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

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(With 2 Figures in the Text)

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 4856 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

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* 1932–5

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.

isolated in this period, and therefore offers a 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

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from V, W, X, Y and Z races as defined by Andrewes & 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 strains. 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 methods 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 strains: 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 stages 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 24 hr. Thereafter 0·2% 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 concentration by spinning, to contain 100,000 million organisms per ml. 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 ml. of serum was absorbed with quantities of organisms varying from 50,000 millions to 500,000 millions (i.e. 0·5–5 ml. of absorbing suspension), the whole being made up to a volume of 10 ml. with normal saline. The mixture was kept in the water-bath at 50° C. for 4 hr., 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 4 hr., 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. Suspensions 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.

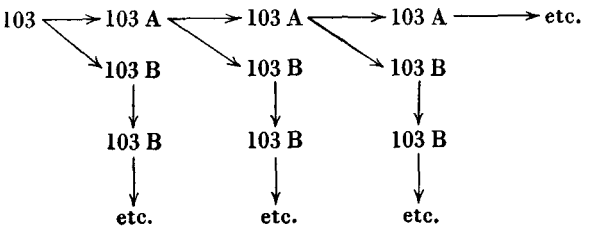

Fig. 1.

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.

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	60	100	100	100	80
103 A „ „	100	1·5	—	—	5	—	—
103 A antiserum against suspension	100	400	25	15	40	40	8
103 A antiserum absorbed 103 A	—	4	—	—	—	—	—
103 A antiserum absorbed 103 B	100	5	—	—	—	—	—
103 A antiserum absorbed YHR	100	5	—	—	—	—	—

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

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 anti-

serum 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 6 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 in 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 group 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% 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 & 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 103B. 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 103B 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 contains (see Table III). Similar complete absorption is not effected by 103B, nor by P119B, another group variant which will be described shortly.

In the process of absorbing W antiserum with YHR, there is a considerable reduction in 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

Table III. *Absorption of W antiserum*

Antiserum	Suspensions						
	V	W	X	Z	103 B	YHR	P119 B
W, control	350	6000	1000	250	1250	10,000	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 in subsequent tables, these figures denote the number of organisms with which 1 ml. of the serum has been absorbed.

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 strong presumptive evidence that this is so.

*Type P*119

A similar form of variation was observed in type P119 after this strain had been kept in artificial culture for approximately 5 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 P119B are more marked than those of 103B. 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 P119 antiserum and the three group strains are shown in Table IV.

P119B 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 P119 A or P119B.

Table IV. *Absorption of P119 antiserum*

Antiserum P119	Suspensions P119A	P119B	V	W	X	Z	103B	YHR
Control	5000	1500	30	50	175	350	600	1000
Absorbed P119B (2×10^{11})	5000	15	—	—	—	—	25	35
Absorbed 103B (2×10^{11})	5000	1000	—	—	10	—	—	25
Absorbed YHR (2×10^{11})	5000	1500	—	—	—	—	—	—

It may, therefore, be concluded that P119B 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 103B 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 subculture, *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).

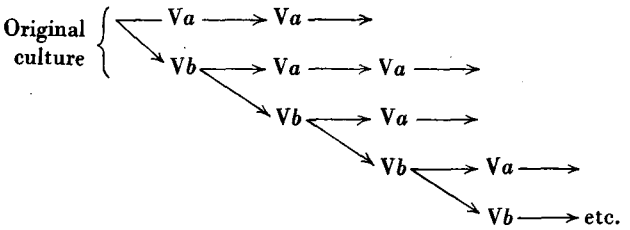

Fig. 2. (After Takita.)

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 investigations 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 variation 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 P119. Both types of colony have been found in certain strains over a period of years. This appears to be characteristic of 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 & 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 P119 respectively. The third is the current edition of the historical strain Hiss and Russell Y, which is believed to be a variant of Andrewes & 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 & 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 & 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 & Warren (1929*a*, *b*) and Downie *et al.* (1933) who described the Newcastle-Manchester bacillus: of Sartorius & Reploh (1932), 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 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 then carried out with the three strains believed to be pure group strains, namely 103 B, YHR, and P119 B. The results are shown in Table V.

Table V. *Cross-absorption of* 103 *B, YHR, and P*119 *B*

Serum	Suspension						
	V	W	X	Z	103 B	YHR	P119 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 P119 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 P119B	—	25	—	150	>250	1250	—
P119 B, control	125	50	250	2500	2500	2500	5000
P119 B, absorbed 103 B	—	—	—	—	—	—	1750
P119 B, absorbed YHR	—	—	—	15	30	—	2500
P119 B, absorbed P119 B	—	—	—	—	—	—	—

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, P119 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 P119 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.

P119 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. dysenteriae *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.

Table VI. *Absorption of V antiserum with* 103*B, YHR, and P*119*B*

Serum V, diluted 1 in 10	Suspensions V	W	X	Z	103B	YHR	P119B
Control	2000	75	275	600	1000	2000	125
Absorbed 103B ($0{\cdot}5 \times 10^{11}$)	1500	50	100	125	—	35	10
(1×10^{11})	1125	35	85	85	—	35	35
($1{\cdot}5 \times 10^{11}$)	1125	30	75	85	—	35	30
(2×10^{11})	1125	25	75	85	—	35	25
Absorbed YHR ($0{\cdot}5 \times 10^{11}$)	1750	—	75	85	40	50	35
(1×10^{11})	1000	—	75	85	35	35	35
($1{\cdot}5 \times 10^{11}$)	1000	—	75	85	30	17	30
(2×10^{11})	1000	—	75	75	30	—	30
Absorbed P119B ($0{\cdot}5 \times 10^{11}$)	750	25	125	150	>250	>250	10
(1×10^{11})	1000	—	125	150	250	>250	—
($1{\cdot}5 \times 10^{11}$)	1000	—	125	125	250	250	—
(2×10^{11})	1000	—	125	100	250	250	—

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%. The greater portion of the heterologous agglutinin is removed by 103B and YHR, and to a lesser extent by P119B. Each removes the agglutinin acting on itself, the last traces of YHR going with reluctance. YHR and P119B exhaust the group agglutinin for W more effectively than does 103B. This is to be expected of YHR: the reason for its occurrence with P119B is not 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.

Table VII. *Absorption of V antiserum with mixed suspensions*

Serums	Suspensions V	W	X	Z	103B	YHR	P119B
V, diluted 1 in 10 absorbed: 103B, YHR, P119B each $0{\cdot}5 \times 10^{11}$	700	—	35	25	—	—	—
V, diluted 1 in 10 absorbed: 103B, $0{\cdot}75 \times 10^{11}$; YHR, $0{\cdot}75 \times 10^{11}$; X, $0{\cdot}5 \times 10^{11}$	500	—	—	—	—	—	—
V, diluted 1 in 2 absorbed: 103B, $0{\cdot}75 \times 10^{11}$; YHR, $0{\cdot}75 \times 10^{11}$; X, $0{\cdot}5 \times 10^{11}$	3000	—	—	40	15	50	50
V, diluted 1 in 2 absorbed: 103B, 1×10^{11}; YHR, 1×10^{11}; P119B, $0{\cdot}25 \times 10^{11}$; X, $0{\cdot}75 \times 10^{11}$	2500	—	—	—	—	—	—

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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 103B (group components 1, 2, 3), YHR (group components 1, 2, 4), P119B (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 agglutinin.

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 immunization, are of interest. 103B was used as the absorbing agent in a massive dose producing complete absorption.

Table VIII. *Absorption of low titre V serum with* 103*B*

	Suspension				
Serum	V	W	X	YHR	Z
V, control	1000	25	75	1000	250
V, absorbed 103B	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 2500 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 D427 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				
Serums	V Oxford	Z Whittington	VZ Stansfield	VZ Massom	VZ D427
V (monospecific)	250	—	125	250	125
Z (monospecific)	—	125	—	—	—

B. dysenteriae *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 P119 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. dysenteriae *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, P119, 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 ab-

sorbed as far as is possible with either 103 B or HRY, 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 HRY, *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 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. dysenteriae *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.

Table X. *Absorption of Z antiserum with group antigen*

Serum	Suspensions						
	V	W	X	Z	103 B	YHR	P119 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 P119 B (1×10^{11})	—	—	50	1000	50	25	—
Z, undiluted, absorbed: 103 B, 1×10^{11}; X, $0{\cdot}25 \times 10^{11}$	—	—	—	2500	10	10	—

Absorption is equal with 103 B and YHR, and less complete with P119 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 & Warren, 1929*a*, *b*), and the Manchester bacillus (Downie *et al.* 1933), found in the United Kingdom, was first pointed out by Scott (Whitehead & 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.

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)	—	—

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 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.

Table XII. *Absorption of* 88 *antiserum with group antigen*

	Suspension							
Serum	88	V	W	X	Z	103 B	YHR	P 119 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 P 119 B (1×10^{11})	1250	50	50	50	50	250	600	50

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 103B left a residue for V and W.

Table XIII. *Absorption of Newcastle antiserum with group antigen*

	Suspensions								
Serum	New.	88	V	W	X	Z	103B	YHR	P119B
Newcastle, control	1000	1500	125	50	250	250	1000	1000	125
Newcastle, absorbed YHR (2×10^{11})	1000	1000	—	—	—	—	—	—	—
Newcastle, absorbed 103B (2×10^{11})	1000	1000	—	—	—	—	—	25	—

The group agglutinin in this particular Newcastle antiserum is completely removed by both 103B and YHR, but in another serum which was tested, a residue of agglutinin for V and W remained after absorption with 103B, 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 6 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 P288 and P143 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 P274 and *B. alkalescens* (Andrewes), and Aoki VII (received from N.C.T.C.). Both *alkalescens* and Aoki VII are agglutinated by P274 serum to its full titre. Each removes its own agglutinin from P274 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 P274 is, therefore, of considerable interest.

Précis of results

The results of this analysis are summarized in Table XIV.

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(?)

It will be seen that V, W, X, and Z, together with 103, P119, 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.

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 have 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 already 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 occurred 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

—P288, P274, D1, and D19—are occasional late fermenters of dulcitol, though it is doubtful if this is of much significance. The relationship borne by one of these strains (P274) 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, P119, 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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