; with V, 3 cases; with Schmitz, 1 case; with P119, 1 case; with E. histolytica, 3 cases.

In cases of dysentery the collection of serum for agglutination tests is often difficult, as the patients usually recover and are discharged from hospital prior to the optimum time for taking serum, which is about the twentieth day after onset of the illness. For this reason many tests have been made before agglutinins had an opportunity to develop to their maximum, and the number of positive results has without doubt been reduced in consequence. Unfortunately records are not sufficiently complete to enable the exact day of disease when the blood was taken to be given. These remarks apply equally to all tests of the kind recorded in this article.

In 58 of the above cases the serum was tested for agglutinins for the homologous organism. Of these, 37 were negative, 5 produced agglutination in a dilution of 1 ; 25, 7 in 1 : 50, 5 in 1 : 125, and 4 in 1 : 250 and over.

Gardner's points (1), (2) and (3) are therefore satisfied.

P119. - As far as can be ascertained, this type does not occur in any of the European classifications, but it has been recognized in Japan, where it figures as No. XII of Aoki's classification. Further reference to this will be made in a later communication.

Like 103, it is very closely related to the members of the V-Z spectrum, and particularly to X. It possesses, however, an antigen peculiar to itself, and as it is only feebly agglutinated by a serum polyvalent for the V-Z series, it must be regarded as a separate type.

This organism was recovered from exactly 100 individuals of whom 98 showed the usual symptoms of bacilly dysentery. Of these cases, 12 were classed as severe, 34 as moderately severe, and 50 as mild; in 2 no record of severity was made. These 2 isolations were from menials undergoing carrier tests, and it is significant that, although they presented no symptoms, mucus was present in the stools of both.

In 83 of the cases, the stool from which the organism was isolated consisted of blood and mucus showing typical bacillary exudate; 12 showed blood and mucus or mucus and indefinite exudate. The records relating to the remaining 5 are incomplete.

Mixed infections occurred as follows: With Shiga, 1 case; with V-Z spectrum, 12 cases; with Sonne, 1 case; with 103, 1 case; with 88, 1 case.

Agglutination tests with the patient's serum against the homologous organism were carried out in 39 cases; 17 were negative; 2 showed agglutination in a dilution of 1 : 25, 8 in 1 : 50, 8 in 1 : 125 and 4 in 1 : 250 or over.

Points (1), (2) and (3) are therefore satisfied. In both 103 and P119, the very close antigenic relationship which they bear to the V-Z series seems a further argument in favour of their pathogenic rôle.

170. - This type cannot be identified in any of the well-known classifications, and does not appear to have been previously described.

It possesses a distinct antigen peculiar to itself, and has no serological relationship either to the V-Z spectrum or to any other dysentery organism investigated by the writer.

Of the 134 individuals from whom it was recovered 132 were suffering from symptoms of bacillary dysentery. Of these cases 5 were severe, 32 were moderate and 93 were mild, and 1 is incompletely recorded. The remaining two isolations were from menials undergoing routine exmination.

Eighty-eight of the cases showed blood and mucus and bacillary exudate, 40 showed blood and mucus or mucus with indefinite exudate, 4 showed no exudate, and 2 are unrecorded

Mixed infections occurred as follows: With V-Z spectrum, 1 case; with P274, 1 case; with Sonne, 3 cases; with E. histolytica, 3 cases.

Agglutination tests, with the patient's serum against the homologous organism, were carried out in 68 cases. 49 were negative, 2 showed agglutination in dilution of 1 : 25, 12 in 1 : 50, and 5 in 1 : 125. As shown by rabbit inoculation, this organism has indifferent agglutinogenetic properties, with which finding the above results are in keeping.

It will be seen therefore that Gardner's points (1), (2) and (3) are fulfilled by this organism.

88. - This organism, which is numerically the most common of all "new" types, presents several features of great interest.

In common with the four types which follow, it differs from the V-Z races and from the three types just described, in being a late dulcite-fermenter. This property is, however, not constant, being absent in about one-third of all strains isolated.

Although differing in its biochemical reactions, 88 has been shown by Dr. W. M. Scott to be identical in its antigenic composition to the organism known at home as the Newcastle dysentery bacillus, which has been proved responsible for several small outbreaks of dysentery in England and elsewhere. This antigenic similarity has been confirmed by workers in India (Lieutenant-Colonels R. F. Bridges and D. T. M. Large). Further, a strain having the biochemical characters of Newcastle, and the usual serological characters, has recently been isolated from a case of dysentery in Bareilly.

Table II shows the more important biochemical reactions of 88 (and of P288, P274, D1, and D19, which are identical in this respect), of Newcastle, and of B. alkalescens (Andrewes). The last of these organisms has never been found in association with cases of dysentery in India, but has been isolated from normal stools.

TABLE II.

	Lactose	Glucose	Mannite	Dulcite	Indol
88, P288, P274, D1, D19	No change	Acid	Acid	Acid (late) 10th day	-
Newcastle	No change	Acid and Gas	No change or Acid and Gas	No change or Acid and Gas	-
B. alkalescens	No change	Acid	Acid	Acid 3rd day	+

The discovery of the Newcastle dysentery bacillus in India is a matter of considerable importance, as it has hitherto been the practice in military laboratories to discard non-motile gas-forming organisms isolated from cases of dysentery. (Repeated observations have led to the conclusion that here B. Morgan is not a cause of dysentery.) The routine method of investigation has now been altered to ensure that such strains do not escape notice.

88 shows a fairly close antigenic relationship to the V-Z spectrum, but is only feebly agglutinated by a V-Z polyvalent serum. It possesses a main antigen peculiar to itself.

This organism was isolated from 240 cases, of which 231 presented the symptoms of bacillary dysentery. Eighteen were severe, 80 were moderately severe, 125 were mild, and 8 are incompletely recorded. Of the remaining cases, 8 were menials undergoing routine examination and 1 was a convalescent case of typhoid fever.

One hundred and thirty-seven of the cases showed typical blood and mucus and bacillary exudate; 54 showed blood and mucus or mucus and indefinite exudate; 10 showed no exudate; and 12 cases are incompletely recorded.

In 1 case 88 occurred in association with V, in another with Schmitz, in another with 170, and in 2 cases with Sonne, while in 9 cases it was associated with E. histolytica.

Agglutination tests with the patient's serum against the homologous organism were carried out in 68 cases. Of these, 52 were negative, 1 showed agglutination in a dilution of 1 : 25, 7 in 1 : 50, 4 in 1 : 125, and 4 in 1 : 250 and over.

This type, therefore, fulfils Gardner's first three criteria. A further point, namely its antigenic similarity to the Newcastle bacillus, which has been shown to be the cause of localized outbreaks of dysentery elsewhere, seems to place the question of its pathogenic action beyond reasonable doubt.

P288. - As far as can be ascertained, this type has not been described outside India. It is relatively uncommon and in the three years under review it was recovered from only twenty-six cases.

It has an antigen peculiar to itself, and shows no cross-agglutination either with the V-Z spectrum or with any of the other types.

Twenty-five of the 26 isolations were from cases of typical bacillary dysentery, of which 1 was severe, 11 were of moderate severity, 13 were mild. The remaining case was a menial undergoing routine examination.

In twenty-two of the cases the stools showed typical blood and mucus and bacillary exudate, and in three blood and mucus or mucus and indefinite exudate.

In one case P288 occurred in association with E. histolytica.

Agglutination tests with the patient's serum and the homologous organism were carried out in eight cases. Five were negative, 1 showed agglutination in a dilution of 1 : 12, 1 in 1 : 50, and 1 in 1 : 500.

It is deemed advisable, for the sake of completeness, to make brief mention of a small isolated outbreak of dysentery apparently caused by P288 which occurred in June, 1935. The Indian platoon of a British regiment stationed in Mingaladon, Burma, was affected, and ten cases occurred between June 4 and 19; from the stools of these P288 was readily isolated, no other pathogenic organism being found. Details of this outbreak given by Major D. A. O. Wilson will be published in this Journal shortly.

P288 therefore satisfies the first, second, third and fifth of the above criteria.

P274. - The history of this strain is somewhat chequered and as investigations are incomplete it is not proposed to give any details at present other than a brief outline of the difficulties which have been encountered.

Subcultures of the original strain of P274, and of an identical strain P500, were sent by the writer to the Enteric Laboratory, Kasauli, for the preparation of high titre serum to be used in the new scheme of classification. A serum was in due course prepared which had a titre of 5,000, and, using this, ten strains were identified in various laboratories between July, 1932, and August, 1933.

About August, 1933, whilst periodic routine tests of the serum were being made at Kasauli, it was discovered that the titre had suddenly fallen to 500. Reflection at a later date indicates that this sudden fall in titre was probably due to the use of a new batch of bacterial suspension for testing the serum. At the time the significance of this point was not appreciated, and it was assumed that the serum had deteriorated.

A fresh serum was accordingly prepared and issued, and between August, 1933, and December, 1934, twenty-three strains of P274 were identified in various laboratories.

Now comes the interesting point. Prior to August, 1933, a number of strains of "inagglutinable Flexner" had been received for investigation at Kasauli. When these were tested with the new P274 serum no fewer than thirteen of them agglutinated to the titre of the serum.

The possibility of these unexpected results being caused by mutation in the strains of P274 was considered, but was discarded in favour of the simpler explanation that the serum issued to laboratories had deteriorated even more quickly than that stored in Kasauli.

Some recent work has challenged this hypothesis in rather a striking way. Since the discovery of the Newcastle dysentery bacillus in India, gas-forming strains are being tested with a serum which is polyvalent for all the dulcite-fermenting strains, of which 88 (antigenically identical with Newcastle) is one. Two gas-forming strains which were agglutinated by this serum were discovered and presumed to be Newcastle bacillus, but when these were tested with monovalent serum it transpired that they were clumped, not by 88, but by P274 serum.

This problem has been carefully investigated by Major F. G. A. Smyth, who will publish full details in due course. It has been found that P274 serum will agglutinate these gas-forming strains in much higher dilution than it will agglutinate its homologous organism; yet this is undoubtedly a heterologous agglutination, as absorption of P274 serum by the gas-forming organism, while it removes all agglutinins for the latter, has little effect on the agglutinin content of the serum for P274 itself.

These facts strongly suggest that the existing strain of P274 is a variant which contains two or more antigens, of which at least one was lacking in the original strain. The mutation which has taken place from the original strain bears points of resemblance to that which has occurred in B. dysenteriae Schmitz (Boyd, 1935), but there are certain anomalous findings which have still to be cleared up. All freshly isolated strains which agglutinate with P274 serum are now being collected and compared. Until the question is settled, the validity of the strains which have been named P274 is open to suspicion, and it is therefore premature to give any analysis of results.

The experience carries a moral which cannot be too strongly emphasized. It is this: No classification of dysentery bacilli is of real value unless it is founded on results obtained from recently isolated strains. Mutation during artificial life especially in the mannite fermenting series is by no means a rare occurrence, and may involve a change in the antigenic pattern of the organism which will completely invalidate any conclusions formed, in so far as they are applied to newly-isolated strains. This is no mere academic criticism, for many attempts at classification have gone astray through lack of appreciation of this point.

D1. - This organism has an antigen peculiar to itself and shows no cross-agglutination with other strains.

Thirty-seven of the 41 cases from which it was isolated were clinically bacillary dysentery. Two were severe, 5 were of moderate severity, 26 were mild, 4 are incompletely recorded, and 3 were menials undergoing routine exminations who showed no symptoms.

In 27 cases the organism was recovered from typical stools of blood and mucus showing microscopically bacillary exudate. In 7 cases the stools contained blood and mucus

or mucus, but microscopically showed indefinite exudate. In 3 cases information is not available.

On no accasion did the organism occur in association with other dysentery bacilli, but twice it was isolated in mixed infection with E. histolytica.

Agglutination tests with the patient's serum and the homologous organism were performed in 8 cases. Six were negative, in one agglutination occurred in a dilution of 1 : 85, and in another 1 : 250.

Although the evidence of serological response by the patient is not so full as might be desired; this is chiefly because of the small numbers tested. Points (1) and (2) are fulfilled.

D19. - This is a rare type which hardly merits individual attention, as it has been isolated on only five occasions in three years. The original strain was one of a collection made by Major W. Walker in Secunderabad. No isolations of this organism were made by the writer during his three years in Bangalore and Poona.

A late dulcite-fermenter, it is unrelated in its antigenic composition to any of the other types.

All five cases from which it was isolated were clinically bacillary dysentery. Two were moderately severe, and 3 mild; 3 showed bacillary exudate. D19 occurred in association with W in one case and with Sonne in another. These were the two moderately severe cases.

Agglutination tests were carried out with the patient's serum and the homologous organism in three cases: two were negative, but in the third (which was not one of the mixed infection cases) agglutination occurred in a dilution of 1 : 250.

It is perhaps worthy of note that this strain has well-marked agglutinogenetic properties. The evidence satisfied Gardner's first three points, but is too scanty to permit of definite conclusions being drawn.

Atypical mannite-fermenting strains (excluding lactose-saccharose fermentors).

Fifty-seven strains of this kind were isolated. Of these, twenty-three were dulcite fermenters, and were not sent to Kasauli. They were not specially investigated, but there is good reason to believe that a proportion were B. alkalescens (Andrewes), although definite evidence on this point is not available.

The remaining thirty-four strains have been investigated by Lieutenant-Colonel R. F. Bridges at the Eneteric Laboratory, Kasauli, and it is by his courtesy that the following results are available.

(1) One strain proved to be the Newcastle bacillus already mentioned. Strictly speaking it should not be classed as atypical, but should be bracketed with 88.

(2) Thirteen strains were proved by agglutination and absorption tests to be identical with P143 one of the less common strains isolated in Poona.[1]

The following is an anlysis of the 13 cases from which P143 was isolated. All were typical cases of bacillary dysentery. One case occurred in Kasauli, 1 in Sialkot, 1 in Razmak, and the remainder in Quetta. One case was severe, 4 were moderate, and 7 were mild; information is not available regarding the remaining case.

The stools contained typical blood and mucus in all cases, and all except one showed bacillary exudate microscopically. In one case E. histolytica was discovered; in the remaining 12 no other organism of a pathogenic nature was found.

[1] See Journal of the Royal Army Medical Corps, 1932, Vol. 59, p.332.
The antiserum for all these strains was transferred to the Enteric Laboratory, Kasauli, to serve as a starting point for further investigations.

Agglutination tests with the patient's serum and the homologous organism were carried out in two cases and were negative.

Gardner's first two points are satisfied but other evidence of pathogenicity is not yet forthcoming.

(3) Five strains proved to have some antigenic relationship to one another, 2 being of one type and 3 of another, the 2 types showing a moderate degree of cross agglutination. Although neither was agglutinated by the usual diagnostic sera, the serum prepared from one of these types (the first) has considerable agglutinating powers against organisms of the V-Z spectrum. This type forms indol, and does not ferment dulcite. The other type (three strains) ferments dulcite, but does not produce indol, differing in this respect from B. alkalescens. All five were from typical cases of dysentery.

(4) The remaining fifteen strains bore no serological relationship to one another or to any known organism.

From the above data it is considered that 103, P119, 170, 88, and P288 may be accepted as being capable of causing bacillary dysentery. The evidence regarding D1, and more especially D19 and P143, is less complete but

nevertheless points definitely in the same direction. P274 is still sub judice.

SUMMARY AND CONCLUSIONS.

(1) A table is given which analyses the dysentery group bacilli isolated in the military laboratories of India in 1932, 1933 and 1934.

(2) Dysentery group bacilli isolated by the writer in Bangalore and Poona of types not described in "A System of Bacteriology" (1929) have proved to be widely distributed throughout India, and with few exceptions have been found only in the stools of cases of clinical dysentery.

(3) Using a system of classification in which these strains are included, it has been possible to identify practically all dysentery group bacilli isolated during this period. No further strains of any importance have been discovered.

(4) The evidence as a whole is in favour of the belief that the majority of these "new" strains (three of which have been found in other countries) are capable of causing dysentery.

ACKNOWLEDGMENTS.

My thanks are due to all officers, R.A.M.C. and I.M.S., who have worked in military laboratories during these three years, as it is only by their loyal co-operation that it has been possible to make this investigation. The final typing of the "new" strains was carried out by Lieutenant-Colonels Dunbar and Large, and Majors Hepple, Scales, and Mearns; the rather thankless task of attempting to classify the lactose-saccharose fermenting strains was undertaken by Major Hepple, and at all times advice and help of every description, together with all the diagnostic sera, were provided without stint by Lieutenant-Colonel Bridges, who also carried out further investigations of "inagglutinable" strains.

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A N N E X U R E IV.

THE ANTIGENIC STRUCTURE OF THE MANITOL-FERMENTING GROUP OF DYSENTERY BACILLI

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In the years 1929-31 a study was made of the mannitol-fermenting dysentery bacilli isolated from cases in the military hospitals of India (Boyd, 1931, 1932). Several new members of this group, previously classified as "inagglutinable", were identified by serological methods, and subsequently a scheme of identification based on these findings was applied throughout the military laboratories of India. Some of the results of this investigation have already been published (Boyd, 1936).

As it will be necessary to refer to these new types in this paper, an analysis of 4,856 strains isolated in the years 1932-5 is given in Table I. This table does not include late fermenters of lactose and sucrose, but, apart from these, embraces all the various types of mannitol-fermenting dysentery bacilli. It includes as far as possible every strain having these characters which was isolated in this period, and therefore offers a

TABLE I.

Analysis of all Mannitol-Fermenting Dysentery Bacilli (except Late Fermenters of Lactose and Sucrose) isolated in Military Laboratories in India in the years 1932-35.

Type of Organism	Number	Percentage
Andrewes' V-Z series ...	3686	75.9
Type 103	199	4.1
P 119	131	2.7
88	371	7.6
170	190	3.9
P 288	64	1.3
P 274	75	1.5
D 1	67	1.4
D 19	8	0.2
P 143	16	0.4
Type unknown	49	1.0
Total ...	4856*	

* Excludes 95 strains discarded before typing had been carried out.

reasonably accurate index of the frequency with which these types occur in India. As a temporary measure, pending the acceptance of a more accurate classification, the new types are named by the index number of the type strain.

In all cases identification was effected by biochemical and serological tests. The V-Z series includes all those which agglutinated with antiserums prepared from V, W, X, Y and Z races as defined by Andrewes and Inman: the others were identified by means of serums prepared from the type strains.

In the course of this work a hitherto undescribed form of variation was observed to occur in certain straihs. A superficial investigation of this phenomenon showed that it threw considerable light on the antigenic structure of the group as a whole, and probably gave a clue to the vexed question of classification. It was, therefore, decided to study the matter more closely.

MATERIALS AND METHOD EMPLOYED

Organisms

(Note.- For brevity, the familiar symbols of the Flexner group are freely used - e.g. V for B. dysenteriae Flexner V, etc. The National Collection of Type Cultures, Lister Institute, is referred to as "N.C.T.C.")

V: V Oxford (N.C.T.C.); V Lentz (Dr. Scott).

VZ: VZ Massom, VZ Stansfield (both N.C.T.C.); VZ/D 427 (Major Bensted).

W: W Cable (N.C.T.C.).

X: X Hughes (N.C.T.C.); X Kelly (Dr. Scott).

Z: Whittington (N.C.T.C.).

Y: Y Hiss Russell (N.C.T.C.); Y Lentz (Dr. Scott).

103: type strain: 103/P 166; 103/Mohd. Zaman; and others; all isolated in India.

P 119: type strain; P 119/493 (India).

88: type strain: 88/Q 6 (India).

Newcastle: Newcastle/Aberdeen (Dr. Scott).

170, P 288, P 274, D 1, D 19, P 143: type strains.

In addition, a large number of strains isolated by the writer, or received from colleagues in India and Egypt, and from Dr. W. M. Scott, Ministry of Health, have been used for confirmatory tests.

Antiserums

Antiserums were prepared by inoculating rabbits intravenously with graded doses of organisms either as broth cultures, or in the later stage of immunization as saline suspensions. In both cases the organisms were killed either by chloroform or by formalin. Owing to the variable agglutinin response of the rabbits, no standard course was followed. Injections were continued until a serum of suitably high titre was produced.

Suspensions for Agglutination

Agglutinable suspensions were prepared by growing the organism in broth for twenty-four hours. Thereafter 0.2 per cent formalin was added to kill the organisms and to act as a preservative, and the suspensions were standardized to a constant opacity.

Suspensions for Absorption

Suspensions for absorbing purposes were prepared by growing the organisms on Gordon's peaflour tryp-agar in Roux bottles, washing off with saline, killing and preserving with chloroform, and standardizing, after concentrating by spinning, to contain 100,000 million organisms per millilitre. Absorbing suspensions were prepared in bulk from 103 B, Y Hiss Russell, P 119 B, V Oxford, W Cable, X Hughes and Z Whittington.

Absorption Tests

1 millilitre of serum was absorbed with quantities of organisms varying from 50,000 millions to 500,000 millions (i.e. 0.5-5 millilitres of absorbing suspension), the whole being made up to a volume of 10 millilitres with normal saline. The mixture was kept in the water-bath at 50° C. for four hours, then placed in the incubator overnight, and if necessary, centrifuged next morning. Controls containing no suspension were placed in the water-bath and

incubator for the same time as the actual tests; these are the controls shown in the tables.

Agglutination Tests

Agglutination tests were performed by Dreyer's technique. The tubes were incubated at 50° C. for four hours, and the results were at once read against a special dark background, using a hand lens to determine the finer degrees of agglutination. Intermediate results were calculated by means of Dreyer's interpolation table, slightly modified. Nil results indicate a titre of less than 1 in 10.

I. - ANTIGENIC VARIATION IN THE MANNITOL-FERMENTING DYSENTERY BACILLI

Type 103

Variation was first observed, and has been studied in most detail, in type 103. This is believed to be one of the original Y strains, probably Y Lentz, of which only group variants now exist.

When newly isolated, 103 did not agglutinate with antiserums prepared from the V, W, Z and Y races, and agglutinated very feebly with X antiserum. After some time in artificial culture, however, it was observed to produce two types of colony. One of these which may be called 103 A, was in all outward respects identical with

the colonies of the freshly isolated strain, being smooth in character, and virtually inagglutinable with antiserums of the V-Z series. It differed only in having acquired the power to reproduce both daughter colonies having its own characters, and variants of the kind about to be described. The second type of colony, which may be called 103 B, could be distinguished by its naked eye appearances, being larger than normal and somewhat rough in outline. These appearances were not, however, accompanied by the characters of roughness defined by Arkwright (1921): cultures from 103 B colonies grew with uniform turbidity in broth, while agar cultures could be evenly suspended in normal saline. Suspension of 103 B proved to be readily agglutinated by the V-Z antiserums. 103 B further differed from 103 A in that it bred true, producing only colonies of its own type; with one unexplained and doubtful exception, this has proved to be a permanent character.

In unselected subcultures from the original strain the variant colonies were at first scarce. In time their numbers increased, and ultimately they dominated the picture to the exclusion of the others. The process was, therefore, a steady progression from a pure 103 A culture to a pure 103 B culture.

Variation of this kind has occurred in the majority of the 103 strains which have been examined. In some cases

the variant appeared in a few days or weeks after the strain was isolated: in others it did not appear for some months; and in certain strains, now several years old, it has not yet been found. In a few strains the variants could not be distinguished by the appearance of the colonies, all of which were smooth and regular; they were to be found only by testing the agglutination reaction of a number of colonies.

The variation is illustrated diagrammatically in fig. 1.

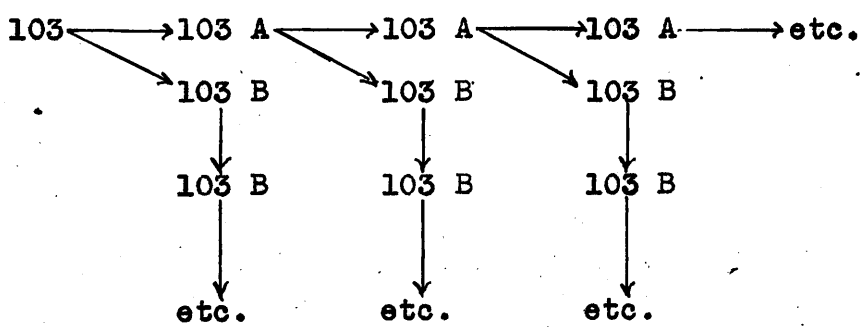

Fig. 1.

TABLE II.

Certain Agglutination and Absorption Tests with 103 A and 103 B.

	103 A	103 B	V	W	X	YHR	Z
103 B suspension agglutinated by antiserum	400	100.0	60	100	100	100	80
103 A " " " "	100	1.5	-	-	5	-	-
103 A antiserum against suspension	100	400.0	25	15	40	40	8
103 A antiserum absorbed 103 A ...	-	4.0	-	-	-	-	-
103 A antiserum absorbed 103 B ...	100	5.0	-	-	-	-	-
103 A antiserum absorbed YHR ...	100	5.0	-	-	-	-	-

Note - The figures in this table are percentages of the titre of the serum in question for its homologous organism.

Cross-agglutination tests with 103 A, 103 B and the V, W, X, Y, Z strains, and also certain absorption tests, are shown in Table II.

The high titre to which 103 B is agglutinated by the antiserums of the Flexner organisms is a striking feature, and indicates that it shares a common antigen with these strains. As far as 103 A is concerned, there is little evidence from the way in which it is agglutinated by these antiserums to suggest that it contains any of this shared antigen: on the other hand, its homologous antiserum has a well-marked action on the allied organisms, a result which indicates that it does in fact possess this antigen.

103 A antiserum, when absorbed by its homologous organism, loses all agglutinins except a trace of group agglutinin. (It may be remarked that the removal of the last traces of group agglutinin from high titre antiserums has always presented considerable difficulties in certain cases. This is probably due to some inherent property of rabbits' serum). When absorbed by 103 B, 103 A antiserum loses all group agglutinin (except these same traces), but retains its power to agglutinate the homologous organism.

From these results it may be concluded that 103 A contains two antigens: one, a specific or type antigen which is peculiar to itself; the other, a group antigen which it

shares with the V-Z series. 103 B, on the other hand, contains only group antigen; it is devoid of any power to absorb type agglutinin from 103 A antiserum, but readily removes the group agglutinin.

The somewhat conflicting observations that 103 A, while almost inagglutinable with group antiserum, is nevertheless capable of producing group agglutinins, may be explained in several ways.

(i) The supposedly pure 103 A suspension used for immunizing the rabbit may have contained a few 103 B organisms. Results similar to those in Table II have, however, been given by a 103 A strain of undoubted purity, which has now been under observation for six years without showing any variant colonies. This explanation, therefore, seems unlikely.

(ii) 103 A bacilli may contain in an accessible position a mixture of large quantities of type antigen and minute quantities of group antigen, the latter insufficient to be clumped by group agglutinin, but sufficient to give rise to agglutinins in the rabbit.

(iii) The type antigen in 103 A may be situated in a superficial or dominating position, while the group antigen may be more deeply seated and in some way protected or

inactivated by the type antigen. Under these circumstances, when the organisms are brought into contact with type agglutinin clumping occurs in the usual way, but when they are exposed to the action of group agglutinin, there is no reaction, as the agglutinin cannot obtain access to its antigen. On the other hand, when the organisms are inoculated into a rabbit, disintegration of the bacterial bodies occurs, accompanied by freeing of the antigens, each of which proceeds to give rise to its appropriate agglutinin.

There are points both for and against the last two hypotheses. The proportion of group agglutinin is so high in 103 A antiserum that it is difficult to understand how it can be produced by quantities of antigen so minute as to be insensitive to agglutination. Conversely, a suspension of 103 A is capable of absorbing practically all group agglutinin from its own antiserum; this is difficult to reconcile with the conception of a concealed group antigen inaccessible to the action of its antibody.

Another observation which has an important bearing on this question is that 103 B has more powerful agglutinogenic properties than 103 A, producing an antiserum with a much higher titre. While this might be explained by the fact

that in 103 B the group antigen is completely freed from type antigen and is thus of enhanced quality, the simpler explanation that 103 B contains an increased quantity of group antigen seems more probable.

As the two hypotheses are not antagonistic, it is probable that both play a part in the phenomenon.

These questions are of importance in reaching an understanding of the nature of the variation in 103. This is clearly a retrogressive process in which there is loss of type antigen, and coincident exaltation of group antigen. It is a general rule that in any process of degeneration the more highly specialized structures are the first to be lost, and it may be concluded that the type antigen, which is a distinctive character of the organism, falls into this category. In contrast with the loss of type antigen, the group antigen obtains prominence in the degraded variant, partly no doubt because of the removal of the protective or inhibitory influence of the type antigen, but also because there is an increase in the quantity of group antigen which may be in the nature of a replacement proliferation. This would suggest that group antigen is of a more primitive nature than type antigen.

This variation of 103 differs radically from the diphasic variation which occurs in the flagellar antigen of the Salmonella group. In the latter the process is

not accompanied by permanent loss of antigen. Reversion from one phase to another may occur at any time, and bacterial cells of both types, each capable of producing its own antigen, are present in all colonies although, according to the phase, one type or the other predominates.

A form of variation which occurs in certain strains of streptococci, notably types 3, 13 and 19, has been described in detail by Griffith (1935). This bears a close resemblance to the variation which occurs in 103. When Streptococcus type 3 was plated on an agar medium in which was incorporated about 2 per cent of homologous agglutinating serum, three types of colony could be recognized, one opaque, another translucent, and a third showing both opaque and translucent sectors. Serological investigation showed that the opaque colonies had type-specific characters, and that the translucent colonies had group characters, while the mixed colonies had both. In a plate made from an actively growing unselected subculture type-specific colonies were rare, group colonies rather more common, and mixed colonies in a majority. When a type-specific (i.e. opaque) colony was subcultured and replated, a more or less similar crop of colonies to that just described was produced. When a group (i.e. translucent) colony was similarly plated, it reproduced mainly translucent colonies, but a few mixed

colonies showing both clear and opaque areas appeared on the plate. Even after oft-repeated subculture from selected translucent colonies this mixed character remained, and from the tiniest opaque focus it was possible to produce on transference to a fresh plate opaque colonies which could be shown to be type-specific. The general tendency, however, was for type-specific cultures to assume group characters in artificial culture. Griffith notes the similarity of this variation to that observed in certain Flexner types (notably W and Y) by Dr. W. M. Scott. It also closely resembles the variation in 103. There is, however, one important difference. In the streptococci, as Griffith observes, there is the same fluctuation of phases which has been observed in the flagellar antigen of the Salmonella group; type specific colonies can be recovered from cultures of the group phase, and vice versa. In 103 the change is permanent; type-specific colonies cannot be recovered from the group phase.

There are also many points of similarity between the 103 variation and that which occurs in the melitensis-abortus group (Pandit and Wilson, 1932). Here a similar permanent loss of specific antigen occurs. Unlike "para-melitensis" and "para-abortus", the 103 variants, and other Flexner variants which will be described later, give negative results with the thermo-agglutination test, and with Millon's reagent.

It is worthy of mention that in the course of this investigation true "rough" variants of the kind described by Arkwright (1921) have occasionally been encountered in cultures of the Flexner races. These had the accepted characters of roughness, and seemed to be almost completely devoid of any antigen capable either of being agglutinated or of producing agglutinin when inoculated into a rabbit. In other words, the degeneration had proceeded a stage further, and both type and group antigen had been lost.

Y Hiss and Russell (YHR) and W.

In carrying out various investigations in connexion with 103, it was observed that the Hiss and Russell Y strain had absorptive properties almost identical with 103 B. This can be seen in Table II. At first the two were believed to be identical, but more recent work has shown that this is not so, and that, as Dr. W. M. Scott has suggested (personal communication) YHR is probably derived from a W strain, and embodies the group antigen of that organism.

Cross absorption tests with 103 B and YHR show that neither completely exhausts the agglutinin in the antiserum of the other.

On the other hand, absorption of W antiserum with YHR almost completely removes the group agglutinin which it

TABLE III.

Absorption of W Antiserum.

Antiserum	Suspensions						
	V	W	X	Z	103 B	YHR	P119 B
W, control	350	6000	1000	250	1250	10000	500
W, absorbed YHR (1×10^{11})* ...	250	2500	500	-	30	125	50
W, absorbed YHR (2×10^{11}) ...	50	1000	50	-	-	50	-
W, absorbed YHR (3×10^{11}) ...	30	1000	50	-	-	10	-
W, absorbed YHR (5×10^{11}) ...	30	1000	-	-	-	-	-
W, absorbed 103 B (5×10^{11}) ...	300	2500	500	-	10	3000	-
W, absorbed P119 B (5×10^{11}) ...	250	5000	750	-	250	3000	-

* Note - In this and subsequent tables, these figures denote the number of organisms with which 1 ml. of the serum has been absorbed.

contains (see Table III). Similar complete absorption is not effected by 103 B, nor by P 119 B, another group variant which will be described shortly.

In the process of absorbing W antiserum with YHR, there is a considerable reduction of the titre of the antiserum for its homologous organism W, suggesting that YHR contains some specific W antigen. This fall in specific agglutinin, however, reaches a point beyond which massive increases in the absorbing dose of organisms produce no further loss. It seems probable, therefore, that the fall in titre for W merely represents the elimination of group agglutinin which reacted to higher titre than did the specific agglutinin.

Absorption of the antiserums of the other members of the Flexner group with YHR gives results resembling those obtained with 103 B, and confirms that YHR is devoid of type antigen.

As far as the writer's experience goes, organisms having the characters of YHR have never been isolated direct from the stools of dysentery cases, and the resemblance which the colonies of this strain bear to those of 103 B suggests that, like the latter, it is a variant. Up to date, no colonies exactly similar to YHR have been recovered from known W strains, but investigations on this point are incomplete. Colonies which show a marked increase in group antigen and a

less obvious decrease in specific antigen can be recovered from certain strains of W, but no colonies completely devoid of specific antigen have so far been found.

There is, therefore, no conclusive proof that YHR is derived from a W strain, but there is a strong presumptive evidence that this is so.

Type P 119.

A similar form of variation was observed in type P 119 after this strain had been kept in artificial culture for approximately five years. When first observed this variation was diphasic, i.e. A and B colonies each produced A and B daughter colonies. The degradation progressed rapidly, and in less than a year no true A colonies could be recovered from any cultures of this organism.

The "rough" characters of the variant P 119 B are more marked than those of 103 B. In broth it grows with a well-marked deposit, but this when shaken up remains in suspension, so that no difficulty is experienced in carrying out agglutination tests. Cultures on agar form an even suspension when washed off with isotonic saline.

In the only other old strain of this type which is available there is no evidence of variation.

The results of absorption tests with P 119 antiserum and the three group strains are shown in Table IV.

TABLE IV.

Absorption of P 119 Antiserum.

Antiserum P 119	Suspensions							
	P 119 A	P 119 B	V	W	X	Z	103 B	YHR
Control	5000	1500	30	50	175	350	600	1000
Absorbed P 119 B (2×10^{11})	5000	15	-	-	-	-	25	35
Absorbed 103 B (2×10^{11})	5000	1000	-	-	10	-	-	25
Absorbed YHR (2×10^{11})	5000	1500	-	-	-	-	-	-

P 119 B almost completely exhausts the group agglutinin in the serum without appreciably affecting the type agglutinin. 103 B and YHR have a similar action, but fail to remove agglutinin for either P 119 A or P 119 B.

It may therefore be concluded that P 119 B has lost the type antigen, but retains the group antigen of the parent organism. This group antigen has much in common with the group antigen of 103 B and YHR, but has in addition an element peculiar to itself.

V, X and Z.

Variants devoid of specific antigen have not yet been isolated from V, X and Z, but colonies with decreased specific antigen and an apparent increase in group antigen are to be found in certain strains of V and Z. It is not at present possible to make a definite statement regarding X.

Takita (1937) has made some interesting observations regarding variation in these types. On examining certain old strains of V, W and Z, he found that two varieties of colony were present in plates made from these organisms. These colonies could be differentiated by agglutination with the antiserums of the group. One of the colonies, which Takita designated a, was agglutinated to high titre both by the antiserum of its own type, and by the group antiserums. The other colony, called b, gave a lower titre than a with the

homologous antiserum, and was only feebly agglutinated by the group antiserums. On sub-culture, a colonies reproduced only a colonies, whereas b colonies, produced both a and b varieties.

This variation, described in detail in the case of the strain V Lentz, can be shown diagrammatically (fig. 2).

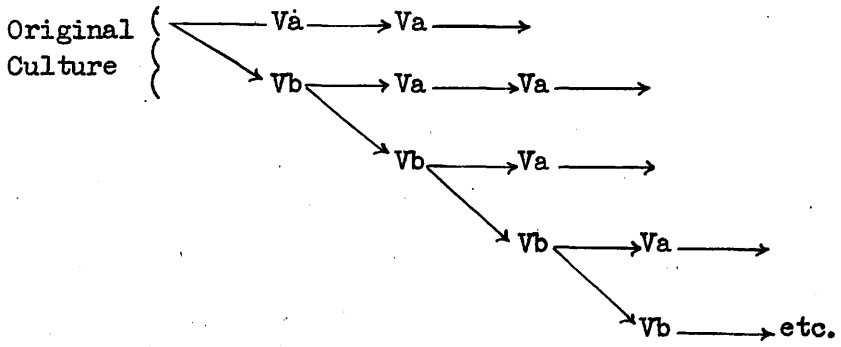

Fig. 2. (After Takita.)

Further experiments were carried out with antiserums prepared from V a, and V b substrains. It was found that the results were not easy to interpret, and were in no sense decisive, but it was concluded that the V a substrain contained both specific and group antigen, whereas the V b substrain had lost all or most of the group antigen.

From these findings Takita suggests that the main difference between the parent V strain and the V a substrain lies in the antigenic instability of the former (which produced both V a and V b forms) and the stability of the latter (which produced only V a forms); while the V b variant has lost, completely or almost completely, the group antigen, but shows a constant tendency to revert to the form in which group antigens are present. He regards this variation of the V b form as a phasic variation of an O antigen of the same type as that described by Andrewes as occurring in the flagellar antigens of the typhoid-paratyphoid group.

In qualification of his conclusions regarding the V a substrain, Takita remarks that the possibility of the original culture containing both a and b forms cannot be excluded. This is, however, much more than a mere possibility. This variation in V Lentz is of long standing, and while from the beginning of his investigation Takita had no difficulty in isolating a and b forms from plates made from his original culture, he produces no evidence of the presence of a third type of colony - the parent colony producing both a and b substrains. Nor, as b forms are constantly producing both a and b forms, does there seem any necessity to postulate the existence of a parent type of colony at this stage in the life of the culture.

While this does not alter the ultimate conclusions reached by Takita regarding the antigenic content of the substrains, it simplifies the interpretation of many of his results, and points to a more probable explanation of the variant he has observed. There can be little doubt that the b forms are the existing representatives of the parent strain, and that they have acquired in artificial culture the property of splitting off degraded variants, the a forms. It is of course possible, but unlikely, that this property of splitting off variants was a primary character of the strain.

Interpreted in this way the variation in V is closely comparable to that occurring in 103. The V b forms correspond to 103 A in that they contain type antigen and, possibly, concealed group antigen, and in that they give rise to similar daughter colonies and to variants. The V a forms correspond to 103 B in that they are rich in group antigen and reproduce only daughter colonies similar to themselves; they differ in that they retain a portion of type antigen.

This explanation of Takita's findings is incompatible with the idea that the variation he has observed is of the nature of a phasic variation of O antigen. On the other hand, if it is correct it confirms and extends the observations already made in respect of 103 by demonstrating

an analogous variation in other members of the group.

One further point is worthy of notice. The variants which appear in cultures of V and Z do not overgrow the parent type of colony in the same way as do the variants of 103 and P 119. Both types of colony have been flund in certain strains in which the variant retains some of the type antigen, as opposed to those in which type antigen is completely lost.

Type 88.

So far no antigenic variation has been observed in 88, a type which is of great interest because its antigen is identical with that of the Newcastle bacillus. There is, however, considerable evidence to suggest that the type K described by Sartorius and Reploh (1932) is derived from 88. On grounds of analogy it is to be anticipated that variants would be formed by type 88.

Other Types.

No variants have been found in cultures of 170 or of any of the other less common types.

Summary and Conclusions.

Variation is of common occurrence in strains of mannitol-fermenting dysentery bacilli when they have been maintained in artificial culture for some time.

The essential features of this variation are a loss, partial or complete, of type antigen, and an increase, apparent or real, of group antigen. The distinctive characters of the organism disappear and retrogression towards a type common to all races occurs.

The process of variation is of a similar nature in all races. Variants, whose characters are permanent, are split off from the parent strain, and are generally, although not always, to be recognized by their altered colony characters as well as by their altered antigenic content. The production of variants is scanty at first. In certain races which produce a variant devoid of type antigen, the process is rapidly progressive, and in time colonies of the parent type completely disappear. In others, where the loss of type antigen is incomplete, a state of balance seems to be reached, and in unselected subcultures both forms of colony occur over long periods of years.

Three strains which possess only group antigen, and are devoid of type antigen, have been examined. Two of these are of known parentage, having taken origin from types 103 and P 119 respectively. The third is the current edition of the historical strain Hiss and Russell Y, which is believed to be a variant of Andrewes and Inman's W. These three strains are very closely related to one another, but display minor differences in their antigenic pattern.

Considered as a whole, the changes which occur in the process of variation favour the hypothesis that type antigen is a recent and specialized individual character which occupies a superficial position or is relatively loosely associated with the bacterial body, while the group antigen is a more primitive and permanent character, more deeply seated in or more intimately blended with the body of the bacillus. Further, it seems reasonable to conclude that organisms which possess this group antigen are closely related to each other. This has, therefore, a very important bearing on questions of classification.

II.- ANALYSIS OF THE ANTIGENS IN THE MANNITOL-FERMENTING GROUP OF DYSENTERY BACILLI, AND ITS BEARING ON THEIR CLASSIFICATION.

Since the discovery of mannitol-fermenting dysentery bacilli at the beginning of the present century, repeated efforts have been made to classify these organisms and to define their relationship to one another. The most complete survey of the subject is that made by Andrewes and Inman (1919), whose classification of the group into the races V, W, X, Y, and Z has undergone little modification, and is still generally accepted as the basis of our knowledge on the subject.

Andrewes and Inman's conclusions may be summarized very briefly as follows: They consider that in these organisms

there are present at least four distinct antigenic components, all of which are represented in any given strain, but to a very different degree. In the races V, W, and Z there is a great preponderance of a single antigenic component, different in each case, together with a minor proportion of the components of the associated types. The race X refuses to agglutinate with any but sera of its own race, save to a trivial degree, but is able to give rise to a serum which will agglutinate X, Z, and V races. The true Y race is believed to be of more primitive antigenic structure than the others, and to present a mixture of V, W, and Z, and to a lesser extent X components: there is no evidence of a fifth, or Y, component. Subraces termed VZ and WX are also recognized.

Andrewes and Inman realized that the number of strains at their disposal was limited, and that in a more extensive collection further types would probably come to light. This has proved to be correct, and these strains, frequently referred to as "inagglutinable Flexner bacilli", have provided a recurring stimulus to the further investigation of this subject. Mention may be made of the work of Aoki (1921, 1923), who described twelve types of dysentery bacilli (including Shiga's bacillus): of Clayton and Warren (1929a,b) and Downie et al. (1933) who described the Newcastle-Manchester

bacillus: of Sartorius and Reploh (1933) who implemented the usual methods of study by investigating reaction to bacteriophage and described certain additional types: and of Waaler (1935), who studied "bacterial dissociation" in the group. These are but a few of the many approaches which have been made to the subject. In none of them is there any important departure from the general ideas enunciated by Andrewes and Inman, and the only new type described which has found general acceptance is the Newcastle-Manchester bacillus.

The recognition of type and group antigen in these organisms, brought to light by the experiments made with the variants of 103, suggested a different conception of the antigenic structure of the various members of the group. It seemed possible that, as suggested by Andrewes and Inman, each race possessed a distinctive type or specific antigen, but that co-agglutination resulted, not because of the presence in each of a minor proportion of the type antigen of the other races, but because each possessed, in addition to its own type antigen, a varying proportion of a common group antigen.

An early series of experiments was carried out by absorbing antiserums of V, W, X and Z, of relatively low titre, with 103 B. The results substantiated this hypothesis, and seemed to show that the common group antigen was present - in

TABLE V.

Cross-Absorption of 103 B, YHR, and P 119 B.

Serum	Suspension V	W	X	Z	103 B	YHR	P 119 B
103 B, control	75	50	125	2500	5000	5000	1000
103 B, absorbed 103 B ..	-	-	-	-	-	-	-
103 B, absorbed YHR ..	-	-	-	20	150	125	-
103 B, absorbed P 119 B	-	-	-	125	250	>250	-
YHR, control	25	50	75	500	1000	2500	600
YHR, absorbed 103 B ..	-	-	-	50	30	500	50
YHR, absorbed YHR ..	-	-	-	-	-	250	-
YHR, absorbed P 119 B ..	-	25	-	150	>250	1250	-
P 119 B, control	125	50	250	2500	2500	2500	5000
P 119 B, absorbed 103 B	-	-	-	-	-	-	1750
P 119 B, absorbed YHR ..	-	-	-	15	30	-	2500
P 119 B, absorbed P 119 B	-	-	-	-	-	-	-

its entirety - in 103 B. Subsequent experiments with high-titre antiserums, however, showed that in certain antiserums the group agglutinin is not completely exhausted by 103 B.

Cross absorption tests were than carried out with the three strains believed to be pure group strains, namely 103 B, YHR, and P 119 B. The results are shown in Table V.

None of these antiserums is completely absorbed except by its homologous organism, and even then traces of agglutinin remain, notably in Y. But whereas YHR almost completely exhausts the group agglutinin in 103 B antiserum and vice versa, P 119 B is less effective in absorbing 103 B and YHR antiserums.

It is therefore possible that 103 B and YHR share an antigen containing two components, which may be designated 1 and 2, of which P 119 B possesses only component 1. Each of the three has a residual component peculiar to itself, so that the content of each may be designated as follows:-

103 B: components 1, 2, 3.

YHR: components 1, 2, 4.

P 119 B: components 1, 5.

Of these components, 1 strongly predominates, and 2, 3, 4 and 5 occur usually in smaller quantities.

As will be seen later, absorption of V, W, X and Z antiserums confirms these findings, but reveals the presence

of yet another group component not found in any of these three group strains. This is present in V, X and Z, and may be called component 6.

Using the three group strains as absorbing agents for components 1, 2, 3, 4 and 5, and either X or Z for component 6, an analysis has been made of the group antigen present in the different races.

B. dysenteriæ Flexner V (Oxford).

The analysis of this strain will be given in some detail, to make clear the methods adopted.

The antiserum used in these tests agglutinated its homologous organism in a dilution of 1 in 20,000. Preliminary absorption tests were carried out with this serum diluted 1 in 10, and the results are shown in Table VI.

It will be seen that the maximum absorbing effect is usually produced by a dose of 100,000 million organisms. The titre for the homologous organism is reduced by about 50 per cent. The greater portion of the heterologous agglutinin is removed by 103 B and YHR, and to a lesser extent by P 119 B. Each removes the agglutinin acting on itself, the last traces of YHR going with reluctance. YHR and P 119 B exhause the group agglutinin for W more effectively than does 103 B. This is to be expected of YHR; the reason for its occurrence with P 119 B is not

TABLE VI.

Absorption of V Antiserum with 103 B, YHR, and P119 B.

Serum V diluted 1 in 10			Suspensions						
			V	W	X	Z	103 B	YHR	P 119 B
Control		..	2000	75	275	600	1000	2000	125
Absorbed 103 B	(0.5×10^{11})	..	1500	50	100	125	-	35	10
	(1.0×10^{11})	..	1125	35	85	85	-	35	35
	(1.5×10^{11})	..	1125	30	75	85	-	35	30
	(2.0×10^{11})	..	1125	25	75	85	-	35	25
Absorbed YHR	(0.5×10^{11})	..	1750	-	75	85	40	50	35
	(1.0×10^{11})	..	1000	-	75	85	35	35	35
	(1.5×10^{11})	..	1000	-	75	85	30	17	30
	(2.0×10^{11})	..	1000	-	75	75	30	-	30
Absorbed P 119 B	(0.5×10^{11})	..	750	25	125	150	>250	>250	10
	(1.0×10^{11})	..	1000	-	125	150	250	>250	-
	(1.5×10^{11})	..	1000	-	125	125	250	250	-
	(2.0×10^{11})	..	1000	-	125	100	250	250	-

TABLE VII.

Absorption of V Antiserum with Mixed Suspensions.

Serum	Suspensions						
	V	W	X	Z	103 B	YHR	P 119 B
V, diluted 1 in 10 absorbed: 103 B, YHR, P 119 B each 0.5×10^{11}	700	-	35	25	-	-	-
V, diluted 1 in 10 absorbed: 103 B, 0.75×10^{11}; YHR, 0.75×10^{11}; X, 0.5×10^{11}	500	-	-	-	-	-	-
V, diluted 1 in 2 absorbed: 103 B, 0.75×10^{11}; YHR, 0.75×10^{11}; X, 0.5×10^{11}	3000	-	-	40	15	50	50
V, diluted 1 in 2 absorbed: 103 B, 1.0×10^{11}; YHR, 1.0×10^{11}; P 119 B, 0.25×10^{11}; X, 0.75×10^{11}	2500	-	-	-	-	-	-

clear. In all cases a considerable residue of agglutinin for X and Z remains.

Absorption was next carried out with mixtures of various strains. The results are shown in Table VII.

When all three group strains are used simultaneously as absorbing agents the only group agglutinin left in the serum is that for X and Z, which in its turn is removed by the addition of X to the absorbing mixture.

For complete absorption of this V antiserum it is therefore necessary to use 103 B (group components 1, 2, 3), YHR (group components 1, 2, 4), P 119 B (group components 1, 5) and X (group components 1, 2, 6, and possibly others), V has therefore, all six components in its antigen. Of these, component 1 predominates.

Attention has already been drawn to the fall in the titre of the absorbed serum for its homologous organism. The explanation of this seems to be that the unabsorbed serum acts by virtue both of its group and of its type agglutinins, and has a higher group titre than type titre. After absorption, clumping occurs only to the titre of the type agglutin.

In this connexion the results of an absorption test of

the same serum, taken from the rabbit at an earlier stage in the process of immumization, are of interest. 103 B was used as the absorbing agent in a massive dose producing complete absorption.

TABLE VIII. - Absorption of Low Titre V Serum with 103 B.

Serum	Suspension V	W	X	YHR	Z
V, control	1000	25	75	1000	250
V, absorbed 103 B ..	1000	-	20	-	50

As can be seen in Table VIII, the titre of the serum for its homologous organism remained unaffected. It would seem that originally the serum had type agglutinin of as high a titre as group agglutinin, and that subsequent inoculation of the rabbit led to the production of group agglutinin in excess of type agglutinin. This has been a frequent experience. In general, type agglutinin never reaches a high level, and titres in excess of 2,500 are the exception. Group agglutinin titres, on the other hand, are much higher, and often reach a figure of 25,000.

Some interesting facts have emerged in connexion with the so-called VZ subrace, believed by Andrewes and Inman to contain relatively large quantities of the distinctive Z antigen as well as distinctive V antigen. Three strains were available for investigation, namely VZ Massom and

VZ Stansfield, which were used by Andrewes and Inman, and VZ D 427 isolated by Major H. J. Bensted in India. Tested with monospecific V serum prepared as above, and monospecific Z serum prepared along similar lines (see hereafter) the results shown in Table IX were obtained.

It will be seen that the VZ strains contain the type antigen of V, but not of Z. Their Z characters are presumably due to the presence of a large proportion of group component 6 which is found in V, X, and Z strains.

TABLE IX. - Agglutination of VZ Subraces with Monospecific Antiserums.

	Suspensions				
	V	Z	VZ	VZ	VZ
Serums	Oxford	Whittington	Stansfield	Massom	D 427
V (monospecific) ..	250	-	125	250	125
Z (monospecific) ..	-	125	-	-	-

B. dysenteriæ Flexner W (Cable)

The results of the absorption of an antiserum prepared from this strain are shown in Table III.

Except for traces of agglutinin for V, all group agglutinins are absorbed by YHR, which contains components 1, 2, and 4. Component 4 is present to a well-marked degree, as can be seen by the failure of 103 B to affect absorption. The proportion of component 2 is shown by the difference in the absorptive powers of 103 B (1, 2, 3) and P 119 B (1, 5).

It is inconsiderable.

Results obtained with two other different batches of W serum are substantially the same.

In one of these groups agglutinin for V was completely removed, in the others a trace remained. The reason for this occasional incomplete removal of agglutinin for V is not clear, but in view of its complete removal from some batches of serum, it seems doubtful if it can be attributed to the occurrence of specific V antigen in W. Whatever the explanation, the quantity is so small that it has no practical significance.

It will be observed that this particular strain, W Cable, contains a high proportion of group antigen. Recently isolated strains of W are usually poor in group antigen, and would hence be preferable for the preparation of type specific serum for diagnostic purposes.

B. Dysenteriæ Flexner X (Hughes).

Several attempts have been made to analyse the antigen of this type but the results have been unsatisfactory.

Andrewes and Inman remark that X strains can be agglutinated only by X antiserum, and react feebly with antiserums of the group, but that X antiserum has a high titre for the other members of the group. This latter observation

refers alike to artificially prepared rabbit antiserum and to the serum of human beings who have been infected with this organism.

The strain X Hughes now appears to be more amenable to group agglutination, and is well agglutinated by the antiserums of the group.

The following points have emerged from investigations made up to date.

X strains are not agglutinated by type specific agglutinins for V, W, Z, 103, P 119, 88, or any of the less common races: they are, however, clumped by group agglutinin, some strains reacting better than others in this respect. X Hughes produces an antiserum of high titre which is rich in group agglutinin, all of which is completely absorbed by the homologous organism. When absorbed as far as is possible with either 103 B or YHR, the titre of the serum for X is reduced to a low level, and varying amounts of group agglutinin remain, particularly for Z. The antiserum may in fact be left by this procedure with a higher titre for Z than for X. Absorption with 103 B and YHR, plus Z, gives a serum with a relatively low titre for X and an appreciable residue of group agglutinin. It has not yet been possible to produce a satisfactory monospecific antiserum.

It seems highly probable that difficulty has arisen because the cultures used for making X antiserum were group

TABLE X.

Absorption of Z Antiserum with Group Antigen.

Serum	Suspensions						
	V	W	X	Z	103 B	YHR	P 119 B
Z (diluted 1 in 2), control	-	-	75	2500	200	300	75
Z (diluted 1 in 2), absorbed 103 B (1×10^{11})	-	-	50	1000	-	-	-
Z (diluted 1 in 2), absorbed YHR (1×10^{11})	-	-	50	1000	-	-	-
Z (diluted 1 in 2), absorbed P 119 B (1×10^{11})	-	-	50	1000	50	25	-
Z undiluted, absorbed: 103 B, (1×10^{11}) X, (0.25×10^{11})	-	-	-	2500	10	10	-

variants poor in type antigen. A number of freshly isolated strains have recently been procured from India, and further investigations are being pursued.

It may, therefore, be concluded provisionally that X contains a distinctive type antigen, and group components 1, 2, and 6. Further investigations may supplement this list, and permit of the production of a pure monospecific serum.

B. dysenteriæ Flexner Z (Whittington).

Unlike antiserums prepared from V, W, and X, that prepared from Z contains relatively insignificant quantities of group agglutinin. A summary of absorption tests is shown in Table X.

Absorption is equal with 103 B and YHR, and less complete with P 119 B. From this it would appear that group components 1 and 2 are present. Agglutinin for X remains, and this can be removed by absorption with X, or with "VZ". This is presumably due to the presence of component 6. The group components present in Z are, therefore, 1, 2, and 6.

Type 103.

It has already been shown (Table II) that 103 B, which contains group components 1, 2, and 3, effects complete absorption of group agglutinin from the antiserum prepared from the parent strain.

Type P 119.

From this type also there has been isolated a group variant which contains all the group antigen found in the present strain (see Table IV). It contains components 1 and 5.

Type 88 and the Newcastle-Manchester Bacillus.

The close relationship which exists between Type 88, found in India, and the Newcastle bacillus (Clayton and Warren, 1929a, b), and the Manchester bacillus (Downie et al. 1933), found in the United Kingdom, was first pointed out by Scott (Whitehead and Scott, 1934). Two strains of 88, originating from Poona, form Sartorius' Group L.

The rather striking range of biochemical reactions shown by these different strains is set out in Table XI.

About one-third of the strains of 88 isolated in India have biochemical reactions identical with the Flexner races. The remaining two-thirds produce acid in dulcitol after some days' incubation.

The Manchester bacillus produces acid and gas in glucose, mannitol, and dulcitol, the reaction in dulcitol being delayed. Newcastle bacillus produces acid with a bubble of gas in glucose, and after some days may have the same action on dulcitol.

It is to be noted that all strains of these organisms

TABLE XI.

Biochemical Reactions of Type 88, Manchester Bacillus, and Newcastle Bacillus.

	Lactose	Glucose	Mannitol	Dulcitol	Sucrose	Indole
Type 88 (33% of strains)	-	Acid	Acid	-	-	-
Type 88 (66% of strains)	-	Acid	Acid	Acid (late)	-	-
Manchester bacillus	-	Acid and gas	Acid and gas	Acid and gas (late)	-	-
Newcastle bacillus	-	Acid and gas	-	Acid and gas (late)	-	-

TABLE XII.

Absorption of 88 Antiserum with Group Antigen.

Serum	Suspension							
	88	V	W	X	Z	103 B	YHR	P119 B
88, control	1250	50	500	400	400	2500	5000	1250
88, absorbed 103 B (1×10^{11})	1250	30	25	-	-	-	10	-
88, absorbed YHR (1×10^{11})	1250	-	-	-	-	-	25	-
88, absorbed P119 B (1×10^{11})	1250	50	50	50	50	250	600	50

TABLE XIII.

Absorption of Newcastle Antiserum with Group Antigen.

Serum	Suspensions								
	New.	88	V	W	X	Z	103 B	YHR	P119 B
Newcastle, control	1000	1500	125	50	250	250	1000	1000	125
Newcastle, absorbed YHR (2×10^{11})	1000	1000	-	-	-	-	-	-	-
Newcastle, absorbed 103 B (2×10^{11})	1000	1000	-	-	-	-	-	25	-

so far found are consistently indole-negative, whereas most strains of the V-Z series produce indole.

In spite of these very diverse and, in the case of the Manchester and Newcastle bacilli, undysentery-like biochemical reactions, the antigen of these three strains is identical. Each is capable of robbing the antiserums of the others of all their agglutinin.

The close antigenic relationship of 88 to the Flexner group was noted when the organism was first described. A similar serological relationship was found in the Newcastle bacillus by Clayton and Warren. This is confirmed for both by absorbing their antiserums with 103 B and YHR (see Tables XII and XIII). Each is in this way shown to possess Flexner group components 1 and 2, and possibly also 4.

Tests carried out with two other batches of 88 antiserum of different origin gave similar results. In both cases YHR effected virtually complete absorption of group agglutinin, whereas 103 B left a residue for V and W.

The group agglutinin in this particular Newcastle antiserum is completely removed by both 103 B and YHR, but in another serum which was tested, a residue of agglutinin for V and W remained after absorption with 103 B, while absorption with YHR was complete.

From a consideration of all these data, it seems clear that 88 and the Newcastle-Manchester bacilli are of one

antigenic type, which embraces a number of strains showing varied biochemical reactions. The non-dulcitol-fermenting form of 88 has all the characters - morphological, biochemical, serological - of the Flexner group, and it is possible that this is the original form from which have been derived strains showing variation in biochemical reactions, but maintaining an unchanged antigenic structure.

Types 170, P 288, P 274, D 1, D 19, and P 143.

These types, with two possible exceptions, do not show any appreciable degree of cross-agglutination either with each other or with any of the types previously described. This character has persisted through some six years of artificial culture, and may be taken to indicate a complete absence of the group antigen common to the Flexner group.

As exceptions to the above general statement, antiserums prepared from certain strains of P 288 and P 143 have shown a limited but suggestive degree of cross-agglutination with the Flexner group. It has not yet been possible to investigate this fully.

Cross-agglutination occurs between P 274 and B. alkalescens (Andrewes), and Aoki VII (received from N.C.T.C.). Both alkalescens and Aoki VII are agglutinated by P 274 serum to its full titre. Each removes its own agglutinin feom P 274 antiserum, but leaves behind agglutinin

for the homologous organism. Similar results have been found with certain gas-forming coliform organisms whose exact nature is unknown. There is little evidence to suggest that B. alkalescens and these gas-forming strains are capable of producing dysentery, and their relationship to P 274 is, therefore, of considerable interest.

Précis of Results.

The results of this analysis are summarized in Table XIV.

It will be seen that V, W, X, and Z, together with 103, P 119, and the 88-Newcastle-Manchester bacilli, contain both type and group antigen. Each has a distinctive type antigen peculiar to itself, which is not shared, even in the minor degree suggested by Andrewes and Inman, with any of the other strains: all contain the principal component of the group antigen: and the minor group components are scattered through the different types, each occurring in some and not in others. In contrast to these seven types, the remaining types shown in Table I each have a distinctive type antigen, but do not contain Flexner group antigen.

TABLE XIV.

Analysis of Antigen in the Mannitol-Fermenting Dysentery Organisms.

Organism	Type antigen	Group antigen components
V and VZ	Specific	1, 2, 3, 4, 5, 6
W	"	1, 2, 4
X	" (?)	1, 2, 6(?)
Z	"	1, 2, 6
103	"	1, 2, 3
P119	"	1, 5
88 Newcastle Manchester	"	1, 2, 4
170	"	nil
P288	"	nil (?)
P274	"	None of the above group components
D1	"	nil
D19	"	nil
P143	"	nil (?)

A considerable number of freshly isolated strains have been tested with type-specific serums from which group agglutinin has been absorbed. All fall clearly into one type or another, and none has been found to contain more than one type antigen. It has not been possible to confirm in the same extensive way the details of the analysis of group antigen components. In making this analysis only a limited number of strains (from one to three) of each type was used. It is possible that more extensive investigations may reveal differences in the quantity and arrangement of the minor group elements in certain of the types. Something of this kind has been seen in the V and so-called "VZ" strains. Differences of this nature are, however, unimportant and are not at variance with the general principles which have been enunciated.

Classification.

Reasons have already been given for regarding the possession of group antigen as a character of fundamental significance, indicating close relationship among the organisms in which it occurs. On these grounds the various types fall naturally into two groups. One comprises the seven organisms in which Flexner group antigen occurs.

The other, which can only be provisional, embraces those strains which have the biochemical reactions of the Flexner bacilli but which lack Flexner group antigen.

It is generally accepted, although on somewhat different grounds to those now put forward, that V, W, X and Z are closely related members of one group. There is little doubt that 103 is identical with one or other of the original Y strains, and that it was overlooked by Andrewes and Inman because variation had previously occured in those strains they examined; in consequence of this the specific element was missed and the variants were grouped with Y Hiss and Russell, itself a variant of W. Dr. W. M. Scott informs me that 103, which he believes to be identical with the original Lentz Y, is fairly common both in the United Kingdom and in other parts of the world. It occurs in Sartorius and Reploh's series where it is classified as Y 2. It has been recovered from Army cases in Egypt as well as India. Although P 119 does not appear to have been described previously in the literature of this country, it is not a newcomer, for it is identical with group G of Sartorius' series, which embraces two strains from Poona, eleven strains from Lagos, and Nos. IV and XII in Aoki's series. P 119 shows well-marked cross-agglutination with

the members of the V-Z series, and is therefore liable to be classed as a weakly agglutinating "Flexner" organism by anyone using only unabsorbed polyvalent serum for its identification. This, coupled with its apparent rarity in Europe, may account for the fact that it has been hitherto overlooked.

The only type whose introduction into this group may be regarded as controversial is the Newcastle-Manchester bacillus, which differs from the others in its biochemical reactions. It is, however, considered that the gap between these strains and the other members of the group is bridged by 88, which, while it has an antigenic structure identical with the Newcastle-Manchester organisms, has also the typical biochemical reactions of the Flexner group. It is considered that under these circumstances the evidence of relationship provided by the antigenic structure should overrule the less important irregularities of biochemical reaction.

It is suggested that this group of seven organisms should be regarded as an extended edition of the familiar Flexner group, and should retain the name. The additional members should, for purposes of identification, either be accorded further letters of the alphabet (103 has a strong

lien on the letter Y) or, preferably, all seven should be accorded roman numerals.

Little is at present known regarding the antigenic structure and relationship of the six types which make up the second group. Their distinctive type antigens make them easy to identify, and observations made in the six years since they were first recognized leave no reasonable doubt that they can cause dysentery.

The placing of these antigenically unrelated organisms in one group is a provisional measure which has little to justify it other than the general similarity of their biochemical reactions. Even this feature is inconstant, as four of them - P 288, P 274, D 1, and D 19 - are occasional late fermenters of dulcitol, though it is doubtful if this is of much significance. The relationship borne by one of these strains (P 274) to other organisms which apparently do not cause dysentery requires further investigation, and may ultimately lead to its removal from this group.

SUMMARY.

(1) Group variants devoid of type antigen, described in the previous section, provide material for ascertaining the various components of the antigen in the mannitol-fermenting dysentery bacilli.

(2) Flexner Y, as defined by Andrewes and Inman, is not a valid type. The strains which are regarded as being of this type, such as Y Hiss and Russell and Y Lentz, are old strains which have lost their type antigen and possess only group antigen. The reason why many newly isolated strains are identified as Y is because the antiserums used for their recognition contain more group than type agglutinin.

(3) The four Flexner types, V, W, X and Z, each possess a distinctive type antigen and share a complex group antigen. They do not, as suggested by Andrewes and Inman, possess minor quantities of each other's type antigen.

(4) 103, P 119, and the 88-Newcastle-Manchester series have also distinctive type antigens, and share the same group antigen with V, W, X and Z.

(5) It is considered that the existing Flexner group should be extended to include all organisms which have a type-specific antigen and share this common group antigen.

The three types in (4) should therefore be placed in the group and named accordingly.

(6) Six types which have the biochemical reactions of the Flexner group, and which have individual type antigens but no Flexner group antigen, are provisionally placed in a separate loose group.

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A N N E X U R E V.

THE LABORATORY DIAGNOSIS OF BACILLARY DYSENTERY.

Transactions of the Royal Society of Tropical Medicine and Hygiene, 1940, Volume XXXIII, pp. 553 to 571.

Bacillary dysentery is an acute inflammatory disease which affects the mucous membrane of the large intestine. The infecting organisms are almost invariably confined to the bowel, although in rare instances the blood stream may be invaded, usually as a terminal phase in fatal attacks. In acute cases laboratory investigations are therefore chiefly concerned with the recovery of the dysentery bacillus from the faeces; in more chronic cases evidence of infection is sometimes to be obtained by examining the patient's serum for the presence of specific agglutinins.

ISOLATION AND IDENTIFICATION OF THE DYSENTERY BACILLUS.

In discussing laboratory methods it is not proposed to enter into details which must be familiar to Fellows of this Society, and it will suffice to stress a few points which have been shown by experience to be of particular importance.

Collection of the Specimen.

First place must be given to the necessity for securing specimens early in the disease. In the acute case, the first few stools consist of brown faecal matter in which mucus cannot as a rule be found. These are followed by watery evacuations containing small shreds of thin mucus from which the infecting bacillus can almost invariably be isolated, sometimes in pure culture. Then comes the most characteristic stage in which frequent small stools are passed consisting almost entirely of sticky mucus. In some text books this mucus is described as resembling red-current jelly, but this is a description with which I cannot agree. The mucus is of a glairy whitish-yellow colour, like raw white-of-egg, and is flecked with streaks of blood. It may in some cases be bathed in blood, but when this is washed off it will be seen that the mucus itself is untinted. From stools of this kind the isolation of the organisms is also comparatively easy.

As the case progresses towards recovery the mucus becomes more viscid, and brown faecal material reappears in the stools. From now onwards recovery of the organism becomes progressively more difficult.

For the successful isolation of the dysentery bacillus it is essential that the specimens examined should be as fresh as possible. The dysentery bacillus is a delicate organism which is very quickly overgrown by Bacillus coli and other allied organisms, which are normal inhabitants of the bowel, and when the specimen is an hour or two old recovery of the pathogen is very difficult. The best arrangement is to have the bedpan containing the newly-passed specimen brought direct from the ward to the laboratory, so that the bacteriologist can himself select the most suitable portion. When this is impracticable, a selected piece of mucus should be preserved, until it can be examined, in a solution of glycerine, such as that recommended by Sachs (1939). This consists of 30 per cent. glycerine in normal saline, adjusted with sodium phosphate to a reaction of pH8, and tinted with phenol-red. This mixture is put up in 10 c.c. quantities in small screw-capped bottles, and should only be used if it is a pink colour. The development of acid, which is liable to occur in glycerine, and is fatal to the dysentery bacillus, is shown by a change in colour from pink to yellow.

In acute cases of dysentery it is usually neither necessary or desirable to obtain specimens other than from the patient's stools, although some report well on the suitability of material obtained by means of rectal swabs. In chronic cases positive results may sometimes be obtained, where other methods fail, by examining the bowel through the sigmoidoscope, and taking swabs direct from ulcerated patches.

Microscopic Characters of the Mucus.

Much can be learnt from the microscopic appearance of the mucus, and with a little experience it is possible to give a fairly definite diagnosis by this means alone. It is unnecessary to stain the mucus before examining it. All that need be done is to place a small portion on a slide, apply a coverslip, seal the edges with vaseline, and examine with a one-sixth objective. The characteristic feature is the remarkable cellularity, each field being closely packed with cells. The number of red blood cells varies, depending on the portion which has been selected: they are scattered throughout the field, and rarely occur in rouleaux. Exclusive of red cells, over 90 per cent. of the others are polymorphs, some of which

may show fragmentation of the nucleus: the remaining 10 per cent. is made up of macrophages (which may resemble amoebae, but should not be mistaken for them), eosinophils, small mononuclear cells, and shed epithelial cells. It is in fact a typical inflammatory exudate. Of course, pus from an abscess has more or less the same microscopic characters, and even an over-strong green-soap enema can produce a good imitation, but with the exercise of a little common sense these pitfalls can be avoided. In the later stages the cellularity of the mucus is less marked. Polymorphs are fewer and degenerating epithelial cells more common.

Isolation of the Organism.

For the isolation of the organism MacConkey's medium and litmus-lactose-taurocholate-agar are equally good: the choice is a matter of personal preference. The selected portion of mucus should first be thoroughly washed in a tube of sterile normal saline, and then well rubbed on to the surface of the medium, using a small pair of forceps or a stiff platinum loop. If the mucus is carefully washed as described, to remove any adherent faecal matter, there is little danger of producing too

thick a growth on the plate, for the organisms are relatively scanty. Attempts at plating from saline in which the mucus has been broken up are usually unsuccessful, and it is much better to rub the mucus directly on to the plate. The greater the area of medium inoculated, the better is the chance of success; if possible two three-inch plates should be devoted to each specimen.

After incubation overnight, dysentery colonies if present can usually be recognized by their non-lactose-fermenting properties and by their delicate translucent appearance. Selected colonies should be picked off the plate and seeded into three tubes of peptone water containing lactose, glucose, and mannitol respectively, and on to an agar slope. Next day the biochemical reactions, supplemented by an agglutination test with organisms washed from the slope, will complete the identification.

Reference to the various diagnostic serums which should be used will be deferred until the classification of dysentery bacilli has been discussed.

CLASSIFICATION OF DYSENTERY BACILLI.

The identification and classification of the different organisms capable of causing bacillary dysentery, which began with Shiga's important discovery in 1898, has been a gradual process and has not yet reached finality. From time to time in the last 40 years new types of suspected dysentery bacilli have been described, and in most cases there has been a good deal of healthy scepticism before their claims have been admitted. This attitude has been adopted because there is no infallible method by which their pathogenic action can be assessed, and because prolonged investigation is usually necessary before a sufficient weight of evidence accumulates to prove or disprove the case.

Criteria of Pathogenicity.

It will be recalled that Koch's postulates on this question are, briefly, that the organisms should be present in all cases of the disease, that it should be capable of being isolated and maintained in pure culture, and that it should reproduce the disease in susceptible animals. In the case of dysentery bacilli, these postulates are difficult to satisfy. As numerous types of

bacilli cause dysentery, the first postulate can never be satisfied as regards any one type; the second is readily fulfilled but in itself is unconvincing; and for the third, animal inoculation in most cases affords no reliable evidence, while the infection of the human subject with cultures is not a practical proposition, though in certain cases accidental laboratory infection has provided evidence of this kind. There are, however, certain characteristics which lend strong presumptive evidence of pathogenicity.

First is the period of the disease during which the suspected organisms are to be found in the stools. In the case of organisms of undisputed authenticity, such as Shiga's bacillus, this follows a very definite sequence. They are present in large numbers, sometimes in almost pure culture, in the early acute stages; they become less common and more difficult to isolate as the case advances; and when recovery ensues they disappear completely. When this sequence is reproduced by organisms whose biochemical and other characters suggest that they are dysentery bacilli, and when after careful investigation no other known pathogenic agent can be discovered, it must be

regarded as presumptive evidence of some value.

Second, the bacillus should not be present in the bowel, and so in the stools, of persons who are not suffering from acute or chronic dysentery. Observations on this subject have been made on a large scale in the military laboratories of India, where native cooks, table-boys, and others of this class are examined prior to engagement to ensure that they are not carriers of enteric or dysentery bacilli. In the course of 3 years I examined over 3,000 such men, making between 8,000 and 9,000 platings of their stools (Boyd, 1932). In no instance was a pathogenic dysentery bacillus, of any of the types to be described later, isolated except from men suffering from acute or chronic dysentery, in whose faeces cellular mucus could be found. This observation has in large measure been confirmed by others who, in the period which came under my control, made some 119,000 platings of the stools of 35,000 menials with similar results (Boyd, 1936). This constitutes a very massive control, the results of which cannot be lightly disregarded.

Thirdly, the development, and especially the progressive development, of agglutinins for the suspected organism in the serum of the patient during the course of

the disease is generally to be accepted as an indication that the defences of the body are being called into action to repel the attacks of an invading organism. This evidence is of value when the agglutinins are for the specific antigen of the bacillus concerned.

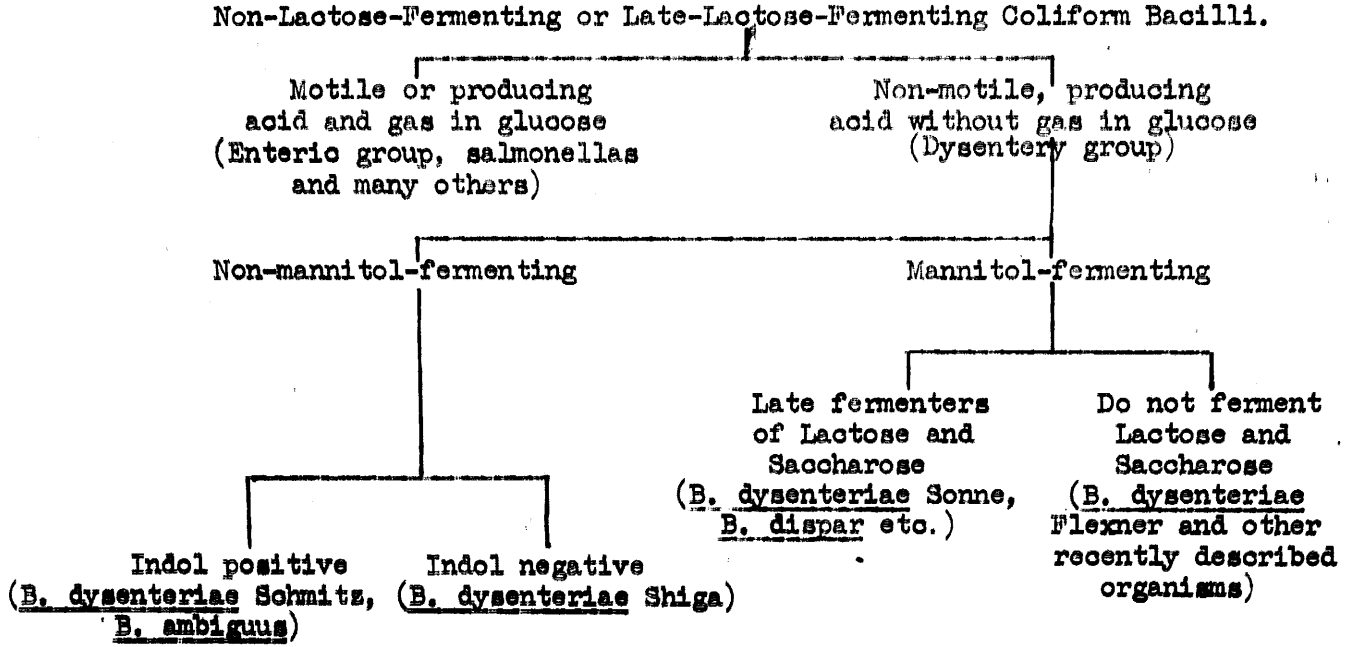
In addition to these three criteria, evidence has been afforded in certain cases by the occurrence of laboratory infections, and by localized outbreaks in which, in the absence of any other pathogenic agent, the suspected organism has been recovered from all or the majority of the cases in the outbreak.

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS OF DYSENTERY BACILLI.

Morphologically, organisms of the dysentery group are indistinguishable from each other. They are Gram-negative, coliform bacilli, non-capsulate, non-sporing, and non-motile.

Differentiation may be effected partly by their biochemical reactions, and partly by their antigenic characters. While their grouping must finally be determined by antigenic structure, it is fortunate that preliminary classification can be made by means of biochemical reactions, which enable them, as was pointed out by

TABLE I.


Non-Lactose-Fermenting or Late-Lactose-Fermenting Coliform Bacilli.
Motile or producing acid and gas in glucose (Enteric group, salmonellas and many others)
Non-motile, producing acid without gas in glucose (Dysentery group)
Non-mannitol-fermenting
Mannitol-fermenting
Late fermenters of Lactose and Saccharose (B. dysenteriae Sonne, B. dispar etc.)
Do not ferment Lactose and Saccharose (B. dysenteriae Flexner and other recently described organisms)
Indol positive (B. dysenteriae Schmitz, B. ambiguus)
Indol negative (B. dysenteriae Shiga)

Murray (1918), to be placed in four main groups. This is shown in Table I.

With one exception, to be detailed later, all dysentery bacilli are either non-lactose-fermenters, or late-lactose-fermenters, and produce acid without gas in glucose. They are divided into two main groups according to their action on mannitol.

Non-Mannitol-Fermenters.

B. dysenteriae Shiga is the most important member of the non-mannitol-fermenting group, and is the only dysentery bacillus known to produce an exotoxin. It usually causes a more severe form of the disease than do the other dysentery organisms. It possesses a distinctive type antigen and has no tendency to undergo variation other than, in old cultures, from smooth to rough.

B. dysenteriae Schmitz (B. ambiguus) is distinguished from Shiga's bacillus biochemically, by the fact that it produces indol from peptone, and serologically, by the possession of a distinct antigen. Contrary to statements made in certain textbooks, it has been my experience that these two organisms show no cross-agglutination whatsoever. In artificial culture Schmitz' bacillus occasionally

produces a variant of simpler antigenic structure than the parent organism (Boyd, 1936), a character which for some time gave rise to considerable confusion.

This organism was first described in detail in 1917 by Schmitz, but was probably known before this date. It was accepted as a dysentery bacillus by Kruse, who included it as Type J in his series. Murray (1918) found it among collections of strains on which he reported so ably, and it has since been recognized in many parts of the world. Evans (1938) found an outbreak of asylum dysentery in an institute in Wales to be attributable to this organism, and even more recently it has been recovered from other cases of asylum dysentery.

Schmitz' bacillus complies with the criteria previously given, and has the further qualification of having been recovered postmortem from dysenteric lesions in the bowel, and of apparently having caused a laboratory infection. (Hirschbruch & Theim, 1918).

In addition to these two recognized types, other organisms having the biochemical but not the antigenic characters of both Shiga's and Schmitz' bacilli are occasionally encountered. So far no conclusive evidence has been produced to show that these strains are pathogenic,

and in any case, their rarity renders them of little importance.

Mannitol-Fermenters

The mannitol-fermenting group is subdivided by the action of its members on lactose and saccharose, as some strains ferment these sugars after a few days' incubation, while others - the majority - lack this property. Some workers regard the late fermentation of saccharose as of doubtful significance, and believe the reaction to be variable, but after careful investigation I am quite satisfied that, working with recently isolated strains and pure saccharose, the results conform with the grouping I have given.

(1) Late Lactose-Saccharose Fermenters.

The only known pathogenic member of this group is B. dysenteriae Sonne, which is of common occurrence throughout the world. The newly isolated smooth organism has an antigen peculiar to itself. It rapidly undergoes mutation, producing a variant with a different antigenic structure, probably of the nature of a group antigen.

Other strains having the biochemical but not the antigenic characters of Sonne's bacillus are by no means uncommon, e.g., B. dispar. Most of them are almost devoid of agglutinogenic properties, and none of them in my experience has any definite relationship to dysentery. It is considered that they can safely be regarded as non-pathogenic.

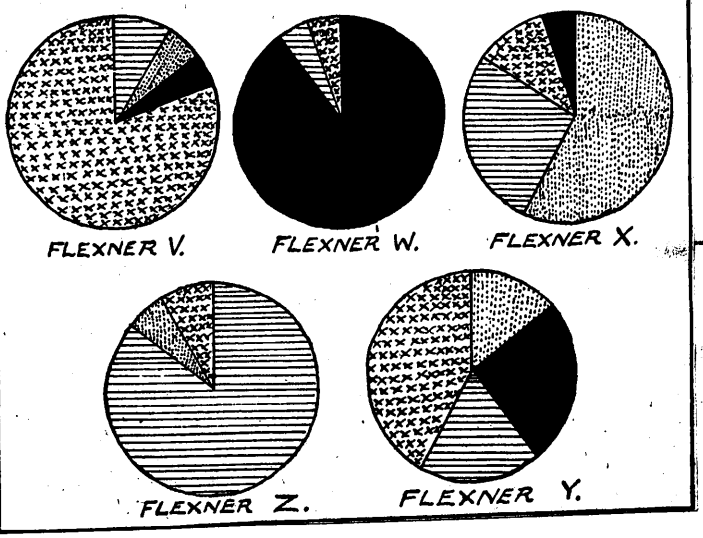
(2) Non-Fermenters of Lactose and Saccharose.

The mannitol-fermenting subgroup which does not acidify lactose and saccharose is generally known as the Flexner group, but it will be shown that this name is not sufficiently comprehensive.

The first member of this subgroup was discovered by and named after Flexner, and shortly afterwards allied but slightly different strains were described by Hiss and Russell, Strong and Musgrave and others. During the 1914-1918 war two diagnostic serums were in general use, one prepared from the original Flexner strain, and the other from the strain then known as Flexner Y. Experience in the Near East, in France, and elsewhere showed that a much more extensive range of antigenic types existed, and Andrewes and Inman (1919) collected and studied a series

FIGURE I.

Antigenic Structure of Flexner Group Bacilli.
(After Andrewes & Inman.)



of 200 strains, and divided them into five races, which they named V, W, X, Y and Z. Andrewes' conception of the antigenic structure of these races is of interest. It is shown diagrammatically in Fig. 1. (after Andrewes).

He believed that V, W, X and Z each possessed a predominating and distinctive antigen of its own, and also contained minor quantities of the antigens of the others. Y, on the other hand, was believed to be a more or less equal mixture of the antigens of V, W, X and Z, with no distinctive antigen of its own.

This work, which was accepted as authoritative, was a very great advance in the elucidation of the problem. Unfortunately, the collection of strains on which it was based was by no means exhaustive - it contained, for example, only one strain from India - and, further, it included strains which had been maintained for some time in artificial culture and which, as we now know, had undergone variation, a phenomenon not recognized at that time.

The inadequacy of the collection is shown by our findings in India. Table II is an analysis of organisms having the Flexner biochemical reactions isolated in

military laboratories in the years 1926 to 1930, and shows that more than a quarter of the Flexner-like organisms isolated were of different antigenic composition from Andrewes' races.

TABLE II.

Relative numbers of agglutinable and inagglutinable mannitol-fermenting dysentery bacilli isolated in military laboratories in India.

Year	Agglutinable	Inagglutinable	Percentage Inagglutinable
1926	271	129	32.25
1927	342	164	32.4
1928	492	306	38.2
1929	922	286	23.7
1930	1,077	360	25.4

It was a comparatively easy matter to make further observations of these inagglutinable strains along the lines followed by Andrewes, and in this way several additional races were identified, of which four were common, three were relatively common, and others were rare. (Boyd, 1931 and 1932).

TABLE III.

Analysis of 7,339 strains of Dysentery Bacilli isolated in the Military Laboratories of India in the Years 1932-1935.

	Non-Mannitol-Fermenters		Mannitol-Fermenters														
			Late Fermenters of Lactose and Saccharose		Non-Fermenters of Lactose and Saccharose												
						Other Types											
	Shiga	Schmitz	Sonne	Inagglutinable with Sonne Serum	V. W. X. Y. Z., etc. (Andrewes' Series)	103	P.119	170	88	P.288	P.274	D.1	D.19	P.143	Total of these other Types	Inagglutinable with any of these Sera	Not investigated
No.	1050	402	801	135	3686	199	131	190	371	64	75	67	8	16	1121	49	95
Per cent.	14.3	5.5	10.9	1.8	50.2	2.7	1.8	2.6	5.0	0.9	1.0	0.9	0.1	0.2	15.3	0.7	1.3

Table III is an analysis of all dysentery organisms isolated in military laboratories in India from 1932 to 1935, and shows the relative frequency of the different types. It must be emphasised that this is a "totalitarian" table and includes every organism of the dysentery group, as defined above, isolated from the stools of cases with symptoms of dysentery.

The identification of these types was, of course, only part of the problem. There remained the question of establishing their pathogenicity, and of determining their relationship to the recognized races and to each other.

Antigenic Variation in the Flexner Group.

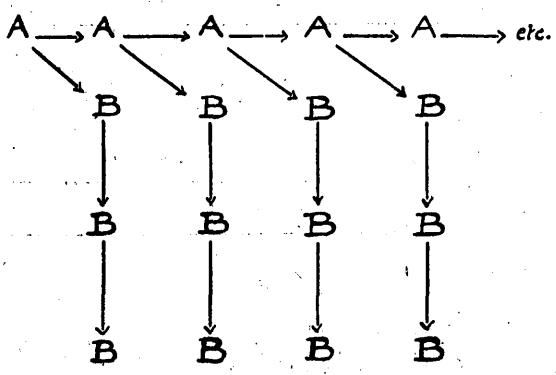
Fortunately, at an early stage in the investigation an observation was made which threw new light on the antigenic structure of these organisms, and enabled a more accurate and scientific form of classification to be adopted. It was found that during life in artificial culture a form of variation or mutation, involving a fundamental change in antigenic structure, frequently occurred in one of the "new" races, Type 103. In parenthesis it may be said that there is now no reasonable doubt that 103 is not a "new" race at all, but is the old

Lentz "Y", which in its original form was not included in Andrewes' series. This strain, when newly isolated, is not agglutinated by serum prepared from Andrewes' V, W, X, Y, Z series, although a serum prepared from 103 is able to agglutinate Andrewes' races to a relatively high titre. After a variable time in artificial culture 103 produces variants, which can be distinguished by their colony characters and which in antigenic structure are almost identical with the Y races in Andrewes' series, being readily agglutinated to a high titre by V, W, X, Y and Z antiserums. At this stage of its life, therefore, two types of colony are to be found on plating 103 - first the original type of colony, which on replating produces both itself and the variant, and secondly the variant, which breeds true and reproduces only colonies of its own type. Naming the original colony A, and the variant B, this process may be represented as in Fig. 2.

Absorption tests show that 103B is capable of abstracting all heterologous agglutinins from 103 serum, so that after absorption this serum will agglutinate only 103A. This is shown in Fig. 3.

FIGURE 2.

Diagram of Variation in Type 103.



Further, 103B and the Hiss and Russell Y strain, which is one of the Y type races in Andrewes' series, are found to be almost, though not exactly, identical. Like 103B, Hiss and Russell Y is also able to rob 103A serum of its heterologous agglutinins, and to render 103B serum almost inert, as shown in Fig. 4.

Not only is this so, but when serums prepared from Andrewes' V, W, and Z races (X will be discussed later) are absorbed with 103B, or Hiss and Russell Y, they, too, lose their heterologous agglutinins and become monospecific.

Subsequent observations have shown that similar variation occurs in all the Flexner races, though not always so complete as that shown by 103. There is, during artificial life, a constant tendency towards the production of variants possessing decreased quantities of specific antigen, and apparently increased quantities of group antigen.

In the light of these findings, Andrewes' conclusions regarding the antigenic structure of the Flexner group must be revised. Each race does not, as he suggests, contain a predominant quantity of a specific antigen and minor quantities of the specific antigens of the other races. Each does in fact possess a distinctive specific

FIGURE 3.

Agglutinin Content of 103 A. Serum

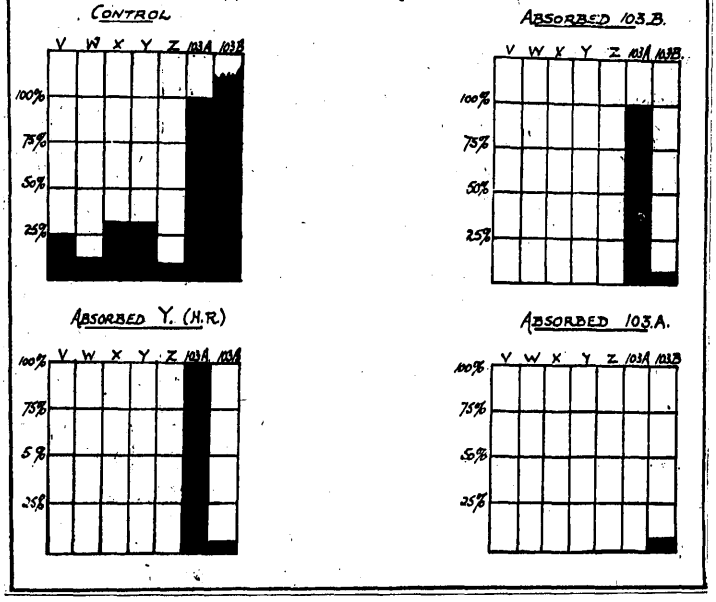
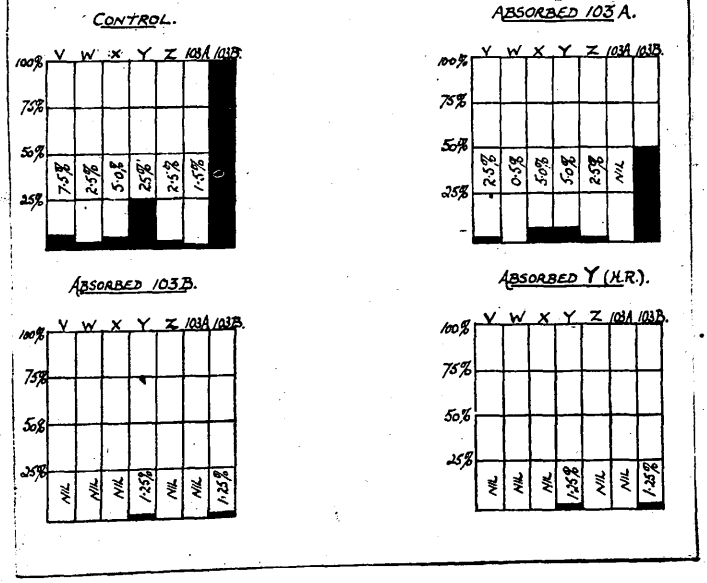


FIGURE 4.

Agglutinin Content of 103.B. Serum.



or type antigen, but all share a common group antigen which occurs, free from type antigen, in the variant 103B, and in Hiss and Russell Y, which, for reasons that need not be detailed here, is believed to be a group variant of W.

This is shown in very diagrammatic form in Fig. 5, in which no attempt is made to indicate the correct proportions of the different antigens.

More detailed investigations (Boyd, 1938) have shown that the group antigen is more complex in structure than was originally supposed, and contains several components, but this does not affect the argument and need not concern us further in this discussion.

To return to the question of classification: it is considered that membership of the Flexner group should be extended to, and limited to, those races which, in addition to possessing a distinct specific antigen, are also endowed with the common group antigen which has been described. The latter character constitutes a bond of relationship which is of much greater significance than less fundamental properties such as biochemical reactions.

Reviewed in this light, the Flexner group is found,

as far as is known at present, to consist of six members, three of Andrewes' series and three others.

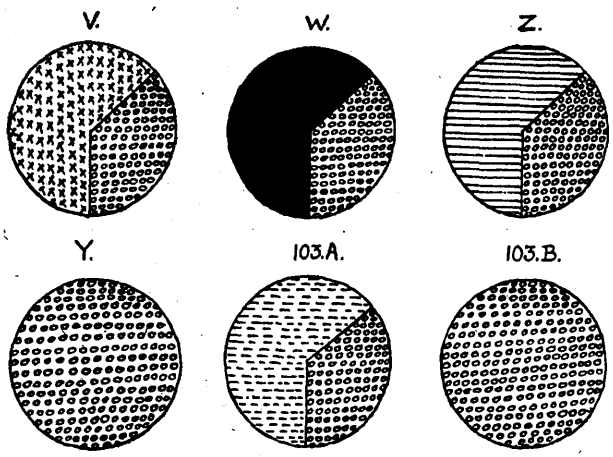
Andrewes' V, W, and Z races are valid types; each has its own type antigen, and all have varying quantities of the common group antigen, which, of course, accounts for the cross-agglutination which the races show. The so-called VZ type is a V race which has the type antigen of V, and an excess of a group component also found in Z.

Neither X nor Y are believed to be valid types. Y has already been discussed. After a long series of experiments, not yet published, it has been concluded that X is an incomplete variant of Z and not a separate race. It must, however, be mentioned that Andrewes' type strains of X, as maintained by the National Collection of Type Cultures, have now entirely different characters from those he described, and it is possible, though perhaps hardly probable, that there exists in Europe a race which has not found its way to India, and which is the original X described by Andrewes.

Type 103 is clearly a member of the Flexner group, although it differs somewhat from the previous three races in that the group antigen in the freshly isolated strain appears to be somewhat obscured, so that the organism has

FIGURE 5.

Antigenic structure of certain Flexner group organisms.



NOTE :- This is a simplified diagram which does not take into consideration the different components of the group antigen. For this reason X is omitted.

little tendency to be cross-agglutinated by the antiserums of the other members of the group. It has a wide distribution, having been found in this country, on the Continent, in the Near East, in West Africa, and, of course, in India.

P119 is another undoubted member of the group. Like 103, it produces a variant which is devoid of type antigen, but rich in group antigen. So far as I am aware this race has not been found in this country nor in Europe, but it occurs in the Far East, and figures as Type XII in Aoki's series. It has also been shown to be common in South Africa (Bevan, Personal communication.).

Type 88 also has a distinctive type antigen, and Flexner group antigen. This race is of particular interest to workers in this country. It differs from all other members of the Flexner group in that its biochemical reactions are inconstant. In India, it was observed that about two-thirds of the strains were late fermenters of dulcite, and its range of biochemical variations was further extended when Scott (1934) showed that its antigen was in every respect identical with that of the biochemically anomalous Newcastle dysentery bacillus, described by Clayton and Warren (1929, a and b), and also

TABLE IV.

Biochemical Reactions of Type 88, Manchester Bacillus, and Newcastle Bacillus.

	Lactose	Glucose	Mannitol	Dulcitol	Saccharose	Indole
Type 88 (33 per cent. of strains)	-	A	A	-	-	-
Type 88 (66 per cent. of strains)	-	A	A	A (late)	-	-
Manchester bacillus ...	-	AG	AG	AG (late)	-	-
Newcastle bacillus ...	-	AG	-	AG (late)	-	-

with the Manchester bacillus of Downie and Wade (1933). The range of biochemical reactions shown by this versatile organism are given in Table IV.

In the last few years strains having the Newcastle biochemical reactions have been found in India, and a few months ago a typical 88 was isolated from a case of dysentery in England by Lieut.-Colonel Leigh. This race has now been found in many parts of the world.

As regards pathogenic action, these three races, 103, P119, and 88, have an equal claim with V, W, and Z to be classed as true dysentery bacilli. They possess all the characteristics of the more familiar races, and would without doubt have been accepted by Andrewes had they been included in the collection he studied.

Of the remaining six Flexner-like organisms shown in Table III, with the possible exception of P143 none appears to possess Flexner group antigen, though each has a characteristic type antigen. Three of them can be regarded as capable of causing dysentery, but the evidence incriminating the other three is less definite.

170 is a relatively common race which fulfils the postulates made previously. Up to date it has not been reported except from India.

P288 is less common, but is nevertheless very definitely a true dysentery bacillus. In addition to complying with the postulates, it was on one occasion the cause of a small isolated outbreak which occurred in the Indian platoon of a British Regiment reported by Wilson (1936). Ten cases occurred, and this race was recovered in typical fashion from the stools of all ten; no other pathogenic organism was discovered.

D1 is also less common, but has all the characters of a true dysentery bacillus. It has recently been found in Sudan by Dr. Horgan of the Stack Laboratories (personal communication).

P288 and D1 are late fermenters of dulcite. At one time it was thought that this character might be used in classification, but this idea has been abandoned.

P274 has certain characters which favour its acceptance as a dysentery bacillus, but on the other hand it has a group relationship with B. alkalescens, Aoki's Type VII, and certain gas-forming strains which are probably non-dysenteric. It had better, therefore, remain sub judice until further evidence is forthcoming.

D19 and P143 are of such rare occurrence that they can at present be disregarded.

Besides these races which have been described, others having the Flexner biochemical reactions, but of distinct antigenic structure, are occasionally found. They have no clear relationship to dysentery, and are probably not pathogenic. In a recent review of these organisms, Bensted (1939) concurs in this opinion.

One type which deserves mention is B. alkalescens, originally described by Andrewes (1918), who believed it to be non-pathogenic. I have never found this organism to be associated with symptoms of dysentery, and in fact it is of rare occurrence in India. It seems, however, to be commoner in this country, and has recently been advanced by Nabarro and others (1939) as a cause of diarrhoea and dysentery in children. Judged by the standards already cited, Nabarro's conclusions must be accepted with caution, especially in view of the fact that B. alkalescens shares a highly agglutinable group antigen with a number of organisms of doubtful nature.

PROPOSED CLASSIFICATION OF THE FLEXNER GROUP.

I have from time to time suggested that some authoritative body should revise the classification of the Flexner group of dysentery organisms, but so far this hasnot been done. As I find that the meaningless index numbers by which my type strains were originally designated are being quoted in various publications, I am going to propose now a more convenient nomenclature. I suggest that two types should be recognized, namely, B. dysenteriae Flexner, comprising these races which possess Flexner group antigen, and B. dysenteriae Boyd[x], embracing those pathogenic organisms which have the Flexner biochemical reactions, but do not possess the group antigen.

The names would then be as shown in Table V.

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x In reading his paper the speaker had proposed the name of B. dysenteriae India for this type, but in the discussion which followed Major-General Perry urged that this should be changed to B. dysenteriae Boyd. The proposal has accordingly been adopted.

TABLE V.

Proposed Classification of Mannitol-Fermenting Dysentery Bacilli.

New Name			Old Name		
B. dysenteriae	Flexner	I	Andrewes and Inman		V
"	"	II	"	"	W
"	"	III	"	"	Z
"	"	IV	Type 103		
"	"	V	" P119		
"	"	VI	88-Newcastle-Manchester group		
B. dysenteriae	Boyd	I	Type 170		
"	"	II	" P288		
"	"	III	" D1		

Other races can if necessary be tacked on to these types when they are finally proved to be pathogenic.

It will be noted that all the organisms described fall within the dysentery group as defined in Table I. From time to time strains such as B. morgani, B. asiaticus, B. columbensis, and others of this nature have been described as causing dysentery. It has never been my experience to find these organisms in circumstances which made me suspicious of their role, and conversely I have frequently isolated them from normal stools.

IDENTIFICATION OF DYSENTERY BACILLI BY SEROLOGICAL METHODS.

It may be asked if it is necessary as a routine measure to determine the race of every dysentery bacillus isolated. This depends on the object in view. The information is of little value to the clinician, but may be of importance to the epidemiologist, bent on tracing the origin and spread of an outbreak. It may also be of interest to those concerned in the manufacture of therapeutic serum.

Our custom in the Army is to make a preliminary classification according to the fermentation of mannitol. For the non-mannitol fermenting group, antiserums for Shiga's and Schmitz' bacillus are provided, and a slide agglutination test suffices to distinguish the two.

For the mannitol fermenting group, three serums are supplied. One is anti-Sonne serum, and contains both specific and group components. The second is polyvalent for Andrewes' races, and the third for 103, P119, 88, and 170. The only reason for having two polyvalent serums for these races is that it is difficult to prepare one serum having a titre of 250 for all seven races; a low-titre serum capable of clumping them all could, however,

easily be made. In India, the arrangement of polyvalent serums is slightly different, and components which clump the less common races are also included. A selected central laboratory is supplied with serums for the indentification of the individual races. The most rapid and most certain way of typing the different races is by means of a series of monospecific serums, which can easily be prepared by absorbing out the group agglutinin. Using these, identification can be effected in a few minutes with a slide agglutination test, and the results obtained in this way are perfectly clear-cut and unmistakable.

SEROLOGICAL DIAGNOSIS OF BACILLARY DYSENTERY.

There remains to be considered the question of diagnosing dysentery by means of agglutination tests with the patient's serum. This is of little practical value in acute cases, as agglutinins do not develop for some time after the onset of the disease, when the patient is usually cured and back at work. In chronic cases, a definite result may be obtained when Shiga's bacillus is the infecting organism; a titre of 1 in 40 or over is usually accepted as indicating infection. The same

probably applies to Schmitz' bacillus and Sonne's bacillus, if care be taken to prepare the agglutinable suspension from the type phase of the organism. In the case of the Flexner group, the problem is beset with difficulties, first because of the multiplicity of strains, and secondly because it is almost impossible to prepare suspensions which are free from group antigen. At first sight it might seem that the problem could be solved by using a suspension of group antigen, but unfortunately serum from normal subjects frequently contains a considerable concentration of natural agglutinin for these group antigens, as can be seen in Table VI.

Strange to say, the same applies to normal rabbits - see Table VII. The diagnosis of Flexner infection as the cause of mucous colitis, chronic enteritis, and allied conditions, based on the power of the patient's serum to agglutinate Flexner suspensions, usually the so-called Flexner Y, has therefore, a very flimsy foundation. To be of any value, the test must be carried out with great care and with a much more elaborate technique than is usually employed. It is essential that, as a preliminary measure, the serum to be tested should be absorbed with

TABLE VI.

Agglutination results given by the serum of normal individuals who have never been abroad and who give no history of dysentery.

	V.	W.	X.	Z.	103B.	H.R.Y.	P119B.	Sonne B.
1.	-	-	-	30	30	150	25	125
2.	-	-	-	-	15	60	-	-
3.	-	-	-	50	40	175	-	-
4.	-	-	-	25	30	60	-	-
5.	-	-	-	25	20	75	-	25
6.	-	-	-	25	40	50	-	50

TABLE VII.

Agglutination results given by the serum of normal young rabbits.

	V.	W.	X.	Z.	103B.	H.R.Y.	P119B.	Sonne B.
1.	-	-	-	75	75	125	-	
2.	25	-	30	75	125	150	30	
3.	-	-	-	50	75	125	-	
4.	-	-	-	35	125	60	-	
5.	-	-	-	40	75	75	-	
6.	-	-	-	50	50	50	-	50
7.	-	-	-	25	20	15	-	-
8.	-	-	-	125	125	250 +	30	125
9.	-	-	-	50	50	125	-	150

pure group antigen, i.e., with strains such as 103B or Hiss and Russell Y. Thereafter any agglutination which the serum produces in suspensions of the different races will be specific in nature, and consequently of some diagnostic value.

If some of the statements I have made in this paper appear rather dogmatic, it is because lack of time prevents me from giving chapter and verse for the underlying reasons. The details will be found in the original articles quoted in the references.

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ANNEXURE VI.

LABORATORY FINDINGS IN CLINICAL DYSENTERY IN MIDDLE EAST FORCE BETWEEN AUGUST 1940 AND JUNE 1943.

Journal of Pathology and Bacteriology, 1946, Volume LVIII, pp. 237 to 241.

Middle East Force was, during the period covered by this communication, an extensive command, and had hospitals with their attached laboratories in Egypt, Sudan, Eritrea, Palestine, Syria, Cyprus, Libya, Cyrenaica, Tripolitania and Malta. The figures given in this paper are compiled from returns received from laboratories in all these countries, and can therefore be claimed to be a reliable cross-section of the dysenteric flora of this area. They were rendered in monthly reports submitted to the Deputy Director of Pathology, Headquarters, Middle East Force, between August 1940 and June 1943, when it was decided that it was no longer necessary to attempt the isolation of dysentery bacilli as a routine procedure in all suspected cases.

Methods

The methods used in the isolation and identification of dysentery bacilli were those taught at the Royal Army Medical College, which have been described elsewhere (Boyd, 1939-40). For the isolation of the organism, McConkey's medium or litmus-lactose-bile-salt-agar medium was used. Sodium desoxycholate was not available until

the end of this period and then only in limited quantities. Its use would probably have increased the percentage of isolations (though a controlled experiment showed that in the acute stages of bacillary dysentery its advantages are not so obvious as in the old or chronic case) but would have made no important difference in the percentages of the different types found. For serological confirmation of the identity of the dysentery-like organisms isolated the following antisera (all supplied by the Emergency Vaccine Laboratory) were used:- (1) Shiga, (2) Schmitz, (3) Sonne, (4) Flexner polyvalent 1 and 2.

Flexner polyvalent 1 serum was a mixture of sera prepared from Flexner types I (V), II (W) and III (Z) in quantities sufficient to give an approximate titre of 1 : 250 for each of these types. Flexner polyvalent serum 2 was a mixture of Flexner IV (103), V (P 119), VI (88-Newcastle-Manchester) and Boyd I (170). The last of these was included because this organism had been found to be of relatively common occurrence in India. Other types identified in India (Boyd, 1932 and 1939-40) were deemed to be too rare to merit inclusion in routine diagnostic sera, but in order to maintain a check on their occurrence, instructions were issued for unidentified dysentery-like organisms to be sent to the Central Pathology Laboratory, where mannitol fermenters were investigated by the author and non-mannitol fermenters by Major J. D. MacLennan.

Results

Tabel I is an analysis of the findings during the period under review. The figures include all cases investigated by laboratories, but do not represent the total incidence of dysentery in the force, as many cases were of necessity diagnosed on clinical grounds only.

TABLE I

Cases of clinical dysentery investigated between August 1940 and June 1943, showing isolations and percentages

	August to December 1940	1941	1942	January to June 1943	Total	Isolations as a percentage of total cases investigated	Percentages of various dysentery bacilli isolated
Total number of cases of clinical dysentery investigated	2381	22,578	31,991	8023	64,972		
B. dysenteriae Shiga	97	1375	2696	348	4516	6.95	18.86
Schmitz	75	455	917	154	1601	2.46	6.68
Sonne	35	567	822	342	1766	2.71	7.37
Flexner I to VI and Boyd I	755	4533	7855	1609	14,752	22.71	61.59
Other non-mannitol fermenters	2	311	143	50	516	0.79	2.16
Other mannitol fermenters	92	354	330	24	800	1.23	3.34
E. histolytica	130	1070	1554	1709	3463	5.33	...

The total number of dysentery bacilli isolated was 23,951. The percentage of isolations (including E. histolytica, 42.19 per cent.) is lower than that usually obtained in peace-time conditions, but this is the inevitable result of the "rush" which occurs during the dysentery season, when it is often impossible to examine more than one specimen from each patient or to devote more than half a plate of medium to each specimen. In practice, the isolation and identification of the organism is not in the majority of cases a matter of much importance to the physician. The experienced pathologist has no difficulty in making an accurate diagnosis of the type of dysentery (whether bacillary or amœbic) from the microscopic characters of the exudate, and no further differentiation is normally required for purposes of treatment.

It is of interest to compare these figures with those of the Army in India (table II). The Indian figures are from a series of 7,339 strains identified in the years 1932-1935. They do not include "other non-mannitol fermenters", but as these were of rare occurrence, the percentages are not significantly raised. The close relationship between the two sets of figures is remarkable.

The vast majority of the "missed" cases were bacillary and not amoebic dysentery. The latter is rarely a self-limiting disease, and, although temporary remissions may

occur in the absence of diagnosis and specific treatment, recurring relapses sooner or later draw attention to the real nature of the disease. For this reason, the true incidence of amœbic dysentery is unlikely to be much greater than

TABLE II

A comparison between the percentages of dysentery bacilli in India, 1932-1935, and Middle East Force, August 1940 - June 1943.

	Shiga	Schmitz	Sonne	Flexner I-VI and Boyd I	Other mannitol fermenters	Other non-mannitol fermenters
Army in India, 1932-1935	14.3	5.5	10.9	62.3	6.9	No record
Middle East Force, August 1940 - June 1945	18.86	6.68	7.37	61.59	3.34	2.16

that shown in table I, i.e. just over 5 per cent. of all cases. Its incidence was slightly higher in the Sudan and Eritrea than in Egypt and Palestine and tended to be greater in troops who had been in the country for some time.

Of the dysentery bacilli isolated, 94.5 per cent. were of recognised types and were readily identified by the standard antisera supplied by the Emergency Vaccine Laboratory. No attempt was made to identify the

individual types of Flexner bacilli, nor are accurate figures available to show the proportions which fell into the groups covered by the Flexner polyvalent 1 and Flexner polyvalent 2 antiserum respectively. It can be said, however, that a very considerable number were in the latter group, indicating that these more recently described types are of common occurrence in the Middle East as in India.

Atypical strains

An account of the atypical non-mannitol fermenters has already been published by MacLennan (1945). Of the 800 mannitol fermenters which were not identified by the standard antisera, a total of 109 strains were received at the Central Pathology Laboratory for investigation.

Fifty-five of these were of types already described (Boyd, 1932) and occurred in the following numbers:- Boyd II (P 288), 13; Boyd III (D 1), 2; P 274, 28; P 143, 5; D 19, 7. Type P 274 has now been shown to have a wide distribution, always in association with clinical bacillary dysentery. Originally described from India, it has been found in the Middle East as noted above, in Australia (Rothstadt et al., 1943) in America (Kuhns, 1943; Wheeler, 1944; and others), and recently a strain was

isolated in the Scottish Command Laboratory. It is therefore proposed that this type should be known as Boyd IV. For convenience, but with less justification, P 143 may be called Boyd V, and D 19 Boyd VI. No evidence other than that they have been isolated only from cases of dysentery can be produced regarding the pathogenic action of the last two types.

Eighteen others were found to be of one antigenic pattern, but subsequent investigation showed that they were not true dysentery bacilli, as they possessed a degree of motility. Their capacity to produce indol in peptone water excluded them from the salmonella group.

Antisera were prepared from several of the 28 residual strains, but only two of these reacted with strains other than the homologous organisms. The two types thus identified accounted for 10 strains, leaving 18 unidentified. By an unfortunate mischance one of these types (Rhemes), of which there were 4 strains, was lost.

The remaining type (1296/7), of which 6 strains were found, was passed on to Lt.-Col. A. E. Francis for further investigation. It was found to contain Flexner group antigen and therefore qualifies to take its place as a member of the Flexner group. Its detailed characters have

been described in a separate communication by Francis (1946) on two new Flexner types, in which 1296/7 is designated Shigella Flexneri, type VIII.

An analysis of these results is given in table III.

Table III

Analysis of 109 strains submitted as atypical mannitol-fermenting dysentery bacilli

Type	No.
Boyd II (P 288)	13
Boyd III (D 1)	2
Boyd IV (P 274)	28
Boyd V (P 143)	5
Boyd VI (D 19)	7
Flexner VIII (strains 1296/7) ...	6
Strain "Rhemes" (lost)	4
B. dispar. types	8
Not true dysentery bacilli (motile)	18
Unidentified	18

It is perhaps worthy of note that B. alkalescens, which by virtue of its biochemical reactions should have found its place in this series, is conspicuous by its absence.

Summary

1. 23,950 strains of dysentery-like organisms were isolated and investigated in the Military Laboratories in Middle East Force between August 1940 and June 1943.

2. 94.5 per cent. were serologically identified by means of the standard sera supplied by the Emergency Vaccine Laboratory.

3. Of the "atypical" non-mannitol fermenters, 50 of a series of 109 were of types which have already been described.

4. A new Flexner type (of which only 6 strains were isolated) has been identified and will be described as B. dysenteriae Flexner VIII.

My thanks are due to the many pathologists in the laboratories of the Middle East Force, without whose willing collaboration it would have been impossible to compile these figures, and to Sgt. J. Pilling, R.A.M.C., who assisted in the investigation of the "other" non-mannitol-fermenting organisms.

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