AGGLUTINATION RESULTS WITH CERTAIN DYSEN- TERY ORGANISMS PLACED AGAINST HOMO- LOGOUS AND HETEROLOGOUS SERA.¹

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THE purpose of these experiments was to ascertain whether or not killed cultures of the various dysentery organisms hereunder mentioned exhibited agglutination-phenomena in homologous and heterologous sera. The alternative method of using living cultures for production of specific sera is reserved for a future communication. The following organisms were tested:—

Flexner (original); Shiga 1, Shiga 2, Y 14, Y 11, Strong 16, Strong 13, Strong 7, Flexner 3 (atypical); Flexner 4, Flexner 8 (typical); Flexner 9 (typical); Flexner 10, Y 12, Unknown 2, Shiga 15, Shiga X, Shiga 17, Flexner 18, Shiga 5.

No. 2 unknown strain was taken in order to test the action of the serums on a bacterium which is not closely related to the dysenteric group. No organism in the table below (Table I.) showed the slightest power of liquefying gelatin even after incubation for several weeks.

Table I. shows the biological reactions of the above.

Technique.—A stock-suspension of the bacillus in physiological salt-solution of the estimated strength of 10,000,000,000 organisms to the c.c. was prepared, and the organisms were killed by heat at 60° C. for an hour. To this suspension, $\frac{1}{10}$ c.c. of 1 in 20 carbolic acid per 10 c.c. of suspension was added. From this stock a dilute suspension of the estimated strength of 1,000,000,000 organisms to the c.c. was made, of which 1 c.c. was injected into the ear vein of a rabbit on two occasions at an interval of seven days.

Seven days after the second injection blood was withdrawn from the rabbit, and the serum tested against a suspension of the organism injected in dilutions (of the serum) of from 1 in 50 to 1 in 500 (equal parts of dilute serum and of suspension in the strength of 1,000,000,000 organisms to the c.c.). Where the result was negative the rabbit was reinjected, the procedure

¹ Received November 15, 1918.

Table I.

SHIGA STRAINS.									
	Motility.	Glucose.	Lactose.	Cane Sugar.	Mannite.	Dulcite.	Maltose.	Milk.	Indol.
Shiga 1 . . .	0	Acid	0	0	0	0	0	0	0
Shiga 2 . . .	0	"	0	0	0	0	0	0	0
Shiga 15 . . .	0	"	0	0	0	0	0	0	0
Shiga X. . . .	0	"	0	0	0	0	0	0	0
Shiga 17 . . .	0	"	0	0	0	0	0	0	0
Shiga 5 . . .	0	"	0	0	0	0	0	0	0
FLEXNER STRAINS.									
Flexner (original) .	0	Acid	0	0	Acid	0	Acid	No change	+
Flexner 9 (typical) .	0	"	0	0	"	0	"	"	+
Flexner 10 . . .	0	"	0	0	"	0	"	"	+
Flexner 18 . . .	0	"	0	0	"	0	"	"	+
Flexner 4 . . .	0	"	0	0	"	0	"	"	+
Flexner 8 . . .	0	"	0	0	"	0	"	"	+
Flexner 3 (atypical) .	0	"	0	0	"	Acid	"	"	+
Y STRAINS.									
Y 12	0	Acid	0	Faintly acid	Acid	0	Acid	No change	0
Y 11	0	"	0	Acid	"	0	"	0	0
Y 14	0	"	0	"	"	0	"	No change	0
STRONG STRAINS.									
Strong 16 . . .	0	Acid	0	0	Acid	0	Acid	0	0
Strong 13 . . .	0	"	0	0	"	0	"	0	0
Strong 7 . . .	0	"	0	0	"	0	"	0	0
UNKNOWN STRAINS.									
Unknown 2 . . .	0	Acid	Acid	Acid	Acid	Very faintly Acid	Acid	Clot	+

outlined being repeated and a second test performed. The agglutinations were carried out macroscopically, the results being noted after the test-tubes had been kept in the incubator at 37°-5 C. for from sixteen to twenty hours.

Where a positive reaction was obtained in the last-named dilution further tests were made up to a dilution of 1 in 2000.

The results obtained with homologous sera are shown in Table II.

Table II.

ORGANISMS AGAINST OWN SERUM.

Organisms (Twenty in number).	Agglutination, 1 in 500.	Agglutination, 1 in 2000.	Remarks.	Control Normal Serum (Rabbit). (All Strengths, 1 in 25 to 1 in 500).
Flexner (original)	+	+	...	0
Shiga 1	+	0	...	0
Shiga 2	+	+	...	0
Y 14	0	0	1 in 300 = +	0
Y 11	+	0	...	0
Strong 16	+	0	...	0
Strong 13	0	0	1 in 25 = +	0
Strong 7	0	0	" "	0
Flexner 3 (atypical)	0	0	...	0
Flexner 4	0	0	1 in 200 = +	0
Flexner 8 (typical)	+	0	...	0
Flexner 9 (typical)	0	0	1 in 100 = +	0
Flexner 10	0	0	" "	0
Y 12	+	+	...	0
Unknown 2	+	+	...	0
Shiga 15	0	0	1 in 25 = +	0
Shiga X	0	0	" "	0
Shiga 17	+	0	...	0
Flexner 18	0	0	1 in 200 = +	0
Shiga 5	0	0	...	0

The table shows that four out of twenty organisms (20 per cent.) were agglutinated by their corresponding sera in a dilution of 1 in 2000; five out of twenty (25 per cent.) in a dilution of 1 in 500; nine out of twenty (45 per cent.) in dilutions below 1 in 500, but of these last, four agglutinated only in the dilution of 1 in 25.

The results given in Table III. were obtained with those sera (4) which gave agglutination with their homologous organisms in a high dilution (1 in 2000), when put against the nineteen heterologous organisms.

From Table III. it appears that in no instance did a serum agglutinate a heterologous organism in a dilution of 1 in 2000; that in one instance, out of the four sera examined, the result was negative, even with a dilution of 1 in 500 (this was in the case of 2 Unknown Serum), in one instance also negative in that dilution except for a "trace" with three organisms; that of the two instances in which a positive result was obtained with that dilution, it occurred with, in the one instance (Shiga 2 serum), only six out of nineteen organisms, in the other (Flexner serum (original)), with only two out of the nineteen.

The Tables show that a trace of agglutination, or (very rarely) a positive result was obtained with low dilutions in some instances.

It remained to be ascertained whether the agglutinin produced by a heterologous organism in the two sera concerned was the same as that produced by the homologous organism or not. Equal quantities

Table III.
AGGLUTINATION (+ OR 0) WITH HIGH-TITRE SERA AGAINST HETEROLOGOUS ORGANISMS.

Organism.	Flexner (Original) Serum.			Shiga 2 Serum.			Y 12 Serum.		Unknown 2 Serum.	
	1 in 500.	1 in 2000.	Traces.	1 in 500.	1 in 2000.	Traces.	1 in 500.	Traces.	1 in 500.	Traces.
Flexner (original)										
Flexner 4 . . .	-	-	...	+	0	...	0	...	0	...
Shiga 5 . . .	0	...	...	0	...	Trace 1 in 50	0	...	0	Trace with 1 in 200
Flexner 9 . . .	+	0	...	0	...	Trace 1 in 100	0	...	0	Trace with 1 in 25
" 8 . . .	+	0	...	+	0	...	-	Trace with 1 in 500	0	...
Unknown 2 . . .	0	...	...	0	...	+1 in 25	0	+1 in 50	-	...
Flexner 18 . . .	0	...	...	0	...	+1 in 200	0	Trace 1 in 25	0	...
" 10 . . .	0	...	...	+	0	...	0	Trace 1 in 300	0	Trace with 1 in 25
Y 12 . . .	0	...	...	+	0	...	-	...	0	Trace with 1 in 50
Strong 7 . . .	0	...	...	0	...	...	0	...	0	Trace with 1 in 25
" 13 . . .	0	...	...	+	0	...	0	...	0	...
Y 14 . . .	0	...	...	0	...	Trace 1 in 50	0	...	0	...
Strong 16 . . .	0	...	...	0	...	"	0	...	0	...
Shiga X . . .	0	...	...	0	...	"	0	...	0	...
" 17 . . .	0	...	...	0	...	"	0	...	0	...
" 15 . . .	0	...	...	0	...	Shiga 15	0	...	0	Trace with 1 in 100
" 1 . . .	0	...	Trace with 1 in 50	0	...	...	0	...	0	...
" 2 . . .	0	...	Trace with 1 in 100	-	-	...	-	Trace with 1 in 500	0	...
Flexner 3 (atypical)	0	...	Trace with 1 in 100	0	...	Trace 1 in 25	0	Trace 1 in 25	0	...
Y 11 . . .	0	...	Trace with 1 in 100	+	0	...	-	Trace with 1 in 500	0	Trace with 1 in 25

of a strong suspension of the organism and a low dilution of the homologous serum were placed in a test-tube and incubated over night; the supernatant fluid was removed from the deposit resulting, centrifuged, and placed in dilutions of 1 in 50, 1 in 100, 1 in 200, 1 in 400, against an equal quantity of a suspension of the heterologous organism (1,000,000,000 to the c.c.), the homologous organism being used as a control. With the exception of a slight deposit in the 1 in 50 tube of Shiga 2 (homologous organism control) the result was negative.

The conditions of this test were then reversed, so that the serum of high titre was treated with strong suspension of the heterologous organism, the supernatant fluid, obtained as described, being placed in the dilutions stated against a suspension of the homologous organism, the heterologous one being used as a control. Two heterologous organisms were used against each of the two sera concerned. The results were entirely negative.

It therefore appears that in the two instances in which this test could be made the agglutinin produced by the heterologous organism was the same as that produced by the homologous one.

CONCLUSIONS.

1. Agglutinin is produced in considerable amount by 20 per cent. only of the organism tested, in moderate amount by from 25 per cent. to 50 per cent.

2. Sera which agglutinate their homologous organisms strongly (in high dilution) for the most part fail to agglutinate heterologous organisms in moderate dilution. In low dilutions traces of agglutination sometimes occur.

3. The agglutinin produced by heterologous organisms is the same as that produced by homologous organisms.

Florand, Bezançon, and Paraf (1918¹) found that twenty-six bacteria of the dysenteric group were not agglutinated by any serum, or only in 1 in 200, or lower dilutions. Similarly, Lancelin and Rideau (1918²) report that agglutination with Flexner and Shiga-Kruse bacilli, when obtained, is so in low dilutions only (1 in 150 and 1 in 50).

REFERENCES.

1. FLORAND, BEZANÇON, AND PARAF Cited in the *Daily Review of the Foreign Press*, issued by the War Office (Medical Supplement, May 1, 1918).
2. LANCELIN AND RIDEAU. . *Ibid.*, June 1, 1918.