AGGLUTININS FOR SHIGELLA PARADYSENTERIAE, FLEXNER IN NORMAL HUMAN SERA

by

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Numerous investigators, including Shiga (1898, 1902), Kruse (1900, 1901), Flexner (1900, 1901), Strong and Musgrave (1900), Martini and Lentz (1902), Vedder and Duval (1902), Park (1903), and Nabarro and Signy (1932) have demonstrated that dysentery is endemic in Europe, Asia, Eastern and Southern United States and the Philippines. On the other hand reports of dysentery outbreaks in the Pacific Northwest are comparatively rare. Investigations have been made of the sera of normal individuals in those sections of the world where dysentery is endemic and in many cases agglutinins for the dysentery organisms were present. Because these agglutinins were demonstrated in the sera of normal persons, the agglutination with the patient's sera for the diagnosis of dysentery was considered of little value. It appeared to us, since dysentery is relatively uncommon in this section of the country that a brief survey of agglutinins in the sera of normal individuals might be profitable with special reference to the value of the agglutination test in the diagnosis of dysentery here.

The causes of bacillary dysentery are: Shigella dysenteriae, S. paradysenteriae, Flexner, and

S. paradysenteriae, Sonne. The general methods of biochemical differentiation are well known; the results of many of these tests are variable making conclusive differentiation difficult. On the other hand the antigenic differentiation by absorption experiments with high titer immune sera is extremely valuable. The S. dysenteriae is an antigenic unit as is the S. paradysenteriae, Sonne. The S. paradysenteriae, Flexner organisms, however, present a very complex antigenic picture.

Kalic (1927) attempted to unify the three main systems of classification of the dysentery organisms--the English (Andrews and Inman); the German (Kruse); and the Japanese (Aoki). Kalic examined samples of types from each classification, comparing them by absorption of agglutinin experiments, using many combinations of specific serum and absorbing organism until the antigenic picture was clear to him. His conclusions are recorded in Table 1. Kalic did not place Aoki's number VII because it was motile, his number XI because it produced gas on sugar broths, and his number VI because the author's strain was rough.

Sartorius and Reploh (1932) followed a similar procedure in studying the antigenic relationship of the Flexner group of organisms. They studied not only the classical organisms but numerous examples of each strain. Their results are shown in Table 2. Several Y dysentery strains were noted. The original Hiss-Russel Y organism, according to Sartorius and Reploh, is identical with the the D of the

TABLE I

COMPARISON OF THE CLASSIFICATIONS
OF DYSENTERY ORGANISMS - KALIC

ENGLISH (Andrews)	GERMAN (Kruse)	JAPANESE (Aoki)
Flexner V	B? or C?	--
" VZ	A	--
" W	--	--
" WX	--	--
" X	--	II
" Y	D	I
" Z	H	X
(Shiga)	(Shiga-Kruse)	VIII (Shiga)
--	--	III and V
--	--	IV
--	E (Sonne)	--

TABLE II

COMPARISON OF THE CLASSIFICATIONS
OF DYSENTERY ORGANISMS - Sartorius
and Reploh

ENGLISH	GERMAN	JAPANESE
Flexner V	B, C	V
" VZ	A	--
" W	D and original Hiss-	I
" X	-- Russel Y	X
Y		
" Y	--	--
Y		
" Z	H	II
		IV and XII
		III and VI
		IX

German classification and the number I of the Aoki classification. The organism they designate as Y is the original Y of the Andrews and Inman classification while the Scott Y organism was designated as Y .

In comparing the two charts it will be noted that though in general the authors agree, a few discrepancies can be observed. In the first place, Kalic believes the original Hiss-Russel Y is the Y of the English classification, while Sartorius and Reploh place the original Hiss-Russel Y in line with the Andrews and Inman W. Secondly the Japanese II and X are reversed in the two tables, and thirdly, Kalic states that the Japanese III and V are identical, while Sartorius and Reploh believe that the Japanese III and VI are identical. These differences in the observations of the three investigators demonstrates the difficulty of straightening out the complex picture of the antigenic structure of the Flexner organisms.

Davison's cross agglutination experiments (1920), summarized in Table 3, with the five English type organisms indicates the antigenic structure of the S. paradysenteriae, Flexner organisms.

TABLE III

CROSS AGGLUTINATION WITH THE
FIVE ENGLISH TYPE ORGANISMS
- DAVISON

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

The first study of the agglutinin content of normal human sera for dysentery organisms dealt with the agglutinins for S. dysenteriae. Shiga (1901) tested thirty-four normal serums with the organism he isolated. The titer in all cases was less than 1-10. Kruse (1900) testing twenty-five normal sera with his organism noted that several of the sera were positive at a 1-10 or 1-20 dilution, and exceptionally as high as 1-50. In a later paper, Kruse (1901) stated that in cases of persons whose serum had a titer of 1-50 he was dealing with those who had previously had dysentery.

With the subsequent isolation of the Flexner organism, the sera of normal persons were tested to determine their agglutinin content for dysentery organisms of this type. The organisms which Kruse isolated from insane patients with dysentery (probably some strains were the Flexner organisms) often agglutinated with normal sera at 1-50.

More recently Ritchie (1916) tested the sera of 792 persons with the Flexner organism. His results are recorded in Table 4.

TABLE IV

RESULTS OF EXAMINATION OF BLOOD
OF 792 NORMAL PERSONS FOR
DYSENTERY AGGLUTININS
- RITCHIE

	Total Number of Sera	- in 1-64 %	+ in 1-64 %	+ in 1-128 %	+ in 1-256 %	+ in 1-512 %
Females	257	16.34	32.68	46.69	2.72	1.55
Males	535	31.77	45.42	22.24	0.37	0.18
Total	792	26.72	41.28	30.17	1.13	0.65

From these investigations, Ritchie concluded that at a dilution of 1-64, the great majority of normal sera agglutinated S. paradysenteriae, Flexner. In general, the sera of women agglutinated the organisms to a higher titer than did the sera of men.

Burgdorf (1925) investigated 200 sera testing them with the D and H organisms of the Kruse classification and with the Kruse type itself. His results are given in the following table, table V.

Table V

Results of Examination of Blood of 200 Normal Persons for Dysentary Agglutinins - Burgdorf

% of Sera Agglutinating at the given dilutions	Organism D %	H %	Kruse %
1-0	--	2.5	6.0
1-5	--	27.5	29.5
1-10	--	37.5	42.0
1-25	7.0	24.5	18.5
1-30	23.5	5.5	4.0
1-100	45.0	2.5	--
1-200	22.5	--	--
1-400	2.0	--	--

Burgdorf's original problem was to discover the relationship, if any, between blood groups and groupings derived from agglutination of certain bacteria with normal sera. The author concluded that persons in all blood groups have dysentery agglutinins.

Burgdorf demonstrated the specifity of the agglutinins by absorption experiments. The Kruse type absorbed out all of its own agglutinins leaving the agglutinins for the D and H strains unaltered. Similarly, the H organism absorbed out all of its agglutinins leaving in original titer the agglutinins for S. dysenteriae and the dysentery D strains. The D type, however, absorbed out not only all of its own agglutinins but those of the H organism as well, leaving those for the S. dysenteriae in original titer. According to the author it is evident that none of the major agglutinins for the H type were present. This fact probably explains the low titer of the sera investigated for the H organism.

Menton (1929) tested the sera of 262 males and 262 females. His results are tabulated in Table 6. Positive results were obtained in 6.1% of sera from males and in 6.48% of those from females, the former, however, generally giving agglutination in higher dilutions.

Table VI

Results of Examination of Blood
of 512 Persons of Dysentary
Agglutinins - Menton

Males		Females	
Number of Specimens	Types	Number of Specimens	Types
1	Plus to Sonne	1	Plus to Sonne
5	Plus to Sonne	6	Plus to V
1	Plus to Sonne	6	Plus to V
1	Plus to V	3	Plus to V
3	Plus to V	1	Plus to Z
1	Plus to V		
1	Plus to W		
1	Plus to Z		
1	Plus to Y		
246	Neg. to all	245	Neg. to all
262		262	

EXPERIMENTAL WORK

The organisms which I used in my experimental work were all of the Shigella paradysenteriae, Flexner type, referred to as 352, 360, and Warden. The 352 type is designated in the collection at the University of Oregon Medical School as S. paradysenteriae, Flexner and was received in 1931 from the Hooper Foundation. This organ- had been sent to the Hooper Foundation by the Alabama State Department of Health in 1930. The 360 organism was obtained from the American Museum of Natural History, in 1918, in which collection it was called S. paradysenteriae, Hiss-Russel No. 200. The strain designated as Warden was isolated by the author from a dysentery case at the Multnomah Hospital. The Shigella dysenteriae organism (340 in our collection, received from the University of California Bacteriology Department in 1928, marked as the American Type Culture Collection #51) was tested with several sera.

In order to be sure that I was working with typical smooth strains, the organisms were plated out on plain agar. A typical isolated smooth colony was transplanted to an agar slant. This culture was tested for its biochemical reactions, and for its antigenic composition with Flexner high titer immune serum. If it reacted typically it was retained as a permanent stock culture. The antigenic inter-relationship of the organisms used is demonstrated in Table 7.

TABLE VII

AGGLUTINATIVE RELATIONSH IP OF THE FLEXNER DYSENTERY STRAINS EMPLOYED

Serum	Antigen	Serum dilutions 1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12800	Control
360	352	++	+	0					0
	360	++++	++++	++++	++++	+++	++	0	0
	Warden	++++	++++	++++	++++	+++	++	+	0
Warden	352	++++	++++	++++	+++	++	+	0	0
	360	++++	++++	+++	+	0			0
	Warden	++++	++++	++++	+++	++	+	0	0
352	352	++++	++++	++++	++++	+++	+	0	0
	360	+	+	0					0
	Warden	+	0						0
352 after absorption Warden	352	++++	++++	++++	+++	++	+	0	0
	360	0							0
	Warden	0							0
Warden after absorption with 352	352	0							0
	360	++	+	0					0
	Warden	++++	++	+	0				0

Before preparing the bacterial suspension for the agglutination tests reported below, a culture was taken from the stock collection and transplanted daily for at least three successive days after which the agar in the Blake bottle was inoculated. After twenty-four hours incubation, the bacterial growth was washed from the agar surface with 0.85% NaCl solution. This suspension of the bacteria was placed in a 55° C water bath for one hour, after which it was tested for sterility by heavy inoculation from the suspension on at least three agar slants. The suspension was then diluted until it was of the same density as Nepholometer tube 3, $BaSO_4$ standard corresponding to approximately 900,000,000 organisms per cc.

The 115 sera tested were, in the main, those drawn primarily for routine Wassermanns at the Multnomah Hospital, but the sera of 12 laboratory workers were also used. All sera were inactivated at 55-56° C for ½ hour.

The titer for each serum was determined by noting the highest dilution in which agglutination occurred. In this dilution a few clumps of bacteria were seen in an otherwise turbid solution. The control tube showed no clumping. A magnifying lens was usually necessary , showing fine and granular clumps rather than flocculent groups, though in the more concentrated solutions, the clumps were often visible to the naked eye. At a dilution of 1-5 and

1-10 complete agglutination frequently occurred. Intermediate degrees of agglutination were noted between the dilutions of 1-5 and that of the titer.

RESULTS

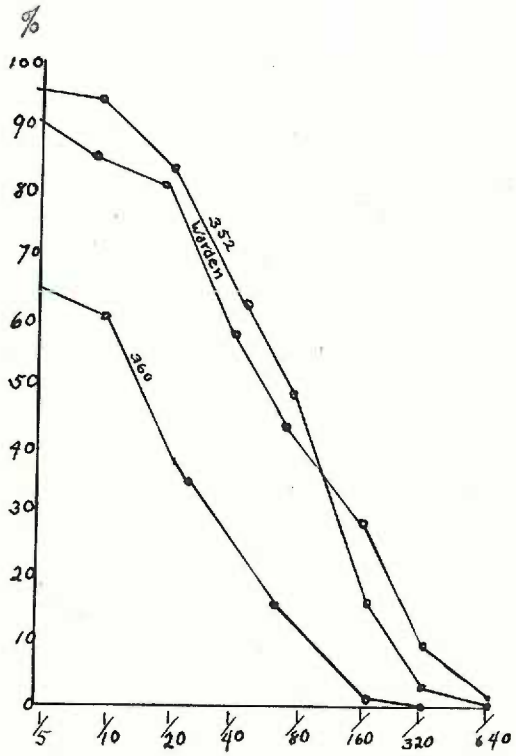
A summary of the results of agglutination tests with normal sera is recorded in Table 8.

TABLE VIII

AGGLUTINATION BY NORMAL HUMAN SERA
OF THREE STRAINS OF
S. PARA DYSENTERIAE, FLEXNER

Strain of Flexner Bacillus	Total no. of sera tested	No. and % of sera giving no agglutination in a dilution of 1-10		No. and % of total sera tested giving agglutination in each dilution							
				1-5	1-10	1-20	1-40	1-80	1-160	1-320	1-640
352	111	No.	3	108	107	96	74	54	21	2	0
		%	3	97	96	87	67	49	19	2	0
360	109	No.	36	73	68	53	29	14	2	0	0
		%	34	66	62	42	27	13	2	0	0
Warden	112	No.	9	103	96	93	66	48	32	9	1
		%	8	92	86	83	59	43	29	8	1

Graph of Table VIII



Dilutions of Sera

SEX DISTRIBUTION OF NORMAL AGGLUTININS

The comparative titer of the sera of men and women presents an interesting problem. Ritchie (1916) tested 792 human sera and concluded that the majority of sera from women agglutinated S. paradysenteriae, Flexner to a higher titer than did the sera from men. Menton, on the other hand, found that while more women's sera agglutinated the Flexner organisms, the men's sera agglutinated them to a higher titer. My results are shown in Table 9.

Table IX

Comparison of the Agglutinative Power of the Sera of the Two Sexes for the Three Strains of S. paradysenteriae Flexner Employed

Organism	Sex	Dilutions of Sera							
		1-5 %	1-10 %	1-20 %	1-40 %	1-80 %	1-160 %	1-320 %	1-640 %
352	Male	96	94	81	67	42	19	0	0
	Female	98	96	91	67	58	20	4	0
360	Male	67	62	48	35	21	2	0	0
	Female	67	62	51	22	7	2	0	0
Warden	Male	96	85	79	62	40	21	8	0
	Female	91	87	75	64	53	40	9	2

My results show relatively little qualitative difference between the agglutinating power of the sera of the two sexes. Approximately the same percentage of sera of men and women showed agglutinins at each dilution, though the 352 and Warden organisms were generally agglutinated to a higher titer than was the 360 organism. Approximately the same percentage of sera of both sexes agglutinated the three organisms employed in the lower dilutions, but the sera of women generally showed antibodies in a higher titer. With the 360 organism more men showed agglutinins in the higher dilutions of sera than did women. It seems likely that the selection of sera and organisms at least partially explains the differences in titers between the sexes demonstrated by the observations of Ritchie and Menton, as well as my own.

AGE DISTRIBUTION OF NORMAL AGGLUTININS

In order to discover the differences, if any, in the agglutinating power of the sera of babies and adults, I tested the cord blood of a few babies born at the Multnomah Hospital. The results with these sera are recorded in Table 10.

TABLE X

AGGLUTINATION BY NORMAL INFANT SERA
OF THREE STRAINS OF
S. PARADYSENTERIAE, FLEXNER

Strain of Flexner Bacillus	Total no. of sera tested	No. and % of sera giving no agglutination in a dilution of 1-10		No. and % of total sera tested giving agglutination in each dilution							
				1-5	1-10	1-20	1-40	1-80	1-160	1-320	1-640
352	10	No.	2	8	8	7	5	2	1	0	0
		%	20	80	80	70	50	20	10	0	0
360	12	No.	8	4	4	2	0	0	0	0	0
		%	67	33	33	17	0	0	0	0	0
Warden	12	No.	5	7	7	4	1	0	0	0	0
		%	42	58	58	33	8	0	0	0	0

Though a much smaller number of babies than adults was tested, the cord sera, like the adult sera, showed a greater percentage agglutinating the 352 and Warden organisms than agglutinated the 360 organism. My observations with the serum of cord blood demonstrated the lower agglutinin content of children's sera, confirming the investigation of various workers (Toomey, 1934; Buchner, 1923). Buchner recorded the percentage of sera of children at various ages possessing agglutinins for the Flexner organisms. A summary of his results is given in Table 11.

Table XI

Results of Testing for Agglutinins
in Sera of 166 Normal Persons
- Buchner

Age (Years)	Dysentary Agglutinins %	No Dysentary Agglutinins %
¼	--	3
½	41.6	7
¾	33.3	8
1	83.3	1
2	80.0	7
3-5	80.4	8
6-13	90.0	4
Adult	64.7	6
Total	73.4	26.6

ABSORPTION EXPERIMENTS WITH NORMAL SERA

One normal serum was absorbed with three different strains of S. paradysenteriae, Flexner, viz. 352, 360, and a third from the collection at the University of Oregon Medical School. The serum was tested before and after each absorption with ten different Flexner strains. The titers for the ten organisms were either lowered or the agglutinins were completely removed by all three strains. The 352 and 360 organisms showed reciprocal absorption. A qualitative difference between normal and immune agglutinins would seem to be indicated by these results.

AGGLUTININS FOR S. DYSENTERIAE IN NORMAL SERA

Forty-four sera were tested with S. dysenteriae. Ten sera gave agglutination in a maximum dilution of 1-10 and two agglutinated in a dilution of 1-20. In every case the organisms were only partially agglutinated. These experiments confirm the work of Shiga (1901), Kruse (1900) and others.

EFFECT OF TYPHOID IMMUNIZATION ON NORMAL AGGLUTININS FOR S. PARADYSENTERIAE, FLEXNER

In order to determine the effect of typhoid immunization, six sera were tested before and after the injection of the vaccine. The serum was drawn just before the first injection was given, 2-13-35. Following the last injection (given 2-26-35), the serum was drawn again (3-12-35) and tested. With the 352 organism the titer was doubled in four cases. In two cases, however, the titer was only half as high as before immunization. With the 360 organism* the titer was increased in every case from four to sixteen times the original titer. With the Warden organism there was a reduction of the titer in three cases, no change in two and an increase in one. In no case were S. dysenteriae agglutinins present before immunization but in four cases partial agglutination was noted in the 1-10 dilution after immunization.

One serum was tested again 4-16-35. With the 352 organism the titer was intermediate between that before and that after typhoid immunization. The titer with the 360 organism remained the same as that after immunization which was sixteen times higher than that before immunization. The titer with the Warden organism also was the same

* It is significant in this connection to note that another organism definitely identified as an E. typhi strain was agglutinated by a 360 high titer serum to the same dilution as by a stock E. typhi serum.

as that after immunization which was $\frac{1}{2}$ the titer before immunization.

There are two possible explanations for the changes of titer. In the first place, the slight variation of the normal agglutinin titer might explain the apparent variation of titer after immunization. On the other hand, the typhoid injection might be the cause of the variations. Davis (1932) noted an antigenic relationship between E. typhi and S. paradysenteriae, Flexner. This fact could account for the increase in titer for the Flexner organism but does not account for the lowered titer in some other instances for the Flexner organism, nor for the increased titer for S. dysenteriae. Fraenkel (1915) reported an increased titer for the Y bacillus following inoculation with typhoid vaccine as well as after an attack of typhoid fever. Kortman (1931), however, stated that "normal agglutinins in children's sera can not be stimulated specifically as with vaccines nor non-specifically as with fevers, etc.".

DISCUSSION

From Table VIII it is apparent that agglutinins for S. paradysenteriae, Flexner are present in the great majority of normal sera in this section of the country. When the sera were diluted 1-40, 67% agglutinated 352, 59% agglutinated Warden, while only 27% agglutinated the 360 organism. If approximately 50% of the normal sera in this section of the country have agglutinins for the Flexner organisms, an agglutination test with the patient's serum carried only to that dilution would be of little value in the diagnosis of dysentery.

Basset (1904) tested the sera of 40 cases of summer diarrhoea, from which the Flexner organism was isolated. Twenty-three or over 50% of the 40 sera tested did not agglutinate the organisms used higher than a dilution of 1-100.

With normal sera I found that at a 1-80 dilution, 43% of the sera agglutinated the Warden organism, 49% of the sera agglutinated 352, and 13% agglutinated 360.

When approximately 50% of the cases of summer diarrhoea, definitely ascertained to be caused by the Flexner organism, do not produce agglutinins to a titer higher than 1-100, and 12-49% of normal people have a titer of 1-80, the value of the agglutination test can readily be seen to be limited. At any dilution between 1-5 and 1-320 not all persons with dysentery will show agglutinins,

while at any of these dilutions some normal persons will show agglutinins.

Besides the dilution of the sera one must consider several things before confirming the diagnosis in a suspected case of dysentery by an agglutination test with the patient's serum. The titer of the patient's serum is naturally significant, but no conclusion can be drawn from the results unless the serum had been tested before the attack of the disease. As is the case in typhoid fever, however, several investigators have shown that the titer for the infecting organism will rise with the continuation of the disease to reach a peak and fall gradually. Since the titer of normal sera remains constant over a period of months varying only in a slight degree, repeatedly testing the titer of a person suspected of having dysentery should aid in the confirmation of the diagnosis.

The choice of dysentery organisms to use in performing the agglutination test is of great importance. If an organism giving the typical biochemical and agglutination reactions of dysentery organisms is isolated from the stool and tested with the patient's serum at intervals of several days showing a rise in titer, excellent confirmation is given to the diagnosis of dysentery. On the other hand, if the organism is not isolated from the stool, other dysentery organisms may be tested with the patient's serum, but the difficulty of interpreting the results is

greatly increased. The variations in antigenic structure of the Flexner group complicates the situation by making it difficult to determine which strain or strains to use in the agglutination test. Provided the infection is caused by a S. paradysenteriae, Flexner organism, the possibility of using a strain in the agglutination test which has an antigenic structure identical with that of the infecting organism is very slight. In such a case only those antigens possessed by the two organisms would be affected by the agglutinins in the sera. A low titer would result which would be poor confirmatory evidence of the diagnosis of dysentery.

Also, some strains regardless of the antigenic structure are more agglutinable than others. Recently isolated strains are generally considered as more readily agglutinable. My results show that the 360 organism is agglutinated less often and to a lower titer than is either the 352 or Warden organisms.

If the agglutinin content of the sera were known, it would be a relatively simple matter to decide whether the lack of agglutinins in the sera or the low agglutinability of the organism explained the low titer of the 360 organism in normal sera.

After determining the antigenic structure of the three organisms used in relation to each other, it became apparent that to some extent one could determine what agglutinins for these organisms were present and in what comparative

amount in normal sera. Because only the three strains of Flexner were tested with normal sera, a thorough study of the agglutinin content of the sera is naturally not possible.

From the cross agglutination and absorption experiments recorded in Table 7, one may conclude that the 360 and Warden organisms have at least one antigen in common. For simplicity, this antigen will be called "B".

The 352 serum does not agglutinate the 360 organism, nor does the 360 serum agglutinate the 352 organism to any significant titer, consequently one must conclude that the 352 and 360 organisms possess no antige in common.

The Warden serum agglutinates the 352 organism to the same titer that it agglutinates the homologous organism. On the other hand the 352 serum does not agglutinate the Warden organism. Obviously the Warden serum contains an antibody for the 352 organism, hence the two organisms must possess a common antigen.* This antigen we have designated as "A".

Besides the A antigen that is possessed by both the 352 and Warden strains, there is an antigen peculiar to the 352 organism alone, demonstrated by the manner in which the specific high titer serum agglutinates the

* Since the 352 serum does not agglutinate the Warden organism this antigen may exist in the 352 organism as only a partial antigen, or haptene.

352 organism but does not agglutinate either the Warden or 360 organisms. This third antigen was designated as "C".

A summary of the possible antigenic structure of the three strains is as follows:

352	AC
360	B
Warden	AB

If a serum were to agglutinate the 352 organism, it must possess antibodies for A or C or both. But if A antibodies were present the Warden organism would also be agglutinated. If C antibodies alone were present only the 352 strain would be agglutinated, while in either case the 360 organism would not be agglutinated because no B antibodies were present. It is thus possible to predict what organisms would be agglutinated if certain antibodies were present.

Antibodies present	Organisms agglutinated
a	Warden and 352
b	Warden and 360
c	352
ab	Warden, 352 and 360
ac	Warden and 352
abc	Warden, 352 and 360
bc	Warden, 352 and 360

If the "a" antibody was the major agglutinin in the serum while the "b" antibody was present in a comparatively small amount, the Warden and 352 organisms should be agglutinated to a high and fairly equal titer while the 360 organism would be only slightly agglutinated.

Calling the major agglutinin by a capital letter and the minor by a small letter, the following combinations would be possible.

A B C Ab Ba AB Ac aC AC Bc bC BC Abc ABc ABC aBc abC AbC aBC abc

With these data in mind, I attempted to classify the sera tested, placing each in one of the above divisions. Though naturally it was difficult in some cases to place certain sera in the table, in many cases it was fairly simple when one considers the probable complexity of the antibody content of the serum. A few examples of sera will probably make the problem clearer.

Dilutions of Sera

Type	Organism	5	10	20	40	80	160
Ab	352	++++	++++	++++	++++	+++	++
	360	++++	++	++	-	-	-
	Warden	+++	+++	+++	++++	++++	++++
C	352	++	++	+	-	-	-
	360	-	-	-	-	-	-
	Warden	-	-	-	-	-	-
Ca	352	++++	+++	++	+	-	-
	360	-	-	-	-	-	-
	Warden	+	+	-	-	-	-
AB	352	++++	++	++	++	-	-
	360	++++	+++	++	-	-	-
	Warden	++++	+++	+++	+	-	-
Cab	352	++++	++++	++++	+++	+++	
	360	+++	++	-	-	-	
	Warden	+++	+++	++	+	-	
Cb	352	++++	++++	++++	++++	++++	
	360	+++	++	-	-	-	
	Warden	+++	+++	+	-	-	

A summary of the classification is recorded in Table 12. The combinations AC, ABC, aBc, etc. were not found.

Table XII

Attempted Classification of Sera According to their agglutinin content

Type of serum	No. of sera	Type of serum	No. of sera
A	13	aC	13
B	1	Bc	1
C	5	bC	5
Ab	27	BC	1
Ba	5	Abc	1
AB	22	abC	5
Ac	1	ABc	1

It will be noted that 42 sera have A as the major agglutinin, present either alone or combined; 7 sera have B as the major agglutinin; 28 have C; 23 have AB; and 1 has BC.

My absorption experiments with normal sera demonstrated that 352 and 360 showed reciprocal absorption. With high titer immune sera, however, no significant cross agglutination between the two organisms was noted. A qualitative difference between normal and immune agglutinins is indicated though there is no dependable method of distinguishing them.

Gibson (1930) concluded, from his absorption experiments, that normal agglutinins were made up of specific and non-specific elements. He considered that within normal sera are present the precursors of all those antibodies which may arise in response to a specific immunizing stimulus.

The sources of these normal agglutinins in human sera for dysentery organisms presents a difficult problem. One hypothesis is that subliminal infection may stimulate antibody formation sufficiently to produce the normal agglutinins. Paul Widowitz (1923) stated that the so-called normal agglutinins are to be considered as "specific residual immune agglutinins". According to him the first infection which is widely endemic in Austria, often attacking infants in the first half year of life, produces a low agglutinin content of the blood which is increased through reinfection. Almost all persons, he believes, after the age of puberty, have passed through an infection of this disease. In addition, co-agglutinins

indicate to him an infection by the homologous bacteria.

G. Barberra (1926) also believes that preformed agglutinins do not exist in the blood serum, but that they are produced each time bacteria enter the body, and remain for an indefinite period.

Buchner (1928) studied the sera of 166 normal persons and 160 persons clinically sick with or suspected of having dysentery. His work is especially valuable because he gives the percentages of positive sera in various age groups. A summary of his results with normal sera is given in Table 11. Buchner demonstrated that though no agglutinins were present in the first few months of life they appeared shortly thereafter. An increasing percentage of children showed agglutinins until adolescence, when the number decreased. Buchner concluded that "normal agglutinins " were the result of infection.

According to Menton (1929) the following facts are evidence that "most of these so-called normal agglutinins are due to subliminal or mild infection".

A. "Shiga infections are rare in this country; agglutinins to this microorganism also rare in healthy individuals.

B. "When dysentery was more frequent, the percentage of healthy people showing agglutination was higher than the present series.

C. "In countries where dysentery is more common the percentage of healthy people showing agglutination was much

much higher than in England.

D. "Mild attacks of diarrhoea were often ignored by the patient.

E. "Most of the positives in the investigation were obtained from people residing in thickly populated industrial districts.

F. "Normal agglutinins are absent at birth."

Havens and Mayfield (1931) studied the "Significance of Agglutination in Normal Persons". They concluded that "agglutination develops as a result of specific antigenic exposure under natural conditions, even without a clinical infection and that in the absence of such antigenic opportunity such antibodies do not develop".

If infection is the source of normal agglutinins as proposed by the foregoing investigators (Barberra, Buchner, Menton and Havens and Mayfield) and according to Havens and Mayfield (1931) "in the absence of such antigenic opportunity such antibodies do not develop" how could one explain the wide distribution of specifically absorbable dysentery agglutinins in the sera of lower animals (L. Schwichtenberg, 1935).

Summer diarrhoea is a relatively common infection of children in the first few years of life. If it could be demonstrated that in the majority of cases the dysentery organism were the causative agent producing agglutinins in the serum of the infected child, summer diarrhoea

might be considered a contributing factor to the presence of normal agglutinins for dysentery organisms.

In order to discover the aetiological factor or factors of summer diarrhoea, the Rockefeller Institute studied several hundred cases publishing their report in 1904. Dr. Flexner directed and summarized the work of bacteriologists in Boston, New York, Philadelphia and Baltimore. Of 412 cases, 279 or 63.2% contained dysentery organisms. Twenty-three were of the Shiga type; in six the Shiga organism was associated with the Flexner; and in the remainder, the Flexner alone was reported. Of this last group, twelve also contained the Y organism of Hiss and Russell.

Other investigators had somewhat similar results. Weaver, Tunnicliff, Heinemann and Micheal (1905) studied the "Etiology of Summer Diarrhoea in Infants". From 102 patients they isolated the dysentery bacillus in 26 cases. All were of the Flexner type.

Nabarro and Signy (1932) included the Sonne organism in their study of dysentery in children. During four years investigation, the Sonne organism was isolated from 82 children and 5 nurses, the Flexner Z from 17 children and 1 nurse, and the Flexner W from 1 child. Facts particularly to be noted are the absence of the Shiga organism and the high percentage of Sonne organisms isolated.

From the above data it seems probable that the normal agglutinins of adults might be the result of infection in infancy and childhood, but several problems are suggested which demonstrate that the picture is not as simple as it might at first glance appear. In the first place, the fact that adult serums seem to be comparatively lacking in agglutinins for the S. dysenteriae organism would be in apparent accord with the hypothesis of infection as a source of normal agglutinins, because S. dysenteriae is only seldom the aetiological factor of summer diarrhea. Flexner strains are very common in summer diarrhoea cases and as such would be expected to show high agglutination in adult serum, as they do. On the other hand, the Sonne organism since its isolation in 1914 has also been demonstrated as the causative agent in many cases of summer diarrhoea in infants. Agglutinins were shown to have been produced. Adult sera, however, very noticeably fail in the great majority of cases to agglutinate the Sonne organism (L. Schwichtenberg, 1935). The question arises as to why the Sonne organism known to be a cause of summer diarrhoea and dysentery in children fails to agglutinate in adult sera if the normal agglutinins of adults are due to previous infections.

The degree of permanency of the agglutinins arising as a result of infection might be utilized as a criterion to demonstrate their ability to act as a source of normal

agglutinins, but unfortunately not much work has been done in this field. Most investigators agree that in the first week of dysentery agglutinins appear in the blood, increasing during the course of the disease, reaching a peak, and decreasing slowly during convalescence.

Louise Cordes (1904) studied the agglutination reactions of the sera of twenty-two children with dysentery. Ten of these sera gave a positive reaction in 4-25 days following the onset of symptoms while 12 failed to give a reaction. Eleven of the 12 sera were tested on the second to eighth day and the other on the twelfth day.

Basset (1904) demonstrated agglutinins in the sera of 30 of 51 dysentery cases caused by the Flexner organisms. The titers varied from 1-20 to 1-5000. Of the sera of patients from whom the dysentery organism was not isolated 3 agglutinated at 1-1000; 1 at 1-500 and 1 at 1-50. Nine were negative at 1-50; 1 was negative at 1-20.

Menton (1929) studied dysentery agglutinins in the apparently healthy. He found that over a period of four months the dysentery agglutinins remained fairly constant.

On the other hand, Rosenthal (1903) investigating persons ill with dysentery found that their serum agglutinated ten to twelve days after the onset of symptoms. By four to five weeks the agglutinating power of the serum was much weaker with generally negative results after fifty-two days.

If infection is the source of normal agglutinins it seems more than likely that repeated infections would be necessary to stimulate the antibody production found in adult sera because it is apparent that the agglutinins in normal persons and those with dysentry are different in their degree of permancy.

Another hypothesis attempting to account for the normal agglutinins for dysentery organisms is that some of the organisms normally inhabiting the intestinal tract stimulate the production of antibodies for dysentery organisms. According to Sonne "it is firmly established, that with all people whose serum agglutinated dysentery bacilli of these groups and whose stools were examined showed organisms which more or less strongly agglutinated the corresponding dysentery immune serum. The contrary is also true".

Flexner, according to Torrey (1905) suggested that the mannit fermenters may be occasional if not constant inhabitants of the normal intestine. On this hypothesis Torrey accounts for the agglutinins for the mannit group in normal blood.

D. C. Browne (1932) examined the stools of fifty healthy people from public clinics. He isolated one S. dysenteriae and five of Duval's lactose fermenters (probably the Sonne organism). From fifty healthy persons

(private practice) he isolated one Hiss Y, one Flexner and a much lower percentage of Duval's organism. From the above data it is apparent that if the hypothesis were correct, the adult serum would contain more agglutinins for S. paradysenteriae, Sonne than for S. paradysenteriae, Flexner,. Several workers have shown that Sonne agglutinins are rarely found in the serum of an adult while our own work confirms the fact that agglutinins for the Flexner organism are found in the majority of adult serums. These facts do not bear out the hypothesis.

Keck (1917) noted that following the injection of a vaccine made from a coli-like organism isolated from a case of cystopyelitis, heterologous agglutinins developed. Agglutinins for the Y dysentery bacillus appeared first and to a relatively high titer. E. typhi and other strains of dysentery bacilli were agglutinated in lower dilutions.

Pinner and Ivancevic (1920) injected rabbits with a non-spore forming air bacillus, guinea-pig bile, and tuberculin. After two weeks the sera were tested with suspensions of typhoid, paratyphoid, dysentery and proteus bacilli. Comparing these results with those for the same reactions before the injections showed that the intravenous injections caused the rabbits to produce substances which agglutinated the non-specific bacteria. The agglutination titers were not above 1-400, but none of

the original titers was above 1-25. These results suggest a method of agglutinin formation by non-specific stimulation.

Another hypothesis attempting to account for normal agglutinins is transmission of some of the agglutinins of the mother through the placental serum. Hausherr (1921), Acel and Acel-Vecsei (1924) and Toomey (1934) all demonstrated agglutinins in the retro-placental blood.

Acel and Acel-Vecsei examined 120 sera of pregnant and parturient women to determine whether the agglutinins for the dysentery Y bacillus found in the retro-placental (maternal) and cord (fetal) bloods are alike. They found that the maternal bloods showed a high percentage (17-98, depending on the strain of Y used) of positive results, while the infant blood showed a much lower percentage (0-27) of positive results. The titers of maternal bloods were also much higher than those of infant blood. According to their observations the retro-placental blood is more stable to heat than is the cord blood.

By absorption with paratyphi B, they found that the agglutinins for Y were removed in cord blood but could not be removed from retro-placental blood. The authors concluded that the agglutinins in the the maternal blood were of the immune type while those of the cord blood

were of the normal type; that agglutinins apparently do not pass from the maternal to the fetal circulation and that the mother and child during pregnancy behave immunologically as two seperate individuals.

If, according to Acel and Acel-Vecsei, retro-placental blood is of the immune type, then the agglutinins of immune sera are more stable to heat than are those of normal sera. Felix and Olitzki, however, (1929) stated that normal and immune agglutinins possess the same resistance to heat.

Toomey examined the sera of 25 mothers and the placental sera of their new born infants, finding that in no instance was there greater agglutination in the infant's than in the mother's serum. Although the placenta contained some agglutinins, the blood of the same infant taken ten days later contained a much greater amount of agglutinins. The author noted that the titer of infant's serum in a general way paralleled that of the mother's milk. From his observations, he concluded that production of antibodies in human beings seems to be brought about by placental transmission and absorption of injested agglutinins.

Relation of Dysentery Agglutinins to Diagnosis of Tuberculosis

Hull and Henkes (1923) observed that "persons in the incipient stage of tuberculosis apparently carry in the blood a substance capable of agglutinating the Flexner dysentery bacillus". They also noted that persons in the advanced stage of tuberculosis as well as normal persons as a rule do not react to this test though the authors stated that some normal persons react with dilutions of less than 1-40. Evidently the organism they used was either a weakly agglutinating strain or did not possess the antigens specific for the agglutinins in the sera, because our results show that 26-63% of sera have titers of 1-40 or above. According to Hull and Henke's observations one would assume that 26-63% of the population would be in the incipient stage of tuberculosis. This statement is obviously incorrect and indicates again the value of using several types of S. paradysenteriae, Flexner organisms in testing for specific agglutinins in normal sera.

SUMMARY

A survey was made of the occurrence of agglutinins for three strains of S. paradysenteriae, Flexner in normal human sera. The majority of the 115 sera tested were those drawn for routine Wassermans at the Multnomah Hospital; the remainder were sera of laboratory workers. The three organisms employed were designated 352, 360, and Warden. Cross agglutination and absorption experiments with high titer immune sera showed that 360 and Warden have a common antigen as do also 352 and Warden, while 352 and 360 possess no antigen in common. The 352 organism contains also an antigen not shown by either of the other two strains. At a 1-5 dilution of the sera, the organisms were agglutinated in 66-97% of the cases depending on the strain used. At a 1-80 dilution, 13-49% of the sera agglutinated, while at a 1-160 dilution 2-19% gave agglutination. Practically the same percentage of the sera of men and women possessed agglutinins at a 1-5 dilution. The sera of women agglutinated 352 and Warden to a higher titer than did the sera of men, while the latter agglutinated 360 to a higher titer. Infant's sera (cord blood) in no case agglutinated in as high dilution as adult sera. Both adults and infant sera consistently agglutinated 360 to a lower titer than was obtained with either 352 or Warden.

Forty-four normal sera were also tested with S. dysenteriae. Agglutinins were not often present. Ten of the

forty-four sera tested gave agglutination of this organism in a maximum dilution of 1-10 and two agglutinated in a dilution of 1-20.

One normal serum was absorbed with three different strains of S. paradysenteriae, Flexner, viz. 352, 360, and a third from the collection at the University of Oregon Medical School. The serum was tested before and after each absorption with ten different Flexner strains. The titers for the ten organisms were either lowered or the agglutinins were completely removed by all three strains. Almost the same after titer was obtained for each of the ten organisms regardless of the absorbing strain. The 352 and 360 organisms showed reciprocal absorption. A qualitative difference between normal and immune agglutinins would seem to be indicated by these results.

The sera of six persons were tested before and after typhoid immunization. With both the 352 and Warden organisms the titer was lowered in some sera and increased in others. With the 360 organism, however, the titer was increased in every case 4-16 times. In none of the sera was S. dysenteriae agglutinins present before immunization, but in four of the six sera partial agglutination was noted in the 1-10 dilution after the typhoid immunization.

Since agglutinins for S. paradysenteriae, Flexner are present in a great majority of normal persons and since these agglutinins are often present in comparatively

high dilutions of sera, the value of using the patient's serum in agglutination tests for the diagnosis of dysentery is obviously limited. Though the evidence indicates these normal agglutinins are not fully specific, there is no dependable method distinguishing between normal and immune agglutinins.

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