

STUDIES ON THE  
PARATYPHOID-ENTERITIDIS GROUP

by

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Approved

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AGGUTINATION TESTS

Preliminary information.

The investigations of which this paper represent a part have been conducted by the Department of Bacteriology of the University of Kansas under the direction of Professor N. P. Sherwood in an attempt to obtain a more complete understanding of the nature of a group of organisms isolated from influenza cases during the winters of 1918-1919 and the year following. During February and March of 1919 there were isolated from the feces of a large percentage<sup>1</sup> of cases of influenza examined, organisms which culturally resembled the group of paratyphoid-enteritidis bacilli, but which failed to agglutinate with *B. paratyphosus* B serum. That they might be more closely related to *B. suipestifer* or *B. enteritidis* was suspected, and with the object of demonstrating such relationships as might exist, work was undertaken to establish the interagglutinability of the members of the group.

The work on agglutination was carried out for a part of the organisms during the summer of 1919 by Sherwood, Downs, and McNaught,<sup>1</sup> rabbits being immunized with *B. enteritidis*,

B. suipestifer, and with strains 78-5, 65-6, 52-1-3, 59-4-3, 56-1-a, 94-5, 72-6, and 42-4. These organisms, together with available stock strains of the paratyphoid-enteritidis group, and other members of the group isolated from the influenza cases, were agglutinated with the immune sera developed, the results of which have been reported.<sup>1</sup> Absorption tests showed that they were not related to B. paratyphosus B, B. suipestifer, typhimurium, cavipesticus, nor paratyphosus A, but that they showed decided cross agglutination with each other and with B. enteritidis.

On the basis of agglutination and absorption tests, 15 of the isolated organisms were classified into four types, as indicated below. Type I (85) is identical with B. enteritidis. Type IV is a heterogenous group. Type III contains some atypical strains which fall into a Type III Atypical.

Classification of strains according to Type.<sup>1</sup>

I	II	III	IIIAtyp.	IV
85-4	65-6	94-5	42-4	51-11-1
	67-5	68-6	56-1-a	52-1-3
		63		59-4-3
		64-11		66-4
		78-5		72-6

### Plan of the Work.

The work herein reported is a continuation of that research, with especial attention directed toward the relationship which seemed to exist between *B. enteritidis* and the organisms isolated from the influenza cases. The work of Macadam<sup>2</sup> in which he reported apparently culturally similar organisms isolated from cases in Bagdad and elsewhere which he found to be heterogenous and inagglutinable, after a time acquiring the character of agglutinating with *B. paratyphosus* B serum in low dilutions, suggested that a change might have occurred among these organisms with regard to their agglutinating characters with *B. enteritidis* serum. In addition to the organisms obtained in 1919, twenty-five strains representing twelve cases from which organisms were isolated in the early part of 1920 were tested with *enteritidis* serum and with the sera listed below. That this type of organisms should be isolated with considerable degree of constancy during the recurring epidemic one year after the first is of great interest, and warrants close observation of the nature of the organisms.

Rabbits were immunized with strains representing each of the four types which had been outlined from the previous work, and with *B. enteritidis* (N.Y.). Thus, Type I

is represented by strain 85-4 (and enteritidis), Type II by strain 65-6, Type III by 94-5, Type III Atypical by 42-4, and 56-1-a, and Type IV by 72-6. Full grown rabbits were used in almost every case. Inoculation in all cases except for rabbits developing E<sub>38</sub>, E<sub>40</sub>, and E<sub>42</sub> sera, was by the intravenous method of Downs<sup>S</sup>, using the ear veins in the administration of antigen. Starting with 0.2 c.c. of suspension of organisms killed at 60°C., three inoculations were made on alternate days, followed by increasing doses (0.2 to 1.5 c.c.) of live culture suspension injected daily for a period of six days. In order to prevent mixing of antigens, individual autoclaved (in some cases boiled) syringes were employed. The suspensions were made in 0.85% sterile salt solution from growth on agar slants, about 2 c.c. of suspension being obtained from the growth of one slant. The rabbits were bled from the heart with a syringe six to nine days after the final inoculation, and the serum obtained by centrifugation. The serum was preserved with 0.5% phenol in test tubes closed with rubber stoppers thru which passed capillary pipets of known calibration which permitted the measuring of desired quantities of serum by the drop method, and prevented contamination of the serum by the introduction of a fresh pipet each time the serum was used.

The agglutination tests were set up in 6 c.c. test tubes with 0.2 c.c. of serum dilution and 0.2 c.c. of suspension of organism in each tube. Especial care was taken to have the tubes clean and free from acid. After being rinsed, swabbed, re-rinsed, and finally rinsed with distilled water, the tubes were inverted and allowed to dry. In running several organisms with one serum, the various dilutions were made up in batches before being distributed to the tubes, thus insuring comparable results. Suspensions of organisms were made in 0.85% saline from agar slants or plates, one slant furnishing enough growth for about 1.5 c.c. of suspension. The tubes containing 0.2 c.c. of dilution and 0.2 c.c. of suspension were placed in the incubator for about three hours, at the end of which time the results were observed. In order to check results, the tubes were placed in the ice box over night and observations again made.

With the immune sera obtained, 42-4, 72-6, 85-4, 56-1-a, Ent., 94-5, and 65-6, cross agglutinations were run with these and other organisms of the group isolated in 1919; with twenty-five representatives of the group isolated in 1920; and with *B. enteritidis* and *B. suipestifer*. The results of these agglutinations tests are given in the following pages:

NOTES ON TABLES

Dilutions:	1	2	3	4	5
	1/20	1/40	1/80	1/200	1/400
	6	7	8	9	C
	1/800	1/2000	1/4000	1/8000	Control

Titration of E sera:

	E38	E40	E42
Dil. up to 1/800	4+	4+	4+
1/2000	3+	3+	3+
1/4000	2+	2+	2+

TABLE 1

Serum: 65-6

Dilutions: 1/20      1/40      1/80      1/200      1/400

Organisms:

42-4	-	-	-	-	-
72-6	-	-	-	-	-
85-4	+ + + +	+ + +	+ +	+	-
56-1-a	-	-	-	-	-
Ent.	+ +	+ +	+	+	-
94-5	-	-	-	-	-
65-6	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +

	1/800	1/2000	1/4000	1/8000	Control
42-4	-	-	-	-	-
72-6	-	-	-	-	-
85-4	-	-	-	-	-
56-1-a	-	-	-	-	-
Ent.	-	-	-	-	-
94-5	-	-	-	-	-
65-6	+ + + +	+ +	+ +	-	-



TABLE 3

Serum: 85 23

Dilutions: 1/20      1/40      1/80      1/200      1/400

Organisms:

42-4	-	-	-	-	-
72-6	-	-	-	-	-
85-4	±	±	-	-	-
56-1-a	-	-	-	-	-
Ent.	+++	++	±	-	-
94-5	-	-	-	-	-
65-6	-	-	-	-	-

	1/800	1/2000	1/4000	1/8000	Control
42-4	-	-	-	-	-
72-6	-	-	-	-	-
85-4	-	-	-	-	-
56-1-a	-	-	-	-	-
Ent.	-	-	-	-	-
94-5	-	-	-	-	-
65-6	-	-	-	-	-

TABLE 4

Serum: E 17

Dilutions: 1/20      1/40      1/80      1/200      1/400

Organisms:

42-4      -      -      -      -      -

72-6      -      -      -      -      -

85-4      + +      +      +      -      -

56-1-a      + +      +      +      -      -

Ent.      + + + +      + + + +      + + + +      + + + +      + + +

94-5      -      -      -      -      -

65-6      + +      +      +      -      -

1/800      1/2000      1/4000      1/8000      Control

42-4      -      -      -      -      -

72-6      -      -      -      -      -

85-4      -      -      -      -      -

56-1-a      -      -      -      -      -

Ent.      + + +      + +      +      -      -

94-5      -      -      -      -      -

65-6      -      -      -      -      -



TABIE 6

Serum: 94<sub>12</sub>

Dilutions: 1/20      1/40      1/80      1/200      1/4000

Organisms:

42-4	-	-	-	-	-
72-6	-	-	-	-	-
85-4	-	-	-	-	-
56-1-a	-	-	-	-	-
Ent.	+	+	+	+	±
94-5	+	+	+	+	+
65-6	+	-	-	-	-

	1/800	1/2000	1/4000	1/8000	Control
42-4	-	-	-	-	-
72-6	-	-	-	-	-
85-4	-	-	-	-	-
56-1-a	-	-	-	-	-
Ent.	-	-	-	-	-
94-5	-	-	-	-	-
65-6	-	-	-	-	-

TABLE 7

Serum: 72 <sub>1</sub>					
Dilutions:	1/40	1/80	1/200	1/400	Control
Organisms:					
42-4	+	+	+	-	-
72-6	+	+	+	+	-
85-4	-	-	-	-	-
56-1-a	-	-	-	-	-
Int.	+	-	-	-	-
94-5	-	-	-	-	-
65-6	-	-	-	-	-
6-2	-	-	-	-	-
43-12-1	+	+	-	-	-
44-1-1	-	-	-	-	-
52-1-3	-	-	-	-	-
59-3-2	-	-	-	-	-
59-4-3	-	-	-	-	-
59-6-2	-	-	-	-	-

TABLE 8

Serum:	72 <sub>1</sub>				
Dilutions:	1/40	1/80	1/200	1/400	Control
Organisms:					
60-7-5	-	-	-	-	-
64-11	-	-	-	-	-
66-4	-	-	-	-	-
67-5	-	-	-	-	-
73-6	+ + +	+ + +	+	-	-
68-7	-	-	-	-	-
71-4	-	-	-	-	-
78-5	+ +	+ +	-	-	-
84-2	-	-	-	-	-
109-5	±	-	-	-	-
109-6	±	-	-	-	-
111-2	-	-	-	-	-

TABLE 9

Serum: 56<sub>24</sub>

Dilutions:    1/40            1/80            1/200            1/400            Control

Organisms:

42-4	-	-	-	-	-
72-6	-	-	-	-	-
85-4	-	-	-	-	-
56-1-a	+ + +	+ +	+ +	-	-
Ent.	+	+	-	-	-
94-5	-	-	-	-	-
65-6	-	-	-	-	-
6-2	+ + +	+ +	-	-	-
43-12-1	-	-	-	-	-
44-1-1	-	-	-	-	-
52-1-3	+ + +	+ +	+	-	-
59-3-2-	-	-	-	-	-
59-4-3	-	-	-	-	-
59-6-2	-	-	-	-	-

TABLE 10

Serum: 56<sub>24</sub>

Dilutions: 1/40      1/80      1/200      1/400      Control

Organisms:

60-7-3	-	-	-	-	-
64-11	-	-	-	-	-
66-4	-	-	-	-	-
67-4	-	-	-	-	-
73-6	+ + +	+ +	+ +	+ +	-
68-7	-	-	-	-	-
71-4	-	-	-	-	-
78-5	+ + +	+ + +	+ +	+	-
84-2	-	-	-	-	-
109-5	-	-	-	-	-
109-6	-	-	-	-	-
111-2	-	-	-	-	-

TABLE 11

Serum: 42-4	85-4		Control		
Dilutions:	1/40	1/80	1/40	1/80	Control
Organisms:					
42-4	+	+	+	+	-
72-6	-	-	-	-	-
85-4	-	-	+	+	-
56-1-a	-	-	-	-	-
94-5	-	-	-	-	-
65-6	-	-	-	-	-
6-2	-	-	-	-	-
43-12-1	+	+	+	+	-
44-1-1	-	-	-	-	-
52-1-3	-	-	-	-	-
59-3-2	-	-	-	-	-
59-4-3	-	-	-	-	-
59-6-2	-	-	-	-	-

TABLE 12

Serum:	42-4		85-4		
Dilutions:	1/40	1/80	1/40	1/80	Control
Organisms:					
64-11	-	-	+	+	-
60-7-3	-	-	-	-	-
66-4	-	-	-	-	-
67-5	-	-	-	-	-
68-7	-	-	-	-	-
71-4	-	-	-	-	-
73-6	-	-	-	-	-
78-5	-	-	-	-	-
84-2	-	-	-	-	-
109-5	+	+	-	-	-
111-2	+	+	-	-	-

TABLE 13

Serum:	94-5		65-6		Control
Dilutions:	1/40	1/80	1/40	1/80	
Organisms:					
42-4	-	-	-	-	- -
72-6	-	-	-	-	-
85-4	-	-	-	-	-
56-1-a	-	-	-	-	-
Ent.	-	-	-	-	-
94-5	+ + + +	+ + + +	-	-	-
65-6	-	-	+ + + +	+ + + +	-
6-2	-	-	-	-	-
43-12-1	-	-	-	-	-
44-1-1	-	-	-	-	-
52-1-3	-	-	-	-	-
59-3-2	-	-	+ + + +	+ + + +	-
59-4-3	-	-	-	-	-
59-6-2	-	-	-	-	-

TABLE 14

Serum:	94-5		65-6		
Dilutions:	1/40	1/80	1/40	1/80	Control
Organisms:					
64-11	-	-	-	-	-
60-7-3	-	-	-	-	-
66-4	-	-	-	-	-
67-5	-	-	+ + + +	+ + + +	-
68-7	-	-	-	-	-
71-4	-	-	-	-	-
73-6	-	-	-	-	-
78-5	-	-	-	-	-
84-2	-	-	-	-	-
109-5	-	-	-	-	-
111-2	-	-	-	-	-







TABLE 18

Serum:	42 <sub>22</sub>			72 <sub>1</sub>		
Dilutions:	1/40	1/80	1/200	1/40	1/80	1/200
Organisms:						
6-2	-	-	-	-	-	-
5-1	-	-	-	-	-	-
5-2	-	-	-	-	-	-
9-1	-	-	-	-	-	-
9-2	-	-	-	-	-	-
9-3	-	-	-	-	-	-
9-6	-	-	-	-	-	-
9-7	-	-	-	-	-	-
9-9	-	-	-	-	-	-
9-11	-	-	-	-	-	-
14-2	-	-	-	-	-	-
14-3	-	-	-	-	-	-
14-4	-	-	-	-	-	-
15-1	-	-	-	-	-	-

TABLE 19

Serum:	42 <sub>22</sub>			721		
Dilutions:	1/40	1/80	1/200	1/40	1/80	1/200
Organisms:						
15-3	-	-	-	-	-	-
15-4	-	-	-	-	-	-
16-4	-	-	-	-	-	-
17-2	-	-	-	-	-	-
17-6	-	-	-	-	-	-
25-1	-	-	-	-	-	-
25-3	-	-	-	-	-	-
32-1	-	-	-	-	-	-
120-2	-	-	-	-	-	-
126-6	-	-	-	-	-	-
132-5	+	+	-	-	-	-
Suipest.	-	-	-	-	-	-
94-5	-	-	-	-	-	-
56-1-a	-	-	-	-	-	-
Ent.	-	-	-	-	-	-

TABLE 20

Serum:	85 <sub>23</sub>			56 <sub>24</sub>		
Dilutions:	1/40	1/80	1/200	1/40	1/80	1/200
Organisms:						
6-2	-	-	-	-	-	+ + + +
5-1	-	-	-	-	-	-
5-2	-	-	-	-	-	+ + + +
9-1	-	-	-	-	-	-
9-2	-	-	-	-	-	-
9-3	-	-	-	-	-	-
9-6	-	-	-	-	-	-
9-7	-	-	-	-	-	-
9-9	-	-	-	-	-	-
9-11	-	-	-	-	-	-
14-2	-	-	-	-	-	-
14-3	-	-	-	-	-	-
14-4	-	-	-	-	-	-
15-1	-	-	-	-	-	-

TABLE 21

Serum:	85 <sub>23</sub>			56 <sub>24</sub>		
Dilutions:	1/40	1/80	1/200	1/40	1/80	1/200
Organisms:						
15-3	-	-	-	-	-	-
15-4	-	-	-	-	-	-
16-4	-	-	-	-	-	-
17-2	-	-	-	-	-	-
17-6	-	-	-	-	-	-
25-1	-	-	-	-	-	-
25-3	-	-	-	-	-	-
32-1	-	-	-	-	-	+ + + +
120-2	-	-	-	-	-	-
126-6	-	-	-	-	-	-
132-5	-	-	-	-	-	-
Suipest.	-	-	-	-	-	-
94-5	-	-	-	-	-	-
56-1-a	-	-	-	-	-	+ + + +
Ent.	+ +	+	-	-	-	+ + +

TABLE 22

Serum:	94 <sub>12</sub>		E <sub>42</sub>	65		
Dilutions:	1/40	1/80	1/80	1/40	1/80	1/200
Organisms:						
6-2	-	-	-	-	-	-
5-1	-	-	-	-	-	-
5-2	-	-	-	-	-	-
9-1	-	-	-	-	-	-
9-2	-	-	-	-	-	-
9-3	-	-	-	-	-	-
9-6	-	-	-	-	-	-
9-7	-	-	-	-	-	-
9-9	-	-	-	-	-	-
9-11	-	-	-	-	-	-
14-2	-	-	-	-	-	-
14-3	-	-	-	-	-	-
14-4	-	-	-	-	-	-
15-1	-	-	-	-	-	-

TABLE 23

Serum:	94 <sub>12</sub>		E <sub>42</sub>		65	
Dilutions:	1/40	1/80	1/80	1/40	1/80	1/200
Organisms:						
15-3	-	-	-	-	-	-
15-4	-	-	-	-	-	-
15-4	-	-	-	-	-	-
17-2	-	-	-	-	-	-
17-6	-	-	-	-	-	-
25-1	-	-	-	-	-	-
25-3	-	-	-	-	-	-
32-1	-	-	-	-	-	-
120-2	-	-	-	-	-	-
126-6	-	-	-	-	-	-
132-5	-	-	-	-	-	-
Suipest.	-	-	-	-	-	-
94-5	+ + + +	+ + +	-	-	-	-
56-1-a	-	-	+ + +	-	-	-
Ent.	+ + +	+ +	+ + + +	+ + +	+ +	+

TABLE 24.

Serum	Titre 123456789	Agglutinated	Dilutions 123456789
42-4	4444430	56-1-a Ent. 43-12-1 109-5 111-2	4431 3321 ?43? ?44? ?44?
	?	132-5	?110
72-6	44321 4321	42-4 42-4 43-12-1 73-6 78-5	440 3220 ?210 ?3210 ?220
85-4	10 ?22? ?	E 64-11 E	3210 ?21? 210
56-1-a	?3220	E 6-2 52-1-3 73-6 78-5	?110 ?320 ?321? ?32220 ?33210
Ent. 17	444433210	85-4 56-1-a 65-6	20 210 210
E <sub>38</sub> E <sub>40</sub> E <sub>42</sub>	444443220	85-4 65-6 59-3-2 73-6 78-5 109-5 9-3	?221? ?100? ?100? ?332? ?011? ?011? ?111?
94-5	432110 43?	E 65-5 E	44310 10 32?
65-6	444444220	85-4 E 59-3-2 67-5	44210 22110 ?44? ?44?

? = No test set up.

0 = No agglutination

TABLE 25

Serum	Titre	Agglutinated	Dilutions	
			1234567890	
E	1/1000	59-4-3	1/100	
		42-4	E	
		94-5	"	
		52-1-3	"	
		78-5	1/100	
	1/500	94-5	1/100	
		66-4	1/1000	
	?	85-4	1/500	
		109-5	1/50	
		66-4	1/1000	
	1/1000	85-4	"	
		85-4	1/1000	
	56-1-a	000	59, 78, 94, 72, 42, 71, 64, 85, Ent, 64, 85,	1/1000
	<u>65-6</u>	1/1000	59, Ent, 78-5, E., 94, 42, 72 67-5	1/1000 ? 1/500
94-5	1/500	78-5, 68-6, 85-4, 109-5	1/1000	
	1/100	Ent.	1/2000	

Suipestifer was agglutinated by no serum except its own.

(Results of 1919 work)

OBSERVATIONS

Table 24 represents a bird's-eye view of the positive results of Tables 1 to 23, and shows what organisms have been agglutinated by each of the seven sera, and the homologous titration of the agglutinating serum at the time. Table 25 gives somewhat the same picture of the positive results of some of the work done in 1919, excluding the sera and organisms which have not been used in the 1920 work.

It is interesting to note that E serum with a titration of 1/4000 agglutinates strain 9-3 in dilutions of 1/40 and 1/80 but does not agglutinate any other member of the 1920 group. On the other hand, E serum agglutinated seven of the 1919 group including strains 85, 65, and 56, which is substantially a repetition of its behavior during the former work. The only other serum agglutinating any of the 1920 group was 42-4 (titre 1/800) which agglutinated strain 132-5 in dilution of 1/80 and slightly in 1/200.

In 1919 E serum of titre 1/1000 agglutinated strain 109-5, 1/50, but in 1920 did not. On the other hand, it did agglutinate strain 109-6 in dilutions of 1/80 and 1/200.

In the 1919 work E serum (1/1000) agglutinated strain 59-4-3 in dilution of 1/1000, but failed in 1920 to agglutinate 59-4-3 and 59-6-2; but did agglutinate strain

59-3-2 slightly in dilution of 1/40.

It should be noted that in selecting strains to use in this work an attempt was made to get representatives of each case from which isolation had been made, and also to get enough members from some one case in each of the two groups to note if differences exist in the several strains isolated from the same case. Thus, in the 1919 group each case is represented, and three organisms from case 59 were selected. From the 1920 group Case 9 is represented complete with 7 strains.

From table 24 it may be pointed out that:

Serum 65-6	agglutinates organisms	85-4
		Ent.
" 56-1-a	"	" Ent.
" 85-4	"	" "
" 94-5	"	" Ent.
		65-6
" E	"	" 85-4
		56-1-a
		65-6

usually in low dilutions.

The former work showed, as noted in table 25, that:

Serum 65-6	agglutinated organisms	E, 72-6, and 42-4
" 94-5	"	" E, 85-4
Serum E	"	" 42-4, 94-5, 85-4
" 56-1-a	"	" 94-5, 72-6, 42-4, 85-4, E.
	(altho not agglutinating itself)	

Thus it will be seen that in the main the results of this work bear out the observations formerly made, and that there has been little or no observable change in the agglutinating characteristics of the organisms studied. The fact that the sera of some organisms agglutinate B. enteritidis but that the enteritidis serum does not reciprocate would indicate that if immune sera were developed from all of the organisms under observation, more relationships between these organisms and enteritidis would be observed than are observed by agglutinating the organisms with enteritidis serum only. And also, just as there seems to be some group relationships among the strains for which immune sera have been developed; so it does not seem improbable that other relationships might be demonstrated to exist if immune sera were developed for all of the organisms.

This work can give little indication of the nature of the group of organisms isolated in 1920, since no sera were developed from any of these organisms.

DISCUSSION

Much work has been done on the paratyphoid-enteritidis group, and the significance and nature of its many members is not yet satisfactorily understood. Jordan,<sup>3</sup> who has done a great deal of work in this group, points out that although *B. paratyphosus* B is accorded by some investigators to be identical with *B. enteritidis* (type Artryck, not Gaertner) and with the bacillus of mouse typhoid, the tracing of relationships within this group is particularly hazardous because of the presence of common agglutinins. The A and B types of paratyphoid bacilli are different and can be distinguished by agglutination as well as by other means. The interrelationships of the hog-cholerae group have not been successfully demonstrated by the agglutination test, and the division into two groups, those bacteria more closely connected with the domestic animals, including the hog-cholerae bacillus and the bacilli found in certain meat-poisoning epidemics, and those more closely related to typhoidal human infections, such as the so-called paratyphoid organisms, is the only definite suggestion from agglutination work. In view of work that has been done since his statement was made some modification to the above is undoubtedly necessary.

Weiss<sup>4</sup> finds that twenty-one of his paratyphoid strains isolated from various sources (but in no case from known paratyphoid fever) fell into a heterogenous group, the members of which reacted culturally like typical paratyphosus but differed from the fixed types in not agglutinating B. paratyphosus A and B sera and failing to show cross agglutination. Three sera made from strains in this group reacted with homologous antigen only. He also includes *B. enteritidis*, *B. Columbensis*, and the Danysz organism in the heterogenous paratyphoid group because they fail to agglutinate para A and B sera, and are culturally like the members of the heterogenous group. According to Weiss, Uhlenhuth and Hübener obtained an organism from a case of meat poisoning which was similar to *B. paratyphosus* but failed to agglutinate with sera of the fixed types. It was agglutinated by patients serum and by *B. enteritidis* serum, however. Other workers have found similar organisms. On the basis of antigenic findings Weiss divides *B. paratyphosus* A into two groups, *B. Paratyphosus* B into seven groups, *B. typhi murium* into two (one agglutinating and one non-agglutinating), and *B. cholerae suis* the same as *B. typhi murium*.

In connection with the discussion of the pathogenicity of various members of the paratyphoid-enteritidis group a quotation from the report of Krumwiede, Kohn and Valentine<sup>5</sup> will be of interest. "By correlating the fermentative results, especially in relation to quantitative reductional differences, well-defined groups result. This grouping correlates host origin and agglutinative characters." It is indicated that *B. paratyphosus* "B" is essentially a human pathogen, and paratyphoid fever produced by this type is probably caused by the transfer of the organism from man to man, not from animals to man.

In a later report Krumwiede, Valentine and Kohn<sup>6</sup> describe fifteen strains isolated from guinea pigs of which thirteen were alike in their agglutination reactions. Other strains from mice, rabbit, and cat identical with the group of thirteen. Others belong to the *B. enteritidis* group. The host origin, they point out, is not necessarily an index to the biological characters of an organism, and the naming according to origin is not justified. None of the rodent strains studied belong in the *B. paratyphosus* group.

That variations in this group do occur is a common observation, and the variations affect cultural and well as agglutination characteristics. Jordan<sup>7</sup> cites several in-

stances of especially variable strains, among which is the *B. paratyphosus* B organism reported by Sobenheim and Seligman which became inagglutinable to *B. paratyphosus* B serum and at the same time acquired the property of being agglutinated by *B. enteritidis* serum. To quote from Jordan's article, "Agglutination reactions in general are subject to a considerable range of variation, and in the paratyphoid-enteritidis group especially are to be accepted only guardedly as criteria of relationship. Perhaps the most striking case of lack of correlation between agglutination reactions and cultural characteristics is the close agglutinative relationship of the culturally diverse avian paratyphoid bacilli and *B. typhosus*. Malsow has shown further that *B. enteritidis* and *B. abortus equinus* also manifest agglutinative affinities to this group. On the other, hand, as well, known, *B. enteritidis* and *B. paratyphosus* B, while agglutinatively distinct, possess the closest cultural similarity."

SUMMARY

Immune sera were developed from antigens consisting of B. enteritidis and seven strains of paratyphoid-enteritidis like organisms isolated from the feces of influenza cases of the 1918-1919 epidemic.

Twenty-four strains representing each case from which organisms were isolated in 1919 were tested for agglutinability with each of the above sera.

Twenty-five strains representing each case from which organisms were isolated in 1920 were tested for agglutinability with each of the above sera.

Comparison was made with agglutination results obtained nine months previously.

### CONCLUSIONS

From the agglutination tests carried out in this work, no marked variation from agglutinative relationships with *B. enteritidis* indicated nine months previously were observed.

Certain interrelationships exist between some members of the group isolated in 1919.

Certain relationships seem to exist between *B. enteritidis* and members of the paratyphoid-enteritidis like organisms isolated from feces of cases of influenza in 1919.

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