THE ANTIGENIC RELATIONSHIPS OF SHIGELLA PARADYSENTERIAE, FLEXNER AND ESCHERICHIA STRAINS

by

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Bordet, Gruber and Durham, and other early investigators observed that a high titer agglutinating serum possesses the power to agglutinate not only the species used for immunization, but also certain related strains. Krumwiede, Cooper and Prevost(1925) add that serum agglutination is only relatively specific, for a serum may agglutinate not only the homologous organism and related strains, but also unrelated strains.

Using typhoid serum a definite serological relationship between Shigella paradysenteriae Flexner strains and the typhoid bacillus was demonstrated by Hiss(1904). Andrewes and Inman(1919) reported the agglutination of Shigella paradysenteriae Sonne in Flexner W serum to a relatively high degree. Shigella alkalescens has been found to share antigenic components with Flexner type strains Y,V, and X by Keter(1938). That a high titer rabbit serum for a pathogenic organism, Shigella shigae, may agglutinate strongly an apparently unrelated non-pathogenic organism from the intestinal flora of rabbits was reported by Ingalls(1937). She attributed the result to the existence of a haptenic fraction common to both

organisms.

Kristensen, Bjelen and Kjaer(1936) found 164 paracolon bacillus strains to be agglutinated in high titer O sera of the Salmonella group strains. In no instance were the paracolon bacilli agglutinated in the more specific H Salmonella sera. Dudgeon(1924) reported that certain hemolytic Bacterium coli strains were agglutinated in high dilutions by ten human typhoid antisera. He found a hemolytic Bact. coli and a non-hemolytic Bact. coli strain to agglutinate to higher titer in a human paratyphoid serum than the isolated organism.

A number of investigators have reported on the presence of minor agglutinins for Bact. coli in the sera of animals immunized to strains of Shigella flexneri.

Park(1904) isolated a Bact. coli strain from a case of dysentery which produced abundant agglutinins in animal sera for a Flexner Manila culture. A serum prepared against the Flexner strain agglutinated the coli culture to a high titer.

Kuhn, Gildermeister and Woithe(1911) found a Bact. coli strain, cultivated from the stools of dysentery patients, to agglutinate to a high titer in a dysentery antiserum. On further investigation a number of Flexner antisera were found to react in high dilution with Bact. coli cultures. The authors termed this reaction 'para-

agglutination', a phenomenon which is characterized by the agglutination of organisms other than the known etiological agent in immune sera. They explained the reaction by suggesting that a Bact. coli culture by close association with the dysentery bacillus in the body of a patient had its receptor apparatus modified to resemble the pathogenic organism.

Paragglutination was the theory used by Culbertson (1929) to explain the agglutination in high titer Flexner antisera of certain Bact. coli organisms isolated from dysentery cases.

Andrewes and Inman(1919) tested a strain of Bact. coli in monovalent Flexner antisera, types V, W, X and Y, finding the coli antigens to agglutinate 1:35 in the W serum. Andrewes (1918) reported that certain strains of Bact. coli agglutinate to the end titer in a Flexner Y serum.

Sievers(1937) found a number of Flexner cultures to agglutinate in five Bact. coli antisera in a dilution of 1:160, but upon absorption of the sera with the Flexner organisms, coli agglutinins were not removed. Absorbing the Flexner sera with Bact. coli antigens, he noted that Flexner agglutinins were not removed.

Hayashi(1938) tested six strains of Bact. coli, which he had selected after many experiments, in mono-

valent antisera of the dysentery races, prepared by the use of ten type strains of Aoki, five of Watanabe and five classified as milk races. His agglutination results are given in chart 1. Upon absorption of the dysentery sera with the coli strains showing agglutination to high titers, he found three coli cultures which completely absorbed the Flexner agglutinins from the three type sera. Bact. coli M48 absorbed agglutinins from the milk race type IV serum; strain M89 removed the agglutinins from milk race serum type V; culture Aty 16 absorbed fully the Watanabe type IV serum. The three Bact. coli cultures gave biochemical reactions typical of their group. The author stated that one may find antigenic variation in some coli strains which causes them to behave serologically as identical to certain Flexner type strains.

Hayashi tested the cultural reactions of the Flexner strains, milk race types IV and V, and found them to exhibit biochemical variations which tended to relate them culturally to the Bact. coli group. The Watanabe type IV also gave cultural reactions which approximated those of the coli strains. Because of the biochemical variation found in the three Flexner type strains, the investigator concluded that such variation might be the reason that the three cultures of Bact. coli are serologically identical to the Flexner races mentioned above.

From the discussion it is seen that Bact. coli strains M48, M89 and Aty 16 are identical antigenically to types 1V and V of the milk races and type 1V of Watanabe respectively, and that these strains are related through the cultural variation of the Flexner races.

Mackie(1939) investigating the specificity of the agglutinin reaction for Shigella dysenteriae, tested thirteen strains of Bact. coli in three monovalent Flexner rabbit antisera. In one instance a Bact. coli culture agglutinated to titer in a Flexner serum. Six coli strains gave relatively high titers,1:1280 and over, in the three sera. The remaining organisms agglutinated in dilutions from 1:160 to 1:640.

Absorption of the Flexner sera with the individual coli strains variously affected the titer for the homologous dysentery antigen. Mackie reported that eleven coli strains were able, in at least one instance, to reduce the homologous titer of a Flexner serum fifty percent or more. It may be noted that a reduction in titer of fifty percent refers only to a drop in titer from one serum dilution to the next highest dilution. One coli culture was able to remove completely the agglutinins for the homologous Flexner strain. Four coli organisms reduced the homologous Flexner titer to one-eighth the original titer. In one-half the cases the dysentery titer was not affected.

CHART 1

Hayashi (1938)

AGGLUTININ TITERS FOR BACT. COLI STRAINS IN FLEXNER TYPE SERA

Cultures	Aoki and Murakami type sera					Watanabe type sera			Milk race type sera		
	I	II	III	V	VI	II	IV	V	III	IV	V
	10000	10000	10000	10000	10000				10000	10000	10000
Coli M200	2000	1000	1000	1000	1000				1000	1000	---
M48	2000	1000	100	2000	2000				1000	10000	---
P51	1000	1000	---	---	8000				---	---	---
P74	2000	2000	---	---	10000				1000	---	---
M89	2000	---	10000	---					---	---	2000
Atyl6	10000	2000				2000	2000	10000	---	---	---

The individual coli strains always removed the corresponding coli agglutinins from the Flexner sera.

Mackie prepared monovalent high titer sera for the 13 coli strains and tested the Flexner bacilli by agglutination and absorption. The Flexner antigens agglutinated from 1:160 to half the serum titer, except one Flexner strain which agglutinated to the full titer, and which absorbed completely the homologous coli agglutinins from that serum. Absorption of the Bact. coli antisera with one or more of the three Flexner cultures reduced the homologous titer of each serum fifty percent or more. The Flexner strains were antigenically distinct from one coli strain, for they agglutinated only in low dilutions of the coli serum and were able to absorb only their own agglutinins, failing to remove the coli agglutinins.

The agglutinogenic cross relationships which he observed might be classed as instances of paragglutination, however, if this phenomenon is dependent upon the alteration of receptor apparatus, it seems unlikely. Mackie reported that the strains of Bact. coli were obtained from patients who never afforded cultural evidence of infection by Shigella flexneri.

The author stated that heterologous absorption presents strong evidenc of fundamental agglutinogenic similarity. He concluded that the general uniformity of

results obtained by reciprocal agglutination and reciprocal absorption in the monovalent antisera are strongly suggestive of a fundamental antigenic relationship between strains of Shigella flexneri and Bact. coli.

HISTORY AND ANTIGENIC STUDY OF THE FLEXNER RACES

The most complete serological classification to date of the antigenic composition of the Flexner group of dysentery bacilli was published by Andrewes and Inman in 1919. Previous to this time confusion existed in regard to the relationships between the strains of the Flexner group and the relationships of Flexner cultures to other dysentery bacilli.

In 1900 Simon Flexner reported the isolation of a bacillus from cases of dysentery in the Philippines which he believed to be identical to the bacillus of Shiga. During the next few years Strong and Musgrave(1900), Kruse (1900) (1901), Duval and Bassett(1902), Wollstein(1903) and Hiss(1904) reported the isolation of dysentery bacilli closely resembling the strains of Flexner and Shiga.

Martini and Lentz(1902) established clear serological differences separating the non-mannite fermenting bacilli of Shiga and Kruse from the mannite fermenting group, which included the strains of Flexner, the pseudo-dysentery organisms of Kruse, the cultures of Hiss and Russell, Strong and Musgrave and other investigators.

The first culture isolated by Kruse(1900) was found to be identical in serological and cultureal reactions to the bacillus isolated in Japan by Shiga. The dysentery strains

isolated the following year by Kruse(1901) and referred to by him as pseudo-dysentery organisms,fell into the group of mannite fermenting organisms. Lentz discovered the importance of the sugar alchohol, mannitol, as a primary differentiating medium for the separation of the Shiga and Flexner bacilli. The results of Park, Collins and Goodwin (1904) substantiate the work of Martini and Lentz.

One of the first men to suggest a working classification of the dysentery bacilli was Kiss(1904). On the basis of fermentation reactions in glucose, maltose, mannitol, sucrose and dextrin he divided them into four groups. He stated that the organisms belonging to the groups differentiated by fermentation tests were also distinctly separated by marked differences in the agglutination tests in high titer sera. Group 1 was represented by a Shigella shigae strain; Groups 2,3 and 4 were mannitol fermenting organisms. The fault of this classification is that the mannitol fermenting bacilli give inconstant reactions in maltose, sucrose and dextrin. Moreover, as Torrey(1905) reported, the biochemical reactions do not parallel the serological findings.

Kruse in 1907) presented his serological classification of the pseudo-dysentery group. He divided the group into eight races by agglutination and absorption tests, finding

that each race differed from the others by the presence of an unshared major antigenic component. He designated each race by letter, from A to H. His classification is not used in this country now, but her merits the distinction of being the first to apply serological methods to the mannite fermenting group and to point out the antigenic complexity of that group.

Andrewes and Inman(1919) drawing from a large source of material, reported that the Flexner group is a heterogeneous group in that well marked serological differences exist among its various strains. A marked tendency to group agglutination was also found to be present amongst the Flexner races. By agglutination, using monovalent rabbit sera, by agglutinogenesis and absorption, indications were given for the existence of at least four distinct antigenic components, all of which are commonly represented in any given strain, but to a very different degree. These components are the V,W,X and Z antigens.

V, W and Z strains behave almost as distinct species because of the preponderance of a single antigenic component. Each requires for its adequate agglutination a serum belonging to its own race. However, each may agglutinate to some degree in the sera of the others.

The X race is the most sharply defined of all in regard to agglutination, for it will agglutinate only with

its own antiserum. The X antiserum is least sharply defined as regard agglutinogenesis, for it will agglutinate V, X and Z races.

The Y race contains a more evenly balanced mixture of the V, W and Z components with a small amount of X. The Y strains form a distinct serological race, but less so than V, W, X and Z, differing somewhat among themselves. The Y strains are weakly agglutinated in the sera of the other races. The authors believe the Y race to be of a more primitive antigenic structure than the others.

Two subraces Vz and Wx were found to exist. They are essentially members of the V and W races respectively, but contain so large a proportion of the second component as to modify their serological behaviour.

Gettings(1919) and Murray(1918) obtained similar results from their investigations, concluding that the Flexner strains of dysentery bacilli fall into four antigenic groups.

Aoki in 1921 presented his general classification of dysentery bacilli, including the bacillus of Shiga and the lactose fermenting organisms. His classification is based on crosse agglutination tests only, and he considered 43 strains. The dysentery bacilli are divided into eight types, designated by Roman numerals 1 to V111.

In 1923 he added three more groups making eleven in all. Groups 1 to VI contain pseudo-dysentery(designated paradysentery in this country) strains; group VII includes bacilli which coagulate milk and show very changeable characters; group VIII corresponds to Shiga's bacillus.

According to Aoki the relationship between the individual groups in regard to agglutination is as follows; the four subgroups I, VI, VII and VIII are sharply defined inter se, and are easily distinguishable from the others; types II, III, IV and V, on the other hand, vary in their mutual reactions and it is sometimes difficult to differentiate between them; types IX, X and XI are also sharply defined and stand apart from the remaining strains.

Using the five English type strains, V, W, X, Y and Z, and monospecific high titer sera, Davison(1920) presented the results of his cross agglutination experiments (chart II) The Y race is seen to agglutinate in V, X, Y and Z sera; the X strain is agglutinated only with the X antiserum; the W strain appears specific as does its serum. Sixty-two cultures isolated from cases of Flexner dysentery and tested in the monovalent sera showed more cross agglutination than the type strains. W, X and Y sera frequently agglutinated the same cultures. Fifteen Flexner strains were not agglutinated by any of the type sera leading Davison to conclude that there are more than four distinct antigens

CHART 11

Davison (1920)

CROSS AGGLUTINATION OF THE 5 ENGLISH TYPES

Serum	Culture	Agglutination titers 1:100	1:125	1:250	1:625	1:1250	1:2500
V	V	+	+	+	+	+	
	W	0					
	X	0					
	Y	+	+				
	Z	0					
W	V	0					
	W	+	+	+			
	X	0					
	Y	0					
	Z	0					
X	V	0					
	W	0					
	X	+	+	+			
	Y	+	+	+			
	Z	+	+	+	+		
Y	V	+	+	+			
	W	0					
	X	0					
	Y	+	+	+	+		
	Z	0					
Z	V	0					
	W	0					
	X	0					
	Y	+					
	Z	+	+	+	+		

CHART 111

Kalic (1927)

COMPARISON OF THE THREE SYSTEMS OF CLASSIFICATION

Andrewes English types	Kruse German types	Aoki Japanese types
V	B or C	---
Vz	A	---
W	---	---
Wx	---	---
X	---	11
Y	D	1
Z	E	X
Shiga	Shiga-Kruse	V111-Shiga
---	---	111,V
---	---	1V
---	E Sonne	---

CHART 1V

Sartorius, Reploh (1932)

Autoren	Rassen der Flexnergruppe der Ruhrerreger									
Kruse	A	BC	H	D						
Andrewes	Vz	V	Z	W	X	Y				
Aoki, Murakami		V	11	1	X		111,V	1X,X11	1X	L
Sartorius Zusammenstellung	A	BC	H	D	X	Y	F	G	K	L

dilutions of other type sera, and other type strains agglutinate more or less strongly in L serum. The results of these investigators indicate the complexity of the antigenic characteristics of the Flexner dysentery organisms.

Kemper(1933), and Sartorius and Frese(1936) investigated further the intermediate races, i.e. those which are not typically of any one race, of the Flexner group. Many strains were found which seemed to have two or more antigenic components in almost equal amount, modifying their serological behaviour so that specific type classification was impossible.

The most simple explanation of the antigenic make-up of the Flexner bacilli was published by Boyd(1932). From serological procedures he concluded that the Flexner types V, W, X and Z each possess a distinctive type antigen and share complex group antigen. They do not, according to Boyd, possess minor quantities of each other's type antigens. A type specific serum, Flexner 88, was absorbed with a Flexner strain,YHR, believed to be a pure group strain devoid of type antigen. The Homologous titer remained the same after absorption. The agglutinins for V, W, X and Z specific strains, present before absorption, were completely removed. He repeated the experiments with two other group strains 103B and R119B, obtaining the same results.

In conclusion Topley and Wilson state that although the Andrewes and Inman classification comprises the majority of the Flexner strains, there are occasional types which appear to be antigenically distinct.

ANTIGENIC STUDY OF ESCHERICHIA STRAINS

The Escherichia coli group is a heterogeneous group which includes a variety of related species widely distributed through nature as intestinal parasites, and water and soil inhabitants. Bacterium coli and Bacterium coli mutabile are members of this group, although Bact. coli mutabile is usually referred to as a paracolon bacillus. The latter organism was first described by Massini in 1907 as a non-lactose fermenting organism of the colon group which gave rise to lactose fermenting variants, the variants showing no tendency to revert ot the parent form.

Bact. coli displays serologically an extreme heterogenieity of antigenic factors. Lepper(1921) reported that each strain of Bact. coli is practically specific, as the Bact. coli sera agglutinate to titer only the homologous organisms, heterologous strains agglutinating in low dilutions. Working with 196 Bact. coli strains, Bredenbroker (1937) arrived at similar conclusions, declaring that there are many serological types. Sievers(1937) prepared five high titer Bact. coli rabbit antisera and tested them with 87 strains of Bact. coli. He concluded that it is impossible to classify or divide the coli strains into specific groups as only the homologous strains will agglutinate

to titer, while the heterologous cultures agglutinated in varying degrees.

Investigating the antigenic make-up of Bact. coli and paracolon bacilli, Merrold and Culver(1921) stated that the paracolon strains are more homogeneous serologically than Bact. coli strains. Eighteen of forty three cultures from the paracolon group agglutinated to titer in two sera, and all the strains agglutinated at least 1:80. The Bact. coli sera were found to be almost strain specific, as only a few heterologous strains gave low cross agglutination in those sera. They add that there is no correlation in the Bact. coli group between fermentation and serological reactions.

The facts reported above have borne out a general statment made by Mackie in 1913, that immune sera to various Bact. coli types have been found to exert little or no action on other strains which correspond in the fermentation reactions. With paracolon bacilli he found the results different. The serum of one strain agglutinated similar strains to a corresponding degree.

Dudgeon(1924) found forty nine slow lactose fermenting cultures to agglutinate to a high titer in antisera prepared against three of the strains. Absorbing the sera with the same strains, he noted that any slow lactose fermenting organism removed its own agglutinins from the antisera and

also those of the other strains. From his results he concluded that these organisms are antigenically of one group. The forty nine cultures were tested in Bact. coli antisera and found to agglutinate to a marked degree.

Dudgeon and Fulvertaft(1927)tested fourteen slow lactose fermenting strains in a high titer serum prepared with one of them, the homologous organism agglutinating 1:5000. Agglutination was positive for the thirteen strains in a dilution of 1:1000. The serum was then absorbed with two related strains resulting in the removal of agglutinins for the fourteen strains. They also found that Bact. coli and slow lactose fermenting cultures are closely related antigenically. A high titer serum of a slow lactose fermenter agglutinated the homologous organism 1:8000 and a Bact coli strain 1:1000. A Bact. coli serum agglutinated the homologous organism 1:20,000 and the lactose fermenting strain 1:1000.

In 1921 and 1924 Dudgeon reported on Bact. coli strains, hemolytic and non-hemolytic, isolated from infections of the urinaty tract. The hemolytic urinary strains are more closely related to each other serologically than are the non-hemolytic urinary strains to each other. The hemolytic and non-hemolytic urine bacilli may show some cross agglutination when treated with the two different types of sera. In one instance a non-hemolytic Bact. coli

was found to agglutinate higher in a hemolytic antiserum than the homologous strain. The common type of hemolytic urinary Bact. coli furnishes an antiserum which is partially or completely desaturated by other hemolytic urinary strains. This antiserum is not absorbed by non-hemolytic Bact. coli. A non-Hemolytic Bact. coli serum is not desaturated by heterologous non-hemolytic strains, the agglutinins being absorbed only by the homologous culture.

The paracolon bacilli of Herrold and Culver and the slow lactose fermenting strains of Dudgeon and Pulvertaft give serological reactions which indicate these strains to be an exception to the well known antigenic diversity of Bact. coli cultures. From the reports of the authors it sis seen that these strains are closely related antigenically as shown by their behaviour in high titer sera. The descriptions of the biochemical tests of the paracolon and the slow lactose fermenting cultures seem to indicate that the investigators were working with Bact. coli mutabile organisms.

From the foregoing reports Bact. coli strains have been shown to be antigenically diverse, with no known method of classification. The Bact. coli mutabile cultures appear to form a more homogeneous serological group than do the Bact. coli organisms. The Bact. coli and Bact. coli mutabile strains are related antigenically.

AGGLUTININ CONTENT OF NORMAL RABBIT SERA FOR SHIGELLA FLEXNERI AND BACT. COLI STRAINS

That natural bacterial agglutinins are widely, yet unequally distributed in the sera of animals has long been known. Mackie and Finkelstein(1930) reported that it has long been recognized that the natural antibodies are specific for various types of antigens. The occurence of natural agglutination of bacteria by normal serum has been attributed to the natural agglutinating antibodies analogous to the immune agglutinins by various workers.

In reviewing the literature the need for further investigation on the agglutinin content of normal rabbit sera for strains of Bact. coli, Bact. coli mutabile and Shigella flexneri is seen necessary before any definite statement can be made concerning the subject.

Gibson(1930) reported that the range of titers for Bact. coli in normal rabbit sera is low. He tested five strains in several sera finding only one serum to agglutinate the cultures in a dilution of 1:16.

Mackie and Finkelstein(1930) tested two strains of Bact. coli in normal rabbit sera. One strain agglutinated in a 1:4 dilution, the other culture did not agglutinate.

Testing normal rabbit sera with Flexner strains, Kruse types A, D and E, Burgdorf(1925) found the A and D races

to agglutinate in dilutions from 1:10 to 1:100. The H type strain gave positive results no higher than 1:25. Gibson (1930) found a normal rabbit serum to agglutinate a Flexner Y culture to 1:128.

Ingalls(1937) published the fact that a high percentage of rabbit sera agglutinated various strains of dysentery bacilli in a dilution of 1:20. However, she did not test those cultures to higher dilutions.

Jordan(1937) reported that while in general the bacterial species which agglutinated in relatively high dilution in one serum behaves in a similar manner with other serums, individual differences are noticed. His findings indicate that Bact. coli cultures agglutinate only in low dilutions of normal sera, if at all; Flexner dysentery strains appear to agglutinate as high as any other organism so tested. In comparing the agglutinin content of normal rabbit sera to the sera of other animals, investigators agree that the agglutinin content of rabbit sera is low.

In order to demonstrate a rise in titer for an organism tested in an immune serum, it is necessary to know first the agglutinin titer for that organism in normal animal serum. For as Ritchie (1916) stated, figures obtained by testing a series of immune sera are of greatest value when those figures are compared with the results obtained with presumably normal sera, the same technique

being employed in each series.

EXPERIMENTAL WORK

Materials

Five Shigella paradysenteriae Flexner strains, designated in the collection of the bacteriology department of the University of Oregon Medical school as S. paradysenteriae Flexner 352, 352A, Warden, W and Z were used in our experimental work. The 352 strain was received in 1931 from the Hooper Foundation, which in turn, had received it from the Alabama State Daprtment of Health in 1930. The strains Warden and 352A were isolated locally from cases of dysentery in 1935 and 1936 respectively. The Andrewes' type strains W and Z were obtained upon request from the Parke, Davis and Co. laboratories in 1939. The W and Z strains were received late in the course of our work and could not be completely studied at the time.

Forty three Bact. coli and 38 Bact. coli mutabile cultures were from the stock collection of the bacteriology department. The exact origin of each of these strains cannot be given, but it is known that the majority of them were isolated from fecal specimens. Six Bact. coli cultures and one mutabile strain were isolated from patients at the Multnomah County hospital in 1939.

Monovalent high titer rabbit sera were prepared for

the Flexner dysentery strains, Bact. coli mutabile strain, 199, and for the Bact. coli strains Mayfield and N.A., Normal serum was taken from each rabbit previous to immunization.

Bacterium coli

Fecal strains	Urine strains	Sputum strain	Unknown origin
Register	Mayfield	Langesen	Grady
Reberson	Lake U		Martin 2
Grunewald	Cruse		Marta
Williams	Martin		Holstrom
R.A.	Richards		5-2W
5	Ebber		Maxhill
Ballman	Moreland		Grewblew
Samson	Flynn		Williams W
Holt	Gordon		Billagur
Swanson			Hiller
2			Murry W
Halen			O.Mebelic
Hogan			
Murry			
Hillman			
Isaacson			
Doyle			
Peterson			
Daly			
Harper			
33B			
32A			
31C			
34B			
22B			
22C			

Bacterium coli mutabile

Fecal strains	Blood culture	Food	Unknown origin	
Lockwood	266	265	14G	257
Hillman M.			G22	262B
Borden			46G	269A
			70	269F
			78A	269I
			100	269J
			1013	269M
			1016	269P
			1025M.R.	331
			1045	269-5
			137-1	351A
			139A	412
			153	Sparks
			C153	Fields
			199	Duncan
			219-1	Moore
			247-2	Hogan

Methods

The cultural characteristics of the Flexner strains, the Bact. coli and Bact. coli mutabile strains were studied. The Flexner cultures were inoculated to tubes of lactose, glucose, mannitol, sucrose and maltose. They were found to give consistent reactions, forming acid in glucose, mannitol and maltose. Indol formation was variable, as was the methyl red reaction in glucose phosphate broth. The results are shown in table I.

All strains of Bact. coli and Bact. coli mutabile were streaked on Endo plates and inoculated in lactose, glucose mannitol, sucrose and maltose media. The strains are grouped in respect to the cultural reactions which are recorded in tables II and III. Bact. coli organisms form nine biochemical groups, the majority falling into the first two groups. Bact. coli mutabile cultures exhibited more variation, resulting in the formation of fourteen groups.

Care was taken to insure the use of smooth strains of Shigella paradysenteriae Flexner and Escherichia for the preparation of antisera, and for the antigens for agglutination and absorption tests. A loopful of each culture was suspended in broth, streaked on an infusion

TABLE 1

CULTURAL CHARACTERISTICS OF 5 STRAINS OF S. PARADYSENTERIAE FLEXNER

Strain	lactose	glucose	mannitol	sucrose	maltose	gelatine liquifact.	Indol	M.R.	V.P.	Colonies on Endo
352A	---	A	A	---	A	---	-	+	-	colorless
352	---	A	A	---	A	---	+	+	-	"
Warden	---	A	A	---	A	---	-	+	-	"
W	---	A	A	A	A	---	-	+	-	"
Z	---	A	A	---	A	---	+	+	-	"

Symbols: A--Acid,24 hour
M.R.--Methyl Red
V.P.--Voges Proskauer

agar plate and incubated for eighteen to twenty hours. Discrete colonies were examined by the use of a hand lens. Smooth, round, glassy colonies were picked and replated until subcultures were uniformily smooth. One smooth colony was chosen and transferred to an agar slant for stock. After a twenty hour incubation, transfers were made from the stock slant to infusion agar slants for the preparation of the antigen for agglutination tests, and, in eight instances, for the preparation of the immune sera. All cultures were tested in .85% sodium chloride solution for spontaneous agglutination.

Standardized antigens, prepared from the smooth infusion agar cultures referred to above and treated with a solution of phenol, were used exclusively for the agglutination tests. The growth was suspended in .85% solution of sodium chloride containing .25% phenol and stored at ice box temperature. The suspensions were standardized to the density of the $BaSO_4$ nephelometer tube number 3, containing approximately 900,000,000 bacilli per cubic centimeter.

Monovalent high titer rabbit sera were prepared by injecting intravenously increasing doses of killed cultures of bacilli standardized to the $BaSO_4$ standard number 4. Injections were given every four days in graded doses of .1cc, .3cc, .5cc and 1cc. Six days after the last injection trial bleedings were made and the titer of the serum

determined. If the titer was sufficiently high the animals were bled to death, the serum preserved with 1:10,000 merthiolate, and stored in the ice box.

Regulation agglutination tubes were used. Serum dilutions were made with .85% NaCl and ranged usually from 1:10 to 1:10240, each dilution being double the one in the preceding tube. A control tube, testing for spontaneous agglutination of the antigen in saline, was included in each test. One-half cubic centimeter each of serum dilution and antigen were mixed and incubated for four and one-half hours in a water bath kept at the constant temperature of 53° C. The tubes were immersed to two-thirds the depth of the contained fluid, generating convection currents in order to assist mixing. The reading recorded was that made after refrigerator storage. The end point of the reaction has been considered to be that dilution in which definite, although incomplete, clumping is visible to the naked eye. If a hand lens was used it is indicated on the tables by the letter L.

Living antigens were used throughout for absorbing the sera and care was taken to consider their complete smoothness. Infusion agar slant cultures were suspended in saline and Blake bottles were inoculated with the suspension. The bottles were incubated for twenty hours, the growth suspended in saline, centrifuged, and freed from medium

constituents by again washing the cells in saline. Packed cells were used in all absorption tests and the minimal absorbing dose of the homologous bacilli determined for each serum.

The serum to be absorbed was added to the cells and enough saline added to give a final dilution of 1:10. The suspension of living cells and diluted serum was held in the water bath at 40° C for two hours and placed in the ice box over night. The serum and bacteria were separated by centrifuge and the supernatant fluid tested in serial dilution with the phenolized antigen corresponding to the culture used for absorption. If the titer for the absorbing strain was positive in a final dilution greater than 1:20 the serum was reabsorbed until the titer fell to 1:20. Usually two absorptions sufficed.

TABLE IV

AGGLUTININ TITERS FOR FLEXNER STRAINS IN NORMAL RABBIT SERA

Normal sera	R.A.	199	Mayfield	Warden	352
Strains					
352A	1:20	1:40	1:40 L	1:20	1:20
352	1:20 L	1:40 L	--	1:40	1:80
Warden	1:20	1:40	1:20	1:40	1:20
W	1:40	1:80 L	1:20 L	---	---
Z	---	1:20	1:10	---	---

TABLE V

AGGLUTININ TITERS FOR BACT. COLI STRAINS IN NORMAL RABBIT SERA

Normal sera	R.A.	199	Mayfield	Warden	352
Strains					
Harper	1:10	1:20	---	---	---
Hillman	1:20	1:20 L	---		
5-2W	1:20	1:20	---	1:20	1:10
R.A.	1:40 L	1:20	---	1:10	1:20
Hogan	1:40	1:160	1:20	1:40	1:40
Murry	1:10	1:10	1:10	---	1:40
34B	1:40	1:20	1:40	1:10	1:20
Cruse	1:40 L	1:160 L	1:40	1:80	---
Mayfield	1:10	1:20	1:20	1:10	1:10
Grunewald	1:10	1:20 L	1:10		
Lake U	1:20 L	1:80	1:20		
Reberson	1:80	1:40	---		
Peterson	1:40 L	1:80	1:20 L		
Holt	1:10	----	1:20		
Hiller	1:40 L	1:80	1:20		
Moreland	1:20	1:20	---		
Isaacson	1:40	1:40	---		1:20
Halen	1:10	1:40 L	1:40 L		
Langesen	1:40	1:20	---		
Marta	---	1:40	1:10		
Williams W	1:20L	1:20	---		
Billagut	1:20 L	1:20			
O.Mebelic	---	---	1:10		
Ebber	---	1:40 L	1:20		
Samson	---	1:20	---		
Gordon	1:20 L	1:20	1:40		
Swanson	1:40 L	1:160	1:80		
Holstrom	1:40 L	1:40 L	1:40	---	1:20
25B	1:20 L	1:20	---		

TABLE V(cont.)

AGGLUTININ TITERS FOR BACT. COLI MUTABILE STRAINS

Normal sera / Strains	R.A.	199	Mayfield	Warden	352
199	1:40	1:40	1:10	1:10	1:10
1045	1:10	1:10	---		1:20
78A	---	1:20	1:10	1:10	1:10
Sparks	1:40 L	1:40 L	1:20	---	1:20
70	1:20 L	1:20 L	1:10	1:10	
Lockwood	1:20	1:20	1:10	---	1:40
Hogan M.	1:20	1:20	1:20	1:20	---
Millman M.	1:160	1:80 L	1:160		
Borden	1:80		1:10	1:40	1:20
1025M.R.	1:160 L	1:80	1:40	---	1:20
Duncan	1:40 L	1:20	1:20		
269A	1:160 L	1:160	1:40 L		
G22		1:20	1:10		
46G	1:20 L	---	1:10		
C153	1:40 L	1:80 L	1:40		

Agglutinin Titers in Normal Rabbit Sera

Tables IV and V indicate the agglutinin content of five normal rabbit sera for strains of Shigella paradysenteriae Flexner, Bact. coli and Bact. coli mutabile. Although a larger number of Escherichia strains were tested in the sera it is seen that the titers for all the cultures show only small variation.

Flexner strains agglutinated in dilutions of 1:10 to 1:80, the majority of strains reacting in serum dilutions no higher than 1:20. The large number of Escherichia strains tested were found to react with the agglutinins in normal rabbit sera. In several instances Escherichia cultures did not react at all with one of the serums. In other instances the titers for several cultures were as high as 1:160, these strains reacting strongly with all the normal sera tested. The greatest number of strains agglutinated in dilutions no higher than 1:20. No difference was noted in the agglutinin titers for Bact. coli and Bact. coli mutabile in normal rabbit sera.

The sera were found to differ somewhat in the normal agglutinin content. Sera K.A. and Mayfield reacted with the bacterial antigens to approximately the same degree. Serum 199, however, gave consistently higher titers with

the same antigens and the agglutination was more marked, for in many instances complete agglutination occured.

It was necessary at times to use the hand lens for accurate readings. The agglutination of strains in normal serum was of an exceedingly granular nature, not usually found in high titer sera.

Antigenic Relationships of the Flexner Strains

TABLE VI

AGGLUTINATION TITERS OF 5 FLEXNER STRAINS IN FLEXNER MONOVALENT ANTISERA

Strain	352A Serum	352 Serum	Warden Serum	W Serum	Z Serum
352A	1:10240	1:160	1:81920	1:5120	1:80
352	1:10240	1:81920	1:20480	1:160	1:10240
Warden	1:10240	1:1280	1:40960	1:2560	1:80
W	1:10240	1:640	1:40960	1:5120	1:80
Z	1:2560	1:40960	1:5120	1:80	1:10240

The Shigella flexneri strains 352A, 352, Warden, W and Z were tested in the high titer sera of these strains by cross agglutination and absorption. The results of the agglutination tests are given above in table VI.

Strain 352A agglutinated in the homologous serum 1:10240, and in the W and Warden sera to titer and higher. Culture 352 agglutinated in its serum 1:81920; to titer in the 352A and Z serums and to half titer in the Warden serum. The Warden strain agglutinated 1:40960 in the homologous serum; to titer in the 352A serum and to half titer in the W serum.

The Andrewes' type strain W agglutinated in the W antiserum in the dilution of 1:5120 and in the 352A and Warden sera to the titer of those sera. The Andrewes' 2 culture agglutinated 1:10240 in the homologous serum, and to half titer in the 352 serum.

From the agglutination results the 352A and Warden strains appear to be members of the W race; culture 352 to belong to the 2 race. The strains 352A, 352 and Warden are not as specific antigenically as the Andrewes' type strains W and 2. The three cultures seem to have a mixture of antigenic components which allow them to cross agglutinate to a marked degree in the three sera of those strains.

Reciprocal absorption tests either confirm or deny the antigenic relationships found among strains by agglutination experiments.

Table VII indicates that the specific antigenic components of strain 352 is different from that of 352A, as absorption of the 352 serum with 352A antigen did not affect the 352 titer, and absorption of the 352A antiserum with culture 352 did not alter the 352A titer. That the two strains share group components is evident by the cross agglutination results. The Warden agglutinin content of the 352 serum remained constant, however there was a small reduction following the absorption of 352A serum with 352.

Reciprocal absorption of antisera 352 and Warden by

TABLE V11

RECIPROCAL ABSORPTION OF FLEXNER SERA BY STRAINS 352, 352A

Serums	352 antigen	352A antigen	Warden antigen
352 serum	1:81920	1:640	1:1280
352 absorbed with 352A	1:81920	0	1:1280
352A serum	1:10240	1:10240	1:10240
352A absorbed with 352	0	1:10240	1:5120

TABLE V111

RECIPROCAL ABSORPTION OF FLEXNER SERA BY STRAINS 352, WARDEN

Serums	352 antigen	Warden antigen	352A antigen
352 serum	1:81920	1:2560	1:640
352 absorbed with Warden	1:81920	0	1:320
Warden serum	1:20480	1:40960	1:81920
Warden absorbed with 352	0	1:40960	1:5120

TABLE 1X

RECIPROCAL ABSORPTION OF FLEXNER SERA BY STRAINS 352A, WARDEN

Serums	352A antigen	Warden antigen	352 antigen
352A serum	1:10240	1:10240	1:10240
352A absorbed with Warden	0	0	1:320
Warden serum	1:81920	1:40960	1:20480
Warden absorbed with 352A	0	1:20480	1:320

those strains shows in table VIII that the organisms are not of the same Flexner race. Warden removes its own agglutinins from the 352 serum, but leaves the 352 titer unchanged. Similarly, strain 352 removes its own agglutinins from the Warden serum, but does not absorb the specific Warden agglutinins. The two strains share group antigenic components which account for the cross agglutination. The 352A agglutinins are partially removed from the 352 serum by the Warden strain, and reduced markedly by the absorption of the Warden serum with the 352 organism.

Table IX gives a different picture, for the 352A and Warden strains appear to be related through a specific antigenic component. The Warden strain agglutinated to titer in the 352A serum and upon absorbing the serum, Warden removed completely the agglutinins for itself and for the homologous organism. Absorbing Warden serum with 352A, a part of the Warden agglutinins were removed, but a large portion remained. It would seem that the Warden strain carries the specific antigenic component of 352A and also an additional component specific for itself. The agglutinin titers for 352 in the two sera were reduced to the same degree by the heterologous absorption.

The Andrewes' type W strain was used by another member of the laboratory to absorb serum Z, resulting in the removal of W agglutinins, but no decrease in the Z

agglutinin titer. The 352 strain was tested in the absorbed serum and no reduction in titer was evident, indicating that 352 is a Z type strain. The W serum was absorbed with the Z type antigen, Z agglutinins being removed, and the W agglutinins remaining. Flexner strains 352A and Warden agglutinated in the absorbed W serum as markedly as they did in the unabsorbed W serum, indicating that cultures 352A and Warden are W type strains.

From the foregoing experiments each strain appears to have a specific antigenic component and one or more additional components which are shared by all the strains. Strain 352A is closely related to strain Warden, as the Warden culture was able to absorb completely the 352A agglutinins from the 352A serum. Strain 352 is distinct from the cultures 352A and Warden as the homologous agglutinins of serum 352 are not absorbed by 352A and Warden antigens, nor can the 352 culture remove 352A or Warden agglutinins from the 352A and Warden monovalent sera.

The results of the absorption tests confirm the observation made from the results of the agglutination tests that the 352A and Warden strains are of one Flexner race, i.e. type W, and the 352 strain of another distinct race, type Z. The agglutination and absorption experiments indicate to some extent the antigenic complexity of the Flexner group.

Agglutination of Escherichia Strains in High Titer Flexner Antisera

Fifty Bact. coli strains were tested in the high titer 352A serum. Those cultures agglutinating to a relatively high titer were tested in antisera 352 and Warden. The results of these experiments are recorded in tables X and XII. From the fifty strains, ten cultures which gave comparatively high titers in the three sera were selected and tested in type antisera W and Z. The agglutinin and titers of this group in the five sera are given in table X.

Thirty eight Bact. coli mutabile strains were tested in a similar manner in the Flexner antisera 352A, 352 and Warden. The agglutination results are given in table XI and XIII. Ten Bact. coli mutabile cultures were selected for testing in the W and Z type sera. These results are included in table XI.

Four Bact. coli strains and five Bact. coli mutabile strains agglutinated from one-eighth to one-fourth the titer of the 352A serum. These titers were the highest observed in this serum. Twenty two Bact. coli and eleven Bact. coli mutabile cultures agglutinated in dilutions of 352A serum from 1:160 to 1:640. One coli strain and

TABLE X

BACT. COLI AGGLUTININ TITERS IN FLEXNER ANTISERA

Strain	Serum 352A 1:10240	Serum 352 1:81920	Serum Warden 1:40960	Serum W 1:5120	Serum Z 1:10240
Holstrom	1:640	1:80	1:40	---	1:160
34B	1:640	1:160	1:0	---	----
Isaacson	1:40	1:160	1:640	---	1:40
Cruse	1:1280	1:320	1:1280	1:640	1:320
5-2W	1:1280	1:160	1:80	1:80	1:20
Hogan	1:160	1:80	1:5120	1:640	1:40
Mayfield	1:1280	1:1280	1:640	1:640	1:320
Murry	1:80	1:640	1:40	1:20	1:80
R.A.	1:2560	1:1280	1:1280	1:320	1:640
Harper	1:320	1:40	1:20	1:80	1:80

TABLE XI

BACT. COLI MUTABILE AGGLUTININ TITERS IN FLEXNER ANTISERA

Strain	Serum 352A 1:10240	Serum 352 1:81920	Serum Borden 1:40960	Serum W 1:5120	Serum Z 1:10240
1045	1:320	1:20	1:40	1:320	---
78A	1:640	1:1280	1:1280		
Hogan M.	1:1280	1:20	1:160	1:80	1:160
1025 M.R.	1:2560	1:160	1:160	1:160	1:160
Lockwood	1:640	1:40	1:320	1:40	1:80
Hillman M	1:640	1:80	1:5120		
Sparks	1:2560	1:1280	1:640	1:160	1:320
199	1:1280	1:2560	1:1280	1:160	1:320
Borden	1:2560	1:1280	1:640		
70	1:40	---	1:2560	---	1:40

TABLE XII

AGGLUTININ TITERS OF 40 BACT. COLI STRAINS

IN FLEXNER ANTISERA

Strains	Serum 352A 1:10240	Serum 352 1:81920	Serum Warden 1:40960
Swanson	1:320	1:80	1:40
2	1:80		
Gordon	1:640		
Williams	1:40	---	---
Murry W	1:640	---	1:40
Miller	1:320	---	---
Moreland	1:80	1:40	1:40
Martin 2	1:160	---	---
Register	1:640		
Williams W	1:160		
C. Mebelic	1:320		
265V	1:80	1:40	---
Grady	1:320	---	---
Sparks V	1:640		
Reberson	1:160		
Mexhill	1:640	1:160	1:80
Lake U	1:160	---	---
5	1:320	---	---
Halen	1:80		
Ebber	1:80	---	---
Grunewald	1:160	1:40	1:40
Grewblew	1:80	1:40	1:40
Samson	1:80	---	---
Marta	1:80	---	---
Ballman	1:160	---	---
Richards	1:80	1:160	---
Martin	1:80	1:40	---
32A	1:160	1:320	1:80
Daly	1:40	1:40	1:40
Peterson	1:80	---	1:40
31C	---	1:40	1:40
Langesen	1:40	---	---
Doyle	1:40	1:40	1:40
33B	1:40		
22B	1:80		
22C	1:40		
Hillman	1:80	1:80	1:20
Flynn	1:40		
Holt	1:80	1:40	---
Billagur	1:160	---	1:40

TABLE XIII

AGGLUTININ TITERS OF 28 BACT. COLI MUTABILE STRAINS

IN FLEXNER ANTISERA

Strains	Serum 352A 1:10240	Serum 352 1:81920	Serum Warden 1:40960
266	----		
265	1:40	----	----
269-5	----		
262B	1:20	1:40	1:40
139A	1:80		
Fields	1:160		
351A	----		
Duncan	----		
257	1:20	1:40	1:40
100	1:20		
269J	1:80		
247-2	1:320	----	1:40
269-I	1:320	1:40	----
269M	1:40	1:40	1:40
269F	1:640		
153	1:640	----	----
1016	----	1:40	1:40
137-1	1:80	1:40	----
C153	1:20		
G22	1:20	1:40	----
46G	1:160		
14G	1:320	----	----
412	1:40	----	----
269A	1:80		
331	----		
269P	----	----	----
1013	----	1:40	----
219-I	1:80	1:40	----

eight mutabile strains failed to agglutinate. The remaining antigens, approximately fifty percent of the total number, agglutinated in dilutions from 1:20 to 1:160.

One Bact. coli strain and one Bact. coli mutabile strain agglutinated in Warden serum I:5120, one-eighth the titer of the serum. Five Bact. coli cultures and two Bact. coli mutabile strains reacted in dilutions of the serum from 1:640 to 1:2560. Three mutabile strains agglutinated in dilutions from 1:160 to 1:320. Sixteen Bact. coli antigens of the forty tested in the Warden serum did not agglutinate; ten Bact. coli mutabile antigens of the twenty four strains tested did not react in any dilution. The remaining cultures agglutinated in dilutions of 1:20 to 1:80.

By observing the agglutination titers of the ten strains of Bact. coli and the ten strains of Bact. coli mutabile in the W and Z type sera, no correlation was found between Flexner agglutinin type specificity and Escherichia agglutination titers, for the strains of Escherichia which agglutinated markedly in the W type serum also agglutinated strongly in the Z type serum. Generally speaking, the same results are seen in the 352A, 352 and Warden antisera, as the Bact. coli and Bact. coli mutabile strains which agglutinated to high titers in one serum seemed to do the same in the other

two sera. There are exceptions in a few cases to this statement, for certain strains agglutinated markedly in only one serum and low in the remaining sera.

The Escherichia end titers were not as high in the W and Z sera, 1:640 the highest, as the end titers in the other three Flexner antisera.

In the study of the antigenic make-up of the Flexner strains, it was concluded that 352A and Warden strains were of the W race, and the 352 strain a member of the Z race. From the tables it will be seen that Escherichia cultures may agglutinate to a high titer in the 352A serum and to a low titer, or not at all, in the Warden serum. In other instances, certain antigens agglutinate strongly in the Warden and 352 sera, and weakly in the 352A serum. In other words, the agglutination of Escherichia strains in the Flexner antisera is not related to the type specificity of the Flexner strains. If the Escherichia and Flexner strains share antigenic components, the antigens are of a group nature.

Absorption of Flexner Antisera with 35 Escherichia Strains

Two Bact. coli strains, R.A. and Mayfield, and one Bact. coli mutabile strain, 199, which agglutinated to high titers in the Flexner sera, were selected for absorption of the Flexner antisera 352A, 352 and Wardem. The findings of the absorption tests are given in tables XIV, XV and XVI.

The Flexner antisera were first absorbed with the homologous organisms until the titers fell to 1:20, then tested with the Escherichia antigens. In every instance the Flexner cultures removed the homologous agglutinins and the agglutinins for the Escherichia strains.

Upon absorbing the Flexner antisera with the Escherichia organisms, the absorbing culture was found to remove its own agglutinins and lower remarkedly the titer for the other two Escherichia strains, yet leave unaffected the titer for the homologous Flexner strain. In table XV it will be noted that after the Warden serum was absorbed with strains 199 and Mayfield, the Warden titer dropped to 1:20480. Because of the fact that the original titer of the serum was high, and because of the necessity of using a large absorbing dose, the drop in titer is not

TABLE XIV

AGGLUTININ TITERS IN 352A SERUM ABSORBED WITH ESCHERICHIA STRAINS

Strains	352A serum unabsorbed	352A absorbed with 352A	352A absorbed with 199	352A absorbed with R.A.	352A absorbed with Mayfield
352A	1:10240	1:20	1:10240	1:10240	1:10240
199	1:1280	1:20	0	1:20	1:40
R.A.	1:2560	0	1:80	0	1:40
Mayfield	1:1280	0	1:320	1:40	0

TABLE XV

AGGLUTININ TITERS IN WARDEN SERUM ABSORBED WITH ESCHERICHIA STRAINS

Strains	Warden serum unabsorbed	Warden serum absorbed with Warden	Warden serum absorbed with 199	Warden serum absorbed with R.A.	Warden serum absorbed with Mayfield
Warden	1:40960	1:20	1:20480	1:40960	1:20480
199	1:1280	1:20	1:40	1:160	1:80
R.A.	1:1280	1:20	1:40	0	1:80
Mayfield	1:640	0	0	1:80	0

TABLE XVI

AGGLUTININ TITERS IN 352 SERUM ABSORBED WITH ESCHERICHIA STRAINS

Strains	352 serum unabsorbed	352 absorbed with 352	352 absorbed with 199	352 absorbed with R.A.	352 absorbed with Mayfield
352	1:81920	0	1:40960	1:81920	1:81920
199	1:2560	0	1:20	1:40	1:80
R.A.	1:1280	0	1:80	1:80	1:80
Mayfield	1:1280	0	0	1:40	1:20

of importance and within the limits of experimental error. The same can be stated concerning the decrease in titer for the 352 organism in the 352 serum following the absorption of that serum with strain 199.

The three Escherichia strains appear to be related antigenically as they gave consistently high agglutinin titers in the Flexner antisera, and each was able to absorb completely its own agglutinins and almost completely the agglutinins for the two remaining cultures from the high titer Flexner sera.

TABLE XVII

AGGLUTININ TITERS IN 352A SERUM ABSORBED WITH

ESCHERICHIA STRAINS 199 AND CRUSE

Bact. coli strains	352A serum unabsorbed	352A absorbed with Bact.coli mutabile,199	352A absorbed with Bact.coli Cruse
Cruse	1:1280	1:640	1:20
5-2W	1:1280	1:1280	1:1280
Mayfield	1:1280	1:320	1:320
R.A.	1:2560	1:160	1:1280
Bact. coli mutabile strains			
199	1:1280	------	1:1280
Hogan M.	1:1280	1:1280	1:320
1025 M.R.	1:2560	1:2560	1:2560
Sparks	1:2560	1:20	1:2560
Borden	1:2560	1:2560	1:2560

Further absorption of the high titer Flexner serum 352A was done with the Bact. coli mutabile strain, 199, and the Bact. coli strain, Cruse. The absorbed serum was tested with the absorbing organisms and seven additional Escherichia strains. The results are given in table XVII.

The mutabile culture, 199, was able to absorb partially the agglutinins from the serum for three Bact. coli organisms and one mutabile strain in addition to the complete removal of agglutinins for the absorbing antigen. From the absorption results the mutabile strain, Sparks, is most closely related to the mutabile organism, 199.

The Bact. coli strain, Cruse, partially removed the agglutinins from the 352A serum for one Bact. coli culture and one Bact. coli mutabile culture, and completely for itself.

From the findings there is no evidence indicating that the Bact. coli mutabile strains form a more homogeneous group antigenically than do the antigenically diverse Bact. coli strains. It is seen that the Bact. coli and Bact. coli mutabile cultures share antigenic components and are thus related.

Agglutination of Flexner and Escherichia Strains in Escherichia Sera

High titer antisera prepared against the Bact. coli strains R.A. and Mayfield and the Bact. coli mutabile strain, 199, were tested with strains of Shigella para-dysenteriae Flexner and cultures of Bact. coli and Bact. coli mutabile.

The antisera were tested first with the strains used for immunization by homologous agglutination and cross agglutination. The results of the tests are recorded in table XVIII, and it is evident that the three cultures share antigenic components as shown by the strong cross agglutination. The mutabile strain 199 agglutinated to titer in the R.A. and Mayfield sera. The Bact. coli antigen Mayfield agglutinated to half titer in the R.A. serum and in a high dilution of serum 199. The coli strain R.A. agglutinated higher than titer in the Mayfield serum and in a high dilution of the serum 199. The homologous titer of the serum 199 is unusually high, being 1:300,000.

The final titers of the Shigella flexneri strains, Warden, 352A, 352, W and Z were low. Comparing the titers of these cultures in normal and immune sera it is seen that only in four instances do the immune sera titers

TABLE XVIII

AGGLUTININ TITERS OF 3 ESCHERICHIA STRAINS AND 5 FLEXNER STRAINS IN HIGH TITER ESCHERICHIA SERA

Escherichia strains	Bact. coli serum, R.A.	Bact. coli serum, Mayfield	Bact. coli mut. serum, 199
R.A.	1:10240	1:40960	1:81920
Mayfield	1:5120	1:20480	1:81920
199	1:10240	1:20480	1:300000
Flexner strains			
352A	0	1:80 L(1:40)	1:80 L(1:40)
352	0	1:40 (-)	1:80 L(1:40)
Warden	0	0	0
W	1:40 L(1:40)*	1:20 (1:20)	1:20 (1:80)
Z	0	1:20 (1:20)	1:20 (1:20)

* Final titer of strain in corresponding normal rabbit serum

exceed those of the normal sera titers. The Varden organism did not agglutinate in any of the sera. The N.A. serum reacted with one Flexner strain only, and in a low dilution. The five Flexner cultures agglutinated no higher than 1:80 in the Bact. coli and Bact. coli mutabile sera.

The Bact. coli and Bact. coli mutabile strains which gave marked agglutination in the Flexner antisera were again tested in the Escherichia sera. In order to make a more conclusive study of the antigenic relationships of the Escherichia strains in our collection, 18 additional Bact. coli and 5 Bact. coli mutabile strains were chosen at random and antigens prepared from them. These antigens were also tested in the three antisera. The agglutination results are given in table XIX.

From the figures in table XIX, giving the end titers of a number of Escherichia strains in sera N.A., Mayfield and 199, the antigenic diversity of the Escherichia group is evident. Four Bact. coli strains, Murry, Miller, Nalen and Hillman, and three Bact. coli mutabile strains, Hillman Mut., Sparks and C153 reacted in high dilutions of the three sera. In some instances these strains agglutinated to the titer or higher of the serum in which they were tested. In exact opposite to the behaviour of the afore mentioned strains, other cultures were found which did not agglutinate. Five Bact. coli and three Bact. coli

TABLE XIX

AGGLUTININ TITERS OF ESCHERICHIA STRAINS IN 3 ESCHERICHIA SERA

Bact. coli strains	Bact. coli serum, R.A. 1:10240	Bact. coli serum, Mayfield 1:20480	Bact. coli mut. serum, 199 1:300000
Holstrom	----	----	1:20
34B	----	1:20	1:40
Isaacson	1:40 L	1:40 L	1:20
Cruse	1:20	1:40	1:160
5-2W	----	1:20	----
Hogan	1:20	1:40	1:160
Murry	1:10240	1:40960	1:20480
Harper	----	1:20	----
Swanson	1:20	1:20	1:20
Gordon	----	----	----
Miller	1:40960	1:40960	1:40960
Moreland	----	1:20	----
O.Mebelic	----	1:20	1:20
Reberson	----	----	----
Lake U	1:80	1:80	1:320
Halen	1:2560	1:10240	1:10240
Ebber	----	----	----
Grunewald	1:40	1:20	----
Samson	----	----	----
Peterson	----	1:80	----
Langesen	----	1:20	----
22B	----	1:20	----
Hillman	1:10240	1:10240	1:40960
Holt	----	1:20	1:40
Billagur	----	----	----
Bact. coli mut. strains			
1045	1:10	----	----
78A	----	----	----
Hogan M.	1:20	1:40	1:80
1025 M.R.	1:20	1:40	1:160
Lockwood	1:20	1:20	1:40
Hillman M.	1:320	1:640	1:2560
Sparks	1:5120	1:40960	1:81920
70	----	----	1:20
Duncan	1:40	1:20	1:40
C153	1:40960	1:5120	1:20480
G22	1:40	1:20	----
46G	----	----	----
269A	1:80	1:40	1:80

mutabile cultures failed to react with any of the sera. Some of the organisms agglutinated in one of the three sera and not in the remaining two, or in two of the sera and not in the third. In such cases the final titers were low; not above the titers found in the normal rabbit serums. The Bact. coli antigen, Lake U, agglutinated to a higher titer in the mutabile serum than it did in the two coli serums. The rest of the cultures agglutinated to approximately the same degree in the three antisera; the titers ranging from 1:20 to 1:80 usually and infrequently to 1:160.

It is seen that those cultures which agglutinated to a high titer in one serum did the same in the other two sera. Those strains which agglutinated low in one serum also agglutinated low in the remaining sera. The Bact. coli strains reacted equally as well in the mutabile sera as they did in the coli sera. The mutabile strains do not appear to be more easily agglutinated by the 109 serum than they do by the R.A. and Mayfield sera.

Reciprocal Absorption of Escherichia Sera with Escherichia Strains

The reciprocal absorption results of the Bact. coli strains Mayfield and R.A. and the Bact. coli mutabile strain, 199, in the corresponding Escherichia antisera are given in tables XX, XXI and XXII. Tables XXIII, XXIV and XXV show the results obtained when the homologous organism was used for absorption.

The Bact. coli R.A. culture is able to reduce the agglutinin titers for the three cultures in the three sera to the normal agglutinin levels. From these findings it is evident that the R.A. organism contains the antigenic components of the 199 and Mayfield cultures. By cross agglutination it was seen that the R.A. strain reacted strongly in the hterologous sera.

The Bact. coli mutabile strain 199 removes the agglutinins for itself and R.A. from the three sera. The Mayfield agglutinins are removed from the 199 and R.A. sera by the 199 organism, but not entirely from the Mayfield serum. The Mayfield antigen still agglutinated 1:640 in its serum after absorption with 199. These results show that the R.A. and 199 organisms are able to completely absorb agglutinins for each other, however, the May-

RECIPROCAL ABSORPTION OF ESCHERICHIA SERA WITH ESCHERICHIA STRAINS

TABLE XX

Bact. coli mutabile Serum, 199

Strains	Serum 199 unabsorbed	Serum 199 absorbed with R.A.	Serum 199 absorbed with Mayfield
199	1:300000	1:40	1:1280
R.A.	1:81920	1:40	1:2560
Mayfield	1:81920	1:20	1:40

TABLE XXI

Bact. coli Serum, R.A.

Strains	Serum R.A. unabsorbed	Serum R.A. absorbed with 199	Serum R.A. absorbed with Mayfield
R.A.	1:10240	1:80	1:640
199	1:10240	1:40	1:320
Mayfield	1:5120	1:80	1:20

TABLE XXII

Bact. coli Serum, Mayfield

Strains	Serum Mayfield unabsorbed	Serum Mayfield absorbed with 199	Serum Mayfield absorbed with R.A.
Mayfield	1:20480	1:640	0
199	1:40960	1:20	1:20
R.A.	1:20480	1:40	0

field organism differs somewhat and seems to have a specific component not shared by 199, but contained in the antigenic make-up of the R.A. strain.

The Mayfield antigen reduces markedly the agglutinin titers of 199 and R.A. in the three sera, but cannot remove completely the agglutinins for them. In absorbing the sera with Mayfield, the titers for R.A. and 199 fell lowest in the absorbed R.A. serum, and to the same degree in the absorbed 199 and Mayfield sera. It would appear that R.A. and 199 contain specific antigenic components not related to the Mayfield antigen. The Mayfield culture is related to the 199 and R.A. strains through similar group antigens.

From the reciprocal absorption results the mutabile culture, 198, and the coli strain R.A. appear to be antigenically identical, with the exception that the R.A. culture is able to remove completely the Mayfield agglutinins from the Mayfield serum, and 199 reduces the titer for Mayfield from 1:20480 to 1:640. The 199 and R.A. strains contain a specific antigenic component not found in the Mayfield organism.

TABLE XXIV

AGGLUTININ TITERS IN MAYFIELD SERUM ABSORBED WITH FLEXNER STRAINS

Strains	Mayfield unabsorbed	Mayfield absorbed with Mayfield	Mayfield absorbed with 352A	Mayfield absorbed with 352
Mayfield	1:20480	1:20	1:20480	1:20480
352A	1:80	1:40	0	1:40
352	1:40	1:80	1:40	1:20
R.A.	1:20480	1:2560	1:40960	1:20480
199	1:40960	1:5120	1:40960	1:40960

TABLE XXV

HOMOLOGOUS ABSORPTION OF R.A. SERUM*

Strains	Serum R.A. unabsorbed	Serum R.A. absorbed with R.A.
R.A.	1:10240	1:20
Mayfield	1:5120	1:20
199	1:10240	1:20

TABLE XXIII

AGGLUTININ TITERS IN 199 SERUM ABSORBED

WITH FLEXNER STRAINS

Strains	Serum 199 unabsorbed	Serum 199 absorbed with 199	Serum 199 absorbed with 352A	Serum 199 absorbed with 352
199	1:300000	1:0	1:300000	1:300000
352A	1:80	1:80	0	1:40
352	1:80	1:40	1:80	1:20
R.A.	1:81920	1:40	1:160000	1:81920
Mayfield	1:81920	0	1:81920	1:81920

*Flexner, Warden strain not used for absorption as it did not agglutinate in Escherichia antisera

Absorption of Escherichia Sera with Flexner Strains

Absorbing the Escherichia sera with the heterologous Shigella flexneri strains, no antigenic relationship between Flexner organisms and Bact. coli and Bact. coli mutabile strains was revealed.

Tables XXIII, XXIV and XXV record the results of these reciprocal absorption tests. In no instance were the Flexner strains capable of absorbing the Escherichia agglutinins, as the specific titers remained at the original high level. In absorbing the Escherichia sera with the homologous organisms, no consistent drop in Flexner titers was evident.

The reciprocal absorption tests here further validate the results of agglutination of Flexner cultures in Escherichia antisera, as in neither instance was evidence given for antigenic relationships between Flexner strains and Escherichia strains.

Comparative Agglutinin Titers of Escherichia Strains in Flexner and Escherichia Sera

In comparing the recorded agglutinin titers of a number of Escherichia strains in high titer Shigella flexneri and Escherichia sera, it is seen in table XXVI that the cultures divide into three groups. The first group contains those Escherichia strains which agglutinated markedly in the six sera. Group II cultures reacted strongly with the Flexner antisera, but not above the normal agglutinin level in the Escherichia sera. Group III strains appear to lack the ability to agglutinate in the dysentery antisera, yet reacted in exceedingly high dilutions of the three Escherichia sera.

TABLE XXVI

AGGLUTININ TITERS OF ESCHERICHIA STRAINS IN FLEXNER

AND ESCHERICHIA SERA

Escherichia strains	Serum 352A 1:10240	Serum 352 1:81920	Serum Warden 1:40960	Serum 199 1:300,000	Serum R.A. 1:10240	Serum Mayfield 1:20480
Group I						
Mayfield	1:1280	1:1280	1:640	1:81920	1:5120	1:20480
R.A.	1:2560	1:1280	1:1280	1:81920	1:10240	1:40960
Murry	1:80	1:640	1:40	1:20480	1:10240	1:40960
Hilman M.	1:640	1:80	1:5120	1:2560	1:320	1:640
Sparks M.	1:2560	1:1280	1:640	1:81920	1:5120	1:40960
199 M.	1:1280	1:2560	1:1280	1:300000	1:10240	1:20480
Group II						
Holstrom	1:640	1:80	1:40	1:20	------	-----
34B	1:640	1:160	-----	1:20	------	1:40
Isaacson	1:40	1:160	1:640	1:20	1:40	1:40
Cruse	1:1280	1:320	1:1280	1:160	1:20	1:40
5-2W	1:1280	1:160	1:80	-----	-----	1:20
Hogan	1:160	1:80	1:5120	1:160	1:20	1:40
Harper	1:320	1:40	1:20	1:0	-----	1:20
1045	1:320	1:20	1:40	-----	1:10	----
78A	1:640	1:1280	1:1280	-----	-----	-----
Hogan M.	1:1280	1:20	1:160	1:80	1:20	1:40
1025 M.R.	1:2560	1:160	1:160	1:160	1:20	1:40
Lockwood	1:640	1:40	1:320	1:40	1:20	-----
Borden	1:2560	1:1280	1:640			
70	1:40	-----	1:2560	1:20	-----	1:20
Group III						
Miller	1:320	-----	-----	1:40960	1:40960	1:40960
Kalen	1:80	-----	-----	1:10240	1:2560	1:10240
Hillman	1:80	1:80	1:20	1:40960	1:10240	1:10240
C153	1:20	-----	-----	1:20480	1:40960	1:5120

DISCUSSION

In this paper certain facts stand out prominently. First, normal rabbit sera are shown often to have the capacity to agglutinate a wide variety of gram negative intestinal bacilli, including strains of Shigella flexneri and Escherichia coli. The titer of such sera, however, is generally low, rarely reacting with any of the antigens in dilutions above 1:80.

Second, the sera of animals immunized with various types of Shigella flexneri not only show a greatly increased titer for the homologous organisms, but also show a strikingly increased ability to agglutinate certain Escherichia strains. In their capacity to stimulate Escherichia agglutinins the various type s of Shigella flexneri exhibit no significant difference. From these sera the homologous organism and often types of the same species, will reduce the Escherichia agglutinin titer at least to the level of the normal titer.

Third, the animals immunized to the strains of Escherichia which are agglutinated to high titer by the Flexner sera, do not produce sera with agglutinin titers for the latter above the normal level.

Every precaution was taken to use cultures which

appeared by the known criteria to be in the smooth phase. A partial S R dissociation of the Escherichia cultures was considered as one possible factor in the marked agglutination of certain Bact. coli and Bact. coli mutabile strains in the Flexner antisera, and yet, the same antigens were found to agglutinate only in low dilutions of the Escherichia antisera, with the exception of two mutabile strains, a fact which lends assurance to the belief that only S phase cultures were used.

The failure of the Escherichia sera to agglutinate Flexner strains suggests at once that the relationships of the two organisms cannot be explained on the basis of simple common antigenic components. Other factors must be present.

Examining the possible factors involved in this relationship, one of the first explanations which presented itself was that of an anamnestic reaction. This reaction is essentially the production in response to a heterologous antigenic stimulus of an antibody that has been produced in the tissues on some previous occasion.

The rabbits which we immunized to Shigella flexneri strains had not been previously immunized against Escherichia cultures. Table V shows that in a number of instances the normal rabbit sera was unable to agglutinate

certain Escherichia cultures, but when the rabbits were immunized to Shigella flexneri strains, the immune sera reacted in strikingly high dilutions with the coli and mutabile antigens. Certain Escherichia cultures which agglutinated only 1:10 in the normal rabbit sera, gave positive tests in the corresponding Flexner antisera to titers of 1:2560. Comparing the agglutinin titers for Shigella flexneri strains in normal rabbit sera and Escherichia sera, no definite difference was noticed.

If the anamnestic reaction is due to non-specific stimulation of the agglutinin forming mechanism, there is no apparent reason why Flexner agglutinins should not be stimulated by the injection of Escherichia antigens, as the Escherichia agglutinins are stimulated by the Shigella flexneri antigens, for the normal titers for the Flexner organisms are as high as some of the normal titers for the Escherichia strains.

In order to study further the possibility that Bact. coli agglutinins might be produced by non-specific antigenic stimulation, Brandon(unpublished thesis) immunized rabbits to horse serum and human serum. The final titers for a number of Bact. coli strains tested in the immune sera were no higher than the titers obtained from testing the same strains in the corresponding normal serum, indicating at least, that coli agglutinins did not rise

in response to the above mentioned non-specific antigens.

The anamnestic reaction, as an explanation for the experimental results we obtained, appears to be inadequate.

The facts would seem to indicate an actual antigenic relationship between strains of Shigella flexneri and Escherichia; again, however, the failure of the Escherichia sera to agglutinate the Flexner antigens above the normal agglutinin level suggests that such relationship is not a simple one. The possibility of a definite relationship through the interaction of related deep and suface antigenic components presents itself. In this hypothesis, and conforming to the experimental evidence, the Shigella flexneri organisms may be thought of as carrying a deep antigen which is serologically similar to a surface antigen belonging to certain Escherichia strains. See diagram 1.

Flexner bacillus

Escherichia bacillus

Diagram 1

The Shigella flexneri antisera would contain agglutinins stimulated by both D and S antigens, the D agglutinins reacting by agglutination with those strains of Escherichia which carried a similar D surface antigen, and being absorbed from the Flexner antisera by the same Escherichia

strains. In absorbing the Flexner antisera, any one of the Escherichia cultures could reduce the titer for the other two strains to the level of the normal agglutinin titers, indicating serological similarity, results which might be expected if the three Escherichia strains were closely related through their D surface antigens. By agglutination and absorption tests, using the Escherichia organisms and the corresponding antisera, definite evidence was given that these strains are antigenically similar.

It is possible that the agglutinin titers for the Shigella flexneri strains in the Flexner antisera would not be changed by Escherichia absorption, for the S agglutinins, responsible in every case for the Flexner agglutination, would not have been removed. The reciprocal absorption results of tables VII, VIII and IX record the antigenic relationships which could be explained by this hypothesis as dependent on the S antigens of the Flexner bacilli.

Still following closely, the fact that the Flexner organisms do not agglutinate above the normal level in the Escherichia immune sera might be explained by suggesting that the D antigens of the Flexner cultures can not come in contact with the D agglutinins of the Escherichia sera due to their position in the bacterial cell.

However, we have seen that the Escherichia agglutinins (D) of the Flexner antisera can be reduced to the normal titer level by absorption of the sera with the homologous Flexner strain or related Flexner strains. One would not expect this to happen unless the D antigens of the Flexner bacilli were exposed to the D agglutinins of the antisera, yet the Flexner absorbing organism is an intact bacterial cell, the surface antigens only being in contact with the serum.

This theory, in its present form, does not seem to explain adequately the results of our experimental work.

There is a possibility that a definite antigenic relationship between strains of Shigella flexneri and Escherichia might be explained by assuming that the sharing of an antigen extends only to a haptene component. The facts would then require that the dysentery bacilli possess a whole antigen of which the haptene component only is shared by certain of the colon bacilli. If this were true, the Escherichia strains would be incapable of stimulating the production of agglutinins which would react with the Flexner organisms, for an isolated haptene is not agglutinogenic. However, possession of a haptene by some of the Bact. coli and Bact. coli mutabile cultures would allow them to agglutinate in Flexner antisera and absorb the agglutinins with which they react, as though

they were whole antigens.

In table XXVI the comparative agglutinin titers for a series of Bact. coli and Bact. coli mutabile cultures in high titer Shigella flexneri and Escherichia sera are recorded.

In order to relate the experimental findings to the suggested presence of a shared haptenic fraction, Group 1 strains may be assumed to carry an isolated hapteno,h, accounting for the high agglutinin titers in the Flexner antisera, and in addition, a complete bacterial antigen or antigens specific for Group 1 strains which we shall designate with the letter A. Group 1 cultures, then, are endowed with the components hA. Group 11 organisms may be assumed to carry a similar haptene, h, to that of Group 1 explaining the marked agglutination in the Flexner antisera, but differing from Group 1 in the specific Escherichia antigenic components of the bacilli, which account for the lack of agglutination in the Escherichia sera; components which we shall call B. Group 11 organisms are essentially hB. The third group cultures are seen to agglutinate to as high titers in the Escherichia sera as did those of Group 1, therefore, the Escherichia antigens responsible for such agglutination may be called A, and since the agglutinin titers were low in the Flexner antisera we may presume that the haptenic fraction carried by

Groups I and II is not present in Group III. Group III strains are given the antigenic component A. We may mention that under the assumptions of this hypothesis the antigens of the Flexner bacilli are composed of a protein, P, with the haptene fraction, h.

Any one of the three Escherichia strains used to absorb the high titer Flexner sera is seen to remove the agglutinins responsible for its own agglutination and to reduce to the normal titer level the agglutinins for the other two strains. Such results could easily mean that the three Escherichia organisms are closely related antigenically, and that the same isolated haptene might be carried by the three strains. By cross agglutination and reciprocal absorption tests of the three Escherichia strains in the Escherichia sera, it is evident that these organisms are related through their specific whole antigens.

We have seen that the absorption of the Shigella flexneri sera with the homologous and related strains reduced the Escherichia agglutinin titers to the normal level. This would be possible if the haptene fraction of the Flexner antigen was similar to that of the Escherichia cultures which agglutinated in the Flexner sera and thus was able to absorb the Escherichia agglutinins from the sera.

A good indication is given, by the results recorded

in table XXVI, that the haptene fraction which we have assumed to belong to the Escherichia strains of Group I and II, is not the same in all the cultures. It could be that the isolated Escherichia haptene differs among strains by a configuration of the chemical grouping, yet remains similar enough to the Flexner haptenic fraction to allow for the marked agglutination.

It is a well known fact that the strains of Bact. coli are antigenically diverse--to the point where they are practically type specific, therefore, in referring to the specific antigenic components as A and B as we have done, it is well to keep in mind that the actual situation is not so simple, but that such designations are sufficient to fit our hypothesis.

Additional experimental evidence in support of the haptene theory is necessary before it can be accepted without reservation. Julianelle(1937) reported on the antigenic relationships of Bact. aerogenes strains to a strain of Pneumococcus type II and a culture of Friedlander's bacillus. He concluded from a series of tests, that the immunological relationships of these organisms depends entirely on the structure of the capsule, and the results confirm the principle that immunological similarities among biologically unrelated organisms depends entirely upon similarities found in the specific

carbohydrate of the capsule. We did not examine our Escherichia cultures for the presence of a capsule, or try to extract specific carbohydrate components. It would be interesting to attempt such procedures and test the resulting extracts in high titer sera of the Flexner and Escherichia strains.

Barnes and Wight(1935) extracted a soluble substance from an encapsulated strain of Bact. coli which reacted with a Pneumococcus type 1 horse serum. Smith(1927) isolated a specific soluble substance from encapsulated Bact. coli strains which proved to be carbohydrate in nature. He added that the soluble specific substance of the coli capsule remains in the capsule and does not diffuse outward into the medium.

Tomcisk(1927) extracted a specific substance from the capsules of a number of Bact. coli strains, each extract reacting in high dilutions with the homologous serum. The isolated substance proved to be non-agglutinogenic. Kozaya(1931) reported that sera prepared from certain organisms may contain two different polysaccharide precipitable antibodies, one specific for the homologous organism, the other non-specific and reacting with a number of unrelated strains.

Ingalls(1937) stated that three or more polysaccharides--om other words specific haptenes--may be isolated from one

organism, and it is seen that the chance for cross reactions would be great indeed. Landsteiner(1936) has gone further and feels that identical haptenes are not necessary for cross reactions between otherwise unrelated substances, but that merely similar molecular grouping suffices.

Finding such a marked antigenic similarity between the three Escherichia cultures which we chose to use for sera preparation and absorption tests, it would be interesting to test the Group lll strains of table XXVl by absorption in the three Escherichia sera already in stock and note if the high agglutinin titers denote specific antigenic relationships. Further information could be obtained if additional sera for Group l and lll strains were prepared and the organisms tested by reciprocal absorption and agglutination methods.

SUMMARY

Eighty eight Escherichia cultures and five Shigella flexneri cultures, the latter including two strains belonging to the W race of Andrewes and one belonging to the Z race, were tested by agglutination in normal rabbit sera, high titer Flexner sera and Escherichia immune sera. Reciprocal absorption experiments were carried out with three of the Flexner and three of the Escherichia strains. The three Escherichia strains appeared to be closely related antigenically through specific and group components.

Normal rabbit sera are shown to have the capacity to agglutinate a majority of the Escherichia and Flexner cultures. The titer, however, of these normal sera was low. Of a total of 164 tests carried out, only 18 showed a maximum titer as high as 1:80. In 8 of these the titer reached 1:160.

The sera of animals immunized with the Flexner cultures not only showed a greatly increased titer for the homologous organisms, but also showed a strikingly increased ability to agglutinate certain Escherichia strains. In their capacity to stimulate Escherichia agglutinins the various types of Shigella flexneri exhibited no significant differences. Absorption of these sera with the homologous organism, or with strains of the same type, reduced the Escher-

ichia agglutinin titer at least to the normal level.

The sera of animals immunized to the strains of Escherichia which were agglutinated to high titer by the Flexner sera, did not give agglutinin titers for the latter above the normal level.

In seeking for an explanation for the above results, three hypotheses were critically examined. The failure of the Escherichia sera to agglutinate Flexner strains suggests that the relationships of the two organisms cannot be explained on the basis of simple common antigenic components.

The concept that the agglutinins for Escherichia strains in Flexner antisera were the result of non-specific stimulation of an already existing antibody forming mechanism, the so called anamnestic phenomenon, seems inadequate to account for the results. The failure of the coli antigens to stimulate dysentery agglutinins is not explained by this hypothesis.

There is the possibility of a definite relationship between strains of Escherichia and Flexner through the interaction of related deep and surface antigenic components. In this hypothesis the Shigella flexneri organisms may be thought of as carrying a deep antigen which is immunologically identical to the surface antigen belonging to certain Escherichia strains. This supposition falls short, however, since it does not account for the

absorption of Escherichia agglutinins from the Flexner antisera by the homologous or related Flexner bacilli.

It might be possible that a definite antigenic relationship between these strains could be explained by assuming that the sharing of an antigen extends only to a haptene component. The facts would then require that the dysentery bacilli possess a whole antigen of which the haptene component only is shared by certain of the Escherichia bacilli. Our own experimental data appears to be adequately explained by this hypothesis which is therefore offered as a basis for further study of the phenomenon herein discussed.

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