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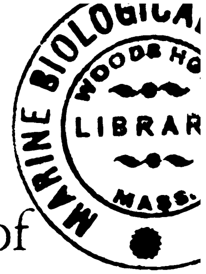

Serological Identification of Dysentery Bacilli*

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THE diagnosis and control of bacillary dysentery is becoming an increasingly important public health problem, especially during the war period.[15] The laboratory is concerned with the problem of diagnosis, and must provide a rapid, accurate, and detailed examination with a report to the control official as quickly as is compatible with accuracy. Because of the nature of the infection, laboratory diagnosis has depended almost entirely on the isolation and identification of the causative organism from fecal specimens. Cultures are seldom isolated from blood and urine; agglutination reactions with patient's serum have been of little use; and bacteriophage tests have value only under special circumstances.[16]

Organisms of the *Shigella* genus constitute a heterogeneous collection both as to physiological properties and antigenic structure. Although several species have recognized pathogenic action, only two types, *Shigella paradysenteriae* (Flexner) and *S. sonnei* are commonly encountered in bacillary dysentery in the United States.[15] *S. dysenteriae* (Shiga) and *S. ambigua* (Schmitz) are relatively rare, while *S. alkalescens* and *S. dispar* are generally considered of doubtful pathogenicity. Considerable evidence has accumulated, however, to show that under certain conditions *S. alkalescens* has distinct pathogenic action,[10, 13] and for the purposes of this discussion it will be considered with the pathogenic types. The Newcastle organism, although it differs somewhat in biochemical properties, will be grouped with the Flexner types.

Identification of the dysentery organisms depends on morphological, cultural, physiological, and serological characteristics. Biochemical reactions are valuable for a tentative identification and especially for separation of the *dysenteriae-ambigua* section from the mannitol fermenting types. Other biochemical properties (fermentation of lactose, sucrose, maltose, rhamnose, arabinose, dulcitol, xylose, sorbitol, and production of indole) are useful for differentiating *S. paradysenteriae, S. sonnei,* and *S. alkalescens* species. Occasionally epidemic strains can be identified by a physiological peculiarity. For type differentation within the paradysentery group, however, fermentation reactions are of little value. In other instances, such as lactose fermentation by *S. sonnei,* many days are required to complete the reactions. Serological methods offer the advantages of accuracy and speed and permit a more complete description than can be obtained from biochemical study. The slide aggluti-

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nation test with specific sera is a simple, rapid, and inexpensive method of serological identification. Within the Flexner group, especially, the advantages of this method are apparent when applied to strain typing.

The basis for the serological differentiation of paradysentery types is the work of Boyd[5, 6] who described six different sero-types each possessing a specific antigen but related by one or more common group antigens. These included the V, W, and Z strains of Andrewes and Inman,[1] the Newcastle or type 88 strain as well as two other types, 103 and P119. Six additional less frequently encountered paradysentery types (170, P288, P274, D1, D19, P143) also were described which possessed no group antigen or only minor antigens of the Flexner organisms. Our examination of the *S. paradysenteriae* strains has confirmed the presence of specific antigens and has identified nine components of the group antigen distributed among the various Flexner types.[17] An additional W serotype was defined by marked differences from the standard strain in group antigen, and epidemiological studies of this variety have shown it to be a valid type. The X and Y strains had no significant type-specific antigens and probably should not be considered as separate types but as variants of other types.

The nomenclature for the Flexner group proposed by Boyd[6] provides a simplification and clarification of terminology and a practical basis for identification. This terminology will be used in the following discussion. The V, W, Z, 103, P119, and 88 (Newcastle) types have been designated by Roman numbers I through VI. It seemed advisable[17] to divide the II or W group into two sections II_a and II_b on the basis of differences in group antigen. Departures have been made from Boyd's scheme in assigning arabic numbers to the components of the group antigen. Four of these components have been used in the strain typing methods although all are not essential for identification of the serological types. These fractions are designated by numbers as follows: 4 present in I, II_a, Y, and VI types; 5 in I and V types; 6 in I, III, and some IV types; 7 in II_b, III, V, and the old type X strains.

The other Boyd cultures gave some cross-reactions with the Flexner types,

TABLE 1

Differentiation of Types of Shigella paradysenteriae (Flexner group) by the Slide Agglutination Test

		Type-specific						Group Specific				
Antigenic Factor →		*I*	*II*	*III*	*IV*	*V*	*VI*	*4*	*5(V)*	*6(I)*	*6(III)*	*7(8)*
Serum →	*Group A (pooled)*	I	*IIa*	*III*	*IV*	*V*	*VI*	*IIa*	*V*	*I*	*III*	*IIb*
Adsorbed with Culture →		*III, IV V, VI*	*I Y*	*I, IIb IV*	*I III*	*I, IIb IV*	*I IIa*	*IIb*	*IIb*	*IIa, V VI*	*IIb*	*I IIa*
Tested with culture												
Type I —(V)	+	+	0	0	0	0	0	+	+	+	+	0
IIa—(W)	+	0	+	0	0	0	0	+	0	0	0	0
IIb—	+	0	+	0	0	0	0	0	0	0	0	+
X	+	0	0	0	0	0	0	0	0	0	0	+
Y	+	0	0	0	0	0	0	+	0	0	0	0
III —(Z)	+	0	0	+	0	0	0	0	0	+	+	+
IV —(103)	+	0	0	0	+	0	0	0	0	+*	+*	0
V —(P119)	+	0	0	0	0	+	0	0	+	0	0	+
VI —(88—Newcastle)	+	0	0	0	0	0	+	+	0	0	0	0

* Strains of Type IV isolated in the United States did not have the 6 fraction but usually agglutinated in the 3 and 4 group sera.

particularly type I. Type P274 had an antigen present in *S. alkalescens* and in some paracolon and coliform bacteria,[13] and the D19 strain shared a fraction in common with *S. sonnei.* Adsorption of these antibodies from the typing sera has simplified the interpretation of the slide test reactions.

The recognized human pathogens of the *Shigella* group are included in the typing scheme devised for diagnostic use. Included in the scheme are two specific sera for the mannitol negative members, *S. dysenteriae* and *S. ambigua,* and for the mannitol fermenting organisms, two pooled typing sera covering the two sections of the *S. paradysenteriae* group and antisera for *S. sonnei* and *S. alkalescens.* By the use of these six sera a tentative serological typing can be made even though fermentation reactions are not completed. If the culture belongs to either section of the paradysentery group, the antigenic type can be determined by testing for the type specific and group antigens with adsorbed typing fluids as shown in Tables 1 and 2.

Cultures used to prepare antisera were tested for smoothness before injection and stock strains have given satisfactory results. Although Glynn and Starkey [7] described two antigenic types of *S. sonnei,* the species is generally considered antigenically homogeneous.[15] In our hands Glynn and Starkey Types I and II cultures appeared to be identical as shown by reciprocal adsorptions. However, we have encountered two antigenically different variants of *S. sonnei.* The variation does not appear to be a " rough " change and for preparing typing sera a strain with broad antigenicity was selected. Antigenic analysis of the several Flexner strains has shown that the same group antibody usually can be obtained in satisfactory titer in antisera from two or three different types. The 6 group agglutinin common to Types I, III, and IV, for instance, could be prepared from each of the three antisera; and I and III sera are shown in Table 1. On the other hand, satisfactory titers for the 7 fraction of II_b, III, V, and the Andrewes and Inman Type X cultures have not been obtained in Type III antisera. Adsorbed typing fluids prepared from sera shown in the tables have proved satisfactory. Use of antisera made from X or Y strains and the use of these strains for adsorption offers some advantages since type-specific reactions are not involved. However, these antisera are not needed and the results presented are based entirely on the Flexner Types I–VI.

TABLE 2

Identification of S. paradysenteriae (Boyd types), S. sonnei and S. alkalescens by the Slide Agglutination Test

Serum →	*Group B (pooled)*	*170*	*P288*	*P274*	*D1*	*D19*	*P143*	*Sonnei*	*Alkalescens*
Adsorbed with →	*I, Sonnei, Alkalescens*	*I*	*I*	*Alkalescens*	*0*	*Sonnei*	*I*	*0*	*0*
Tested with culture									
S. paradysenteriae									
Type 170	+	+	0	0	0	0	0	0	0
P288	+	0	+	0	0	0	0	0	0
P274	+	0	0	+	0	0	0	0	±
D1	+	0	0	0	+	0	0	0	0
D19	+	0	0	0	0	+	0	+	0
P143	+	0	0	0	0	0	+	0	0
S. sonnei	0	0	0	0	0	0	0	+	0
S. alkalescens	0	0	0	0	0	0	0	0	+

METHODS

Antisera were prepared by injecting rabbits with formalinized (0.3 per cent) broth cultures at 5–7 day intervals in increasing doses until satisfactory agglutinin titers were reached (3–5 injections). *S. dysenteriae* were washed before injection. When titers of 1:5,120 or higher were reached or when no further increase occurred after additional injections the animals were bled and the serum preserved with 0.5 per cent phenol or by desiccation by the lyophile process.

Antigens for the slide test were made by suspending the growth from 18–24 hour agar slants in formalinized saline (0.3 per cent) and adjusting the density approximately to Gates 1.0. With most cultures of *S. alkalescens* it was necessary to heat the antigen at 60–70° C. for 1 hour before agglutination would occur. Since this degree of heating did not affect reactions of the other *Shigella* strains studied it was done as a routine procedure with all types.

The slide tests were performed on 5–7 in. glass slides divided by paraffin markings into sections approximately 1 x 2.5 cm. A total of 72 tests could be made on one slide. Paraffin rings as made for the syphilis slide tests (Mazzini, Kline) are also satisfactory. Equal quantities (about 0.02 ml.) of serum and antigen are added to the square, mixed, agitated mechanically or by hand with a rotary-rocking motion for 2 minutes, and then the degree of agglutination is read.

Standardization of the antisera must be done before they can be used for typing. *S. dysenteriae, S. ambigua, S. sonnei, S. alkalescens,* and the pooled sera were used without adsorption but in dilution sufficient to eliminate the minor cross-reactions as shown by tests with representative organisms of all the types. In each of the pooled paradysentery sera six types were included. The typing fluids were made by combining the individual sera and diluting according to titer. Final dilutions in the mixture varied from 1:10 to 1:100.

Type- and group-specific typing fluids were prepared by adsorption with appropriate cultures as indicated in Tables 1 and 2. Sera were adsorbed at 37° C. in 1:5 or 1:10 dilution with formalinized culture until the reactions of the adsorbed sera were specific. The completed typing fluids were preserved with 0.5 per cent phenol.

Methods employed in the Bureau of Laboratories for the isolation of enteric pathogens have been outlined previously.[2] SS agar and MacConkey's agar are used for isolation of *S. paradysenteriae* and *S. sonnei* strains. The classification of the organisms as determined by the agglutination test should be confirmed by fermentation reactions.

RESULTS

A summary of the cultures examined is presented in Table 3. Included in the total figures are stock collection strains received from a number of sources as well as recently isolated cultures of most of the types. The strains isolated in Connecticut and studied at the Bureau of Laboratories of the Connecticut State Department of Health since 1940 are listed separately to show the incidence of the various types in this locality.

Seventeen strains of *S. dysenteriae* have been identified by the slide test although none were isolated in Connecticut. Five cultures of *S. ambigua* were typed including one from a Connecticut case. The majority of strains were in the Flexner group, distributed among the I, II_a, II_b, III, IV, V, and VI varieties as indicated in the table. The frequency of Type III strains is high because of the large numbers isolated from one state institution where this type infection is endemic with occasional active outbreaks. If cultures

from this hospital are excluded the incidence of Type III cases was 27, or about the same as that for Types I, II_a, and II_b. One Type IV case was recently encountered within the state, and this type is present elsewhere in the United States since 8 strains received from Texas and 12 from Mississippi were identified as Type IV. One instance of Type V infection was recognized in Connecticut and one outbreak of Type VI (Newcastle) infection occurred from which ten isolations were made. Representatives of only two of the remaining Boyd types have been studied which were isolated in the United States. We identified ten strains of Type 170 and two of Type P274 isolated in Mississippi.

The incidence of *S. sonnei* types is high and 264 strains were identified of which about 90 per cent were isolated within the state. The figures for *S. alkalescens* does not represent the incidence of this species over the same time period since many strains that were isolated were not identified serologically.

A number of cultures which were tentatively classified as dysentery organisms on the basis of biochemical reactions could not be typed by the diagnostic sera. Some of these have been subsequently classified in the paracolon group, but a number remain, which by physiological tests resemble the pathogenic *Shigella types*. The Eijkman and trimethylamine reactions of some of these strains have been determined and are negative, similar to *S. dysenteriae* and *S. paradysenteriae* cultures.[12, 18] Some of these were mannitol-negative and may be like strains recently described by Sachs [11] isolated from cases of bacillary dysentery in India and Egypt. Other untyped strains were typical *S. paradysenteriae* as defined by *Bergey's Manual*, while some differed only by fermentation of xylose or dulcitol. Cultures identified by fermentation reactions as *S. ceylonensis* or *S. dispar* have not been included in Table 3.

DISCUSSION

The results show that satisfactory serological identification of the pathogenic *Shigella* organisms can be made

TABLE 3

Cultures of Shigella Classified Serologically by the Slide Agglutination Test

Shigella Type	*Old Designation*	*Number of Strains Examined*	*Number of Strains Isolated in Connecticut, 1940–1943*	*Number of Individuals with Positive Cultures in Connecticut, 1940–1943*
Dysenteriae	*Shiga*	17	0	0
Ambigua	*Schmitz*	5	1	1
Paradysenteriae I	Andrewes and Inman V	45	26	23
IIa	" " " W	68	45	37
IIb		27	23	21
	" " " X	16	0	0
	" " " Y	8	0	0
III	" " " Z	371	357	122
IV	Boyd Type 103	25	1	1
V	" " P119	6	1	1
VI	" " 88 (Newcastle)	18	10	10
	" " 170	13	0	0
	" " P274	5	0	0
	" " P288	3	0	0
	" " D1	2	0	0
	" " D19	2	0	0
	" " P143	2	0	0
Sonnei		264	237	198
Alkalescens		86	68	66
Atypical forms		22	..	..

by the slide test. The test is not as complex as might be assumed. The procedure outlined uses 16 or 17 immune sera for the entire group, but a great majority of the isolations could be identified with only 8 or 9 antisera. With the simple method of immunization the necessary antisera can be produced with little trouble. Titrations can be done in a short time, and the adsorption procedures for the preparation of type-specific and group sera are simple provided cultures of the proper antigenic structure are available. Antigens for the slide test are easily and quickly made and 1 ml. of typing fluid should be sufficient for nearly 50 tests. In our own laboratory we feel that the test is an excellent diagnostic aid. Furthermore, this method is similar to that used for *Salmonella* typing and can be incorporated into the routine procedure with little difficulty.

Strain identification in the detail that has been carried out may seem unnecessary and impractical at first thought. The majority of public health laboratories go only so far as to classify into the Flexner group, primarily because typing sera are not available. However, strain typing is essential if satisfactory epidemiology is to be done and detailed serological study is the only reliable means of typing. Occasionally a particular strain can be identified by its fermentative reactions; however, we have had strains of Type I, II_a, II_b, III, IV, and V which gave identical reactions in the usual differential media. Typing has been applied in the study of several small outbreaks of *S. paradysenteriae* infection. For instance, during an outbreak of Type III infection in a state hospital three strains of Type II were found. Two were II_b and one was II_a. Follow-up of these cases showed that the two II_b types were new transfers to the building where the Type III outbreak occurred. One was a recent admission to the hospital and the second had been associated with her for a short time in another section of the hospital. The Type II_a was isolated from an attendant. All three were unrelated to the active outbreak of Type III infection. Other instances similar to this have been encountered.

Boyd's scheme of labeling the paradysentery strains has a number of advantages, and provides a good working basis for classification. The differentiation is based on the presence of the specific antigen, and X and Y strains are not considered as separate types. The common relationship through the group antigen is shown for the Flexner Types I through VI. If marked and consistent differences in group antigen among strains having the same type antigen are found, the strains can be identified by a subgrouping of the type as we have done for the W or II group. Inclusion of the Type 88 or Newcastle organism with the Flexner group appears justified on the basis of serological structure. It seems logical that greater weight should be placed on antigenic structure than on physiological properties. Varieties which differ in gas production or fermentation of mannitol or dulcitol as do the Manchester, Newcastle, and Type 88 strains [6] can properly be considered as one type and included with the other Flexner organisms.

The essential differences between the types are the specific antigens, and therefore methods for typing should be concerned first of all with the detection of these type-specific antigens and secondarily with identification of the major group components. Methods utilizing unadsorbed sera and based on group reactions, such as that published recently by Hardy and associates,[8] overlook this fundamental difference between types. The use of adsorbed sera is imperative if satisfactory dis-

tinction is to be made between the type-specific and the group reactions. Furthermore, the various group components that are of differential value cannot be identified readily except by previous adsorptions of the antisera.

The possibility of loss of the type-specific antigen by variation to the group phase as described by Boyd has caused no difficulty in typing newly isolated Flexner cultures. On the other hand, it has been our experience that all of the newly isolated strains have contained some group antigen and the several group components could be identified in them. We have found variation in group components, however, particularly among the 103 strains. The 21 strains isolated in this country all have differed from the type culture since they did not have the principal 6 component of the type strain but agglutinated with the 3 and usually the 4 group antisera. This difference probably is a result of variation of the type strain.

Among the Flexner types we have found some biochemical differences which have correlated to a considerable degree with serological structure. Some of these seem worth mention. Type VI (Newcastle) and about 50 per cent of the II_a strains were the only indole-negative cultures encountered. Approximately two-thirds of the I and II_b strains, grown in 1 per cent tryptone broth, gave weak or trace reactions at 24 hours with Kovac's reagent and were negative with the Gnezda test. After 5–7 days' incubation, however, all gave distinct reactions by both tests. In contrast to all other types only 4 per cent of Type III strains fermented arabinose. The great majority of III cultures (97 per cent) fermented sorbitol, however, in contrast to the I, II, and IV strains which were uniformly negative. Reactions in sucrose, maltose, and rhamnose have been of no differential value.

On the basis of Boyd's findings we have included his additional types with the *S. paradysenteriae* group. They show little serological relationship to the Flexner types and some of them are slow fermenters of dulcitol or xylose. According to *Bergey's Manual* the xylose fermenting strains would be excluded from *S. paradysenteriae* species. However, they are similar in other characteristics and, considering their probable rôle in dysentery infection, should be included with the pathogenic paradysentery organisms. Furthermore, these strains resemble the other *S. paradysenteriae* in their Eijkman reaction and in their failure to reduce trimethylamine oxide.[12, 18] As yet little information is available on the presence of these types in this country. A low incidence is expected since Types P288, D19 and P143 comprised a total of only 1.2 per cent of the 7,339 strains studied by Boyd.[6] However, Type P274 has been reported in a food handler outbreak[9] and we have identified strains of P274 and 170 types. Possibly the position of the Boyd strains should be left undefined for the present, but it seems probable that the scheme of labeling the Flexner types should be extended eventually to include the type-specific antigens of the additional Boyd strains.

It is impossible that any practical classification of the *Shigella* group will include all types since new ones will be encountered from time to time. In Table 3 a number of strains are listed which could not be classified by the present scheme. Some of these were old stock strains in which variation had doubtless occurred; possibly some of the others will subsequently turn out to be additional pathogenic types. It may be pertinent in this connection to suggest that a service for laboratory study of *Shigella* cultures somewhat like that rendered by Dr. P. R. Edwards at the National Sal-

monella Center for the *Salmonella* group, for instance, would meet a need that is felt often by public health laboratory workers.

Not infrequently, coliform and paracolon cultures have shown antigens in common with members of the *Shigella* group. Usually the reactions were minor and occurred with unadsorbed sera. We have also obtained agglutination of many of the paradysentery strains with certain *Salmonella* sera. These reactions have not been thoroughly investigated but the reciprocal reactions of *Salmonella* cultures in *S. paradysenteriae* antisera have usually been negative, and they could not be attributed to the type or group components that have been identified in the paradysentery organisms. Antigens similar to the *Salmonella* VI and XIII components have already been noted.[4] A broad distribution of the antigens of *S. alkalescens* in coliform and paracolon types [13] and in the P274 strain also occurs. Numerous and broad antigenic relationships have also been described for *Salmonella* organisms and other nonpathogenic types.[3, 14] A similar overlapping of physiological characteristics has been pointed out between the various groups of the enteric organisms. The fact that organisms of the *Enterobacteriaceae* appear to be a continuous series of types without sharp demarcation either as to physiological or antigenic properties between the various sections, constitutes a problem to the " practical diagnostic bacteriologist." At present his best approach seems to be to use all available criteria for differentiation. Typical forms should offer little difficulty. It is up to him to recognize the atypical forms but the study of these can best be handled by the research laboratory.

SUMMARY

A method for serological identification of the pathogenic dysentery bacteria has been presented which utilizes a slide agglutination test employing formalinized antigens and titrated and adsorbed typing fluids. The test has the advantages of accuracy and speed, is similar to *Salmonella* typing methods, and can be readily incorporated into routine procedures.

Shigella organisms which have been identified by the slide test are *S. dysenteriae* (Shiga), *S. ambigua* (Schmitz), *S. paradysenteriae* (Flexner, Newcastle and Boyd types), *S. sonnei,* and *S. alkalescens*.

Within the paradysentery group, type identification is accomplished by the use of suitably adsorbed sera to show the type-specific antigens and certain group antigens of differential value. Type identification which can be done only by serological methods is essential for satisfactory epidemiological study.

The classification of the Flexner group proposed by Boyd in which V, W, and Z strains, Boyd Types 103 and P119, and the Newcastle organism have been designated as Types I through VI respectively on the basis of type-specific antigens provides a practical working basis for identification of the six types. It seems advisable to divide the II or W group into two sections since cultures have been encountered which had the same type-specific antigen but which were distinct epidemiologically and which exhibited marked differences in group components.

The Boyd Types 170, P288, P274, D1, D19, and P143 can be identified by their type-specific antigens. Some cultures have been encountered resembling pathogenic dysentery organisms which could not be classified into the recognized types by serological methods.

Results of the serological typings of 1,005 *Shigella* strains were presented. Strains isolated in Connecticut during a 3 year period included *S. ambigua, S. paradysenteriae* Types I, II_a, II_b, III, IV, V, and VI, *S. sonnei,* and *S.*

alkalescens. Strains of *S. paradysenteriae*, Boyd Types 170 and P274 were identified among cultures isolated in other parts of the United States.

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