AGGLUTINATIVE EFFECT OF NORMAL HUMAN AND ANIMAL SERA ON SHIGELLA PARADYSENTERIAE, SONNE

by

LILIAN SCHWICHTENBERG

A Thesis

Presented to the Department of Bacteriology
and the Graduate Faculty of the University of Oregon
in partial fulfillment
of the requirements for the degree of
Master of Arts

September 1935

APPROVED:

[redacted]

Major Adviser

[redacted]

For the Graduate Committee

of the University of Oregon

Medical School

CONTENTS

INDEX OF TABLES

AGGLUTINATIVE EFFECT OF NORMAL HUMAN AND ANIMAL SERA ON SHIGELLA PARADYSENTERIAE, SONNE

Normal human and animal sera possess agglutinins for a variety of different organisms including the dysentery bacilli. Reviewing the abundant literature dealing with the agglutination of the dysentery organisms, H. Schwichtenberg (1935) found that Shigella paradysenteriae, Flexner was frequently agglutinated in human and animal sera, whereas Shigella dysenteriae was seldom agglutinated. Investigations on the occurrence of agglutinins in human sera for Shigella paradysenteriae, Sonne have been few. Similar investigations with animal sera have not been reported in this country.

Carl Sonne originally isolated and described the Sonne organism in 1915. Though he reported the late appearance of an acid reaction in milk, it was Thjotta (1919) who emphasized the importance of the reaction in lactose broth as a means of differentiating this organism from Shigella paradysenteriae, Flexner. The late production of acid in sucrose and raffinose containing media is likewise characteristic of the Sonne organism, though not significant as a means of differentiation from the other mannite fermenters. The reaction in sucrose media was discussed in a recent article by Sears,

Bilderback, Ashley, and Rolmer (1935). A study of the raffinose fermentation reaction has recently been made by Sears and Schoolnik (1935). The delayed fermentation of lactose and the failure of the organism to produce indol from tryptophane broth serve, biochemically, to distinguish this organism from the Flexner strain.

The specificity of the Sonne antigen, demonstrated originally by Sonne (1915), differentiates the Sonne organism from other members of the dysentery group. Though the absorption of agglutinin test may be used, agglutination in immune serum is sufficient for identification of the organism.

There have been few investigations concerning the incidence of agglutinins in normal human sera for Shigella paradysenteriae, Sonne. However, practically all investigators agree that agglutinins for this organism are not common. Using his own strains, Sonne (1915) ran an agglutination reaction on the sera of 245 hospital patients. In one instance only did he find agglutinins present, and that in a serum dilution of 1:25. Tajetta (1919), Wiseman (1927), and Kerrin (1928) reported complete absence of agglutinins in the sera they tested. The number of sera examined was not recorded. Fraser, Kinloch, and Smith (1929) tested the sera of twenty normal individuals and found agglutinins present

in three instances. The highest dilution giving agglutination did not exceed 1:25. Kenton (1929) tested the sera of 594 normal individuals, only one of whom gave a history of an attack of dysentery. The attack in this individual had taken place thirty years previously. Agglutinins were found in 6.39% of the sera. The sera of eight of the 262 females tested produced agglutinins for the Sonne organism, whereas agglutinins were demonstrated in the serum of only one of the 262 males examined. Smith and Fraser (1930) tested the sera of 138 hospital patients not suffering from dysentery. Thirty-three, or 24%, gave agglutination in a dilution of 1:50 or above. This is the highest incidence of agglutinins for the Sonne bacillus yet recorded. It is possible that the authors' choice of normal sera might account for the high percentage of positive results. In the same article the authors reported the occurrence of an epidemic of dysentery involving forty-eight cases. All were caused by Shigella paradysenteriae, Sonne. Since the disease spreads rapidly and cases are often so mild as to miss diagnosis, it is possible that many of the so called normal sera were from recovered cases, or from patients suffering from a mild attack of the disease. A summary of these authors' results is presented in Table 1.

TABLE 1

Age distribution of agglutinins in normal sera Shigella paradysenteriae, Sonne'

Age group	Number of sera tested	Number of sera positive	Positive %
0-½ years	43	4	9
½-1 "	31	14	34
1-2 "	28	2	7
2-3 "	18	2	11
3-4 "	5	0	-
4-5 "	0	0	-
5-10 "	2	2	-
10-15 "	1	1	-
15-25 "	3	2	-
25-45 "	5	4	-
45-+ "	2	2	-

'From data given by Smith and Fraser

Investigations of the occurrence of agglutinins in animal sera for Shigella paradysenteriae, Sonne have not been reported in this country. That agglutinins are present for other members of the colon and dysentery groups has been demonstrated repeatedly. Gibson (1930) undertook an extensive investigation on the occurrence and nature of natural agglutinins in normal animal sera for the common intestinal organisms, both pathogenic and non pathogenic. The Sonne organism was not included.

EXPERIMENTAL WORK

Materials Used

An 0.85% sodium chloride solution in distilled water was used as a diluent throughout this investigation.

The human sera tested were chosen at random from a series of specimens taken for the routine diagnosis of syphilis at the Multnomah hospital. The sera, previously inactivated in a fifty-seven degree water bath for thirty minutes, were always over twenty-four hours old when tested. When not in use, they were kept in the refrigerator.

Saline suspensions of twenty-four hour growths on meat extract agar in Blake bottles were used as antigens. The density of the suspensions was made to correspond to tube III of a barium sulfate nephelometer, corresponding to approximately 900,000,000 bacteria per cubic centimeter. Little difficulty was encountered with the spontaneous clumping of the bacteria in freshly prepared suspensions, except with the American Type Culture Collection strain, our number 355, which has been in our possession for four years. On plating this strain out on agar distinctly rough colonies were obtained. By selective transplantation of the smoothest colonies

suspensions were obtained that did not clump spontaneously. The degree of smoothness of the suspensions no doubt varied as new quantities of antigen were made up. This factor should be kept in mind in the interpretation of results with this organism. No preservatives were added to the antigen preparations. Heating in a 56 C water bath for one hour was sufficient to produce sterility.

The source of our organisms was as follows: No. 353 was received from the Hooper Foundation for Medical Research, San Francisco, in 1931. No. 355 is no. 31 of the American Type Culture Collection obtained by us in 1931. Nos. 356 and 357 were received from the University of Michigan Medical School laboratory in 1933. Nos. 358, 359, and 359a were isolated from an epidemic of dysentery occurring in this city in 1933. Nos. 366, 367, and 368 were obtained from the Department of Bacteriology of the University of Southern California Medical School, and identified in this laboratory as Sonne strains. All strains bearing names instead of numbers were isolated from an epidemic of dysentery occurring in the hop fields in the vicinity of Portland, Oregon in the fall of 1934. The organisms were identified in this laboratory.

Technique

The dilutions used in the macroscopic agglutination test with normal sera ranged from 1:10 to 1:640. The intermediate dilutions, five in number, were arranged so that each was double the dilution of the preceeding tube. The sera in contact with antigen were kept in a forty to forty-five degree water bath for eighteen to twenty-four hours.[1] With this prolonged incubation period the agglutination was complete and easy to read. Antigen controls, using the suspension of the organism and an equal amount of salt solution, were included with each test.

In the absorption test whole normal serum was treated with a washed suspension of bacteria of such a density that only a drop or two needed to be used. Thus the necessity of the ordinary serum dilutions was avoided. The mixture of serum and bacteria was well shaken and placed in a forty to forty-five degree water bath for two or more hours. It was then centrifuged and the absorption re-

[1] To determine the effect of prolonged incubation, a set of thirty-six beef sera were incubated for two hours, placed in a refrigerator over night and the results recorded the following day. Of the thirty-six sera two showed no increase in titre; twenty-seven showed very fine agglutination in the next highest dilution; seven showed fine agglutination in two higher dilutions.

posted with the supernatant fluid. After further incubation for one to two hours it was again centrifuged and the serum withdrawn and used to agglutinate the desired organisms.

In the absorption tests in which immune sera were used it was found expedient to use diluted serum. A 1:5 dilution of the serum absorbed three times with an equal amount of a heavy suspension of the organism produced a final serum dilution of 1:40. Upon further dilution of the serum by addition of antigen the primary dilution became 1:80. The following dilutions were increased by doubles until a final dilution of 1:25,600 was obtained. Best results followed overnight incubation of the absorption mixture.

For the preparation of immune sera young rabbits weighing three and one half to four pounds were injected intravenously with increasing amounts of heat killed Somne antigen. The highest dilutions in which the homologous organisms were agglutinated ranged from 1:1600 to 1:25,600.

Agglutinins in Normal Sera

The capacity of the sera tested to agglutinate the Sonne organism in the dilutions used is shown in Table 2 and figure 1. Table 2 gives the number of sera agglutinating in the separate dilutions the three Sonne strains used. Figure 1 gives only the average percentage of the three organisms agglutinated in the recorded dilutions. By reference to the latter it is evident that the agglutinating capacity of the sera of the different species falls into the definite series; guinea pigs, rabbits, sheep, swine, cattle, and horses. Though only two horse sera were available it was evident that they possessed stronger agglutinating power than any of the sera tested. Human sera, on the other hand, do not agglutinate the Sonne organisms readily. In a dilution of 1:20, 100% of horse, 100% of cattle, 96.2% of sheep, 93.5% of swine, 46.4% of rabbit and 22.3% of guinea pig sera produced agglutinins for the strains tested. In this same dilution only 1.6% of normal human sera showed agglutination of this member of the dysentery group.

The results of absorption experiments in normal sera are given in Tables 3, 4, and 5.

TABLE 2

Incidence of agglutinins in normal sera for three strains of Shigella paradysenteriae, Somne

Source of serum	Number of sera tested	Organisms used	Number of sera giving agglutination in the following dilutions						
			1:10	1:20	1:40	1:80	1:160	1:320	1:64
Human	121	355	7	3	0	0	0	0	0
		357	5	2	0	0	0	0	0
		359	3	1	0	0	0	0	0
Beef	43	355	43	43	43	43	43	40	27
		357	43	43	43	43	38	29	12
		359	43	43	43	42	41	30	14
Swine	51	355	51	51	51	50	48	41	35
		357	51	51	48	38	35	33	29
		359	42	41	36	35	31	22	12
Sheep	97	355	97	97	96	92	72	17	2
		357	92	91	84	69	39	1	0
		359	94	92	88	76	31	2	0
Guinea pig	16	355	8	4	3	0	0	0	0
		357	4	3	2	1	0	0	0
		359	6	4	2	1	0	0	0
Rabbit	28	355	24	17	13	5	2	0	0
		357	16	8	7	1	0	0	0
		359	14	14	6	3	0	0	0
Horse	2	355	2	2	2	2	2	2	0
		357	2	2	2	2	2	1	1
		359	2	2	2	2	2	2	1
Mole	1	355	0	0	0	0	0	0	0
		357	0	0	0	0	0	0	0
		359	0	0	0	0	0	0	0

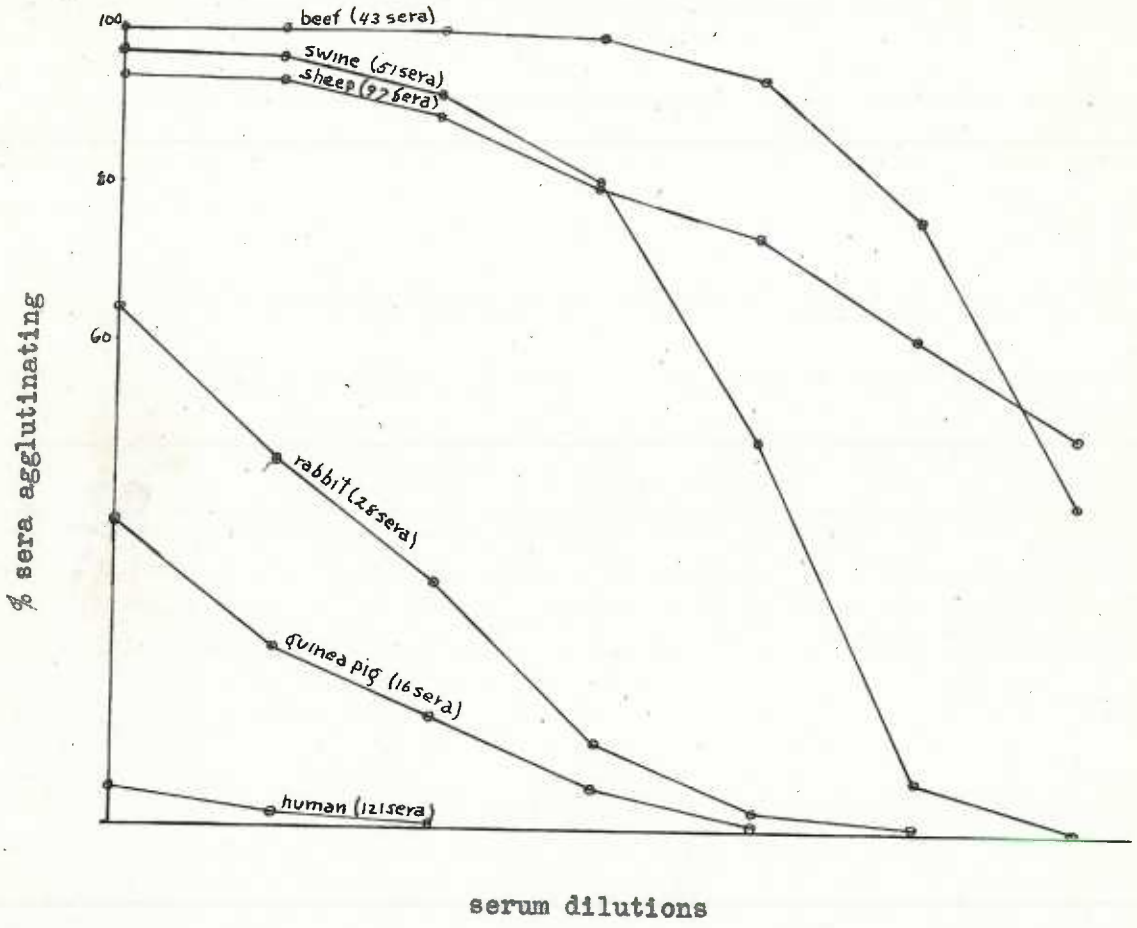

Fig. 2 Graphic Representation of the Comparative Capacity of the Sera of Different Species to Agglutinate S. paradysenteriae, Sonne The percentages ploted are the average for the three antigens used

TABLE 3

Limits of strain and species specificity of agglutinins in normal horse serum, indicated by absorption experiments

organism	original titre of horse serum	titre of serum after absorption with the following organisms											
		355	VH	GB	EF	T	EB	356	K	359	B. typhi	S. paratyphi	B. coli
355	1:320	1:20	1:40	1:20	1:320	1:80	1:20	1:320	1:320	1:160	1:320	1:640	1:320
358	1:320	1:20	-	-	-	-	-	-	-	-	1:320	1:320	1:320
VH	1:320	1:40	-	-	-	1:320	-	-	-	-	1:320	1:320	1:320
GB	1:320	1:20	-	-	-	1:320	1:20	-	-	-	1:320	1:320	1:320
EF	1:320	1:40	-	-	-	1:20	-	1:320	-	1:40	1:320	1:320	1:320
EB	1:640	1:20	-	-	-	-	-	-	-	-	1:640	1:320	1:160
JH	1:640	1:20	-	-	-	-	-	-	-	-	1:320	1:320	1:320
T	1:320	1:80	-	-	-	-	-	-	-	-	1:320	1:320	1:320
DL	1:160	1:40	-	-	-	-	-	-	-	-	1:320	1:160	1:320
359a	1:320	1:40	-	-	-	-	-	1:80	-	-	1:320	1:320	1:640
LS	1:320	1:40	1:40	1:40	-	1:40	1:20	-	-	-	1:320	1:160	1:320
359	1:640	1:80	-	-	1:40	-	-	1:40	-	-	1:640	1:320	1:320
RB	1:640	1:20	-	1:40	-	-	-	-	-	-	1:320	1:320	1:320
356	1:320	1:40	1:80	1:20	-	1:160	1:20	-	1:20	1:80	1:320	1:320	1:320
HH	1:640	1:20	-	-	-	-	-	-	-	-	1:320	1:320	1:640
J B	1:320	1:20	-	-	-	-	-	1:80	-	-	1:320	1:320	1:80
353	1:320	1:40	-	-	-	-	-	1:40	-	-	1:320	1:320	1:160
H	1:640	1:40	-	-	1:20	1:320	-	-	-	1:40	1:320	1:320	1:320
357	1:640	1:80	-	-	-	-	-	1:80	-	1:20	1:640	1:320	1:640
368	1:640	1:80	1:80	1:40	-	1:80	-	1:320	-	1:20	1:320	1:320	1:320
366	1:320	1:80	-	-	1:20	-	1:20	1:40	-	1:20	1:320	1:320	1:320
367	1:640	1:80	-	-	-	1:40	1:20	1:80	-	-	1:640	1:640	1:640
B. typhi	1:320										-	1:40	1:160
S. paratyphi	1:320										1:40	-	1:160
B. coli	1:320										1:80	1:40	-

TABLE 4

Limits of strain specificity of agglutinins in the combined sera of 37 swine, indicated by absorption experiments

organism	original titre of swine serum	titres of serum after absorption with the following organisms						
		355	VH	KB	K	GB	H	357
355	1:320	1:40	1:20	1:80	1:160	-	1:20	1:20
358	1:640	1:80	1:80	1:80	1:320	1:320	-	1:80
VH	1:640	1:80	1:80	1:80	1:40	1:40	1:80	1:320
GB	1:640	1:40	-	1:20	-	1:40	-	-
HF	1:640	1:40	1:20	1:40	1:80	1:160	1:20	-
KH	1:640	1:320	1:320	1:160	1:320	1:80	1:320	1:80
JH	1:320	1:40	1:40	1:20	1:80	1:160	-	-
K	1:640	1:80	1:20	1:80	1:20	1:40	1:20	-
DL	1:320	1:40	1:40	1:20	1:20	1:40	1:40	-
359a	1:640	1:40	1:20	1:40	1:320	1:160	1:20	-
LG	1:640	1:40	-	1:20	1:160	1:320	-	-
359	1:640	1:40	1:40	1:80	1:320	1:640	1:40	1:80
KB	1:640	1:80	1:40	1:20	1:40	1:160	-	1:20
356	1:640	1:40	1:80	1:20	1:80	1:80	1:20	1:40
KH	1:640	1:40	1:80	1:40	1:320	1:320	1:20	-
JB	1:320	1:40	1:20	1:20	1:160	1:160	-	1:20
353	1:640	1:80	-	1:80	1:320	1:320	-	-
H	1:640	1:80	1:80	1:80	1:40	1:80	1:20	-
357	1:1280	1:40	1:40	1:40	1:320	1:160	1:20	1:20
368	1:320	1:40	1:40	1:80	1:160	1:80	1:20	1:20
366	1:1280	1:80	-	1:40	1:20	1:40	-	-
367	1:640	1:80	1:40	1:40	1:320	1:160	1:20	1:80

TABLE 5

Species specificity of agglutinins in normal sheep serum, demonstrated by absorption experiments

Serum	Organisms tested	Original titre of serum	Titre of serum after absorption with 355
sheep 1	355	1:160	-
	357	1:160	-
	359	1:160	-
	352'	1:40	1:40
	360'	1:40	1:40
	Warden'	1:40	1:40
	S. dysenteriae	1:10	-
	E. typhi	1:40	1:40
	S. paratyphi	1:20	-
	S. schottmuelleri	1:20	-
	E. coli	1:20	1:20
sheep 2	355	1:80	1:10
	357	1:40	-
	359	1:40	-
	352'	1:20	1:20
	360'	1:20	1:20
	Warden'	1:20	1:20
	S. dysenteriae	-	-
	E. typhi	1:10	1:10
	S. paratyphi	-	-
	S. schottmuelleri	1:10	1:10
	E. coli	-	-

'Shigella paradysenteriae, Flexner strains

TABLE 6

Antigenic relationship among 22 strains of S. paradysenteriae, Sonne as indicated by specific absorption of immune sera

organism	titre with 353 serum	titre after absorption with KR	titre after absorption with VH	titre with K serum	titre after absorption with K	titre after absorption with RM	titre with VH serum	titre after absorption with VH	titre after absorption with KP	titre with 355 serum	titre after absorption with 359
355	1:1600	-	-	1:6400	-	-	1:6400	-	-	1:25,600	-
358	1:800	-	-	1:12800	-	-	1:3200	-	-	1:800	-
VH	1:3200	-	-	1:25600	1:1280	1:640	1:6400	-	-	1:6400	-
GB	1:800	-	-	1:12800	-	-	1:6400	-	-	1:12800	-
KP	1:1600	-	-	1:3200	-	-	1:3200	-	-	1:800	-
RM	1:1600	-	-	1:25600	-	-	1:3200	-	-	1:6400	-
JH	1:800	-	-	1:6400	-	-	1:3200	-	-	1:6400	-
K	1:1600	-	-	1:25600	1:5120	1:5120	1:6400	-	-	1:6400	-
DL	1:800	-	-	1:25600	-	-	1:3200	-	-	1:12800	-
359a	1:1600	-	-	1:6400	-	-	1:3200	-	-	1:3200	-
LG	1:800	-	-	1:6400	-	-	1:3200	-	-	1:12800	-
359	1:3200	-	-	1:12800	-	-	1:6400	-	-	1:1600	-
KB	1:1600	-	-	1:12800	-	-	1:6400	-	-	1:12800	-
356	1:1600	-	-	1:25600	-	-	1:6400	-	-	1:25600	-
RH	1:3200	-	-	1:12800	-	-	1:800	-	-	1:12800	-
JB	1:1600	-	-	1:12800	-	-	1:6400	-	-	1:12800	-
353	1:1600	-	-	1:3200	-	-	1:1600	-	-	1:3200	-
H	1:3200	-	-	1:6400	-	-	1:6400	-	-	1:12800	-
357	1:800	-	-	1:1600	-	-	1:3200	-	-	1:6400	-
368	1:1600	-	-	1:6400	-	-	1:3200	-	-	1:25600	-
366	1:800	-	-	1:6400	-	-	1:3200	-	-	1:6400	-
367	1:3200	-	-	1:6400	-	-	1:6400	-	-	1:6400	-

When a single horse serum (Table 3) was absorbed with a Sonne organism the agglutinins for that organism were removed. However the agglutinins for many other strains were scarcely reduced. This phenomenon is better demonstrated in the experiments with the combined swine sera (Table 4).

The results of absorption of immune sera are given in Table 6. It is clear that with a single exception the absorption of immune sera with any one Sonne strain will remove the agglutinins for all Sonne strains. The exception occurred with K immune serum. Absorption of this serum with either H or RM strains failed to remove all the agglutinins for K and VM.

The type of agglutination produced by normal sera was characteristic, provided the incubation was allowed to continue for eighteen hours in a forty-five degree water bath. In the low dilutions the clumps were finely granular, and the spaces between opaque, whereas in the higher dilutions the clumps were finely granular and the interspaces decidedly cloudy. Furthermore, the former type of agglutination was readily visible to the naked eye, whereas the latter required magnification and good lighting conditions for adequate observation. Another, though less satisfactory criterion for determining the presence of agglutination, was the degree and type of

sedimentation on standing. In the low dilutions the bacteria completely settled out, leaving a clear or slightly opaque supernatant fluid. The bacteria did not collect as a homogenous mass in the apex of the tube, but as a fine lacy film covering the rounded bottom of the tube. The film in some tubes even folded over upon itself. In the higher dilutions the film was more delicate and the supernatant liquid more opaque. Even in the highest dilutions in which agglutination was found, the delicate film was present covering the entire bottom of the tube. If any settling out occurred in the controls, it appeared as a compact homogenous mass of bacteria lying at the very apex of the tube.

A microscopic examination of the suspensions giving flocculent agglutination revealed large loosely aggregated clumps. The granular type of agglutination was often as complete as the flocculer type, though in the higher dilutions there were many organisms found that were not clumped.

The type of agglutination observed in immune sera is essentially the same as that found in normal sera. The general observation that agglutination in immune sera is very fine in character was confirmed in my investigations. However, in the lower dilutions a finely flocculent

agglutination was found, identical in all respects to that seen in normal sera.

A series of experiments was performed to determine the distribution of agglutinins for members of the dysentery, typhi, and colon groups in sera previously tested with the Sonne organism. A comparison of the titres with these different organisms is given in Table 7.

It was evident that the sera would not agglutinate the other enteric strains as well as strains of Shigella paradysenteriae, Sonne. However, Shigella paradysenteriae, Flexner was agglutinated in a few instances in titres nearly as high as those witnessed with the Sonne organisms.

TABLE 7

Incidence of agglutinins in normal sheep and beef sera for Shigella paradysenteriae, Sonne and other intestinal organisms

Serum		Titre of the serum with the following organisms										
		S. paradysenteriae Sonne			S. paradysenteriae Flexner			S. para-dysenteriae	S. typhi	S. para-typhi	S. schot-tmelleri	E. coli
		355	357	359	352	360	Warden					
Sheep	41	1:160	1:80	1:80	1:40	-	1:20	-				
"	43	1:160	1:160	1:160	1:40	1:20	1:80	-				
"	45	1:160	1:160	1:160	1:80	1:80	1:80	-	1:160			
"	61	1:160	1:160	1:160	1:80	1:20	1:160	-				
"	g	1:160	1:80	1:160	1:40	1:40	1:40	1:10	1:40	1:20	1:20	1:20
"	e	1:80	1:40	1:40	1:20	1:10	1:10	-	1:10	-	1:10	-
Beef	16	1:640	1:640	1:320	1:320	1:80	1:640					
"	17	1:320	1:320	1:160	1:320	1:160	1:160	1:20	1:160	1:40	1:40	1:320
"	20	1:640	1:640	1:320	1:320	1:80	1:320	-				
"	21	1:640	1:320	1:640	1:320	1:80	1:160	-				
"	24	1:320	1:160	1:320	1:160	1:40	1:160	1:10				
"	26	1:640	1:640	1:640	1:320	1:160	1:160	-	1:80			1:160
"	34	1:640	1:320	1:640	1:80	1:40	1:40	1:10	1:80	1:40	1:80	1:80
"	36	1:640	1:640	1:640	1:320	1:40	1:320	-				
"	37	1:320	1:320	1:320	1:320	1:80	1:160	-				
"	41	1:320	1:160	1:320	1:160	1:80	1:80	1:10	1:80	1: 10	1:20	1:20
"	42	1:640	1:320	1:320	1:320	1:160	1:320	-	1:80	1:20	1:20	1:20

Discussion

Animal sera possess agglutinins in high dilutions for Shigella paradysenteriae, Sonne, and Shigella paradysenteriae Flexner, though animals are not disposed to infection by these organisms. Human sera possess agglutinins for the Flexner organisms in dilutions as high as those found with animal sera, but do not possess agglutinins for the Sonne strains. These organisms are equally capable of producing the disease in man. These observations are not in accord with the view that antibodies occur only as the result of previous infection.

Investigations concerning the general occurrence of normal agglutinins in the serum of animals for members of the enteric group other than the Sonne organisms have been made on several occasions. The investigators include Bergey, (1903), Park and Carey, (1903); Hiss, (1904), Park and Collins, (1904); Park, Collins, and Goodwin, (1904); Torrey, (1905); Gibson, (1930); Mackenzie, (1930).

The extensive distribution of agglutinins in normal animal sera for the paradysentery organisms must always be considered when an animal is selected for immunization. If the sera is not previously tested for these antibodies a subsequent agglutination of the serum with an unrelated

organism would suggest a false antigenic relationship between the organisms.

The agglutinins for Sonne are specific because this organism will remove the agglutinins for the strains used in absorption and leave unaltered the titre for other enteric organisms. However, the agglutinins for all other Sonne strains will not be removed by this absorption in normal sera, while the agglutinins will practically all be removed from immune sera.

The difference in behavior of the normal and immune sera is obviously related to the antibody composition of the sera. The immune sera contain antibodies which are specific for the Sonne antigen, normal sera produce antibodies which react to certain antigenic components of the organism only. These reactions suggest a possible difference in mode of formation; the immune antibodies being formed by a specific stimulus of the antibody producing mechanism, and the normal antibodies by some non-specific agent. Such an assumption is strengthened by the fact that animals are not normally subject to bacillary dysentery infection. J. Bamforth, (1933) was unable to produce agglutinins in the sera of rabbits by feeding the animals live, virulent cultures of this strain.

Shigella paradysenteriae, Flexner was agglutinated in high dilutions of homal sera, whereas the Sonne strains were seldom clumped. These observations would seem to indicate that either the Sonne organism is not as potent as Flexner in stimulating antibody production, or dysentery due to this organism in not as widespread. Upon the acknowledgment of Shigella paradysenteriae, Sonne as an important etiological agent in dysentery and mucus diarrhea it has been demonstrated that this organism is widely distributed. Likewise, this culture is apparently very capable of producing agglutinins in cases of infection

Agglutination of known Sonne strains with patients' serum was first attempted by Sonne with his type III organism in 1915. Using the serums of twelve patients he observed titres ranging from 1-10 to 1-250, half giving titres of 1-50. Strains of Mita, (1921) types A and B, agglutinated the serum of the patients in dilution of 1-300 to 1-500 and not infrequently in a dilution of 1-1000. Looky in 1931 ran agglutination tests with patients' sera, obtaining positive results in only one out of seven cases. Though the results are interesting they are not of practical value because the time of receiving the serum in relation to the onset of the disease was not recorded. Bamforth (1923) agglutinated three of his organisms with the serum of two of his patients twelve days after the appearance of symptoms.

The results are recorded in Table 6.

Fraser, Kinloch, and Smith (1926) made a more extensive study of the agglutination reaction of the sera of thirty-three cases of dysentery caused by the Sonne organism. Seventeen of the sera gave agglutination of his stock culture in dilutions of 1-50 to 1-6400. The rise of agglutinins was observed in three cases, as illustrated in table 99.

Wiseman, working in 1927, obtained similar results. The serum of twelve patients was tested the third and ninth days after the onset of illness against his organism "K" and on the tenth day with his organism "T." Agglutination was observed in dilutions of 1-12 to 1-50 on the third day; in 1-50 to 1-250 on the ninth day; and once 1-50 to 1-250 on the tenth day. From the ninth to the tenth day the titre rose in two instances from 1-200 to 1-250, and fell in one instance from 1-100 to 1-50. The entire absence of agglutination was not observed in any case.

A more systematic survey of this reaction was attempted by Smith and Fraser (1930). Fifteen of the forty-eight patients' sera tested failed completely to agglutinate his stock culture in a dilution of 1-50 at the time examined, ranging from a period of two to twenty-two days after the onset of the disease. The time for the appearance of agglutinins in the positive tests was aptly summarized

by the authors; up to the third day after the onset of symptoms 33% of the cases gave a positive reaction in a dilution of 1-50 or over; after 4 to 6 days, 57% were positive; 7 to 9 days, 73%; 10 to 12 days, 88%; 13 to 15 days, 100%; 16 days or over, 83%; the highest titre observed was in a dilution of 1-640. That the agglutination titre tends to be reduced on recovery was also observed by these investigators. Testing a number of sera on successive days, they noted the following results indicated in table 20. Conn and DeNevesques tested with the S and R forms of their own organism the sera of seventeen persons ill from Sonne dysentery. They observed that agglutinins for the S form were more common and present in higher dilution than the agglutinins for the corresponding R form of the organism. They also noted that agglutinins may be present for both forms at the same time. Five of the seventeen sera produced no agglutination in a dilution of 1-20 or above for either the S or the R form of the homologous organism. The highest titre recorded was in a dilution of 1-100.

Sears, Bilderback, Ashley, and Fohner (1935) tested the serum of ten children suffering from Sonne dysentery for Sonne agglutinins. Two of these sera failed completely to give agglutination in a dilution of 1-10. In neither of these instances, however, were the organisms recovered in the stool. In the other eight cases

TABLE 8

Agglutinating ability of patients' serum twelve days after onset of dysentery[1]

Case number	Organisms tested	Maximum serum dilution giving agglutination
1	1	1:50, trace 1:100
	2	1:50, trace 1:100
	3	trace 1:25, faint trace 1:50
2	1	faint trace 1:25
	2	1:50, trace 1:100
	3	-

[1] From data given by J. Bamforth

TABLE V

Occurrence of agglutinins for Shigella paradysenteriae, Sonne in the serum of patients suffering from dysentery caused by this organism'

Case number	Days after onset of illness	Maximum dilution of serum giving agglutination of stock culture
A	8	1:1600
	14	1:1600
B	8	negative in 1:50
	20	1:200
C	4	1:50
	14	1:200

'From data given by Fraser, Kilnloch and Smith

TABLE 10

Occurrence of agglutinins in patients' serum for the Sonne organism'

Case number	Number of days after onset of symptoms	Titre of serum
2	7	1:50
	17	-
3	21	1:200
	27	-
4	3	1:800
8	8	1:1600
	16	1:200
14	16	1:1600
	29	1:1600
22	1	1:50
	10	1:400
29	5	1:200
	12	1:200
	14	1:50
46	9	1:50
	21	1:50

'From data presented by Smith and Fraser

agglutinins were present in dilutions ranging from 1-120 to 1-640. The sera, except one, were taken a week or more after the cessation of the symptoms. This one serum was taken four days after the onset of symptoms and agglutinated the homologous organism in a dilution of 1-160. In one instance sera were taken nine and thirty days after onset of the disease. Between these dates the titres of the serum fell from 1-640 to 1-160.

It was apparent, therefore, that in cases of infection there is a rise and subsequent fall in the agglutinating ability of the patients' serum for this organism.

If it could be demonstrated that the fall in titre of the serum is so great that there is finally no demonstrable agglutination in a dilution of 1-10 we would be in a better position to explain the apparent lack of agglutinins for this organism.

However the absence of agglutinins in normal sera has much significance. If only 1.6% of the sera of normal people agglutinate Shigella paradysenteriae, Sonne in a dilution of 1-20, and none in a dilution of 1-40 (pegell) this reaction can be safely used as a means of diagnosis. Sonne (1915) accepted a titre of 1-10 in normal serum as significant of dysentery infection. However, in view of the higher titres recorded by other investigators it would be advisable to accept the titre of 1-40 as diagnostic.

SUMMARY

Normal human and animal sera were tested for agglutinins for Shigella paradysenteriae, Somne. The strains used in the routine examination of sera were our numbers 355, 357, and 359. Number 355 is strain number 31 from the American Type Culture Collection. Number 357 was received in 1933 from Dr. Seule's laboratory in Michigan. Number 359 was isolated by this laboratory from a case of dysentery in this city in 1933. Only 1.6% of the 121 human sera, diluted 1:20, agglutinated these organisms. In this same dilution 100 % of the forty-three beef, 100% of the two horse, 96.2% of the ninety-seven sheep, 93.5% of the fifty-one swine, 46.4% of the twenty-eight rabbit, and 22.3 % of the sixteen buinea pig sera agglutinated the Somne organisms. The same strains were agglutinated by 49.6% of swine, 41.4 % of cattle, and 0.7% of sheep sera in a dilution of 1:640. Not all the Somne strains used in the routine examination of the sera were agglutinated to the same titre. The strain from the American Type Culture Collection was Agglutinated in higher dilutions than the other strains used.

The type of agglutination produced by normal and immune sera could not be distinguished. The agglutination ranged from a finely flocculant clumping readily visible to the naked eye to a finely granular type seen best with a magnifying lens. The latter type of agglutination was most conspicuous in immune sera. For production of maximum agglutination overnight incubation in a forty to forty-five degree water bath was necessary.

Normal horse and swine sera were absorbed by different strains of the Sonne organism to determine of the agglutinins in these sera were specific for the Sonne antigen. Though the agglutinins were removed for the organism used in absorption as well as for many other strains, they were not entirely removed for all strains. Absorption of the same sera with Eberthella typhi, Salmonella paratyphi, and Escherichia coli did not alter the titre of the sera for the Sonne strains. Similarly, immune sera were absorbed by different strains of Shigella paradysenteriae, Sonne. Each strain used in absorption removed agglutinins for all other strains except in one immune serum. This latter serum still retained agglutinins, though in lower dilutions, for two of the twenty-two strains tested. These results indicate a possible qualitati e difference in the agglutinins of normal and immune sera.

To determine the incidence of agglutinins for other enteric organisms in relation to the distribution of agglutinins for Shigella paradysenteriae, Sonne, a few normal sheep and beef sera were tested with a variety of gram negative organisms. The organisms that were most frequently agglutinated in serum dilutions above 1:160 were S. paradysenteriae, Sonne, S. paradysenteriae, Flexner, and E. coli. Those agglutinated in the lower dilutions only were E. typhi, S. schottmulleri, S. paratyphi, and S. dysenteriae. The latter organism was agglutinated only occasionally, and never in a dilution exceeding 1:20.

The extensive distribution of agglutinins in animal sera for the paradysentery organisms has much significance in relation to the immunization of animals with any of the intestinal organisms. If the initial titre of the serum is not recorded for these strains, a subsequent agglutination by the serum of an unrelated organism would suggest a false antigenic relationship among the strains.

Since agglutinins for Shigella paradysenteriae, Sonne occur in less that two per cent of normal human sera, the agglutination reaction, using patients' serum, is a reliable means of diagnosing dysentery caused by this organism.

BIBLIOGRAPHY

Bamforth, J., "A Small Outbreak of Dysentery Associated with an Unusual Bacillus," J. Hyg., 1924, 22:343

Bergey, D.H., "The Reaction of Certain Water Bacteria with Dysentery Immune Serum" J. Med. Research, 1903, 10:21

Cann, L.W. and De Navarques, S., "Epidemic Dysentery in the Nursing Staff Due to B. Dys., Sonne," J. Hyg., 1931, 31:361

Fraser, A.M., Kinloch, J.P. and Smith, J., "Sonne Dysentery in Aberdeen" J. Hyg., 1926, 25:453

Gibson, H.J., "Observations on the Occurrence, Characteristics and Specificity of Natural Agglutinins," J. Hyg., 1930, 30:337

Hiss, P.H., "On Fermentative and Agglutinative Characters of Bacilli of the Dysentery Group" J. Med. Research, 1904, 13:1

Kerrin, J.C., "An Investigation of B. dysenteriae, (Sonne Type III)" J. Hyg., 1928-1929, 28:4

Leahy, A.D., "Report on 31 Strains of B. Dys. Sonne Isolated During an Epidemic in Roch., N.Y." Am. J. Pub. Health, 1931, 21:1126

Mackenzie, G.M. and Batt, L.W., "Antigenic Analysis of Cultures of B. paradysenteriae and B. morgani, Isolated During an Epidemic of Summer Diarrhea," J. Immunol., 1930, 9:371

Menton, J. "Agglutinins to B. Dysenteriae in the Apparently Healty" Brit. M. J., 1929, 1:1115

Mita, K., "Dysentery-like Diseases in Children and their Causes," J. Infect. Dis., 1921, 29:580

Park, W.H. and Carey, H.J., "The Presence of the Shiga Variety of Dysentery Bacilli in an Extensive Epidemic of Dysentery, with Notes upon the Serum Reactions Obtained," J. Med. Research., 1903, 9:180

Park, W.H. and Collins, H.B., "Specific or Non Specific or Group Agglutinins," J. Med. Research., 1904, 12:491

Park, W.H., Collins, H.B., and Goodwin, M.E., "The Dysentery Bacillus Group and the Varieties which Should Be Included in it," J. Med. Research, 1904, 11:553

Schwichtenberg, M., "Agglutinins for Shigella paradysenteriae, Flexner in Normal Human Sera (Unpublished Report), 1935

Sears, H.J., Bilderback, J.B., Ashley, C.G., and Rohmer, M., "An Outbreak of Dysentery Caused by the Sonne Type Bacillus" Northwest Med. 1935, 34:37

Sears, H.J. and Schoolnik, M., "Metabolic Variation of S. paradysenteriae, Sonne" (Unpublished Report), 1935

Smith, J.M. and Fraser, A.M., "Agglutinative Reactions in Relation to Sonne Dysentery" J. Hyg., 1930, 30:216

Sonne, C., "Ueber die Bakteriologie der giftarmen Dysenteriebacillen" Centralbl. f. Bakteriol. (etc.) 1 Abt., Jena, 1915, Orig., 75:408

Sonne, C., "Die diagnostische Bedeutung der Agglutination der giftarmen Dysenteriebacillen in Patientenserie," Centralbl. f. Bakteriol. (etc.), 1 Abt., Jena, 1915, Orig., 76:65

Thjotta, Th., "On the Bacteriology of Dysentery in Norway" J. Bact., 1919, 4:355

Torrey, J.C., "A Comparative Study of Dysentery an Dysentery Like Organisms," J. of Exp. Med., 1905, 7:365

Wiseman, H.R., "Unusual Dysenteric Infection," J. Hyg., 1927, 26:187