THE ANTIGENS OF SHIGELLA PARADYSENTERIAE

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Introduction.

The genus Shigella, as defined in Bergey's "Manual of Determinative Bacteriology" (1), consists of gram negative non-motile rods which attack a number of carbohydrates with the formation of acid but no gas, and do not produce acetylmethyl-carbinol. It includes all of the bacteria which are definitely known to be capable of causing dysentery in man. Bergey's Manual subdivides the genus into species on the basis of the fermentation of certain carbohydrates, of which the most important are lactose and mannitol. S. dysenteriae, S. ambigua, and S. sonnei, three of the four Shigella species which are accepted as etiological agents of dysentery, are homogeneous both biochemically and serologically. The fourth, S. paradysenteriae, with which this paper is concerned, differs from the others in being not completely homogeneous biochemically and very heterogeneous in its antigenic characters.

This species, as defined in Bergey's Manual, includes the members of the genus Shigella which ferment mannitol and fail to ferment lactose, xylose and dulcitol. The first description

of bacteria possessing such biochemical characters was made by Flexner (2) in 1900. Within the next few years similar strains were described by Strong (3) and by Hiss and Russell (4). Because both of the latter strains differed from the one described by Flexner, both in their antigens and in minor biochemical respects, they were for many years considered separate species. The three were, by common consent, designated "Strong", "Hiss-Russell Y" and "Flexner" bacilli respectively. Later more careful serological analysis established the antigenic relationship between the strains, and their biochemical differences were found to be inconstant. The term "Flexner bacilli" was extended to include the "Hiss-Russell Y" and "Strong" bacilli, as well as other antigenically and biochemically related strains. It is now used commonly as a synonym of S. paradysenteriae, and will be used in this paper in that sense.

Because of the extreme diversity of antigenic and biochemical characters which are found among different strains included in the broad definition of the Flexner species, many attempts have been made to subdivide the species into biochemical or serological varieties or races. The attempts to create biochemical varieties were made chiefly early in the study of the group, and none was successful because of the inconstancy of the characters on which the varieties were based. Antigenic structure was found to be much more stable, to correlate better with

pathogenicity, and to demonstrate more satisfactorily the fundamental relationships between all of the strains of the species. For many years most studies of the classification of the Flexners have been based on serological relationships, with biochemical characters occupying a secondary place.

One of the earliest thorough analyses of the Flexner antigens was made by Andrewes and Inman (5) in 1919. They placed 93% of 116 strains in one or another of 5 serological races, which they called V, W, X, Y and Z. They also recognised 2 subraces, VZ and WX. All of the strains were represented as containing varying proportions of V, W, X and Z antigens, the races being named for the predominating antigen. The Y race was believed to consist of a fairly equal mixture of the antigens of the other races, without any specific antigen of its own. The position of the X race was left in question. It was classified as a separate race, but was not as clearly defined as the V, W and Z races. The VZ subrace differed from both V and Z in the relative proportion of the two antigens, but absorption experiments showed it to be more closely related to V than to Z. The WX subrace bore a similar relation to W and X. Andrewes and Inman believed that the classification made by them included the major antigenic factors found in their series of strains, but recognised the possibility that others might exist.

Several similar analyses were made by other workers, notably by Kruse (6) and by Sartorius and Reploh (7) in Germany, and by Aoki (8) in Japan. Most of the races described by these authors duplicated those of Andrewes and Inman, though each worker used a different system of nomenclature. Kruse and Sartorius and Reploh classified VZ as a separate race, distinct from both V and Z.

No new antigenic races were found which were generally accepted in the English-speaking countries until the work of Boyd (9). He found that of nearly 5000 strains of mannitol-fermenting, non-lactose-fermenting dysentery bacilli isolated in India, only 76% belonged to the Andrewes and Inman races. An extended analysis of the remainder enabled him to classify all but 1% of the total by creating 9 new races. Three of these, which he originally called 103, P119 and 55, were closely related antigenically to the Andrewes and Inman races, though each possessed its own distinctive antigen. Though the strains of the 55 race which were isolated in India were biochemically typical of the Flexner species, they were found to be identical antigenically with the "Newcastle" and "Manchester" bacilli, which had been described previously by Clayton and Warren (10) and by Downie (11) respectively, and which differ from the other Shigellae in producing small amounts of gas from glucose. Boyd included both the "Newcastle" and "Manchester" bacilli among the

Flexners, as members of the S8 race. The other new races, called 170, P288, D1, D19, P143, and P274, were serologically distinct, possessing few or no antigenic components in common with each other or the older races.

Boyd's findings have been widely confirmed by other workers, and representatives of most of the new races have been identified in many parts of the world. Boyd (12) has proposed a new terminology for the entire group, in which roman numerals are used as race names. His terminology, as well as several others which have been proposed by other workers, is given in Table I at the end of this section. He omits three of his new races from his classification because of their infrequent occurrence, and places 170, P288 and D1 in a new species with the prefix Boyd.

Boyd's work on antigenic variation in two of the new races, which will be discussed later, led him to believe that each Flexner race contained a specific antigen and a series of group antigens, the serological relationship between the races being due to common group antigens. He described several of the group antigens and designated them by arabic numerals. He also prepared monospecific serums for several of the races by absorbing the group agglutinins and leaving only the specific ones. He omits the Andrewes and Inman races X and Y from his classification because he was unable to prepare monospecific serums for them, and considers them as group variants of Z

and W respectively.

Two American workers, Wheeler (13) and Weil (14), have recently published studies of the antigens of the Flexner species, including the new races described by Boyd. They both used the agglutinin absorption technic, and attempted to prepare monospecific serums for each race. Wheeler's conclusions agreed closely with those of Boyd. He was able to produce monospecific serums of high titer for V, W, Z, 103, P119, and 88, but was unsuccessful in his attempts to produce one for Y. Though he could absorb heterologous agglutinins from an anti-X serum, the titer which remained for X was very low. He omitted X and Y from his list of specific races. He also analyzed the Flexner group antigens, identifying several which had been described by Boyd and three additional ones. He did not recognize the VZ and WX subraces, but divided the W race into two subraces on the basis of different group antigens. The terminology which he proposed, and which is given in Table 1 is similar in most respects to Boyd's, except that he includes in the paradysenteriae species the races which Boyd places in the Boyd species, and also includes the three races omitted by Boyd.

Weil's approach to the problem differs markedly from that of Boyd and Wheeler. He considers all of the antigenic components found in the related races to be qualitatively equal, instead of being

divided into specific and group factors. The primary antigen of one race appears as a secondary antigen in other races, according to his concept. In this respect he agrees with the original theory of Andrewes and Inman. Weil differs from all of the other workers in finding Y to have a primary antigen and in considering it as a true race. He also finds a primary X antigen and produced monospecific serums for both X and Y. The VZ and WX subraces are termed dual races, containing two primary antigens. He describes two new dual races, one having X and P119 as its primary antigens, the other having Z and 103. Weil considers Wheeler's type strain for the 103 race to be a Z -103 dual race, and Wheeler's type V strain to be a VZ. His proposed terminology differs from both Boyd's and Wheeler's, because of the inclusion of X and Y as races. Like Wheeler, he includes all of Boyd's races in the Flexner species.

Kauffmann (15) used a very small number of strains, which did not include X or any of Boyd's races. He also apparently groups W and Y together. He classifies V as an incomplete variant of VZ, lacking the Z antigen. He uses the same method in analyzing the Flexner antigens as the one used for the salmonellas, assigning to each race an antigenic formula, without distinction between group and specific antigens. His nomenclature is the one originated by Kruse, and generally used since in the German literature.

Another recently suggested terminology is that of Meter (16). He compares the terminology of several earlier authors with that of Boyd, and suggests a numerical nomenclature using arabic numerals. This is similar to the later suggestions of Weil and Wheeler in including all of the Boyd races, but the numbers do not correspond to those of any other classification.

Hardy (17) has published an antigenic analysis of the Flexners based on differences shown by direct agglutination, which he considers sufficient to distinguish the races. He worked with only a few strains, which did not include any representatives of Boyd's races or of any subraces.

The precipitin reaction has been used by a few workers as a method of antigenic analysis of the Flexner species. Goebel, Binkley and Perlman (18), by extracting the bacteria with glycol or pyridine, obtained antigenic substances which they characterise as lipocarbohydrate proteins. They use the term "specific antigen" in referring to the substance, but their protocols indicate that both group and specific factors are present. The injection of an antigen prepared in this way from a strain of the V race resulted in the production of antibodies both against V and other races in much the same proportions as those obtained by the injection of whole bacteria.

Gonzales and Morales Otero (19), using formamide as an extracting agent, found their extracts to be markedly specific antigenically when prepared from freshly isolated cultures. They typed 896 cultures successfully by the precipitin method, using unabsorbed antiserums.

The subject of S-R variation among the Flexners has been studied by MacKenzie and Fitzgerald (20) and MacKenzie and Batt (21). They concluded that the S-R change involved the acquisition of a new antigen and the loss of part of the original antigen of the S strain.

A different kind of variation was originally described by Takita (22) and later studied extensively by Boyd (9). Takita found that cultures of V, W and Z gave rise to two types of colonies. One of these types contained both group and specific antigens and bred true, while the other contained only specific antigen. The second type did not breed true, subcultures of the original colonies giving rise to a mixture of the two types. Boyd observed the same kind of variation in two of his new races, 103 and P119. He found that freshly isolated strains of the 103 race were highly specific in their antigenic composition. They were agglutinated only slightly by antiserums prepared from the V-Z races, though antiserums produced from 103 strains agglutinated the other races moderately well. After varying periods of growth on artificial media, a variant was produced which was agglutinated strongly by the serums of other races. It also removed agglutinins for all other races from serums made from the original 103 strain, but left a high titer of agglutinins for the original specific 103 strain. The original specific antigenic type, which Boyd called 103a, continued to produce variants, while the variant, which he called 103b, bred true. He believed that 103b had lost its specific antigen, with a consequent enhancement of its content of group antigen. The

behavior of the P119 variant was similar. He found that the b variants of the two races were similar but not identical in their antigens, and showed that they were both closely related to the Y race of Andrewes and Inman. He believes Y to be a b variant of the W. race.

The distinction between 103a and 103b is commonly made in the English literature, but in the American literature the only reference to them is made in a recent article by Weil (23). He reports the identification of several strains of 103b which had been recently isolated from patients. All of the workers who have studied the a-b type of variation agree that it is different from the S-R variation. Though the b variants often produce larger and more irregular and opaque colonies than do the a strains, they do not conform to the other criteria of roughness.

Topley and Wilson (24) give a thorough discussion of the antigenic structure of the Flexners and propose a tentative serological classification. Their classification differs from Boyd's in including X and in excluding the entire 88 race on the ground that the non-gas-producing strains of this race are variants of the gas-producing Newcastle and Manchester bacilli, which they do not consider acceptable members of the Flexner species because of their biochemical reactions. Their inclusion of X is tentative, and they suggest that the final classification of the group be postponed until the status of X is decided.

A study of the conclusions reached by the authors whose work has been reviewed shows that there are several important questions still undecided. These may be summarized as follows:

1. The question whether all of the complex antigenic factors of the Flexner races are equal in quality but differ quantitatively from one race to another, or whether they should be separated into qualitatively distinct specific and group antigens. Andrewes and Inman, Kruse and Weil consider them qualitatively equal, while Boyd and Wheeler differentiate between group and specific factors.

2. The status of the subraces, such as VZ and WX. The American Type Culture Collection and the Army Medical School (personal communications) make no distinction between them and normal strains of the V and W races. Hardy and Wheeler do not mention them, though Wheeler notes differences among the group antigens of strains of the V and W races, and subdivides W on this basis. Boyd studied the VZ race separately, and Andrewes and Inman and Weil give the subraces a prominent place in their discussions.

3. The status of the Newcastle-Manchester-88 strains. Boyd includes all of them among the Flexners, as do Wheeler and Weil. Topley and Wilson exclude all of them because of the production of gas by the Newcastle and Manchester strains.

4. The status of Boyd's races 179, P286, D1, D19, P143 and P274. Boyd and Topley and Wilson place the first three in a separate species and do not classify the others. Wheeler, Weil and Meter include all of them in the Flexner species.

5. The status of the X and Y races. Boyd and Wheeler consider neither of them valid races. Weil considers both of them true races, and Topley and Wilson believe that the status of X requires further examination, but agree to the exclusion of Y.

6. The nomenclature of the races. Seven different systems are being used currently by English and American workers, and none of the seven agrees exactly with any of the others.

The original purpose of the present study was the serological classification of 17 locally isolated Flexner strains which had accumulated in this laboratory over a period of years, and of 29 strains which had been received from New Guinea in 1943. All of the strains were biochemically typical of the Flexner species, and had been isolated from cases of clinical dysentery, but their antigenic race had not been determined.

As a preliminary to the classification of these strains, several cultures of serologically identified strains of all of the races were obtained from various other laboratories to serve as "type strains" for the preparation of antisera and as standard antigens for the agglutination and agglutinin absorption tests. It soon became apparent that supposedly typical strains of the same race received from different sources were not always identical serologically. In some instances, notably among strains of the V and 103 races, the antigenic differences were very marked. These observations led to an extension of the original problem to include a detailed study of the antigens of all of the races as represented by the cultures in our

possession in order to determine which of the previously classified strains was really typical of each race as described by the original investigator, and the antigenic basis for the atypical reactions of the strains which had been classified as belonging to the same race but which differed antigenically from the type strains. Thirty-five previously classified strains were used for this purpose. From among them a type strain was selected for each race and subrace. In two instances strains from our own collection were used as type strains, in one case because no previously classified strain of that race could be obtained, and in the other because our strain was identical antigenically with a previously classified one, and was somewhat more sensitive to specific agglutination. The study of the atypical named strains and the subsequent study of our own previously unclassified strains led to discoveries which seemed to pertain to and to throw some light upon some of the controversial subjects which were listed above in the discussion of the literature. The findings and the conclusions derived from them are presented in the body of the paper and in the accompanying tables.

Because of the confused state of the nomenclature, the original names of Andrewes and Inman and of Boyd will be used throughout the paper.

TABLE 1

CLASSIFICATION OF THE FLEXNER GROUP AS PROPOSED BY DIFFERENT AUTHORS

Andrewes and Inman	BOYD			WHEELER				Topley and Wilson
	Original name	Proposed name	Group antigens	Type	Group antigens	Weil	Meter	
V		Flexner I	1.2.3.4.5.6	I	1.2.4.5.6.9	I	1	i
VZ						I.III		
W		" II	1.2.4	IIa	1.3.4	II	2	ii
WX				IIb	1.7.8.9	II.(VII)		
X					1.7.8.9	VII	3	iii
Y					1.3.4	VIII		
Z		" III	1.2.6	III	1.6.7.8.9	III	4	iv
	103	IV	1.2.3	IV	1.6	IV	6	v
	P119	V	1.5	V	1.5.7.9	V	7	vi
	86	VI	1.2.4	VI	1.2.4	VI	5	
	170	Boyd I	-	VII		IX	8	Boyd i
	P288	" II	-	VIII		X	9	" ii
	D1	" III	-	X		XI	11	" iii
	D19		-	XI		XII	12	
	P143		-	XII		XIII	13	
	P274		-	IA		XIV	10	
						III. IV		
						V. VII		

Sources of material.

Serologically classified strains were received from several sources. A number of strains from the British Type Culture Collection were obtained through the courtesy of Dr. J. H. Glynn, and others directly from the Lister Institute. Two of Glynn's strains, Oxford V and Whittington Z, have the same strain names as those used by Andrewes and Inman and by Boyd. Dr. K. M. Wheeler kindly supplied representatives of the Boyd races, and, in addition, duplicate strains of several races were obtained from the American Type Culture Collection, from the Army Medical School, from Dr. A. J. Weil, and from the Parke-Davis laboratories. In all, 81 strains were investigated, 35 of them having already been classified by other workers. Seventeen locally isolated strains and 29 isolated in New Guinea by Dr. Howard Richardson were also studied.

A list of all of the strains used in the study, with their source and the race to which they were assigned as a result of this investigation, is given in Table 2. Strains selected as type strains for each race are marked with an asterisk.

TABLE 2

LIST OF STRAINS USED IN THE PRESENT STUDY.

Name of Strain	Source	Original label of strain	Date of Isolation	Race ****	Comments
*Oxford V	Glynn	same	unknown	V	
1 Weil	Weil	67-104-V	unknown	V	
Kayto	New Guinea		1943	V	
Army V	Army Medical School	43-G-71	unknown	VZ	
V ATCC	ATCC**	9380	unknown	VZ	
Peterson	local		1936	VZ	
*O'Hara	New Guinea		1943	VZ	
Abbett	"		"	VZ	
Manieri	"		"	VZ	
Norwack	"		"	VZ	
*W London	BTCC***	Willesden 1935. No. 4833	1935?	W	
WDJ	Glynn		unknown	W	
Moller	local		1946	W	
Carey	"		1942	W	
352a	"		1936	W	
Warden	"		1935	W	
Weaver	"		1941	W	
360	unknown		unknown	W	
360a	local		1933	W	
363	local		1933	W	
Cromer	local		1944	W	
WPD	Parke-Davis		unknown	W	
*X Tomar	Glynn		unknown	WX	
*X London	BTCC	Staveley 1935 No. 4834	1935?	X	

TABLE 2 (Continued)

Name of Strain	Source	Original label of strain	Date of Isolation	Race ****	Comments
Z London	ETCC	Staveley 1935 No. 4835	1935?	X	
7 Weil	Weil	66-1-411	unknown	X	
X ATCC	ATCC	9470	unknown	X	Contains antigen not present in other X strains.
*Ledingham Y	Glynn		unknown	Y	
Y ATCC	ATCC	9473	unknown	Y	
*Whittington Z	Glynn		unknown	Z	
Z ATCC	ATCC	9403	unknown	Z	
350	local		1933	Z	
352	U.of Cal.		unknown	Z	
Zook	local		1941	Z	Group antigens differ from those of type strain.
Hess	local		1941	Z	Group antigens similar to those of Zook.
Beal	New Guinea		1943	Z	
Olsen	"		1943	Z	
ZPD	Parke-Davis		unknown	Z	
*103 ATCC	ATCC	9748	unknown	103	Contains chiefly type antigens.
3 Weil	Weil	63-143-Z	unknown	103	Contains only group antigens.
570	Wheeler	570	unknown	103	Contains Z group antigen.
2003	Wheeler	2003	unknown	103	Contains both type and group antigens.
4 Weil	Weil	66-1-1430	unknown	103	
Arnold	local		1941	103	Contains X group antigen.
Vinton	local		1934	103	"
Kerr J	local		1940	103	Contains X group antigen
Kerr S	local		1940	103	"
XPD	Parke-Davis		unknown	103	"
Scott	New Guinea		1943	103	
*P119 Wheeler	Wheeler	566	unknown	P119	

TABLE 2 (Continued)

Name of Strain	Source	Original label of strain	Date of Isolation	Race ****	Comments
5 Weil	Weil	63-143-119	unknown	P119	
5-7 Weil	Weil	63-143-X	unknown	P119	Contains large amount of X group antigen.
Camillari	New Guinea		1943	P119	
88 Wheeler	Wheeler	575	unknown	88	
* Quiazda	New Guinea		1943	88	
Phillips	"		"	88	
Klimalzewsky	"		"	88	
Finn	"		"	88	
Moeller	"		"	88	
Bedlek	"		"	88	
O'Dierna	"		"	88	
Streaker	"		"	88	
Holsey	"		"	88	
Johnson	"		"	88	
Stacey	"		"	88	
Rasmussen	"		"	88	
Wachowick	"		"	88	
Blumstein	"		"	88	
Blanchko	"		"	88	
Koel	"		"	88	
Steinberry	"		"	88	
Lipschitz	"		"	88	
Collins	"		"	88	
Messick	"		"	88	

TABLE 2 (Continued)

Name of Strain	Source	Original label of strain	Date of Isolation	Race ****	Comments
P274 Wheeler	Wheeler	563	unknown	P 274	
P288 Wheeler	Wheeler	564	unknown	P 288	
170 Wheeler	Wheeler	565	unknown	170	
D1 Wheeler	Wheeler	571	unknown	D 1	
D19 Wheeler	Wheeler	572	unknown	D 19	
P 143 Wheeler	Wheeler	573	unknown	P 143	

* Indicates strains used as type strains. Unless otherwise indicated, these strains were used throughout the tables as antigens for agglutination, and for the preparation of the type antiserums.

** American Type Culture Collection.

*** National Type Culture Collection, Lister Institute, London.

**** As determined in this study

Methods

The culture medium used throughout the work were beef extract broth and beef extract agar. Antigens were prepared by inoculating Blake bottles of agar with young broth cultures and removing the growth after 18 hours with physiological saline containing 0.3% formalin. The saline suspensions were incubated for 18 hours, tested for sterility, then centrifuged and resuspended in a small amount of saline containing 0.3% formalin. The heavy suspensions were stored in the refrigerator and used both as absorbing antigens and as suspensions for agglutination. For the latter purpose the suspensions were diluted to a density corresponding to the $BaSO_4$ standard no. 3. (MacFarland Nephelometer). These stored heavy suspensions retained their antigenic sensitivity for many weeks, and provided a supply of antigen of uniform character for a large series of tests.

Antiserums were prepared by injecting rabbits intravenously either with formalinized saline suspensions prepared in the above manner, or with young broth cultures preserved with 0.3% formalin. Ordinarily four injections were given at three to four day intervals, the amounts of the injections being 0.25, 0.5, 1.0 and 2.0 ml. respectively. The animals were bled seven days after the last injection and the serum was preserved by adding an equal quantity of glycerine. The titers of the serums obtained in this way varied from 1-2560 to 1-20,000 after the addition of the glycerine.

Agglutination tests were performed by making serial dilutions of serum in tubes and adding an equal quantity of antigen to make a total volume of 1 ml. The final dilution of serum in the first tube was usually 1-40, and the dilutions were carried out to the titer of the serum for the homologous organism. The tubes were incubated for 18 hours in a waterbath at 50°C. and examined immediately after the incubation period. The titer of the serum was considered to be the highest dilution giving agglutination which was visible without the use of a hand lens. Physiological saline was used for making the serum dilutions.

Agglutinin absorption tests were carried out as follows: a portion of the heavy saline suspension of antigen was placed in a 15 ml. centrifuge tube graduated in tenths of a ml., and centrifuged for half an hour at approximately 2500 rpm. The supernatant fluid was then discarded and the appropriate amount of antiserum, diluted 1-10 with saline, was added and thoroughly mixed with the antigen. Usually, a volume of bacterial cells equal to one-half the volume of undiluted antiserum was sufficient to remove all agglutinins for the absorbing strain. It was rarely necessary to repeat the absorptions. The serum-antigen mixture was incubated for 4 hours at 37°C., centrifuged, and the supernatant fluid tested in serial dilution for the presence of agglutinins. When more than one absorption was carried out on a single sample of serum, the same procedure was followed consecutively with the different antigens, the partially absorbed serum mixture being used to

resuspend the packed bacteria in subsequent absorptions.

During the investigation of variants, antiserums prepared from whole cultures were absorbed with antigens made by inoculating Blake bottles with broth cultures grown from single colonies, and the absorbed serum was tested for agglutinins against the whole culture. If the single-colony antigen removed all agglutinins for the whole culture, the culture was assumed to be antigenically homogeneous. If the single-colony antigen failed to remove all agglutinins, the original culture was plated out, a number of isolated colonies transferred to broth, and after 18 hours' incubation, the living broth cultures were tested for agglutination with the absorbed serum. Cultures which were agglutinated, and therefore differed from the antigen used for absorption, were used to inoculate Blake bottles. Cultures of the single colonies of both antigenic types were then preserved by the lyophile process. The antigenic characters of the single-colony cultures proved to be moderately stable, however, even when kept on agar slants under ordinary laboratory conditions.

Antigenic relationships between the Flexner Races as indicated by agglutination titers in unabsorbed antiserums.

Each of the strains was first tested for agglutination in antiserums of all of the races. There was marked cross-agglutination among most of the races, but a few of them were sufficiently specific in their reaction to be identified readily without the use of absorption procedures. The titers given by the antiserums of each race with representative strains of all of the races are shown in Table 3. All of the strains

TABLE 3

AGGLUTININ TITERS OF UNABSORBED SERUMS OF THE FLEXNER RACES.

Antigen	Serum V	W	X	Y	Z	103a	P119	88
V	2560	640	1280	1280	320	320	640	80
W	320	5120	-	5120	-	320	-	40
X	2560	160	2560	1280	1280	640	2560	80
Y	2560	2560	2560	5120	320	640	1280	640
Z	640	-	1280	320	5120	80	1280	40
103a	320	640	640	1280	-	2560	320	-
P119	80	-	160	160	160	-	2560	-
88	-	-	-	-	-	-	160	2560

of the 55 races were so specific in their reactions that they were omitted from the later absorption studies. P119 strains gave slightly stronger cross-reactions, but were agglutinated strongly only by their antiserums.

Antigenic Analysis of the Flexner Races V and VZ.

The lack of standardisation of type strains is especially evident among V and VZ strains. Though English publications ordinarily differentiate between V and VZ, American authors, with the exception of Weil, do not do so, and consider both as V. Of 4 strains labelled V which were received from different laboratories, 2 proved to be VZ, and it is apparent from the protocols of several recent publications that the authors were using VZ strains as type V's.

The VZ subrace was described by Andrewes and Inman in their monograph, and was distinguished from V by its relatively high content of Z antigen. Andrewes and Inman found that a V serum could be exhausted of all of its agglutinins by sufficiently large absorbing doses of VZ antigen, but that V antigen could not absorb all agglutinins from VZ serum. Combined absorption of VZ antiserum with V and Z antigens did, in their opinion, give complete absorption, though in their work they did not ordinarily attempt to obtain complete removal of all agglutinins from their absorbed serums.

The relationship of VZ to the V and Z races was also studied by Boyd and by Weil. Boyd found that a monospecific V serum prepared by his method still agglutinated VZ, but that a monospecific Z serum

did not. Weil did not attempt to remove VZ agglutinins from either his V or his Z monospecific serums. Neither Boyd nor Weil tested VZ serums by combined absorption with V and Z suspensions.

The German authors, Kruse and Sartorius and Reploh, consider VZ as a separate race, which is called A in their system of nomenclature. Kauffmann classed his V strain as a subgroup of VZ, lacking one of the antigens present in VZ.

We studied 4 strains labelled V which were received from other laboratories. Two of these were found to be VZ's, the other 2 were true V strains. One locally isolated VZ strain, and 1 V and 4 VZ strains from the New Guinea collection were also studied.

The agglutination reactions of the V and VZ strains and serums and the results of absorption of the two serums with various antigens are shown in Table 4. The differences in the antibody content of the two serums become very evident after the serums are absorbed with X and Y antigens. The antigen responsible for the cross-agglutination between VZ and Z was shared by the atypical 103 strain 570, as was shown by both Wheeler and Weil. Absorption of VZ serum with either Z or the 570 strain removed agglutinins for both, but no other antigens could remove agglutinins for either from VZ serum.

The only unexpected results of the analysis of the V and VZ strains were obtained in our attempt to demonstrate the identity of the specific components of the two types. It has been generally assumed since the work of Andrewes and Inman that the same specific V antigen

TABLE 4

DATA SHOWING THE COMPARATIVE ANTIGENIC COMPOSITION OF FLEXNER RACES V AND VZ

I Agglutinin titers of unabsorbed serums of the Flexner races with V and VZ suspensions.

	Serum								
Antigen	V	VZ	W	X	Y	Z	103a	103b	P119
Oxford V	2560	5120	640	1280	1280	320	320	5120	640
O'Hara VZ	5120	10,240	640	640	640	2560	160	2560	320

II Agglutinin titers of V and VZ serums with antigens of the Flexner races.

	Titer with Antigen								
Serum	V	VZ	W	X	Y	Z	103a	103b	P119
Oxford V	2560	5120	640	2560	2560	640	320	1280	80
O'Hara VZ	5120	10,240	80	1280	2560	2560	160	1280	-

III Agglutinin titers of V serum after absorption with X, Y and VZ antigens.

	Titer with Antigen								
Oxford V serum absorbed with	V	VZ	W	X	Y	Z	103a	103b	P119
X antigen	1280	1280	160	-	640	-	-	160	-
Y "	1280	1280	-	80	-	40	-	-	-
X+Y "	640	640	-	-	-	-	-	-	-
VZ "	640	-	40	640	640	40	80	1280	40
X+Y+VZ "	-	-	-	-	-	-	-	-	-

IV Agglutinin titers of VZ serum after absorption with X, Y, V and Z antigens.

	Titer with Antigen							
O'Hara VZ serum absorbed with	V	VZ	W	X	Y	Z	103a	103b
V antigen	-	2560	-	40	-	640	-	-
X+Y "	2560	5120	-	-	-	2560	-	-
Z	2560	2560	80	-	80	-	80	160
V+Z "	-	640	-	-	-	-	-	-

is present in both V and VZ races, and that VZ strains contain no antigen which is not shared by either V or z strains. If this assumption is correct, it should be possible, after removing all agglutinins except the specific V agglutinin from V serums by appropriate absorptions, to absorb all of the remaining agglutinins from the serum by absorbing it with VZ suspensions. It should also be possible to remove all agglutinins from VZ serums by absorption with V and Z suspensions.

Two V antiserums, prepared from different strains, were absorbed with X and Y antigens, which removed all agglutinins except those for V and VZ strains. The absorbed serums were then absorbed with VZ suspensions. This procedure removed from the serums all detectable agglutinins for our three V strains, and demonstrated that the specific V antigen was present in the VZ strains, as had been assumed.

When VZ antiserums were absorbed with V and Z suspensions, all agglutinins for V and Z strains, as well as those for strains of the other races and subraces, were removed, but a moderately high titer of agglutinins for VZ strains remained. The experiment was repeated several times, with three different VZ serums, with the same results.

Two interpretations may be offered for the results which were obtained. The first, which is in agreement with the usual concept of the structure of the VZ race, is that the results were due to purely quantitative differences in the amounts of specific V antigen present in our strains, and to corresponding differences in the specific antibody

content of the serums. If the V strains which were used as antigens for agglutination and absorption were unusually low in their content of specific antigen and our VZ strains unusually high in their content of of the same antigen, it might be possible for VZ strains to remove all of the specific antibodies from a V serum without the V strains being able to produce the same result in VZ serums. In favor of this interpretation is the long period, at least 25 years, which has passed since the original isolation of the Oxford V strain, our type strain of the V race. Such a period of artificial cultivation may have caused a decrease in the content of specific antigen. Much shorter periods have been shown by Boyd to result in complete loss of specific antigen by strains of the 103 and P119 races. Also in favor of this interpretation is the relatively low level of residual agglutinins in the absorbed VZ serums. The titer of the residual agglutinins varied in different experiments and with different serums from 3 to 12% of the original titer of the serum. Andrewes and Inman considered their absorptions complete even when a small percentage of agglutinins remained in the serum.

The second interpretation of the differences in the reactions of absorbed V and VZ serums is that VZ strains contain, in addition to the specific V antigen, another which is different from any found in typical strains of the V and Z races. This interpretation is supported by several observations. First, residual agglutinins for VZ suspensions could be demonstrated consistently in all three VZ serums which were tested. The agglutinins reacted against all 7 VZ strains in our collection, though at least one of the strains had been growing under laboratory conditions for

nine years. One V strain, and 2 Z strains which had been isolated only two years previously failed to be agglutinated in a 1-20 dilution of absorbed VZ serum in which the titer for VZ strains was 1-640. Absorbing doses of V and Z antigens of more than twice the amount needed to remove completely all agglutinins for the absorbing strains failed to lower the titer for VZ suspensions. Though the residual titers were low when considered as percentages of the original titer of the serums, they were equal to those remaining in completely monospecific Z serums when these were evaluated in the same way.

The conclusion which was drawn was that our VZ strains possessed a specific antigen which was not present in the V or Z strains which were examined. The small number of strains and antiserums which were available for study prevent our making a more generalized statement. An examination of many more strains by other investigators will be necessary in order to determine whether an individual antigen is characteristic of VZ strains in general. The marked differences in the minor antigens of V and VZ strains, which are shown by our results as well as the previous work of Andrewes and Inman and of Weil, justify a distinction between the two types on that basis alone, even if differences in specific antigens are not found to be constant.

Antigenic Analysis of the Flexner Race W.

Eight of the seventeen strains isolated in this community were found to be members of the W. race. The locally isolated strains as well as the one selected as a type strain corresponded well with the usual description of the race in regard to their agglutinability in the serums of other races. Their cross-reactions with other races are shown in Table 1.

W antiserums absorbed with Y suspensions were found to agglutinate only members of the W race, demonstrating that all of the heterologous antigens of the W strains were present in the Y race. This is in accord with the results of Boyd and Wheeler, and with Boyd's theory that Y is the group variant of W. Five antiserums which were tested varied markedly in their residual titers for W strains after absorption with Y. In one of the serums the titer for the homologous strain dropped after absorption from 1-10,000 to 1-320, in another from 1-2560 to 1-160. The other serums did not show such a marked reduction in titer. Since all of the strains used in the preparation of antiserums had been isolated many years previously, the results were interpreted as indicating a reduction in their content of specific antigen and a proportionate increase in group antigen which brought them closer to the antigenic composition of the Y race. Since all of them were agglutinated to some extent by serums from which all agglutinins for Y had been removed, they had not lost completely the specific characters of their race.

Antigenic Analysis of the Flexner Subrace WX.

The WX subrace was first described by Andrewes and Inman and was discussed recently by Weil, who believes that it corresponds with Wheeler's subgroup IIb. The only strain in our series which seems to belong to the WX subrace is X Toner, which was received from another laboratory as a typical X. It differed from the other X strains in its strong cross-agglutination with W and was considered by us to be a WX for this reason. Its agglutination reactions in the antiserums of other races were identical with those of X strains except in W antiserum. The WX strain reacted to titer with W antiserum, and W strains reacted to titer in serum prepared from it, whereas our X strains and serums gave no cross-reaction at all with W. The antiserum prepared against the WX strain agglutinated all of our strains of the Flexner and Boyd races which have common antigenic components, with the exception of those of the 55 race. With this exception, WX antiserum served as an excellent "polyvalent" antiserum for all of the races.

When the WX strain was tested with absorbed serums of other races, it reacted like a mixture of X and Y. Neither X nor Y was ordinarily capable of removing agglutinins for WX from serums of other races. Absorption of such serums with X would usually leave the titers for WX and Y relatively untouched, and absorption with Y would leave corresponding titers for X and WX. WX suspensions, on the other hand, could always absorb all agglutinins for both X and Y from heterologous serums. WX failed to absorb agglutinins completely from X, Y and W

antiserums, but it did reduce the homologous agglutinins to a very low level.

Neither W, X nor Y was capable alone of exhausting the WX serum of agglutinins. Several combinations of absorbing antigens, however, readily removed all agglutinins from the serum, W+X and Y+Z both being effective. Table 5 shows the principal reactions of the WX strain.

The behavior of this strain seems to be most effectively explained on the basis of Boyd's theory. It behaved consistently like a pure "group phase" organism. The high content of group antigen related both to X and to Y could account for its marked agglutinability in heterologous serums and the strong agglutination of other organisms in its serum. Its lack of a distinctive specific antigen is shown by the ease with which different combinations of heterologous antigens were able to remove agglutinins from a WX antiserum. The relation of X and Y antigens to the group-specific theory will be discussed more in detail later in this paper.

TABLE 5.

DATA SHOWING THE ANTIGENIC COMPOSITION OF THE FLEXNER SUBRACE WX

I Agglutinin titers of unabsorbed serums of the Flexner races with WX suspensions.

	Serum									
	V	W	WX	X	Y	Z	103a	103b	P119	88
WX antigen	2560	2560	10,240	2560	1280	640	640	160	1280	640

II. Agglutinin titers of WX serum with antigens of the Flexner races.

	Titer with Antigen									
	V	W	WX	X	Y	Z	103a	103b	P119	88
WX serum	1280	10,240	10,240	5120	5120	2560	2560	5120	640	-

III. Agglutinin titers of WX serum after absorption with antigens of Flexner races.

	Titer with Antigen								
WX serum	V	W	WX	X	Y	Z	103a	103b	P119
absorbed with									
X antigen	160	640	640	-	640	40			-
W "	320	-	1280	1280	1280	1280			320
Y "	-	-	320	640	-	640	-	-	320
W+X "	-	-	-	-	-	-	-	-	-
Y+Z "	-	-	-	-	-	-	-	-	-

Antigenic Analysis of the Flexner Race X.

Five X strains from different laboratories were studied. One of these, Toner X, has already been discussed as a WX. Another, XPD, will be discussed in the chapter on the Arnold subrace of 103. The other three strains were closely related to each other. Two of them, X London and 7 Weil, appeared to be identical in their antigens. The third, XATCC, differed slightly from the others. Its reactions will be described later. The reactions of the X London strain and the antiserum prepared from it are shown in Table 6.

The status of the X race has been the subject of a great deal of debate among various workers. Andrewes and Inman reported it as a separate race, though they found it less sharply defined than the other races. Boyd doubts its status as a true race, since he was unable to prepare satisfactory monospecific serums for it. In his latest publication he stated that he believed it to be an incomplete variant of Z, but he has not yet published his reasons for this conclusion. Wheeler leaves its status in doubt, finding that though it was possible to prepare monospecific X serums, they were left with a much lower titer than were those of the other races. Weil considers it a valid race, and appears to have been able to produce satisfactory monospecific serums for it.

Study of the strains in this collection leads to a conclusion more in agreement with those of Boyd and Wheeler than that of Weil. The antigenic composition of our X strains is complex. They share antigens especially with the V, Y and Z races. None of these races alone could

absorb all agglutinins from London X antiserum. A combined absorption with Y and Z, however, lowered the titer of the serum for X strains to 1-80. This result, together with the strong agglutination of X strains in serums of other races suggests that X is a race with large quantities of group antigen and little or no specific antigen.

The XATCC strain showed some evidence of an antigen not possessed by the other two strains. It reacted in heterologous serums like X London and 7 Weil, since absorption of the serums of the other races with any of the three X strains removed agglutinins for both of the others. XATCC also completely absorbed agglutinins from serums prepared from the other X strains. When XATCC antiserum was absorbed with either of the other two strains, variable results were obtained. On one occasion, absorption with X London removed agglutinins for X London and for 7 Weil, but left a titer of 1-320 for XATCC. The original titer of the serum was 1-20,000. When the absorption was repeated, using a slightly larger absorbing dose of X London, the titer for XATCC was reduced to 1-80. Absorption with 7 Weil removed all agglutinins from the XATCC serum. In both of the experiments in which agglutinins for XATCC remained after removal of those for the other strains, the 5-7 Weil strain, which we believe to be a P119 with an unusually large group antigen content, was agglutinated to the same titer as XATCC. Since the residual titer was always low and could not be demonstrated consistently, it was interpreted as being due to a slight difference in the relative proportions of the antigens present in the three strains, rather than to the presence of a qualitatively different antigen in the XATCC strain. The reactions

TABLE 6.

DATA SHOWING THE ANTIGENIC COMPOSITION OF THE FLEXNER RACE X.

I. Agglutinin titers of unabsorbed serums of the Flexner races with X suspensions.

	Serum										
London	V	VZ	W	WX	X	Y	Z	103a	103b	P119	88
X antigen	2560	1280	160	5120	2560	1280	1280	640	5120	2560	80

II. Agglutinin titers of X serum with antigens of the Flexner races.

	Titer with Antigen										
London	V	VZ	W	WX	X	Y	Z	103a	103b	P119	88
X serum	1280	640	-	2560	2560	2560	1280	640	2560	160	-

III Agglutinin titers of X serum after absorption with antigens of the Flexner races.

London X serum absorbed with	Titer with Antigen								
	V	VZ	WX	X	Y	Z	103a	103b	P119
Z antigen	80	-	80	320	160	-	-	160	-
Y "	40	320	80	640	-	320	-	-	80
WX "	-	-	-	80	-	-	-	-	-
Y+Z "	-	-	-	80	-	-	-	-	-

of our X strains in a commercial "monospecific X serum" suggest that the "specific" agglutinin of this serum is of a similar character. Only one of our strains, 7 Weil, was agglutinated well by the serum, X London was agglutinated slightly, and the XATCC strain was not agglutinated at all.

Antigenic Analysis of the Flexner Race Y.

The Y race has been generally considered to lack a distinctive antigen, or, according to Boyd's concept, to be a pure "group" race. Weil is the only worker who has claimed to have found a distinctive antigen for Y. In this series, two Y strains were used. They appeared to be identical in all respects, and gave complete reciprocal absorption.

The most notable feature of our Y antisarums was the difficulty of absorbing all heterologous agglutinins from them. Antigens of all of the other races, except F119 and 55, were agglutinated well by Y serum, but none of them could remove more than a small fraction of the agglutinins for other races from the serum. Several combinations of absorbing strains were used. In several cases three or four different antigens were used on a single sample of serum, without complete absorption either of Y antibodies or of these for the other races. Combined absorption of a Y serum with Oxford V, the Zook strain of the Z race, and the 2003 strain of the 103 race lowered the titer for Y from 1-5120 to 1-160, but left a t iter of 1-80 for W, and 1-40 for X strains. This experience differed markedly from that encountered with serums of the other races, in which a combination of two antigens usually succeeded in absorbing all heterologous agglutinins. The conclusion drawn from these results was that the Y strains had an unusually complex antigenic structure, containing group elements common to all of the other races,

but no specific antigen of its own. This is in agreement with the conclusions of Andrewes and Inman, Boyd and Wheeler.

In a commercial "monospecific Y serum" both of our Y strains were agglutinated, but a strain of 103b and the WI strain were also agglutinated, indicating the presence of a group agglutinin in the serum.

Antigenic Analysis of the Flexner Race Z.

Three strains representative of the Z race were obtained from other laboratories, and 4 locally isolated strains and 2 from New Guinea were included in the study. The strain called Whittington Z was used for most of the absorption experiments and for preparing an antiserum. A serum was also prepared from one of the local strains, Zook, since it differed antigenically from Whittington Z.

The race was found to be a distinct and homogeneous one, though some strains were not agglutinated in as high a titer as others by serums of other races. The reactions of Whittington Z and Zook are shown in parts I and II of Table 7. The degree of agglutination of other races by the Z serums was much more marked than that recorded by Boyd, though he also used Whittington Z as a type strain.

The heterologous agglutinins of the Whittington serum were related chiefly to X, V2 and to Wheeler's strain 570, which is an atypical member of the 103 race. The X component was distinct from the other two, while the V2 and 570 components were very closely related to each other.

X was the most efficient of the three in removing agglutinins of totally unrelated races from Z antiserum, but was entirely ineffective against the other two main components. Absorption of Z serum with either VZ or the 570 strain markedly reduced the titer for the other, but did not affect the X component. All three of the components could be removed by absorption with the three antigens, leaving a serum completely monospecific for Z, demonstrating that none of them contained the specific Z antigen. Part III of Table 7 shows the results of absorption of a Whittington antiserum.

All 9 of the Z strains which were examined were agglutinated equally well by monospecific Z serum, and therefore contained the same specific antigen. They also were agglutinated to the same titer by unabsorbed Z serums prepared from three different strains. Two of the strains, Hess and Zook, were identical with each other, but differed greatly from the others in their minor antigenic components. The Zook strain was studied in detail. By plating out the original culture and examining antigens prepared from single colonies, it was shown that the culture was composed of a mixture of two antigenic types, one of which was identical with Whittington, and the other, which predominated greatly in the original culture, had an entirely different proportion of minor antigens. An antiserum was prepared against the antigenic form which differed from Whittington. The reactions of Whittington and of the antigenic form of Zook which differed from it are shown in parts I, II, and IV of Table 7. It can be seen that though the only unabsorbed

serums whose titers with the two suspensions differ significantly are those for Y and 103a. In absorbed serums of V, X and Y, and in the two Z antiserums, there are striking differences in the reactions of the two strains.

Since the differences in the serological reactions of Whittington and Zook do not involve a loss or even a decrease in the amount of specific antigen in either of them, neither represents the group phase of the race. The variation seems explained best on the basis of a difference in their amounts of X and Y group antigens. Whittington contains a much higher proportion of the X group factor, and relatively much less of the Y group factor than does Zook. Neil also noted that the Z strains studied by him varied in their degree of relationship to Y.

Seven of the 9 strains studied were similar to Whittington in the proportions of the above two factors. There was no correlation between the length of time since the isolation of the strains and their antigenic proportions.

The problem of the relationship of the Z race to the VZ subrace and strains of the 103 race such as the 570 strain is one which needs clarification. Strains belonging to the VZ race are encountered frequently, judging from our experience and from reports of other workers who have differentiated it from V strains. Whether 103 strains with marked antigenic relationship to Z are as common as VZ strains cannot yet be determined. Several widely different methods of preparing monospecific Z serums are in current use. Boyd's method of preparing such serums removed agglutinins for VZ. Wheeler's method removes

TABLE 7

Data Showing the Comparative Antigenic Composition of the Flexner Z Strains Whittington and Zook.

I. Agglutinin titers of unabsorbed serums of the Flexner races with Whittington and Zook suspensions.

	Serum								
Antigen	V	VZ	W	X	Y	Z(Whit)	103a	103b	P119
Whittington	640	2560	-	1280	320	2560	80	2560	1280
Zook	1280	5120	80	640	5120	5120	1280	2560	640

II. Agglutinin titers of Whittington and Zook serums with antigens of the Flexner races.

	Titer with Antigen								
Serum	V	VZ	W	X	Y	Z(Zook)	103a	103b	P119
Whittington	320	2560	-	1280	320	5120	80	2560	160
Zook	640	2560	40	1280	5120	10,240	320	2560	160

III. Agglutinin titers of Whittington serum after absorption with antigens of the Flexner races.

	Titer with Antigen							
Whittington serum absorbed with	V	VZ	X	Y	Z(Whit)	103b	P119	103(570)
X antigen	-	1280	-	-	2560	80	-	1280
Y "	80	1280	640	-	2560	-	40	1280
WX "	-	1280	-	-	2560	-	-	1280
VZ "	80	-	640	160	640	160	40	160
570 "	80	160	640	160	640	160	40	-
X + VZ + 570"	-	-	-	-	320	-	-	-

IV. Agglutinin titers of absorbed Flexner serums with Whittington, Zook, X and Y suspensions.

		Titer with Antigen			
Serum	absorbed with	Whittington	Zook	X	Y
V	Whittington	-	640	640	1280
V	Zook	40	-	160	640
X	Whittington	-	-	320	160
X	Y	320	-	640	-
Y	Whittington	-	640	1280	5120
Y	Zook	-	-	320	1280
Y	X	80	640	-	5120
Whit.	Zook	640	-	640	-
Zook	Whittington	-	320	-	160

agglutinins both for VZ and for 570. Weil's method leaves agglutinins for both of the subraces in his monospecific Z serum. His method has the advantage of differentiating such strains from those having the usual V and 103 antigenic character, and also eliminates the extra absorptions necessary to remove agglutinins for them from Z antiserum. However, since agglutinins for such strains can be removed from Z serums and a satisfactory titer for strains of the Z race retained, it seems very doubtful that their relationship to the Z race is through the specific antigen. If this is true, an antiserum which agglutinates VZ and 570 cannot properly be called a monospecific Z serum. It seems highly desirable that absorbed serums to be used for the identification of the Flexner races should be standardized as to agglutinin content so that the term "monospecific" should represent the same definite factor for each race.

Antigenic Analysis of the Flexner Race 103.

Boyd's 103 race proved to be the most complex of the races which we studied. Four supposedly typical strains of 103 obtained from different laboratories proved to be dissimilar in many respects, and all of them reacted much more strongly in antiserums of other races than did the strain originally described by Boyd. Since all of them contained a similar predominant antigen different from those of the other races, it was assumed that this factor represented the distinctive antigen of the 103 race. 103 ATCC, one of the two strains in which

the distinctive antigen was present in the largest amount, was chosen as the type strain of 103a.

Since we were unable to obtain a strain classified as a 103b, an attempt was made to find one among the 103 strains which were available. One of them, 2003, appeared at first to fulfill most of the requirements of the 103b variant. It was agglutinated more strongly by serums of the other races than was the 103a strain, and it was able to absorb all heterologous agglutinins from 103a serum without markedly diminishing the titer for 103a. Its chief difference from the reactions described by Boyd for 103b was its lack of close relationship for Y, a character which was stressed by Boyd. Absorption of either 103a or 2003 serum with Y failed to lower the titer for 2003 to a significant extent, and 2003 was unable to absorb all agglutinins from a Y antiserum.

By the selection of single colonies, a variant was isolated from the 2003 strain which conformed more nearly to Boyd's description of 103b. The variant removed all heterologous agglutinins from the serums of 103a and 2003. Agglutinins for it in 103a and 2003 serums were removed by absorption of these serums with Y and absorption with Y of a serum prepared from the variant removed all but traces of agglutinins for other races and reduced the titer for the variant to 3% of its original value. Thevariant was not as effective in removing agglutinins from a Y antiserum, but it did remove heterologous agglutinins and lower the titer for Y to a greater extent than did any of the other absorbing antigens except WX. The variant was therefore selected as the 103b type strain.

Comparative reactions of the 3 strains, 103a, 103b and 2003, are shown in Table 8. The results indicate that 2003 stands midway between 103a and 103b in its antigenic composition, containing less specific and more group antigen than 103a, but retaining an appreciable amount of specific antigen which is totally lacking in 103b. Single colony antigens of 2003 which were not of the pure 103b antigenic structure were like the parent strain in their reactions.

Wheeler's strain 570, which has already been discussed in connection with the Z race, differs from all of the strains shown in the table. It is not entirely homogeneous, giving rise to colonies which differ in some of their reactions, but since the differences between them were not as striking or as consistent as those shown by other variants, they will not be analyzed in detail. The reactions of the 570 strain in unabsorbed serums and the titers of 570 antiserum after absorption with related antigens are shown in Table 9. It will be noted that cross-reactions between 570 and other races are very slight except for those with VZ, Z and the 103 strains. The components which 570 shares with VZ and Z are similar, though not identical. The reasons for considering them group rather than specific components have already been discussed in the section on the Z race. The 103 component is clearly a specific one, since 570 serums can be absorbed to leave only agglutinins for 103a and 570. The agglutinins for 103a cannot be removed from such an absorbed serum without removing those for 570 as well.

TABLE 5.

DATA SHOWING THE COMPARATIVE ANTIGENIC COMPOSITION OF THREE STRAINS OF THE FLEXNER RACE 103.

I. Agglutinin titers of unabsorbed serums of the Flexner races with 103a, 2003 and 103b suspensions.

	Serum								
Antigen	V	W	X	Y	Z	103a	2003	103b	P119
103a	320	640	640	1280	-	2560	2560	1280	320
2003	1280	640	1280	2560	160	1280	5120	2560	640
103b	5120	1280	2560	5120	2560	2560	5120	10,240	1280

II. Agglutinin titers of 103a, 2003 and 103b serums after absorption.

	Absorbed	Titer with Antigen								
Serum	with	V	W	X	Y	Z	103a	2003	103b	P119
103a	103a	-	-	-	-	-	-	-	-	-
103a	2003	-	-	-	40	-	640	-	40	-
103a	103b	-	-	-	-	-	1280	1280	-	-
103a	Y	-	-	-	-	-	1280	640	-	-
2003	103a	320	160	640	640	80	-	640	640	-
2003	103b	-	-	-	-	-	640	1280	-	-
2003	Y	-	-	-	-	-	640	2560	80	-
103b	103a	640	80	640	640	320	-	320	2560	80
103b	2003	-	-	40	-	-	-	-	160	-
103b	Y	-	-	-	-	-	-	160	320	-

TABLE 9

DATA SHOWING THE ANTIGENIC COMPOSITION OF THE FLEXNER 103 STRAIN 570.

I. Agglutinin titers of unabsorbed serums of the Flexner races with 570 antigen.

	Serum									
Antigen	V	VZ	W	X	Y	Z	103a	103b	570	P119
570	640	2560	160	160	640	2560	2560	5120	2560	160

II. Agglutinin titers of unabsorbed 570 serum with antigens of the Flexner races.

	Titer with Antigen								
Serum	V	VZ	W	X	Y	Z	103a	103b	P119
570	80	2560	-	80	320	2560	2560	2560	-

III. Agglutinin titers of 570 serum after absorption.

570 serum	Titer with Antigen								
absorbed with	V	VZ	W	X	Y	Z	103a	103b	570
Z antigen	-	-	-	-	-	-	1280	40	640
103a "	-	640	-	-	80	640	-	160	1280
103b "	-	640	-	-	-	640	1280	-	1280
103bpZ "	-	-	-	-	-	-	1280	-	1280

Study of the strains described above, as well as those which will be discussed in the following section, indicate that the 103 race is more complex than Boyd's description shows. The change from the specific to the group form is not always as complete as that represented by the 103a to 103b type of reversion, since the 2003 strain demonstrates a partial change, with the retention of part of the characteristics of both 103a and 103b. The intermediate character of the antigenic structure of 2003 is not due to a mixture of colonies of the two types, since single-colony antigens have the same structure as the parent strain. The alteration in the antigenic pattern of the race which is found in the 570 strain is entirely different from the 103a to 103b variation. The 103b antigens are very weakly represented in this strain, whereas the group antigen shared with Z is the dominant factor in its group antigen content. Still another example of the antigenic complexity of the 103 race and its capacity for different antigenic combinations will be discussed in the following section.

Antigenic Analysis of the Flexner 103 Subrace Arnold.

Five strains, 4 of which were isolated in this laboratory, showed characters which set them apart from the others being studied. Their chief distinction was their capacity for antigenic variation. Three phases were found during the course of the investigation, two of them indistinguishable from the X and 103 races respectively, and the third a combination of the other two phases. In this phase these organisms differed from any other representatives of the Flexner or Boyd races

in our collection in their combination of major and minor antigens. Though the major antigen of the 5 strains places them in the 103 race, the appearance in the same cultures of phases representing a complete change in group antigens is sufficiently unusual to warrant a description of their behavior.

Three of the strains, Arnold, Kerr S and Kerr J, were studied by direct agglutination at the time of their isolation. The other two strains, Vinton and XPD, had been growing on artificial media for several years before being studied. All of the strains showed evidence of lack of antigenic homogeneity at the time they were first studied by us. The variability of their antigenic structure was first noticed in their reactions to direct agglutination with serums prepared from the Andrewes and Inman races. Antigens were prepared by suspending agar slant cultures in saline. Suspensions made on some occasions would react only in low dilutions with antiserums of the V, W, Y and Z races, and to 25-50% of the titer of anti-X serums. Suspensions prepared in the same way from cultures made on other occasions would be partially agglutinated by V, Y and Z antiserums to titers approximating those for the homologous races, but without being agglutinated completely even in low dilutions. When antigens were prepared from subcultures of single colonies, clear-cut differences were obtained. Antigens originating from some colonies were agglutinated in high dilution by V, Y, and Z antiserums, those from other colonies only in low dilutions, but in all cases agglutination, when it occurred, was clear-cut and complete. The antigenic structure of the cultures originating from single colonies

remained constant for many weeks, making it possible to study them in detail. Colonies having the antigenic structure which will be called phase 1, and others having the structure called phase 2 were found repeatedly in all 5 strains. Phase 3 has been found in four of the five strains, Arnold, Kerr S, Kerr J, and Vinton. The Arnold strain was used as the type culture for the subrace. In each of its phases it was apparently identical with similar phases of the other strains.

Suspensions having the antigenic structure of phase 1 were characterised by their failure to agglutinate in Y antiserum in a dilution of 1-320. Later, colonies were considered to be in phase 1 if antigens prepared from them were agglutinated by a phase 1 antiserum absorbed with X, and failed to agglutinate in an X serum absorbed with phase 1 antigen.

The agglutination reactions of phase 1 antigens in unabsorbed serums of the Flexner races are shown in part 1 of Table 10, and the reactions of the Flexner races in phase 1 antiserum are shown in part II of the same table. It will be noted that though the phase 1 suspension was agglutinated to 25% or less of the titers of all of the Flexner serums except that of P119, all of the Flexner antigens except W were agglutinated to relatively much higher titers in anti-phase 1 serum. The races to which phase 1 strains showed the closest relationship were X, 103 and P119. Antiserums of these

races were absorbed with phase 1 antigen and also with antigens of other races showing antigenic relationship to phase 1. Phase 1 serum was also absorbed with antigens of the other races. The results are shown in part III of Table 10. Only the reactions of the races most closely related to phase 1 are recorded in the table, though all of the strains used in the investigation were tested. It will be noted that phase 1 was not effective in removing agglutinins from any of the serums prepared from other races. In no case did it markedly lower the titer for the homologous antigen, or remove all heterologous agglutinins. Its efficiency as an absorbing agent was not greater than that of several totally unrelated races.

The close relationship of phase 1 to the 103 race was the most striking result obtained from these experiments. Though it was not agglutinated to a high titer in the unabsorbed serums of 103a and 2003, and though it did not absorb agglutinins from these serums effectively, it proved to be impossible to remove agglutinins for phase 1 from the 103 serums by absorption with any other race, or with 103b. Absorption with either Y, WX or 103b removed all agglutinins from 103a and 2003 serums except those for 103a, 2003 and phase 1. The titer for phase 1 remained almost as high as that for the homologous strain. Agglutinins for phase 1 were removed from 103a serum only by absorption with 2003.

The absorption of phase 1 antiserum, shown in part III of table 10, gives further indication of the antigenic relationships of the races under discussion. None of the 4 races whose absorption

TABLE 10

DATA SHOWING THE ANTIGENIC COMPOSITION OF THE FLEXNER 103 STRAIN ARNOLD PHASE 1.

I. Agglutinin titers of unabsorbed Flexner serums with Arnold phase 1 suspensions.

	Serum									
Antigen	V	W	X	Y	Z	103a	2003	103b	Arnold	P119
Arnold	80	40	640	160	320	640	640	640	5120	1280

II. Agglutinin titers of unabsorbed Arnold phase 1 serum with antigens of the Flexner races.

	Titer with Antigen								
Serum	V	W	X	Y	Z	103a	2003	103b	P119
Arnold	1280	80	5120	2560	2560	640	2560	2560	1280

III. Agglutinin titers of absorbed Flexner serums.

	Absorbed	Titer with Antigen							
Serum	with	V	X	Y	Z	103a	2003	103b	Arnold	P119
103a	X antigen		-	320		1280	640	640	640	
103a	Y "		-	-		1280	640	-	640	
103a	2003 "		-	40		640	-	40	-	
103a	103b "		-	-		1280	1280	-	640	
103a	Arnold "		-	80		1280	160	160	-	
2003	X "		-	1280		640	2560		640	-
2003	Y "		-	-		640	2560	80	640	-
2003	103a "		640	640		-	640	640	-	-
2003	103b "		-	-		640	1280	-	640	-
2003	Arnold "		640	1280		640	640	1280	-	-
P119	X "		-	160		40	40	640	-	1280
P119	Y "		640	-			80		320	1280
P119	2003 "		640	-		-	-	40	80	1280
P119	Arnold "		640	320			320		-	2560
X	Y "		640	-		-	-	-	320	80
X	2003 "		640	-		-	-	-	160	-
X	103b "		640	-		-	-	-	160	160
X	Arnold "		640	640		40	320	-	-	-
X	P119 "		1280	1280		80	640	1280	80	-

TABLE 10 (Continued)

Serum	Absorbed with	Titer with Antigen								
		V	X	Y	Z	103a	2003	103b	Arnold	P119
Arnold	X antigen	-	-	-	-	320	2560	2560	1280	-
Arnold	103a "	640	640	640	640	-	320	640	640	640
Arnold	2003 "	640	1280	-	1280	-	-	-	1280	1280
Arnold	103b "	320	640	-	640	640	640	-	1280	640
Arnold	P119 "	80	320	80	320	640	640	640	640	-
Arnold	X+2003 "	-	-	-	-	-	-	-	40	-

reactions are shown in the table removed all agglutinins from phase 1 antiserum. Combined absorption with X London and 2003 completely removed all agglutinins except those for phase 1, and reduced those to a low level. The results in repeated tests varied between complete absence of agglutination at a dilution of 1-40 and partial agglutination only in a dilution of 1-80. It was decided that if such agglutinins represented a specific antigenic factor, it played a very minor role in the entire antigenic complex of the strains. For practical purposes, the antigens present in phase 1 were all represented either in the X race or in 103, with those related to 103 being the major ones.

Phase 2 colonies of the 5 strains were differentiated from those of phase 1 by their ability to agglutinate in anti-Y serum in a dilution greater than 1-320, by their agglutination in X antiserum absorbed with phase 1, and their failure to agglutinate in phase 1 antiserum absorbed with X. Direct agglutination with antisera of the Flexner races, absorption of Flexner antisera with phase 2 antigen, and, finally, reciprocal absorption between X London and phase 2 proved that phase 2 was antigenically identical with X London.

Phase 3 was detected late in the course of the experiments and has at present been found in only 4 strains. Colonies which yielded antigens with this structure differed from those of phase 1 and 2 in being agglutinated by both of the absorbed sera used in differentiating the phases. A series of direct agglutination and absorption tests similar to those outlined for analyzing the other two phases showed phase 3 to be identical antigenically with the 2003 strain of the 103 race.

The tendency of the three phases to change into one of the others has not been fully determined. One phase 2 culture of Vinton was kept for a period of a year, and examined after that time for the presence of other phases. It was found to have become a mixture of phase 1 and phase 2, with phase 1 colonies predominating. A phase 1 culture of the same strain, kept for the same length of time, contained chiefly phase 1 colonies, with a few in phase 3. The original culture of Arnold gradually lost its phase 2 colonies. When the culture was first isolated the phase 2 colonies were easily found. Two years later the proportion of phase 2 colonies had decreased until extended search was necessary in order to find them. Four years after the original isolation of the strain, an examination of 100 colonies failed to reveal any in phase 2. Examination of cultures made from single colonies and kept for periods varying from a few days to several weeks revealed no change from the original phase. No phase 3 cultures have been kept for sufficient periods of time to determine whether they are capable of reversion. The studies made up to the present time suggest that the trend of reversion is from phase 2 toward phase 1, and then to phase 3. None of the cultures which have been studied have demonstrated any return to phase 2 in subcultures of colonies which were originally in one of the other phases. Not more than two phases have been found in any culture at one time. The cultures which now contain phase 3 have no detectable phase 2 colonies.

There was ordinarily no visible difference in the appearance of the colonies of the three phases. The one exception was found

in the Vinton strain. The phase 2 colonies of this strain were more opaque than those of phase 1, though both kinds of colonies appeared smooth. In none of the strains were the phases biochemically different.

The interpretation of the reactions of the Arnold strains can be made most logically on the basis of an alteration in the character of the group antigen. According to this theory, the strains belong to the 103 race, but, in their original form, had the X group antigen in place of the usual 103b group antigen. Phase 2 is the group variant, containing only the X group antigen. In phase 3 the X group antigen is replaced by the usual 103b group antigen, leaving phase 3 a normal member of the 103 race, with a mixture of group and specific antigens like those of the 2003 strain. Such a combination of antigens has its counterpart in other strains. The 570 strain is analagous to phase 1, differing only in the nature of the substituted group antigen, and the fact that the 570 strain appears to be stable in its antigenic composition. An example of the substitution of one group antigen for another is also found in the Zook strain of the Z race. The Arnold strains differ from 570 and Zook in undergoing a slowly progressive series of changes, in one phase of which the specific antigen of the race is entirely lost.

ANTIGENIC ANALYSIS OF THE FLEXNER RACE P119

The analysis of the P119 race was limited to the study of three strains, two received from other laboratories as typical representatives of the race, and one from the New Guinea collection. Though the New Guinea strain was agglutinated more strongly than was the type strain by the serums of other races, it completely absorbed agglutinins from a serum prepared from the type strain, and was therefore not a group variant.

The reactions of the type strain of P119 in serums of all of the Flexner races, and the reactions of representatives of the other races in P119 serum are shown in Table 1. The P119 strain was agglutinated so weakly by serums of the other races that its identification in unabsorbed serums could be made without difficulty. Its chief group components were those associated with X, and absorption with a combination of X and Y antigens removed all heterologous agglutinins from P119 serum. Weil's dual race V-VII also removed all heterologous agglutinins from P119 serum, but reduced the titer for P119 much more than did absorption with X and Y. The V-VII strain was not studied sufficiently to determine its exact relationship to the other races, but it clearly did not contain all of the specific antigen of P119, and probably was a P119 strain with a large amount of group antigen and relatively little specific antigen, corresponding to the 2003 strain of the 103 race.

Antigenic Analysis of the Flexner Race 88.

The Boyd 88 race was represented in our series by a strain of a gas-producing Newcastle bacillus and a non-gas-producing 88 strain, both of which were received from other laboratories, and by 20 strains from New Guinea. Sixty-eight percent of the New Guinea strains belonged to the 88 race, but no strains of this race were found in our small collection of locally isolated strains. None of the New Guinea cultures produced gas, and all fermented mannitol and failed to ferment xylose. The strains varied only slightly in their degree of agglutination in the antiserums used in the investigation. They showed such scant cross-reactions with other races that they were not included in the absorption experiments.

The position of the 88 race is complicated by its serological relationship with the Newcastle and Manchester bacilli. The Newcastle bacillus ferments glucose with the formation of a small amount of gas, and fails to ferment mannitol. The Manchester bacillus ferments both glucose and mannitol with the formation of small amounts of gas. Neither organism is, therefore, acceptable as a Flexner on biochemical grounds. Both are antigenically indistinguishable from the non-gas-producing strains of the 88 race, which conform to the biochemical criteria of the Flexner species. Boyd considers all of the organisms which possess the 88 antigen as Flexners, because they possess the Flexner group antigen. He states that the race differs from all other members of the Flexner group in that its biochemical reactions are inconstant. Boyd's viewpoint has been accepted by most workers, who include both gas-producing and non-gas-producing strains in the 88

race. Topley and Wilson take the opposite position, considering the non-gas-producing strains as variants of the gas-producing ones, and excluding all of them from the Flexner species. Since strains which possess the biochemical characteristics of the Flexners appear to be encountered more frequently than those of the Newcastle and Manchester types, the production of gas by some strains does not seem a completely valid reason for excluding all of them from the Flexner species. Both Bergey and Topley and Wilson include the Newcastle bacillus among the shigellae, though this genus by definition includes only non-gas-producing strains.

The Races Bearing no Antigenic Relationship to the other Flexner Races.

Only one previously classified strain of each of the races 170, P288, D1, D19, P143 and P274, was available for study. Antisera were prepared against each of the strains, and were used for testing all of the strains in our collection. None of the other strains was agglutinated by any of the six antisera.

Discussion

The conclusions which were drawn from the results of the study recorded in the body of this paper will be discussed and summarized in relation to the controversial questions listed in the introduction.

1. The question of the presence of qualitatively distinct group and specific antigens. Our results led to the conclusion that such qualitatively distinct antigens do exist. This conclusion was based on the observations that all of the heterologous agglutinins could be removed from all of our serums by absorption with one or more of three antigenic complexes. The agglutinins which remained in each serum were found only in members of one race. This was interpreted to mean that each race has a specific antigen peculiar to itself, and that the remainder of the antigens, which cause the cross-reactions between the races, fall into three groups. The first two group antigens were present in X and Y strains, and were named the X group antigen and Y group antigen respectively. The third, which was called the Z group antigen, occurred in strains of the Z race, the YZ subrace, and the 570 strain of the 103 race. The X and Y strains were composed solely of group antigen, but the strains containing the Z group antigen contained, in addition to it, the specific antigen of their race. Each of the three group antigens is a complex composed of many separate factors, some of which are present in only one of the three, others present in more than one. An attempt to classify the separate factors, as was done by Boyd and Wheeler, was abandoned when it became evident that a complete classification would be too cumbersome to be of practical use. The consideration of each of the three

complexes as a unit was found to convey the information necessary for an understanding of the serological reactions of our strains. The antigenic composition of our strains according to this method of analysis is shown in Table 11. All of the strains which were not typical of their race were distinguished either by possessing a different group antigen or by their relative proportions of group and specific components.

The Z group antigen differed from X and Y in being much less widely distributed than the other two, and in being present in relatively large amounts when it appeared at all. Its influence on the serological behavior of strains containing it was so strong that Weil considered it the major antigen of the Z race, and considered the antigen which we classify as the specific Z antigen to be a minor and unimportant factor. The specific Z antigen of Boyd and Wheeler corresponds to ours. Our Z group antigen is identical with Wheeler's group antigen 6, and almost identical with Boyd's 6, though we do not find it in X strains as he did.

2. <u>The status of the subraces</u>. Our collection included several strains and groups of strains which differed sufficiently from the normal pattern of their race to justify classification as subraces. None of them contained more than one specific antigen characteristic of a recognized race. They could not, therefore, be called dual races. The antigenic pattern of the atypical strains was described in the preceding section. The WX and 103b strains contained no specific antigen, and the WZ strains showed evidence of the possession of a

TABLE 11.

The Specific and Group Antigens of the Flexner Races and Subraces, as determined in the present study.

Race	Specific Antigen	Group Antigens
V	V	* (X)Y
W	W	Y
X	-	X
Y	-	Y
Z	Z	X,(Y),Z
103a	103	Y
103b	-	Y
P119	P119	X,(Y)
Subraces		
VZ	V,(VZ?)	(Y),Z
WX	-	X,Y
570	103	Z
Arnold	103	X

* Parentheses indicate group antigens which are present only in small quantities in normal members of the race.

distinctive specific antigen. The other ten atypical strains differed from the ordinary members of their race only in the character or proportion of their group antigens. If monospecific serums alone were used for the classification of strains, the distinctive characters of these strains would not be revealed. We have no epidemiological evidence concerning the significance of subraces except the occurrence of strains of the Arnold type in two members of the same family at the same time. Since it is possible that they may be significant, and since their identification would add very little to the work involved in classifying strains, we feel that they should be differentiated from normal members of the serological races. The addition of three serums to the ones used for race identification would permit a classification of the group antigens of all of the strains in our collection. The best serums for the purpose were WX serum absorbed with Y, which demonstrated the X group antigen, the same serum absorbed with X to show the Y group antigen, and Z serum absorbed with WX for the identification of the Z group antigen. The same result could be obtained, in slightly less clear-cut fashion, by X serum absorbed with Y, Y serum absorbed with X, and Z serum absorbed with X. The use of "group" serums would have the additional advantage of permitting the recognition of strains which lack a specific antigen and thus fail to agglutinate in monospecific serums.

3. <u>The status of the Newcastle-Manchester-55 strains</u>. All 19 of the strains of the 55 race in our collection were biochemically typical Flexners. Gas-producing strains corresponding to the Newcastle and Manchester varieties were apparently not encountered by Boyd.

Wheeler, or Weil among their previously unclassified strains. From these reports, and others which have not been specifically discussed in this paper, we obtain the impression that non-gas-producing strains containing the S8 specific antigen are much more frequently found than are gas-producing ones. The antigenic relationship between our strains of the S8 race and the other Flexner races was not as striking as that between the V, W, X, Y, Z, 103, and P119 strains. They do, however, share some antigens with the races just mentioned, and have more claim to inclusion in the Flexner species than do the antigenically distinct Boyd races, which will be discussed in the next section. The Newcastle bacillus is included in the Shigella genus both by Bergey and by Topley and Wilson, in spite of its production of gas. If it belongs in the Shigella genus at all, it belongs, because of its antigenic characters, in the Flexner species. The presence of a few gas-producing strains among larger numbers of non-gas-producing ones does not seem to us a valid reason for excluding any of them from the Flexner species.

4. <u>The status of the Boyd races 170, P288, D1, D19, P143 and P274</u>. On this subject no personal opinion can be justifiably made. Only one strain of each of these races was available for study. None of our previously unclassified strains belonged to any of the six races.

5. <u>The status of X and Y</u>. Our results agreed with those of Boyd, and Wheeler in failing to demonstrate a specific antigen in either the X or the Y race. They therefore are not entitled to the

status of true races. Our conviction that the group antigens should be included in antigenic analyses of the Flexner races makes us believe that some place should be found for X and Y in the system of nomenclature because they contain the two most important group antigens in pure form.

6. The nomenclature of the Flexner races. To suggest another system of nomenclature would simply add to the existing confusion. The only satisfactory solution to the problem would be the establishment of an international commission such as was created for the nomenclature of the salmonellas, with authority to decide upon a system of classification which could be universally accepted as official. Our personal opinion is that the choice of roman numerals was unfortunate because of the possibility of confusion concerning the symbols V and X, which appear as letters in the Andrewes and Inman classification, which is still frequently used, and as numbers in the newer systems. For the present, the use of the original terminology of Andrewes and Inman and of Boyd, both of which are universally understood, or the interpretation of the new classification by an explanation of its relation to the older system are the safest courses to follow. We agree with Wheeler that some provision should be made for group antigens in any scheme of classification, but prefer the use of three group antigens instead of the nine that he suggests. Such a plan would eliminate X and Y from the specific races, but would include them among the group antigens. The group antigens could be designated by letters, if numbers are used for the specific antigens, or as arabic numerals if roman numerals are

used for the specific races. If numbers are used, our personal opinion is that either uncapitalized roman numerals, such as are used by Topley and Wilson, or arabic numerals such as Neter used, are preferable to the ordinary roman numerals, for the reasons given above.

Summary

Eighty strains of Flexner bacilli were studied antigenically by the agglutinin absorption method. Qualitative as well as quantitative differences in the antigens were found. Specific antigens were found in all races except X and Y. These last two races were believed to consist wholly of group antigens. The relationships between the races appeared to be due chiefly to the almost universal presence of the X and Y group antigens. Another, the Z group antigen, played a dominant part in the antigenic behavior of certain strains and accounted for the distinctive behavior of two subraces. None of the strains contained more than one specific antigen. The three group antigens were all complex, each containing several antigenic factors. For the sake of simplicity each was considered as a unit.

Strains were found both among the "type" strains and those locally isolated, which varied from the normal members of their race in their group antigens. Variants which lacked a specific antigen were also found among strains of the 103 race. The VZ strain showed evidence of the existence of a distinctive antigen not shared by V or Z.

The problem of the nomenclature of the Flexner races was discussed. The limitations of the use of monospecific serums as the sole method of identifying new strains as members of the Flexner species and for assigning them to specific races was criticised because of its failure to reveal the difference between typical strains and those belonging to subraces. The addition of three group serums to the series of monospecific serums was proposed, in order to permit the identification of subraces and strains lacking a specific antigen.

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