S U B J E C T.

The agglutination reactions of typhoid bacilli isolated from the body; with a discussion of typhoid bacilluria, and an account of certain bacilli, hitherto undescribed, found in the urine in enteric fever.

A Thesis for the degree of Doctor of Medicine presented by Adam Patrick, M.A.,M.B.,Ch.B.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

INTRODUCTION.

A series of experiments was carried out in the City of Glasgow Fever Hospital, Ruchill, for the most part between August, 1911, and June, 1912, to elucidate, if possible, certain points in connection with the reactions of typhoid bacilli.

It is well known that typhoid bacilli, freshly isolated from enteric fever patients, vary in their capacity to undergo agglutination. The primary object of the inquiry, therefore, was to discover whether these differences in agglutinability are dependent on the stage of the disease at which the bacillus is isolated, or on the body substance from which it is obtained.

The bacilli were grown chiefly from blood, faeces, and urine, but also from vesicular rose-spots, and (post mortem) from spleen, bile,and mesenteric glands. They were tested by such fermentative and other methods as were considered sufficient to establish their identity as typhoid bacilli. An agglutinating serum was obtained by the inoculation of a rabbit with the stock typhoid bacillus in use in the hospital laboratory. For purposes of comparison the bacilli were tested also with the sera of rabbits immunized in the same way against 4 strains of paratyphoid organisms.

In addition, reciprocal experiments were carried out in nearly every case with the patients' sera and these 5 bacilli. In most instances also in which a bacillus was isolated, it also was tested with the patient's serum and the result compared with the results of agglutination by the stock antisera.

Finally, the bacilli were examined according to the method described by Michaelis, of agglutination by means of acid solutions of varying strengths.

In the course of the inquiry it was discovered that in 6 cases of bacilluria, the bacilli in the urine were neither typhoid bacilli nor contaminating organisms, but were different from any which have hitherto been described. This fact has not previously been noted in connection with enteric fever. The second part of my thesis, therefore, has been devoted to a discussion of typhoid bacilluria. The cases which came under my personal observation are described, and a full account is given of the atypical bacilli.

PART I.

Section I. Methods employed for isolation of bacilli.

Section II. Agglutination reactions carried out with the sera of patients and stock bacilli, typhoid and paratyphoid.

Section III. Agglutination experiments on bacilli with artificial antisera.

Section IV. Agglutination reactions of bacilli with the serum of the patient from whom each was obtained.

Section V. Discussion of variations in agglutinability. Conclusions from experiments.

Section VI. Agglutination of bacilli by acid solutions (Michaelis).

SECTION I.

Methods employed for isolation of bacilli.

The methods employed to isolate the bacilli are described in this section. In all bacilli were obtained 76 times, - from blood 26 times, from faeces 19 times, from urine 17 times, from vesicular rose-spots 3 times, and, post mortem, from the spleen 7 times, from the gall-bladder 3 times, and from a mesenteric gland, once.

Cultures from blood.

These were made as a rule on the morning after the patient's admission to hospital. Two methods were employed. First 1 cc. of blood was withdrawn from a vein at the elbow and added to an amount of bouillon which varied from 100 cc. to 250 cc., though 100 cc. was the quantity most usually employed. This was incubated for 24 hours at 37°C. Later 5 cc. of blood was taken, added to 10 cc. of sterilized ox-bile, and incubated.

The fluid of the culture medium was examined in a hanging drop next day. If only motile bacilli were visible, or no organisms, a sub-culture was made directly on agar. In some instances where no growth was visible in the hanging drop, the sub-culture on agar showed that bacilli had been present. If, as happened on a few occasions, there was contamination by cocci, a sub-culture was made on the

modified Endo medium described in the paragraph on "Cultures from faeces" (page 6), and the bouillon was transferred thence to an agar slope.

There is a pretty general agreement among later authors that the second method used, i.e. to add 5 c.c. of blood to 10 c.c. of bile, or one resembling it, gives the best results in cultures from blood. The first investigator to make a series of successful Cultures from blood was Kühnau[1] who used large quantities of blood (10-20 c.c.) which he mixed with bouillon:- 27% of his cultures were positive. Castellani [2] mixed a few c.c. of blood with a large quantity of bouillon (300 c.c.) and obtained the bacillus in 12 out of 14 cases. Conradi[3] in 1906 introduced ox-bile as a culture medium. The object of the use of bile was to prevent coagulation of the blood, and to it was added 10% of peptone for better growth of the bouillon, and 10% of glycerin to inhibit the growth of any contaminating organisms which might be present. Kayser[4] showed that the use of ox-bile alone as a culture medium gave excellent results. He proved also that the degree of success depended to a large extent on the amount of blood taken, the results with 2.5 c.c. being much better than with .5 c.c.

All authors are agreed that the cultures made in the first week are almost always positive, and that the longer after this the blood is taken, the less is the chance

of obtaining a bacillus. My cases are too few to give a regular series of results, but it may be noted that the 8 cultures made with 5 c.c. of blood in 10 c.c. of bile in the first fortnight were all positive.

In two instances a diagnosis of enteric fever was made from the blood culture on the 3rd day of illness. The Widal reaction in both was negative.

Cultures from faeces.

The faeces were collected from the ward vessels in small sterilized glass tubes by means of a sterilized pipette. The cultural method used was practically that described by Kendall and Day[5] and was as follows :-

The isolating medium was a modified Endo medium* which contained 1.5% of agar instead of Endo's 4% and which was made just alkaline to litmus instead of strongly alkaline (.2% of acidity to phenolphthalein is said to be best (Russell[6])). Plates were made of this medium and were used fresh. A platinum loopful of faeces was well mixed in 10 c.c. of bouillon at incubator temperature, the tube was incubated for an hour, and 3 loopfuls of this bouillon were then spread on a plate by means of a right-angled glass rod. The plate was incubated over-night. After 18-24 hours' growth the colonies of B. typhosus were 1-1.5 mm.

---

* Endo's medium contains lactose (1%) with fuchsin, decolorized by sodium sulphite as an indicator. When acid is produced by splitting of the lactose, the red colour of the fuchsin appears around the colonies of organisms fermenting the lactose.

in diameter, were circular and colourless, and had caused no alteration in the colour of the medium around them. B. coli in 24 hours produced red colonies, about 4 mm. in diameter with a broad red halo in the medium. B. typhosus was easily recognised, and it seldom happened that a bacillus judged from the Endo plate to be typhoid turned out to be something else.

According to Kendall and Day, the preliminary incubation in bouillon does two things - "The clumps of bacteria are thrown down, leaving a more uniform suspension of bacteria in the supernatant fluid for inoculation, and the bacteria undergo a slight development in a medium particularly suited for their growth ..... The transition from faeces to artificial media involves a marked change in the nutritive environment of the bacteria, and experience has shown that cultures grow more readily if the transition be made from faeces to fluid culture media than if the change be made from faeces to solid media direct."

The number of colonies obtained on a plate by this method varied considerable, but 30-40 was common.

Cultures from urine.

These were made when bacilluria was evident to the naked eye. The method described for isolation of bacilli from faeces was used also here. A loopful of the turbid urine was added to 10 c.c. of bouillon at 37°C, the tube was incubated for an hour, and then 3 loopfuls were

spread on a modified Endo plate. This was incubated for 24 hours, and subcultures were made on agar slopes.

Cultures from vesicular rose-spots.

The skin was cleansed with methylated spirit, the vesicle ruptured, and the fluid touched with the point of a platinum needle, after which a bouillon tube was then inoculated. One patient had a single vesicular spot, and another had 2, and from each a pure culture of B. typhosus was obtained.

Cutures from spleen, gall-bladder, and mesenteric gland (post mortem)

The surface of the organ was branded with a hot copper spatula, and a cut made in it with a sterile scalpel. A platinum needle was pushed into the interior, and a tube inoculated. The organism was obtained each time in pure culture.

Cultures from other organs and fluids (post mortem)

B. typhosus was obtained once from the pus of small kidney abcesses. A culture from the other kidney, which was free from abcesses, yielded only B. coli.

I failed to obtain B. typhosus from sputum, lungs, liver, and cerebro-spinal fluid. In the case of a typhoid patient who was 5 months pregnant and who died, cultures from the amniotic fluid, and from the foetal blood and foetal gall-bladder failed to shew the presence of B. typhosus.

All the bacilli were examined after isolation. The following tests were considered sufficient to establish their identity as typhoid bacilli :-

The production of acid without gas in glucose, maltose, and mannite:

the production of slight permanent acidity, without clotting, in litmus milk:

non-fermentation of lactose and saccharose:

non-production of indol in peptone water after 7 days' growth:

non-liquefaction of gelatin:

colourless growth on potato:

presence of motility.

TABLE I.

Cultures from blood 1st Series (in bouillon)

	Name	Age	Days ill.	Rose Spots?	1 c.c.blood in bouillon c.c.	Result.	Result of culture from faeces.
1.	E.B.	20	8	+	150	+	
2.	J.T.	33	8	+	100	+	+
3.	G.B.	6	8	-	100	-	-
4.	W.P.	23	9	+	100	-	-
5.	J.L.	36	9	+	100	-	-
6.	J.F.	10	9	+	100	+	-
7.	J.P.	24	10	+	100	-	-
8.	J.M.	31	10	+	100	-	+
9.	P.K.	12	11	-	200	-	
10.	R.R.	23	12	+	125	+	
11.	L.P.	25	13	-	100	-	-
12.	E.G.	20	13	-	100	-	-
13.	F.B.	30	13	+	100	-	+
14.	G.H.	12	14	-	125	-	
15.	B.J.	5	14	-	200	-	
16.	W.B.	35	14	+	100	+	-
17.	C.W.	23	14	+	100	+	-
18.	W.W.	37	15	+	200	-	
19.	D.M.	20	15	+	100	+	-
20.	A.W.	50	15	+	100	+	+
21.	J.R.	15	15	+	100	-	-
22.	A.R.	52	15	+	100	-	-

TABLE I.

Cultures from blood 1st Series (Contd)

	Name	Age	Days ill.	Rose Spots?	1 c.c.blood in bouillon c.c.	Result.	Result of culture from faeces.
23.	P.J.	29	15	+	100	-	-
24.	D.R.	18	15	-	100	+	-
25.	T.K.	20	16	+	100	-	+
26.	J.O.	12	16	-	100	-	-
27.	T.H.	18	16	+	100	-	-
28.	C.M.	27	17	-	150	-	
29.	M.K.	47	17	+	100	+	
30.	T.J.	42	17	+	100	-	-
31.	R.S.	21	17	+	100	-	-
32.	E.H.	17	19	+	100	+	
33.	T.W.	35	19	+	220	+	
34.	H.O.	38	19	+	250	+	
35.	P.M.	37	21	+	100	-	
36.	M.J.	37	21	-	100	-	-
37.	M.I.	20	22	+	100	+	
38.	C.D.	35	22	-	100	-	-
39.	L.P.*	25	22	+	100	-	-
40.	J.B.	19	22	+	100	-	-
41.	W.M.	40	28	+	100	+	
42.	F.A.	16	28	-	100	-	-
43.	H.J.	35	29	+	200	-	
44.	W.C.	19	29	+	100	-	

* 2nd culture.

TABLE I.

Cultures from blood 1st Series (Contd)

	Name	age	Days ill.	Rose Spots?	1 c.c.blood in bouillon c.c.	Result.	Result of culture from faeces.
45.	J.S.	28	29	-	100	+	-
46.	W.M.	40	31	+	200	+	
1.	J.B.	19	4th day of relapse	-	100	-	-
2.	M.S.	20	15th day of relapse	-	150	-	-

Summary of Results.

Week	Number +	Number -
1st	0	0
2nd	6	11
3rd	5	13
4th	2	4
5th	2	2

				Number of cases.
Blood	+	Faeces	+	2
Blood	+	Faeces	-	5
Blood	-	Faeces	+	4
Blood	-	Faeces	-	18

TABLE I.

Cultures from blood 2nd Series (in bile)

	Name	Age	Days ill.	Rose Spots.	Blood in 10 c.c. bile.	Result.
1.	J.H.	23	3	-	5 c.c.	+
2.	N.P.	22	3	-	5 c.c.	+
3.	G.A.	17	7	+	5 c.c.	+
4.	J.O.	28	7	+	5 c.c.	+
5.	M.B.	57	7	+	5 c.c.	+
6.	A.B.	24	8	+	5 c.c.	+
7.	F.W.	29	13	+	5 c.c.	+
8.	M.K.	38	14	+	5 c.c.	+
9.	D.O.	23	17	+	6 c.c.	-
10.	T.Y.	20	21	-	3 c.c.	-
11.	R.K.	36	22	+	5 c.c.	+
12.	B.O.	35	25	+	5 c.c.	+
13.	K.E.	32	29	-	5 c.c.	-
14.	A.L.	24	Convalescent	-	5 c.c.	-
15.	S.N.	30	Convalescent	-	6 c.c.	-
1.	M.B.	57	6th day of relapse.	-	5 c.c.	+

Cultures from blood 2nd Series (in bile)

Summary of results.

Week	Number +	Number -
1st	5	0
2nd	3	0
3rd	0	2
4th	2	0
5th & after	0	3

Summary of results (both series)

Week	Number +	Number -	Percentage.+
1st	5	0	100%
2nd	9	11	45%
3rd	5	15	25%
4th	4	4	50%
5th & after	2	5	28%
	25	35	

TABLE II.

Cultures from faeces.

	Name.	Age.	Days ill.	Result.	Result of culture from blood.
1.	M.K.	3	6	-	
2.	J.T.	33	8	+	+
3.	G.B.	6	8	-	-
4.	A.B.	24	8	-	+
5.	I.E.	21	8	+	
6.	M.E.	23	8	+	
7.	F.M.	23	8	+	
8.	F.J.	10	9	-	+
9.	W.P.	23	9	-	-
10.	J.L.	36	9	-	-
11.	S.T.	6	9	-	
12.	E.C.	11	9	+	
13.	J.F.	11	9	+	+
14.	I.M.	9	9	+	
15.	J.P.	24	10	-	-
16.	J.M.	31	10	+	-
17.	C.T.	4	10	+	
18.	T.H.	28	11	-	
19.	T.M.	3	11	-	
20.	E.M.	18	11	+	
21.	S.M.	13	12	+	
22.	S.J.	7	12	+	
23.	L.P.	25	13	-	-

TABLE II.

Cultures from Faeces (Contd)

Name.	Age.	Days ill.	Result.	Result of culture from blood.
24. E.G.	20	13	-	-
25. F.B.	30	13	+	-
26. M.G.	28	13	+	
27. G.I.	40	13	-	
28. F.A.	25	14	-	
29. M.A.	20	14	-	
30. W.B.	35	14	-	+
31. C.W.	23	14	+	+
32. D.M.	20	15	-	+
33. A.W.	53	15	+	-
34. J.R.	15	15	-	-
35. A.R.	52	15	-	-
36. P.J.	29	15	-	+
37. D.R.	18	15	-	+
38. J.O.	12	16	-	-
39. T.H.	18	16	-	-
40. T.K.	20	16	+	-
41. T.J.	42	17	-	-
42. R.S.	21	17	-	-
43. K.C.	20	16	-	
44. A.T.	22	20	-	
45. M.D.	27	21	+	
46. M.H.	35	21	-	

TABLE II.

Cultures from faeces (Contd)

	Name.	Age.	Days ill.	Result.	Result of culture from blood.
47.	M.J.	37	21	-	-
48.	P.M.	37	21	-	-
49.	C.D.	35	22	-	-
50.	L.P. ⊠	25	22	-	-
51.	J.B.	19	22	-	-
52.	M.M.	37	27	+	
53.	F.A.	16	28	-	-
54.	W.C.	19	29	-	-
55.	J.G.	28	29	-	+
56.	E.L.	30	35	-	
1.	J.B.	19	4th of relapse	-	-
2.	E.H.	20	10th " "	-	
3.	M.S.	20	15th " "	-	

⊠ 2nd culture.

Summary of results.

Week.	Number +	Number -	Percentage +
1st	0	1	0%
2nd	15	15	50%
3rd	3	14	17%
4th	1	4	20%
5th	0	3	0%

TABLE III.

Cultures from Urine (cases of bacilluria)

Name	Age	Days ill	Days from apyrexia.	Result.	Pus?	Albumin?	Diazo.	Bacilli grown also from.
1. M.B.	57	10	17	+	-	+	+	blood.
2. J.L.	36	15	8	+	-	+	-	
3. H.M.	16	17	5	+	-	-	+	
4. J.M.	31	18	9	+	-	-	-	faeces.
5. M.J.	37	21	12 before death	+	+	-	-	
6. E.B.	20	23	19	+	-	-	-	blood
7. C.W.	23	25	6	+	-	-	-	blood
8. W.P.	23	26	4	+	-	-	-	
9. B.O.	35	26	11 before death	+	+	+	-	blood
10. W.B.*	35	28	0	+	-	-	-	blood
11. R.S.*	21	30	3	+	-	-	-	
12. L.P.	25	55	0	+	-	-	-	
13. P.M.*	37	5th	of normal tem.	+	-	-	-	
14. A.R.*	52	6th	" " "	+	-	-	-	
15. T.B.	24	10th	" " "	+	+	+	-	
16. C.D.*	35	10th	" " "	+	-	-	-	
17. W.C.	19	16th	" " "	+	-	-	-	

* Not B. typhosus.

SECTION II.

Agglutination reactions carried out with the sera of patients and stock bacilli, typhoid and paratyphoid.

A series of agglutination reactions was carried out with the sera of 51 patients, and 5 stock bacilli - a typhoid strain, and 4 paratyphoid strains. Where a bacillus was isolated from the patient, this also was tested with his serum along with the stock bacilli. The microscopic method was used for the estimation of the agglutination tests, which were performed as follows :-

Blood was taken from the ear or finger in the usual way in capillary tubes. These were allowed to stand for a little to permit the fibrin to separate, and were then centrifugalized. The serum was drawn directly from the capillary tubes into a 1/10 c.c. measuring pipette and was the diluted to 1:12½ with normal saline solution in a watch-glass. Further dilutions were made by putting .05 c.c. of saline solution in each of a row of hang-drop slides or watch-glasses, adding .05 c.c. of the 1:12½ dilution of serum to the first glass, mixing, adding .05 c.c. from the first glass to the second glass, and so on. Thus a series of dilutions in geometric progression was obtained, beginning at 1:12½ and going up to a dilution as high as was necessary to reach the limit of agglutination: thus

1:12½, 1:25, 1:50, 1:100, 1:200 .....

The bacilli were used in an 18-24 hours' bouillon culture. A platinum loopful of the serum dilution was put on a cover-glass, and to it was added a loopful of the bouillon culture. Thus all the serum dilutions were doubled.

Dilution of serum.	Dilution after addition of bouillon culture.
1 : 12½	1 : 25
1 : 25	1 : 50
1 : 50	1 : 100
1 : 100	1 : 200
1 : 200	1 : 400
.....	.....

Hanging drop preparations were made in the usual way by fixing the cover-glasses on hollow slides with vaseline. These were kept at room temperature for an hour, and were then examined microscopically. It was found that unless the laboratory was very cold, agglutination reached its maximum within this time. A figure was then put down to represent the amount of clumping present, and the results were tabulated.

The dilutions obtained by mixing the diluted serum with bacillary emulsion by means of loops are perhaps not always strictly accurate, but probably the error is not more than 1/10 of the amount stated. The only method which

gives quite accurate dilutions - to measure also the amounts of bouillon culture used and mix it with the diluted serum - was found to occupy too much time when large numbers of hanging drops (e.g. 150) had to be examined microscopically. The tests shown on pages 50-51 however, make it clear that the results are accurate, and comparable with one another.

The method of using bouillon cultures of the bacilli instead of emulsions of agar cultures in normal culture solution had with us in the hospital always given satisfactory results.

The following were the bacilli used for the tests :-

(1) B. Typhosus, a stock strain used for about 5 years in the Glasgow Fever Hospitals for Widal reactions, and obtained originally from the spleen of a patient who died of enteric fever. This had proved a trustworthy organism, and the results of agglutination tests carried out over a course of years corresponded closely with the clinical features of the cases in which it was used; that is to say, it was agglutinated well with typhoid serum, and was unaffected by the sera of non-typhoid patients.

(2) B. paratyphosus A (Brion-Kayser), which I received along with (3) and (5) from the Glasgow Corporation Public Health Laboratory. These 3 strains had been obtained from Kral some time previously by Dr. R.M. Buchanan, City Bacteriologist.

(3) B. paratyphosus A. (Schottmüller)

(4) B. paratyphosus B. (Schottmüller), obtained from Leeds, and brought originally from Vienna by Professor Grünbaum.

(5) B. paratyphosus B. (Achard)

All these organisms were actively motile and were not agglutinated spontaneously, or on the addition of normal saline solution, or by the serum of a healthy person.

The limit of agglutination was regarded as the dilution in which the largest clumps present contained 2-4 bacilli. A trace of agglutination, as shown by a tendency of the bacilli to adhere in pairs, was reckoned as not agglutination.

In many cases with active sera larger clumps than those mentioned occurred in the highest dilution in which agglutination was present, and so the limiting dilution was calculated as lying between this dilution and that next above it, in proportion to the amount of agglutination present. The degree of agglutination was originally estimated as a fraction of 10 and this made the calculation easier. For instance, if at 1:800 the agglutination was reckoned as $\frac{2}{10}$, and at 1:1600, it was absent, 1:1200 was considered to be the limit of agglutination. In the tables, however, a more graphic and sufficiently accurate method has been

substituted for the figures.

The serum dilution at which complete or practically complete agglutination occurred varied, and bore no constant relation to the limiting dilution.

General remarks.

Widal reactions were carried out with the sera of 51 patients and the 5 bacilli mentioned. In the majority of these cases more than one estimation was made, and in 7 to whom an autogenous vaccine was given, the serum was tested several times both with the bacillus injected, and with the stock typhoid bacillus. In addition,37 bacilli, isolated from patients, were tested with the respective sera, but a description of these experiments is deferred to Section IV.

In one case only, that of a nurse, did the patient come under observation before agglutinins were present in the blood. On the 3rd day, in a dilution of 1:25, her serum agglutinated none of the stock bacilli, but her illness was diagnosed as enteric fever from a positive blood culture. By the 7th day agglutinins had appeared, and early in the 2nd week the clinical signs of enteric were well marked. In the 50 other cases sufficient agglutination was found on the day on which the blood was first examined to make the diagnosis certain. In every case but one, group

agglutination was present, that is to say, one or more of the paratyphoid bacilli were agglutinated at 1:25, or in higher dilution, as well as B. typhosus. The exception occurred in the case of a girl of 10 (B.E.) who was admitted to hospital after 16 days of an illness characterised by headache, abdominal pain, diarrhoea, and slight vomiting. She was evidently recovering, for her temperature was normal, and nothing abnormal could be made out by physical examination. Her serum, however, on the 17th day agglutinated B. paratyphosus B (Achard) to 1:6,400, while the other bacilli, including B. typhosus, were unaffected by the serum at 1:25. 10 days later the Achard B bacillus was agglutinated to 1 in 200, while the others were unaffected as before.

In 3 other cases a paratyphoid bacillus (Brion-Kayser A) was agglutinated at a higher dilution than B. typhosus; in the remainder, the typhoid agglutination was predominant.

With a view to prevent relapse an autogenous vaccine was given in 7 cases. These injections, however, seemed to have little effect on the agglutinating power of the serum.

Agglutination of B. typhosus.

This occurred in a regular manner. There was complete agglutination in the lower dilutions, and a gradual

falling off in higher dilutions until the limit of agglutination was reached.

The most active sera tested agglutinated to -

1 : 30,000 (F.B. 29th day)

1 : 25,000 (W.P. 28th day)

1 : 20,000 (D.R. 20th day)

The first patient had an attack of moderate severity, the second a severe attack, and the third died from toxaemia and heart failure. On the whole, the severe cases showed the most active sera, and of the 51 patients, 8 who died exhibited an agglutination limit of -

1:20,000, 1:15,000, 1:13,000, 1:12,000, 1:6000, 1:5000, and 1:400.

It may be said in general that the limit of agglutination tended to fall as convalescence advanced, and where it was estimated on the patient's dismissal from hospital, was found commonly to be considerably lower than the recorded maximum. The most striking example of this occurred with the serum of W.P., which on the 28th day agglutinated to 1:25,000, and at the end of 6 weeks' convalescence only to 1:100.

The maximum dilutions at which agglutination was present are shown on Table IV (page 26).

TABLE IV.

Agglutination of B. typhosus (stock) by patients' sera.

Highest dilution at which agglutination was present.	Number of cases.
No agglutination	1
- 1:500	6
1:500 - 1:1000	7
1:1000 - 1:2000	11
1:2000 - 1:3000	8
1:3000 - 1:4000	1
1:4000 - 1:5000	1
1:5000 - 1:6000	2
1:6000 - 1:7000	2
1:7000 - 1:8000	2
1:8000 - 1:9000	1
1:9000 - 1:10,000	1
1:10,000 - 1:12,000	2
1:12,000 - 1:14,000	2
1:14,000 - 1:16,000	1
1:16,000 - 1:18,000	0
1:18,000 - 1:20,000	1
1:20,000 - 1:25,000	1
1:25,000 - 1:30,000	1
Total :-	51

Agglutination of B. paratyphosus A (Brion-Kayser)

The tests carried out showed this to be an unreliable organism for agglutination purposes. It was not agglutinated spontaneously or with normal serum, but frequently agglutination was slight in low dilutions and increased when higher dilutions were used. This anomalous reaction did not occur at all with B. typhosus, and only once with one of the other paratyphoid organisms. In such cases the upper limit was noted, irrespective of the behaviour of the bacillus in lower dilutions.

Agglutination occurred with this organism in much higher dilutions than with any of the other paratyphoid organisms, the highest being 1:14,000, with agglutination complete at 1:3,000. B. typhosus was grown from the urine of this patient. One serum only failed to agglutinate the organism at 1:25.

An outstanding fact with regard to the agglutinative properties of this bacillus was that in 11 out of 13 cases in which the serum was examined towards the end of the patient's stay in hospital, the bacillus was agglutinated in higher dilutions than at an earlier stage in the illness.

TABLE V.

Agglutination with B. paratyphosus A (Brion-Kayser) at 2 stages of disease.

	Name.	Day.	Limit	Day.	Limit.
1.	J.T.	8th	1:200	42nd of normal temp.	1:1600
2.	S.M.	13th	1: 50	" " " "	1:4000
3.	D.M.	14th	1: 50	39th " " "	1: 400
4.	A.W.	15th	no agglutination	42nd " " "	1: 50
5.	F.B.	17th	1:400	" " " "	1:3500
6.	P.M.	21st	1:400	37th " " "	1: 700
7.	J.B.	22nd	no agglutination	40th " "	1:1000
8.	J.F.	23rd	1:120	42nd " " "	1: 25
9.	C.M.	24th	1: 40	" " " "	1: 25
10.	W.C.	28th	1: 25	34th " " "	1:1600
11.	W.P.	28th	1:500	42nd " " "	1:3000
12.	G.U.	30th	no agglutination	20th " "	1: 25
13.	E.B.	9th of normal temp.	1:100	42nd " "	1:1600

TABLE VI.

Agglutination of B. paratyphosus A (Brion-Kayser) by patients' sera

Highest dilution at which agglutination was present.	Number of cases.
No agglutination	1
- 1:100	10
1:100 - 1:200	6
1 1:200 - 1:300	3
1:300 - 1:400	3
1:400 - 1:500	0
1:500 - 1:600	2
1:600 - 1:700	1
1:700 - 1:800	4
1:800 - 1:900	0
1:900 - 1:1000	2
.....	.....
1:1400 - 1:1500	1
1:1500 - 1:1600	6
.....	.....
1:1700 - 1:1800	2
.....	.....
1:1900 - 1:2000	1
.....	.....
1:2500 - 1:3000	2
1:3000 - 1:3500	2

TABLE VI (Contd)

Agglutination of B. paratyphosus A (Brion-Kayser) by patients' sera.

Highest dilution at which agglutination was present.	Number of cases
1:3500 - 1:4000	2
.....	.....
1:5000 - 1:6000	2
1:6000 - 1:7000	1
Total :-	51

Agglutination of B. paratyphosus A (Schottmüller)

With this organism the agglutination limit was commonly considered lower than with Brion-Kayser A, nor did it tend to rise as convalescence advanced. The highest dilution at which agglutination was found was 1:1000, but no other occurred above 1:500, and many were under 1:100. In 2 cases there was no agglutination.

TABLE VII.

Agglutination of B. paratyphosus A (Schottmüller) by patients' sera.

Highest dilution at which agglutination was present.	Number of cases.
No agglutination.	2
- 1:100	24
1:100 - 1:200	15
1:200 - 1:300	4
1:300 - 1:400	4
1:400 - 1:500	1
1:500 - 1:600	0
1:600 - 1:700	0
1:700 - 1:800	0
1:800 - 1:900	0
1:900 - 1:1000	1
Total :-	51

Agglutination of B. paratyphosus B (Schottmüller)

The agglutination of this organism resembled that of Schottmüller A and of Achard B. The highest dilution in which agglutination occurred was 1:1000, in the same case as with the preceding organism, but it failed to be agglutinated in a larger proportion of cases (8).

TABLE VIII.

Agglutination of B. paratyphosus B (Schottmüller) by patients' sera.

Highest dilution at which agglutination was present.	Number of cases.
No agglutination	8
- 1:100	17
1:100 - 1:200	9
1:200 - 1:300	6
1:300 - 1:400	7
1:400 - 1:500	2
1:500 - 1:600	0
1:600 - 1:700	0
1:700 - 1:800	1
1:800 - 1:900	0
1:900 - 1:1000	1
Total :-	51

Agglutination of B. paratyphosus B (Achard)

As has been mentioned, one serum agglutinated this organism in a dilution of 1:6400, while not affecting the other bacilli at 1:25. Apart from this, the highest dilution at which agglutionation occurred was 1:1600. Agglutination shewed a tendency to fall off more quickly as higher dilutions were reached than with any of the other bacilli. In one case, agglutination was complete at 1:50, and quite absent at 1:100, and the same result was obtained in 3 successive tests. This bacillus was acted on by every serum employed.

TABLE IX.

Agglutination of B. paratyphosus B (Achard) by patients' sera.

Highest dilution at which agglutination was present.	Number of cases.
- 1:100	17
1:100 - 1:200	17
1:200 - 1:300	2
1:300 - 1:400	7
1:400 - 1:500	2
1:500 - 1:600	0
1:600 - 1:700	0
1:700 - 1:800	3
1:800 - 1:900	0
1:900 - 1:1000	1
.....	.....
1:1500 - 1:1600	1
.....	.....
- 1:6400	1
Total : -	51

Agglutination by the serum of a person vaccinated with B. typhosus.

A note may be added here on the difference found between the serum of a person artificially inoculated with dead typhoid bacilli, and that of a patient suffering from enteric fever. The strain use by the R.A.M.C. for their prophylactic inoculation (B.typhosus (Rawlings)) was obtained from the R.A.M. College and a vaccine was made to inoculate a member of the hospital staff. He was given 2 subcutaneous injections (1) of 500,000,000 dead bacilli, grown for 36 hours in bouillon and killed at 53°C. (2) 10 days later, 1,000,000,000 bacilli similarly prepared.

It was found that this serum, when in its most active condition 10 days after the second injection, agglutinated the stock typhoid bacillus in a much higher dilution than the serum of any of the enteric patients tested. The most powerful enteric fever serum had an agglutinative limit of 1:30,000, while the serum of the inoculated person agglutinated practically completely at 1:25000, and had its limit at 1:200,000. In addition to this, it was more specific than any of the enteric sera, for at 1:25 it agglutinated none of the paratyphoid bacilli. When tested four months later, however, the agglutinative power of B. typhosus had fallen off greatly (to 1:1600), and there was now slight agglutination with 3 of the paratyphoid bacilli, - with

Brion-Kayser A to 1:50, and with Schottmüller A and Achard B to 1:25. 11 months after the inoculation the condition was practically the same as at the end of 4 months.

In this case the Widal reactions were carried out with the stock typhoid bacillus, and not with the bacillus used for the inoculation. The latter organism was clumped to some extent spontaneously and was unsuitable for agglutinative experiments.

It is known that if an animal is inoculated repeatedly with typhoid bacilli at the usual intervals of 10 days, group agglutinins are produced to a greater and greater extent; that is to say, agglutination of paratyphoid bacilli occurs in an increasing degree. This suggests that the difference between this artificial serum, which did not agglutinate the paratyphoid bacilli, and the serum of a typhoid patient, which commonly does, even early in the disease, may be due to the fact that in one case agglutinins were called forth by 2 definite injections of bacilli, whereas in the disease, a continual immunization is going on.

TABLE X.

Agglutination of B. Typhosus by serum of person inoculated with this organism.

SUMMARY OF RESULTS.

May 17. No agglutination.

" 22. 500,000,000 bacilli injected subcutaneously.
(36 hours' growth in bouillon, killed at 53°C)

" 25. No agglutination.

" 28. Limit of agglutination 1 : 50

June 1. " " " 1 : 25,000

" 2. 1,000,000,000 bacilli injected subcutaneously.

" 3. Limit of agglutination 1 : 100,000

" 5. " " " 1 : 50,000

" 8. " " " 1 : 200,000

" 12. " " " 1 : 100,000

(No agglutination with paratyphoid strains)

Oct.24 Limit of agglutination with B.typhosus 1 : 1600

" " " " Brion-Kayser A.1 : 50

" " " " Schottmüller A.1 : 25

" " " " Achard B. 1 : 25

No agglutination with Schottmüller B.

(for full table, see Appendix A)

TABLE XI.

Summary of results of Widal reactions.

Explanation of symbols in the table.

N 4	means 4th day of normal temperature.
R 4	means 4th day of relapse.
$R^2 9$	means 9th day of second relapse.
$\frac{400}{3000}$	means that complete agglutination occurred in a dilution of 1:400 : and the limit of agglutination in a dilution of 1:3000
$\frac{-}{50}$	means that agglutination was not complete at 1:25 and that the limit of agglutination occurred at 1:50
$\frac{?}{3200}$	means that the limit occurred at 1:3200, but that the dilution at which complete agglutination occurred was not investigated.
0	means that there was no agglutination in a dilution of 1:25.

(For full table see Appendix B)

TABLE XI.

Name. Age.	Days ill.	Bac. from	Bac. from.	B. typhosus.	B. paratyphosus. Br-Ka. A	Schott. A	Schott. B	Ach. B
1. J.H. 23	4		Blood	0	0	0	0	0
	7		- / 200	25 / 100	100 / 1500	50 / 400	50 / 350	50 / 200
	8		- / 100	25 / 175	100 / 800	30 / 175	75 / 200	30 / 100
	9			30 / 175				
	10			50 / 180				
	11			50 / 400				
	12			50 / 375				
2. J.T. 41	8		Blood	200 / 3500	50 / 200	25 / 200	- / 50	50 / 175
	23		25 / 200	50 / 300	200 / 1500	25 / 75	25 / 100	50 / 350
	26		? / 400	? / 3000	(2 days after vaccine)			
	28(N1)		? / 800	? / 12000				
	N.42		- / 50	200 / 800	100 / 1600	- / 40	0	50 / 75
3. H.M. 16	10		Urine	50 / 800	- / 1600	- / 25	0	- / 300
	R.21(N.0)		100 / 6000	800 / 8000	- / 1000	- / 100	- / 150	100 / 350

TABLE XI (Contd)

Name. Age.	Days ill	Bac. from	Bac. from	B.typhosus	B. paratyphosus. Br-Ka. A	Schott. A	Schott. B	Ach. B
4. M.D. 27	12		Faeces 0	400/1500	100/800	0	-/50	50/200
5. I.E. 21	13			1600/5000	-/250	-/25	-/400	50/400
6. S.M. 13	13		Faeces	400/3000	-/50	-/75	-/200	-/100
	25		100/400	100/600	-/25	0	-/75	-/150
	N.42		30/400	200/600	400/4000	-/75	-/100	-/50
7. E.M. 18	14		Faeces 1600/6400	1600/7000	100/1600	-/200	25/400	100/500
8. D.M. 20	14		Blood	200/?	-/50	0	0	200/?
	28		100/4000	1600/6400	-/100			400/1600
	36		?/1600	?/3200				
	41 (N.4)		?/400	?/7000	(Vaccine on 36th day)			
	N.7		?/400	?/2000				
	N.8		?/400	?/2000	(Vaccine on N.7)			
	N.10		?/800	?/1500				

TABLE XII. (Contd)

Name. Age.	Days ill	Bac. from	Bac. from	B. typhosus	B. paratyphosus. Br-Ka. A	Schott. A	Schott. B	Ach. B
8. D.M. (Contd)	N.12		?/400	1000/8000	?/800	?/300	?/400	2/700
	N.15		?/400	1/1600				
	N.19		200/1000	400/1400				
	N.39		50/400	400/3000	-/400	-/200	-/300	50/100
9. A.B. 24	14		Blood 800/12000	1600/12000	50/1800	25/200	30/200	100/500
10. A.W. 53	15			50/800	0	-/50	0	50/200
	23(N.2)		Faeces	?/400				?/200
	42 N		-/250	50/250	-/50	-/100	-/100	50/200
11. J.R. 15	14			100/3000	-/200	25/120	-/180	-/100
12. E.C. 11	15		Faeces 100/1600	200/1600	200/3500	-/400	-/100	50/120
13. M.F. 25	15		Spleen -/800	25/400	-/600	-/100	-/30	-/50

TABLE XI (Contd)

Name	Age	Days ill	Bac. from	Bac. from	B.typhosus.	B. paratyphosus. Br-Ka. A	Schott. A	Schott. B	Ach B
14. W.A.	8	15			100/1200	-/50	0	0	-/50
		23		B.typhosus(A.W)	?/800	?/100			
		46(N.20)		100/800	100/1000	-/100	-/100	-/800	25/120
		N.21		?/800	?/800	(Vaccine on No.20)			
		N.23		400/800	400/1000				
		N.25		?/1000	400/1000				
		N.29		?/400	?/1600	(Vaccine on N.27)			
		N.32		?/400	?/800				
		N.38		?/600	?/1600				
		N.40		?/400	?/1000	(Vaccine on N.38)			
		N.42		?/400	?/1600				
		N.68		100/800	200/3200	-/25	50/1000	100/1000	25/180
15. L.P.	25	15		Urine			?/100		100/?
		56(N.1)		-/25	25/1600	-/25	0	-/100	25/120
		N.18			?/400	0	?/100	?/100	?/400

TABLE XI. (Contd)

Name Age	Days ill	Bac. from	Bac. from	B.typhosus	B.paratyphosus. Br.Ka. A	Schott. A	Schott. B	Ach B
16. A.R. 52	16		Urine*	25/200	50/800	-/50	0	-/25
	N.42		-/50	100/800	100/1000	-/25	-/50	-/70
17. T.K. 20	16	R-spot	Faeces	800/3500	-/25	-/70	0	-/25
	24	800/10000	25/800	800/9000				
	27	?/9000	?/800	?/4000	(Vaccine on 25th day)			
	29	?/6000	?/3000	1600/4000				
	35	?/9000	?/1200	?/9000				
	37(N.0)	?/6000	?/2000	?/9000	(Vaccine on 35th day)			
	N.2	?/6000	?/800	?/6000				
	N.42	200/1000	25/200	50/250	-/25	0	0	0
18. B.E. 10	17(N.0)			0	0	0	0	200/6400
	N.10			0	0	0	0	100/1200
19. J.L. 36	17		Urine 200/3000	400/1600	-/25	0	0	-/30
	26(N.3)		-/400	-/900				

* Not B. typhosus.

TABLE XI. (Contd)

Name	Age	Days ill	Bac. from.	Bac. from	B.typhosus	B. paratyphosus. Br-Ka. A	Schott. A	Schott. B	Ach B
19.J.L. (Contd)	36	28(R.2)		- / 800	- / 1000	(Vaccine on R.1)			
		R.3		- / 800	- / 800				
		R.4		- / 600	- / 700				
		R.5		- / 600	- / 600				
		R.6		- / 350	- / 700	(Vaccine on R.5)			
		R.7		- / 200	- / 400				
		R.8		- / 200	- / 400				
		R.9		- / 200	- / 350				
20. F.B.	30	17		Faeces 200 / 12000	6000 / 20000	- / 400	- / 200	- / 200	25 / 50
		29(N.0)		? / 25000	? / 30000				
		N.1		? / 20000	? / 12000	(Vaccine on N.0)			
		N.2		? / 15000	? / 12000				
		N.4		? / 30000	? / 25000				
		N.5		? / 10000	? / 12000	(Vaccine on N.4)			
		N.6		? / 12000	? / 10000				
		N.7		? / 15000	? / 35000				

TABLE XI (Contd)

Name	Age	Days ill	Bac. from	Bac. from	B.typhosus	B. paratyphosus. Br.Ka. A	Schott. A	Schott. B	Ach B
20. F.B. (Contd)		N.8		? / 15000	? / 10000				
		N.9		? / 5000	? / 5000	(Vaccineon N.8)			
		N.10		? / 12000	? / 4000				
		N.11		? / 10000	? / 5000				
		N.42		400 / 3000	800 / 12000	- / 3500	- / 80	- / 60	25 / 60
21. I.M.	9	17(N.0)	Faeces.	- / 1600	400 / 1400	- / 800	50 / 250	25 / 250	50 / 400
22. C.T.	4	18	Faeces	100 / 1300	200 / 1100	- / 800	- / 70	- / 40	25 / 80
23. S.T.	6	19			200 / 1600	50 / 1600	- / 30	0	- / 40
24. E.H.	17	19	Blood		800 / ?	50 / ?		0	
		N.42		50 / 1600	800 / 2000	25 / 250	- / 100	- / 250	25 / 200
25. M.G.	28	19	Faeces 400 / 6000		6000 / 13000	100 / 6000	25 / 200	25 / 120	100 / 200
26. J.M.	31	20	Urine 200 / 800		200 / 700	- / 150	- / 100	- / 80	25 / 80

TABLE XI (Contd)

Name	Age	Days ill	Bac. from	Bac. from blood	B.typhosus.	B. paratyphosus. Br-Ka. A	Schott. A	Schott. B	Ach B
27. D.R.	18	20		800/18000	3000/20000	200/2000	50/200	50/400	50/180
28. M.E.	23	20(N.O)		Faeces 25/4000	1600/10000	-/600	25/400	25/400	50/250
29. A.T.	22	21			400/2500	-/150	-/50	-/80	50/220
30. P.M.	37	21		Urine ■	-/60	50/400	0	0	25/50
		N.37		-/200	200/1200	200/700	25/120	-/250	-/60
31. F.M.	23	21		Faeces 100/1600	200/3000	-/400	-/40	0	-/100
32. J.B.	19	22			-/250	0	-/1000	-/50	-/150
		N.40			50/600	-/1000	-/40	-/100	50/200
33. J.F.	10	23		Blood ?/800	?/800	-/120	0	0	?/800
		N.42		200/1000	200/1000	-/25	-/25	-/30	25/100
34. R.R.	23	24		Blood			?/100		?/100
		N.42		-/100	-/150	-/400	0	0	0

■ Not B. typhosus.

TABLE XI. (Contd)

Name	Age	Days ill	Bac. from	Bac. from	B.typhosus	B. paratyphosus. Br-Ka. A	Schott. A	Schott. B	Ach. B
35. C.M.	27	24			- / 40	- / 40	0		50 / ?
		N.42			- / 100	- / 25	- / 25	0	50 / 50
36. T.H.	18	24			800 / 6000	- / 3000	- / 200	- / 800	200 / 1000
37. M.H.	35	25			200 / 3000	- / 40	- / 40	- / 50	- / 50
38. C.W.	23	27	Blood - / 800	Urine - / 3000	800 / 6000	50 / 200	50 / 400	50 / 200	50 / 150
39. W.C.	19	28		Urine*	100 / 500	- / 25	- / 100	25 / 200	25 / 90
		N.34		- / 70	100 / 500	100 / 1600	- / 300	- / 500	50 / 150
40. W.P.	23	28		Urine 800 / 12000	7000 / 25000	25 / 500	25 / 500	50 / 500	50 / 800
		N.42		- / 120	25 / 100	400 / 3000	- / 120	- / 120	- / 60
41. J.G.	28	29		Blood 1600 / 15000	1600 / 15000	1600 / 6000	25 / 180	50 / 180	100 / 800
42. G.A.	11	30			- / 50	0	50 / ?	- / 50	50 / ?
		N.20			100 / 1600	- / 25	- / 160	50 / 250	25 / 200

* Not B. typhosus.

TABLE XI. (Contd)

Name	Age	Days ill.	Bac. from Blood	Bac. from Urine*	B.typhosus	B. paratyphosus. Br-Ka A	Schott. A	Schott. B	Ach B
43. W.B.	35	30	200/3500	100/6000	200/3000	-/250	-/40	-/250	50/120
44. M.M.	37	30		Faeces 50/3500	1600/14000	200/4000	-/25	-/60	-/50
45. M.J.	37	32		Urine -/80	50/3000	3000/14000	-/200	-/400	100/400
46. R.S.	21	32		Urine* 200/600	800/1800	50/200	-/300	-/300	25/180
47. E.H.	20	101(R^{29})			200/900	-/400	-/50	-/50	25/120
48. J.F.	11	N.5		Faeces 100/200	100/180	100/100	-/110	-/110	50/120
49. T.B.	24	N.24		Urine 800/3000	400/3000	400/1800	25/200	50/100	-/60
50. M.K.	47	N.42		Blood 200/900	200/800	-/100	-/30	0	-/25
51. E.B.	20	N.4		Blood ?/400	800/7000				?/1000
		N.9			?/1600	?/100			?/200
		N.42		800/1800	800/2000	50/1600	-/60	-/50	25/400

* Not B. typhosus.

SECTION III.

Agglutination experiments on bacilli with artificial antisera.

Agglutination experiments on the majority of the bacilli isolated were carried out by means of antisera to determine

(1) whether the time of isolation exercised any influence on the power of the bacilli to undergo agglutination:

(2) whether bacilli obtained from different body substances, such as blood, faeces, and urine, or from different parts of the body, reacted differently:

(3) whether a bacillus was acted on in the same way as the stock typhoid bacillus by the serum of the patient from whom it was isolated, and the antityphoid serum:

(4) whether any facts could be ascertained with a bearing on non-agglutinability of bacilli.

Antisera were prepared from the stock typhoid bacillus and the 4 paratyphoid bacilli used for the Widal reactions, according to the following method :-

A 5 c.c. tube of bouillon was inoculated with the bacillus to be injected and incubated for 24 hours. It was then sterilized by heating to 53°C. for an hour, and 1 c.c. of this bouillon culture was injected into the peritoneal cavity of a rabbit. 10 days later, 2 c.c. of a similar culture was given in the same way, and after a further interval of 10 days, 4 c.c. The rabbit was killed and its blood withdrawn 10 days after the 3rd injection. The blood was allowed to stand over-night, and then the serum which had separated was drawn up into sterilized quill tubes. The tubes were sealed and were immersed in a water bath at 55°C for 30 minutes as an additional safe-guard against contamination.

All 5 sera were obtained and treated in precisely the same manner, and 3 of the rabbits used (for B.typhosus, Brion-Kayser A, and Achard B) belonged to the same litter.

With a view to eliminate any inhibitory action which the isolating medium might exercise on the agglutin-ability of the bacilli, each strain was sub-cultured 6 times on agar, and the bacilli examined were those of the 6th sub-culture. The following experiment showed that this procedure did not lower the power of a bacillus to undergo agglutination when this was good.

Two strains were tested, one isolated from blood in bouillon, and the other from faeces on a modified Endo plate.

1. B. typhosus (W.M.) isolated from blood in bouillon and transferred to agar.
   (a) first culture on agar.
   (b) 6th sub-culture on agar.

2. B. typhosus (M.G.) isolated from faeces on a modified Endo plate and transferred to agar.
   (a) first culture on agar.
   (b) 6th sub-culture on agar.

A patient's serum was used.

Serum dilution.	B. typhosus (Stock)	W.M. 1st	W.M. 6th	M.G. 1st	M.G. 6th
1 : 25	+ + +	+ + +	+ + +	+ + +	+ + +
1 : 50	+ + +	+ + +	+ + +	+ + +	+ + +
1 :100	+ + +	+ + +	+ + +	+ + +	+ + +
1 :200	+ + +	+ +	+ + +	+ + +	+ + +
1 :400	+ + +	+ +	+ +	+ +	+ +
1 :800	+ +	+ +	+ +	+ +	+ +
1 :1600	+	-	-	+	+
1 :3200	-	-	-	-	-

In the 6th sub-culture no deterioration had occurred in the capacity of the bacilli to undergo agglutination.

The following test showed that growth on the modified Endo medium did not affect agglutinability. The stock

typhoid strain was grown on Endo's medium and a sub-culture from this in bouillon was agglutinated side by side with an ordinary sub-culture in bouillon. A patient's serum was used.

Serum dilution.	B. typhosus (Stock)	Same after growth on Endo's medium.
1 : 25	+ + +	+ + +
1 : 50	+ + +	+ + +
1 : 100	+ + +	+ + +
1 : 200	+ + +	+ + +
1 : 400	+ + +	+ + +
1 : 800	+ +	+ +
1 : 1600	+	+
1 : 3200	-	-

These 2 experiments showed also that the method used for estimating agglutination was to be relied on for giving comparable results.

46 strains of B. typhosus were tested -

From blood	17
" faeces	14
" urine	8
" vesicular rose-spot .	1
" spleen	4
" gall-bladder.....	1
" mesenteric gland.	1
Total :	46

The 46 bacilli were isolated from 42 patients. In 3 instances more than one bacillus was grown. In the first, a bacillus was obtained from blood and from urine, in the second from a rose-spot and from faeces, and in the third from the spleen, from the gall-bladder, and from a mesenteric gland.

In addition, each of the 5 stock bacilli, and also a strain of B.coli from urine were agglutinated with the 5 antisera.

In the study of the results obtained, attention was paid not only to the day of the disease on which a bacillus was isolated, and to its source, but also to the length of time which elapsed between its isolation, and the days on which the tests were made. Certain observers have reported variations in agglutinability dependent on this circumstance, and bacilli found to be non-agglutinable, or only slightly agglutinable on isolation, have sometimes become completly agglutinable after standing for 2 or 3 months.

Agglutination by antityphoid serum.

The limit of agglutination of the stock typhoid bacillus with its own antiserum occurred at 1 : 80,000.

The 46 typhoid bacilli from patients were divided

into 3 classes -

(1) Those agglutinated approximately as well as the stock typhoid bacillus (limiting dilution 1:80,000 - 1:45,000)

(2) Those in which there was moderate agglutination (limiting dilution 1:25,000 - 1:1600)

(3) Those in which agglutination was slight (limiting dilution 1:100 - 1:25), or absent at 1:25.

Class(1)include 22 strains
" (2) " 17 "
" (3) " 7 "

Of the 22 bacilli in class (1), 5 were agglutinated quite as well as the stock typhoid bacillus. One of these was grown from a vesicular rose-spot, 1 from blood, 1 from the spleen, and 2 from faeces. 2 bacilli showed no agglutination at 1:25, one from blood, and the other from the spleen.

In the <u>1st week</u> 1 bacillus was isolated -
Class (3) includes 1

In the <u>2nd week</u> 17 bacilli were isolated -
Class (1) includes 11
" (2) " 4
" (3) " 2

In the 3rd week 19 bacilli were isolated -

Class (1) includes 9
" (2) " 8
" (3) " 2

In the 4th week 6 bacilli were isolated -

Class (1) includes 2
" (2) " 3
" (3) " 1

In the 5th week and after 3 bacilli were isolated -

Class (1) includes 0
" (2) " 2
" (3) " 1

There was thus a distinct tendency for the earlier isolated bacilli to be agglutinated better than those obtained later. The earliest bacillus, however, grown on the 3rd day, was not agglutinable.

From the blood 17 bacilli were grown -

Class (1) includes 6
" (2) " 7
" (3) " 4

From the faeces 14 bacilli were grown -

Class (1) includes 11
" (2) " 3
" (3) " 0

From the urine 8 bacilli were grown -

Class	(1)	includes 2
"	(2)	" 5
"	(3)	" 1

Class (1) includes also 2 bacilli from spleen and the bacillus from a rose-spot.

Class (2) " the bacillus from the gall-bladder, and the bacillus from a mesenteric gland.

Class (3) " 2 bacilli from spleen.

A striking difference was evident between the agglutinability of the bacilli from faeces, and those from blood and urine, the bacilli from faeces being agglutinated much better than those from the two other sources.

The resemblance in respect of agglutinability between the bacilli from blood and those from urine suggests that the former come from the blood into the urine (which is the accepted view), and do not pass directly from the intestine to the bladder, as has been suggested by Blumer[7].

It is worthy of note that in the case where 3 strains were obtained from one individual after death, the bacillus from a mesenteric gland was agglutinated to 1:25,000, that from the gall-bladder to 1:15,000, and that from the spleen not at all.

The length of time which elapsed between the isolation and the testing of the bacilli appeared to have a slight influence on those obtained from blood. The average number of days in this period for the 17 bacilli from blood was

in class (1) (6 bacilli) 139 days.
" " (2) (7 ") 120 "
" " (3) (4 ") 79 "

In the case of the 14 bacilli from faeces the average number of days was

in class (1) (11 bacilli) 61 days
" " (2) (3 ") 70 "

The bacilli in the other groups were too few to give comparable results.

The average time between the isolation and the examination of the bacilli from the blood was 117 days, and of the bacilli from the faeces 63 days. If then agglutinability developed with the lapse of time, the difference in the agglutination reactions of these 2 groups of bacilli must originally have been even greater, for the bacilli from the faeces, which were more agglutinable, had been kept a shorter time.

The average age of all the bacilli in class (1) was 88 days, of those in class (2) 93 days, and of those in class (3) 72 days.

Agglutination by antiparatyphoid A (Brion-Kayser) serum.

This serum was much less active than the others and showed the limit of agglutination for its own bacillus at 1:3000. As has been mentioned the organism gave unreliable results in Widal reactions.

The stock typhoid bacillus was not affected at 1:25.

The highest dilution in which agglutination was present with a patient's bacillus was 1:100. 31 bacilli were unaffected at 1:25.

The 46 typhoid bacilli isolated from patients were divided into 3 classes

(1) those with the limit of agglutination between 1:100 and 1:50

(2) " " " " " " " 1:40 and 1:25

(3) those showing no agglutination at 1:25.

Class (1) includes 1
" (2) " 14
" (3) " 31

In the 1st week

Class (2) includes 1

In the 2nd week

Class (1) includes 1
" (2) " 7
" (3) " 9

In the 3rd week

Class	(1)	includes	0
"	(2)	"	5
"	(3)	"	14

In the 4th week

Class	(1)	includes	0
"	(2)	"	1
"	(3)	"	5

In the 5th week and after

Class	(1)	includes	0
"	(2)	"	0
"	(3)	"	3

Here again there was a tendency for the earlier isolated bacilli to be better agglutinated.

Of 17 bacilli from the blood

Class	(1)	includes	0
"	(2)	"	3
"	(3)	"	14

Of 14 bacilli from the faeces

Class	(1)	includes	1
"	(2)	"	8
"	(3)	"	5

Of 8 bacilli from the urine

Class (1) includes 0

" (2) " 1

" (3) " 7

Class (1) includes also 1 bacillus from spleen and that from a mesenteric gland.

" (2) " 3 bacilli from spleen, that from a rose-spot and the bacillus from the gall-bladder.

As with the antityphoid serum, the bacilli from faeces were better agglutinated than those from blood or from urine, and the bacilli from urine again resembled those from blood rather than those grown from faeces.

The results were independent of the age of the bacilli. The average age of the bacilli in class (1) was 33 days, of those in class (2) 93 days, and of those in class (3) 100 days.

Agglutination with antiparatyphoid A (Schottmüller) serum.

This serum agglutinated its own bacillus in a limiting dilution of 1:200,000.

The stock typhoid bacillus was agglutinated at 1:60.

The highest dilution in which agglutination was present with a patient's bacillus was 1:350. 6 bacilli were

not affected at 1:25.

The 46 bacilli were divided into 3 classes -

(1) those with the limit of agglutination between 1:350 and 1:200

(2) " " " " " " " 1:190 and 1: 50

(3) " " " " " " " 1: 40 and 1: 25

and those showing no agglutination at 1:25.

Class (1) includes 6 bacilli
" (2) " 28 "
" (3) " 12 "

It will be seen from the table that the degree of agglutination was independent of the time of isolation of the bacillus.

Of 17 bacilli from the <u>blood</u>

Class (1) includes 0
" (2) " 9
" (3) " 8

Of 14 bacilli from the <u>faeces</u>

Class (1) includes 3
" (2) " 10
" (3) " 1

Of 8 bacilli from the <u>urine</u>

Class (1) includes 1

Class (2) includes 4

" (3) " 3

Class (1) includes the bacillus from the gall-bladder and that from the mesenteric gland.

" (2) " all 4 bacilli from the spleen and the bacillus from a rose-spot.

With this serum also, the bacilli from faeces were agglutinated considerably better than those from blood.

The time between the isolation and the testing of the bacilli in class (1) was 82 days, of those in class (2) 97 days, and of those in class (3) 133 days. Agglutinability seemed to be diminished rather than increased with increase in the age of the organism.

Agglutination by antiparatyphoid B (Schottmüller) serum.

This serum was the most active obtained, and agglutinated its own bacillus to 1:800,000.

The stock typhoid bacillus was agglutinated to 1:150.

The highest dilution in which agglutination was present with a patient's bacillus was 1:400. 2 bacilli were unaffected at 1:25.

The 46 bacilli were divided into 3 classes

(1) those with the agglutinative limit between 1:400 and 1:200

(2) " " " " " " 1:190 and 1:50

(3) those with the agglutination limit between 1:40 and 1:25

and those not agglutinated at 1:25

Class (1) includes 8 bacilli
" (2) " 32 "
" (3) " 6 "

Of 37 bacilli isolated in the first 3 weeks

Class (1) includes 8
" (2) " 26
" (3) " 3

Of 9 bacilli isolated in the 4th week and later

Class (1) includes 0
" (2) " 6
" (3) " 3

The bacilli obtained in the first 3 weeks were agglutinated better than those grown later.

Of 17 bacilli from blood

Class (1) includes 2
" (2) " 12
" (3) " 3

Of 14 bacilli from faeces

Class(1)includes 3
" (2) " 10
" (3) " 1

Of 8 bacilli from urine

Class (1) includes 1

" (2) " 5

" (3) " 2

Class (1) includes also 1 bacillus from spleen, and the bacillus from a mesenteric gland.

" (2) " 3 bacilli from spleen, and the bacillus from a rose-spot, and the bacillus from the gall-bladder.

The bacilli from faeces were agglutinated in somewhat higher dilutions than those from blood, but the difference was not so marked as with the 3 preceding bacilli.

The average age of the bacilli in class (1) was 91 days, of those in class (2) 111 days, and of those in Class (3) 138 days. Agglutinability thus did not increase with age.

Agglutination by antiparatyphoid B (Achard) serum.

This serum agglutinated its own bacillus in a limiting dilution of 1:70,000.

The stock typhoid bacillus was agglutinated to 1:400. This was also the highest dilution in which agglutination occurred with a patient's bacillus. Some agglutination was found in every case at 1:25.

The bacilli were divided into 3 classes -

(1) those with the limit of agglutination between 1:400 and 1:200

(2) " " " " " " " 1:190 and 1: 50

(3) " " " " " " " 1: 40 and 1: 25

Class (1) includes 11 bacilli.

" (2) " 28 "

" (3) " 7 "

Of 37 bacilli isolated in the <u>first 3 weeks</u>

Class (1) includes 10

" (2) " 23

" (3) " 4

Of 9 bacilli isolated in the <u>4th week and after</u>

Class (1) includes 1

" (2) " 5

" (3) " 3

The bacilli isolated in the first 3 weeks were agglutinated somewhat better than those obtained later.

Of 17 bacilli from <u>blood</u>

Class (1) includes 4

" (2) " 9

" (3) " 4

Of 14 bacilli from faeces

Class	(1)	includes 4
"	(2)	" 9
"	(3)	" 1

Of 8 bacilli from urine

Class	(1)	includes 1
"	(2)	" 5
"	(3)	" 2

Class (1) includes also the bacillus from a rose-spot and that from a mesenteric gland.

Class (2) " 4 bacilli from spleen and the bacillus from the gall-bladder.

The bacilli from faeces were agglutinated a little better than those from blood, but the difference was too slight to justify any conclusions.

The average age of the bacilli in class (1) was 95 days, of those in class (2) 84 days, and of those in class (3) 122 days. Agglutinability did not increase with the age of the organisms.

General conclusions from the results of the experiments with antisera.

(1) The bacilli which were isolated earlier in the disease tended to be agglutinated better by artificial antisera than those isolated later, the difference being most marked between those obtained in the first 3 weeks, and those grown after the end of that time.

(2) The bacilli isolated from faeces were agglutinated much better by antityphoid serum, and somewhat better by antiparatyphoid serum, than those grown from blood.

(3) The bacilli isolated from urine resembled those grown from blood rather than those from faeces.

(4) The length of time which elapsed between the isolation of the bacilli and their examination exercised no appreciable influence on their power to undergo agglutination.

TABLE XII.

Agglutination by antityphoid serum.

(Limit of agglutination of B.typhosus (stock) 1:80,000)

Week of isolation of bac.	Aggl. good (1:80,000 - 1:45,000)	Aggl.moderate. (1:25,000 - 1:1600)	Aggl. slight or absent (1:100 and less)	Total
1st	0	0	1	1
2nd	11	4	2	17
3rd	9	8	2	19
4th	2	3	1	6
5th	0	1	0	1
.....				
8th	0	1	0	1
.....				
2nd of conv.	0	0	1	1
Total.	22	17	7	46
Source of bac.				
blood	6	7	4	17
faeces	11	3	0	14
urine	2	5	1	8
rose-spot	1	0	0	1
spleen	2	0	2	4
g.bladder	0	1	0	1
mes.gland	0	1	0	1
Total	22	17	7	46

TABLE XII (Contd)

(Days between isolation and testing)

Source of bacillus.	Aggl.good. (1:80,000-1:45,000)		Aggl.moderate. (1:25,000-1:1600)		Aggl.slight or absent. (1:100 and less)	
	No.	days	No.	days	No.	days
blood	(6)	139	(7)	120	(4)	79
faeces	(11)	61	(3)	70	(0)	-
urine	(2)	85	(5)	80	(1)	7
rose-spot	(1)	96	(0)	-	(0)	-
spleen	(2)	86	(0)	-	(2)	90
gall-bladder	(0)	-	(1)	63	(0)	-
mes. gland.	(0)	-	(1)	63	(0)	-
Average ...	(22)	88	(17)	93	(7)	72

TABLE XIII.

Agglutination by antiparatyphoid A (Brion-Kayser) serum.

(Limit of agglutination of B.paratyphosus A (Brion-Kayser) 1:3000)

(No agglutination of B.typhosus (stock))

Week of isolation of bac.	Limit of agglutination 1:100 - 1:50	1:40 - 1:25	No aggl.	Total.
1st	0	1	0	1
2nd	1	7	9	17
3rd	0	5	14	19
4th	0	1	5	6
5th	0	0	1	1
.....				
8th	0	0	1	1
.....				
2nd of conv.	0	0	1	1
Total	1	14	31	46
Source of bac.				
blood	0	3	14	17
faeces	1	8	5	14
urine	0	1	7	8
rose-spot	0	0	1	1
spleen	0	1	3	4
gall-bladder	0	0	1	1
mesen.gland	0	1	0	1
Total	1	14	31	46

TABLE XIII (Contd)

Days between isolation and testing.

Source of bacillus.	Limit of agglutination 1:100 - 1:50		1:40 - 1:25		No agglutination	
	No.	days.	No.	days.	No.	days.
blood	(0)	-	(3)	122	(14)	127
faeces	(1)	33	(8)	80	(5)	68
urine	(0)	-	(1)	71	(7)	83
rose-spot	(0)	-	(0)	-	(1)	106
spleen	(0)	-	(1)	147	(3)	79
gall-bladder	(0)	-	(0)	-	(1)	70
mesenteric gland	(0)	-	(1)	70	(0)	-
Average	(1)	33	(14)	93	(31)	100

TABLE XIV.

Agglutination by antiparatyphoid A(Schottmüller) serum.

(Limit of agglutination of B.paratyphosus A(Schottmüller) 1:200,000)
(" " " " B.typhosus(Stock) 1:60)

Week of isolation of bac.	Limit of agglutination 1:350 - 1:200	1:190 - 1:50	1:40 and less.	Total
1st	0	1	0	1
2nd	3	10	4	17
3rd	2	13	4	19
4th	1	2	3	6
5th	0	1	0	1
.....				
8th	0	0	1	1
.....				
2nd of conv.	0	1	0	1
Total ...	6	28	12	46
Source of bac.				
blood	0	9	8	17
faeces	3	10	1	14
urine	1	4	3	8
rose-spot	0	1	0	1
spleen	0	4	0	4
gall-bladder	1	0	0	1
mes.gland.	1	0	0	1
Total ...	6	28	12	46

TABLE XIV(Contd)

Days between isolation and testing.

Source of bacillus.	Limit of agglutination 1:350 - 1:200		1:190 - 1:50		1:40 and less.	
	No.	days.	No.	days.	No.	days.
blood	(0)	-	(9)	125	(8)	143
faeces	(3)	81	(10)	77	(1)	116
urine	(1)	91	(4)	72	(3)	112
rose-spot	(0)	-	(1)	113	(0)	-
spleen	(0)	-	(4)	104	(0)	-
gall-bladder	(1)	79	(0)	-	(0)	-
mesenteric gland.	(1)	79	(0)	-	(0)	-
Average	(6)	82	(28)	97	(12)	133

TABLE XV.

Agglutination by antiparatyphoid B (Schottmüller) serum.

(Limit of agglutination of B.paratyphosus B(Schottmüller)serum 1:800,000)
(" " " " B.typhosus (stock) 1:50)

Week of isolation of bac.	Limit of agglutination 1:400 - 1:200	1:190 - 1:50	1:40 and less	Total
1st	1	0	0	1
2nd	3	13	1	17
3rd	4	13	2	19
4th	0	4	2	6
5th	0	1	0	1
.....	.....	.....	.....	.....
8th	0	0	1	1
.....	.....	.....	.....	.....
2nd of conv.	0	1	0	1
Total ...	8	32	6	46
Source of bac.				
blood	2	12	3	17
faeces	3	10	1	14
urine	1	5	2	8
rose-spot	0	1	0	1
spleen	1	3	0	4
gall-bladder.	0	1	0	1
mes. gland	1	0	0	1
Total ...	8	32	6	46

TABLE XV (Contd)

Days between isolation and testing.

Source of bacillus.	Limit of agglutination 1:400 - 1:200 No.	days.	1:190 - 1:50 No.	days.	1:40 and less No.	days.
blood	(2)	74	(12)	150	(3)	150
faeces	(3)	77	(10)	85	(1)	123
urine	(1)	104	(5)	82	(2)	127
rose-spot	(0)	-	(1)	120	(0)	-
spleen.	(1)	160	(3)	93	(0)	-
gall-bladder	(0)	-	(1)	84	(0)	-
mesenteric gland.	(1)	84	(0)	-	(0)	-
Average ...	(8)	91	(32)	111	(6)	138

TABLE XVI.

Agglutination by antiparatyphoid B(Achard)serum

(limit of agglutination of B.paratyphosus B (Achard) 1:70,000)

(" " " " B.typhosus (stock) 1:400)

Week of isolation of bac.	Limit of agglutination 1:400 - 1:200	1:90 - 1:50	1:40 and less	total
1st	0	1	0	1
2nd	5	10	2	17
3rd	5	12	2	19
4th	1	3	2	6
5th	0	1	0	1
.....				
8th	0	0	1	1
.....				
2nd of conv.	0	1	0	1
Total ...	11	28	7	46
Source of bac.				
blood	4	9	4	17
faeces	4	9	1	14
urine	1	5	2	8
rose-spot	1	0	0	1
spleen	0	4	0	4
gall-bladder	0	1	0	1
mes. gland.	1	0	0	1
Total ...	11	28	7	46

TABLE XVI (Contd)

Days between isolation and testing.

Source of bacillus.	Limit of agglutination 1:400 - 1:200		1:190 - 1:50		1:40 and less	
	No.	days.	No.	days.	No.	days.
blood	(4)	145	(9)	106	(4)	131
faeces	(4)	53	(9)	70	(1)	105
urine	(1)	82	(5)	64	(2)	109
rose-spot	(1)	102	(0)	-	(0)	-
spleen	(0)	-	(4)	92	(0)	-
gall-bladder	(0)	-	(1)	66	(0)	-
mesenteric gland	(1)	66	(0)	-	(0)	-
Average	(11)	95	(28)	84	(7)	122

days.

(For full table see Appendix O)

TABLE XVII.

Summary of results of agglutination by antisera.

Explanation of symbols, etc.

N 4 means 4th day of normal temperature.

R 4 " 4th day of relapse.

$\frac{400}{3000}$ means that complete agglutination occurred in a dilution of 1:400, and the limit of agglutination in a dilution of 1:3000.

$\frac{-}{50}$ means that agglutination was not complete at 1:25, and that the limit of agglutination occurred at 1:50.

0 means that there was no agglutination in a dilution of 1:25.

The column "age of bacilli when tested" shows the time which elapsed between the isolation of each bacillus and its being tested with the antityphoid serum. The tests with the other sera were performed at intervals of about 6 days.

(For full table see Appendix C)

TABLE XVII.

AGGLUTINATION BY ANTISERA.

Name	Source	Age of patient.	Days ill when isolated.	Isolation medium.	Age of Bac. when tested (days)	Anti Typhoid.	Anti Br-Ka.	Anti A.Sch.	Anti A.Sch.	Anti B.Ach
B.typhosus (Stock)						8000/80000	0	-/60	-/150	25/400
B.paratyphosus A(Br-Ka)						-/2000	800/3000	16000/60000	15000/120000	-/50
" " (Schott.)A						0	400/1800	15000/200000	10000/60000	0
" " B. (Schott)						0	400/2000	800/12000	40000/800000	0
" " B (Achard)						-/1600	-/70	50/3000	50/400	10000/70000
B. coli	Urine			Endo		0	0	0	-/25	-/25
1. J.H.	blood	23	3	bile	8	0	-/25	25/100	50/250	-/150
2. J.T.	"	33	8	bouillon	108	3000/60000	0	25/120	50/240	25/200
3. E.B.	"	20	8	"	167	-/3000	-/50	25/100	-/80	25/150
4. M.E.	faeces	23	8	Endo	77	800/50000	-/25	-/50	-/120	-/80

5. F.M.	faeces	23	8	Endo	57	3000/60000	-/25	50/200	50/180	50/250
6. A.B.	blood	24	8	bile	22	-/30	0	0	0	-/30
7. I. M.	faeces	9	9	Endo	12	3000/60000	0	-/70	25/200	-/80
8. F.J.	"	10	9	"	20	800/50000	25/100	100/350	50/400	100/350
9. E.C.	"	11	9	"	58	1600/50000	-/25	50/180	25/100	50/250
10. J.F.	blood	10	9	bouillon	139	0	0	-/40	25/100	-/25
11. C.T.	faeces	4	10	Endo	54	3000/50000	-/25	25/150	25/180	50/200
12. E.M.	"	18	11	"	21	12000/70000	0	-/50	-/80	-/180
13. R.R.	blood	23	12	bouillon	162	3000/60000	0	25/80	25/100	-/100
14. M.S.	faeces	28	13	Endo	39	1600/60000	-/40	50/80	25/100	-/100
15. C.W.	blood	23	14	bouillon	86	3000/25000	0	0	25/80	-/150
16. W.B.	blood	35	14	"	97	400/25000	0	-/25	-/100	25/150
17. F.B.	faeces	30	14	Endo	72	-/25000	0	-/80	-/100	-/80

TABLE XVII (Contd)

Name	Source	Age of patient.	Days ill when isolated.	Isolation medium.	Age of Bac. when tested (days)	Anti Typhoid.	Anti Br-Ka.	Anti A.Sch.	Anti A.Sch.	Anti B.Ach.
18. S.M.	faeces	13	14	Endo	116	1600/50000	-/25	25/200	25/180	25/180
19. D.M.	blood	20	15	bouillon	133	1600/25000	0	-/100	-/100	25/200
20. A.W.	faeces	53	15	Endo	131	3000/60000	-/25	25/80	50/200	25/160
21. J.L.	urine	36	15	"	80	400/25000	0	50/180	25/170	-/80
22. D.R.	blood	18	15	bouillon	39	12000/45000	0	-/80	25/100	25/100
23. T.K.	faeces	20	16	Endo	99	25/1600	0	-/25	-/30	-/40
24. H.M.	urine	16	17	"	80	-/3000	0	-/60	25/200	-/80
25. M.K.	blood	47	17	bouillon	140	800/8000	0	-/25	25/80	-/80
26. M.F	spleen	25	17	agar	33	3000/60000	0	-/80	-/50	-/80
27. J.M.	urine	31	18	Endo	60	800/25000	-/50	50/150	50/180	-/100
28. P.J.	spleen	29	18	Agar	63	0	0	25/160	50/180	50/180

29. P.J.	gall-bladder	29	18	Agar	63	$\frac{800}{15000}$	0	$\frac{50}{200}$	$\frac{50}{150}$	$\frac{-}{80}$
30. P.J.	m.gland	29	18	"	63	$\frac{3000}{25000}$	$\frac{-}{30}$	$\frac{50}{200}$	$\frac{50}{200}$	$\frac{50}{400}$
31. E.H.	blood	17	19	bouillon	147	$\frac{-}{1000}$	0	$\frac{-}{80}$	$\frac{-}{80}$	$\frac{50}{200}$
32. H.O.	"	38	19	"	172	$\frac{-}{50000}$	0	0	$\frac{-}{80}$	$\frac{50}{200}$
33. T.W.	"	35	19	"	173	$\frac{3000}{60000}$	$\frac{-}{25}$	$\frac{-}{80}$	$\frac{25}{120}$	$\frac{-}{100}$
34. T.K.	rose-spot	20	20	bouillon	96	$\frac{12000}{80000}$	0	$\frac{25}{100}$	$\frac{25}{100}$	$\frac{50}{400}$
35. J.P.	spleen	24	20	Agar	140	$\frac{3000}{70000}$	$\frac{-}{25}$	$\frac{25}{150}$	$\frac{25}{250}$	$\frac{25}{150}$
36. M.J.	urine	37	21	Endo	94	$\frac{1600}{50000}$	0	0	$\frac{-}{40}$	$\frac{-}{40}$
37. M.D.	faeces	27	21	"	87	$\frac{1600}{70000}$	0	$\frac{-}{80}$	$\frac{25}{140}$	$\frac{-}{150}$
38. M.I.	blood	20	22	bouillon	171	$\frac{1500}{6000}$	0	0	$\frac{-}{25}$	$\frac{-}{25}$
39. K.M.	spleen	11	23	Agar	118	0	0	$\frac{-}{50}$	$\frac{-}{50}$	$\frac{-}{100}$
40. C.W.	urine	23	25	Endo	75	$\frac{800}{25000}$	0	$\frac{-}{40}$	$\frac{-}{50}$	$\frac{-}{200}$
41. W.P.	"	23	26	"	77	$\frac{800}{60000}$	0	$\frac{25}{200}$	$\frac{-}{100}$	$\frac{-}{80}$

TABLE XVII (Contd)

Name	Source	Age of patient.	Days ill when isolated.	Isolation medium.	Age of Bac. when tested (days)	Anti Typhoid.	Anti Br-Ka.	Anti A.Sch.	Anti A.Sch.	Anti B.Ach. B
42. M.M.	faeces	37	27	Endo	39	-/25000	-/25	25/100	-/100	-/50
43. W.M.	blood	40	28	bouillon	185	1600/80000	0	-/25	-/25	-/40
44. W.B.*	urine	35	28	Endo	82	0	-/25	-/25	-/25	0
45. J.G.	blood	28	29	bouillon	48	100/1600	0	-/80	25/180	-/150
46. R.S.*	urine	21	30	Endo	64	0	-/25	0	0	0
47. L.P.	"	25	55	"	108	100/25000	0	0	0	-/30
48. P.M.*	"	37	N.5	"	163	-/50	-/100	-/25	-/60	-/250
49. C.D.*	"	35	N.9	"	166	-/80	0	-/25	0	-/100
50. A.R.*	"	52	N.10	"	61	0	0	0	0	0
51. T.B.	"	24	N.10	"	7	-/50	0	25/80	25/150	-/150
52. W.C.*	"	19	N.16	"	61	0	0	-/50	0	0

* Not B.typhosus.

SECTION IV.

Agglutination reactions of bacilli with the serum of the patient from whom each was isolated.

The results of the agglutination reactions carried out with the patients' sera and the respective bacilli come now to be considered.

37 bacilli were investigated in this way, -

from blood	13
" faeces	14
" urine	8
" rose-spot	1
" spleen	1

In this series of experiments no fixed standard of comparison in respect of a limiting dilution was available, as the sera varied in activity. The results obtained were therefore compared quantitatively with the results of agglutination tests with the same sera and the stock typhoid bacillus. It a few instances it was found that the patient's bacillus was agglutinated rather better than the stock bacillus by his own serum, but the difference was so slight that I found it convenient to regard the result of agglutination with the stock bacillus as the maximum in each case.

In these cases the tests were carried out within a few days of the isolation of the bacilli.

The 37 bacilli were divided into 3 classes :-

Class (1) includes these bacilli which were agglutinated approximately as well as the stock typhoid bacillus.

Class (2) those which were agglutinated distinctly less well than the stock typhoid bacillus.

Class (3) those which were agglutinated much less well than the stock typhoid bacillus, or were not agglutinated.

In Class (1) the ratio of the agglutination limit of the autogenous bacilli to the stock bacillus was from a little above 1 to $\frac{1}{2}$.

In Class (2) the ratio was from $\frac{1}{2}$ to $\frac{1}{6}$

In Class (3) the ratio was below $\frac{1}{16}$.

Class (1) includes 26 bacilli.
" (2) " 8 "
" (3) " 3

Class (1) includes 1 bacillus isolated in the <u>1st week</u>

Of 17 bacilli isolated in the <u>2nd week</u>
Class (1) includes 13.
" (2) " 4
" (3) " 0

Of 13 bacilli isolated in the 3rd week

Class (1) includes 10

" (2) " 1

" (3) " 2

Of 6 bacilli isolated in the 4th week and after

Class (1) includes 2

" (2) " 3

" (3) " 1

From a consideration of the tables it is evident that the capacity of the bacilli to undergo agglutination was independent of the time of isolation.

Of 13 bacilli isolated from blood

Class (1) includes 12.

" (2) " 1.

" (3) " 0.

Of 14 bacilli isolated from faeces

Class (1) includes 8

" (2) " 5

" (3) " 1

Of 8 bacilli isolated from urine

Class (1) includes 4

" (2) " 2

" (3) " 2

Class (1) includes also the bacilli from rose-spot and spleen.

The bacilli isolated from blood were very much better agglutinated than those from either faeces or urine.

Results of agglutination tests with the patients' sera and the artificial serum compared.

The 37 bacilli tested with their respective patient's serum were included in the 46 tested with the artificial sera. A comparison of the results of agglutination tests on the bacilli with the 2 kinds of serum, the patient's and the antityphoid, shows strking differences.

With regard to the time of isolation, as has been seen, the antityphoid serum agglutinated bacilli isolated in the earlier stages of the disease much better than those obtained later; whereas with the patients' sera no such difference was found to exist. No connection could be made out in the latter instance between the time of isolation of the bacillus and the degree of agglutination present.

A consideration of the bacilli from the point of view of their origin showed the following results. The classes were arranged as before (see pages 53 and 84)

Class (1) includes bacilli agglutinated approximately as well as the stock typhoid bacillus.

Class (2) includes bacilli agglutinated distinctly less well than the stock typhoid bacillus.

Class (3) includes bacilli agglutinated much less well than the stock typhoid bacillus, or not at all.

Of 13 bacilli isolated from blood

	with the antityphoid serum.	with the patient's serum.
Class (1) includes	3	12
" (2) "	6	1
" (3) "	4	0

Of 14 bacilli isolated from faeces

	with the antityphoid serum	with the patient's serum
Class (1) includes	11	8
" (2) "	3	5
" (3) "	0	1

Of 8 bacilli isolated from urine

	with the antityphoid serum	with the patient's serum
Class (1) includes	2	4
" (2) "	5	2
" (3) "	1	2

Class (1) includes also the bacilli from rose-spot and spleen.

While with the antityphoid serum the bacilli from blood were less well agglutinated than those from faeces, with the serum of the respective patients they were agglutinated very well indeed; and very much better than those from faeces. The latter responded less well indeed to the agglutinative action of the autogenous serum than to that of the antityphoid serum. Here again the bacilli grown from urine resembled those obtained from blood rather than those from faeces, agglutination with the patient's serum being decidedly better than with the antityphoid serum.

General conclusions from result of experiments with patients' sera.

(1) With the patient's serum no connection could be made out between the time of isolation of a bacillus and the degree of agglutination present.

(2) The bacilli from blood were agglutinated very well indeed, and very much better than those from faeces.

Summary of comparison of results of experiments with antityphoid serum and with patients' sera.

(1) With the antityphoid serum, the bacilli isolated earlier in the disease were agglutinated much better than those obtained later; with the patients' sera, no such difference was found.

(2) With the antityphoid serum, the bacilli from faeces were agglutinated much better than those from blood. With the patients' sera the bacilli from blood were agglutinated very much better than those from faeces. These results were obtained with the same strains of bacilli.

(3) The bacilli from faeces were agglutinated rather less well by the patients' sera than by the antityphoid serum.

TABLE XVIII.

Agglutination of bacilli by the corresponding patient's serum.

Week of isolation of bac.	As well as B.typh.(Stock) Ratio 1 - $\frac{1}{2}$	Less than B.typh.(Stock) Ratio $\frac{1}{2}$ - $\frac{1}{6}$	Much less than B.typh.(Stock) Ratio $\frac{1}{16}$ & less.	Total
1st	1	0	0	1
2nd	13	4	0	17
3rd	10	1	2	13
4th	0	3	0	3
5th	1	0	0	1
.....				
8th	0	0	1	1
.....				
2nd of conv.	1	0	0	1
Total ...	26	8	3	37
Source of bac.				
blood	12	1	0	13
faeces	8	5	1	14
urine	4	2	2	8
rose-spot	1	0	0	1
spleen	1	0	0	1
Total ...	26	8	3	37

TABLE XIX.

Agglutination by antityphoid serum and respective patient's serum.
(Cross table)

..... Antityphoid serum .....

..... Patient's serum .....

Agglutination.	Week of isolation of bac.	Agglutination: Good	moderate	slight or absent
Good	1st			1
	2nd	7	3	2
	3rd	5	5	1
	4th			
	5th		1	
	.....			
	8th			
	.....			
	2nd of conv.			1
	Total ...	12	9	5
Moderate	1st			
	2nd	3	1	
	3rd		1	
	4th	1	1	1
	5th			
	.....			
	8th			
	.....			
	2nd of conv.			
	Total ...	4	3	1
Slight or absent	1st			
	2nd			
	3rd	2		
	4th			
	5th			
	.....			
	8th		1	
	.....			
	2nd of conv.			
	Total ...	2	1	0

TABLE XIX (Contd)

Agglutination by antityphoid serum and respective patient's serum

..... Antityphoid serum .....

..... Patient's serum .....

Serum	Source of bacillus.	Agglutination Good	moderate	slight or absent
Good	blood	3	5	4
	faeces	7	1	-
	urine	-	3	1
	rose-spot	1	-	-
	spleen.	1	-	-
	Total ...	12	9	5
Moderate	blood	-	1	-
	faeces	3	1	1
	urine	1	1	-
	rose-spot	-	-	-
	spleen	-	-	-
	Total ...	4	3	1
Slight or absent.	blood	-	-	-
	faeces	1	-	-
	urine	1	1	-
	rose-spot	-	-	-
	spleen.	-	-	-
	Total ...	2	1	0

SECTION V.

Discussion of variations in agglutinability.

Facts from the literature.

It is known that under certain circumstances bacilli which normally are well agglutinated by an appropriate serum, may become non-agglutinable[8]. One of these circumstances is the passage of the bacillus through the body of man or an animal. According to Porges and Prantschoff[9], "lessened agglutinability is chiefly observed in cultures freshly isolated from the body, or passed through animals: in bacilli from exudates: and in bacilli which have been passed through media containing agglutinins."

The cause of this phenomenon has been variously regarded. Porges[10] showed that typhoid bacilli which had been rendered completely non-agglutinable by heating to 80°C had their agglutinability restored by washing in normal salt solution. He supposed that the nuclein split off the nucleo-protein of the organisms was the substance which inhibited agglutination, and that when this was removed by washing, the bacilli again became agglutinable. The capsulated bacteria such as Friedländer's bacillus are normally non-agglutinable, and Porges and Prantschoff[11] attributed this to increased formation of protein. In 4 non-agglutinable strains of typhoid bacilli, isolated from

spleens, these observers thought they could detect the presence of a capsule.

Another suggestion which has been made (Paltauf[8]), is that certain strains of typhoid bacilli are really composite strains containing both agglutinable and non-agglutinable members. Under certain circumstances, the latter strain may come to predominate.

Culture in agglutinin - containing media brings about non-agglutinability. Sacquépée[12] caused strains of typhoid bacilli to become less agglutinable by growing them in collodion sacs in the peritoneal cavity of rats immunized against B. typhosus. The change, however, took place slowly, and it was only after treating a series of subcultures in the same way that the agglutinability at the end of 5 months was reduced to 1/6 of its original standard. He concluded that non-agglutinable strains were produced in man by growth of the organism in an infected or immunized body.

Numerous observers have recorded the isolation of typhoid bacilli which were agglutinated only slightly, or not at all, (Horton Smith[13], Remy[14], Sacquépée[15], Cambier[16] Emery[17], Nicolle and Trenel[18]). These bacilli have fulfilled all the other tests for typhoid bacilli, and in certain cases it has been found that animals immunized against feebly agglutinable strains resulted in the production of

a serum which agglutinated laboratory strains.

According to Paltauf[8], not infrequently agglutination is lessened with the patient's serum as well as with an artificial antityphoid serum.

A slow rise on the part of these bacilli to a normal standard of agglutinability has been described by most authors as taking place, either after a certain number of sub-cultures, or simply by the lapse of time, without sub-culture (Cambier[16], Emery[17], Porges and Prantschoff[11], Lipschutz[19]) Porges and Prantschoff found that 4 non-agglutinable strains from spleens were agglutinated as well as stock bacilli after about 15 sub-cultures on agar, and Lipschutz noted a similar rise in the case of 3 typhoid strains isolated from urine. In the latter instance, these 3 strains were not definitely agglutinated in a dilution of 1:200 by an active serum(agglutinating to 1:20,000,), whereas, 3 months later, without sub-culturing, they were agglutinated by the serum to 1:20,000.

A change in the characters of an organism by its presence in an animal body is referred to by Besredka [20] in a criticism of work done by Aronson on streptococci. Aronson[21] had endeavoured to prove the identity of streptococci from various sources by means of experiments on animals. He immunized horses with a streptococcus which

he had rendered extremely virulent by passage through a series of mice, and found that streptococci from other sources, also rendered virulent by passage through mice, were acted on by the serum of these horses equallywith the original strain used for immunization. From this he concluded that all the streptococci used were essentially the same. Besredka criticised Aronson's conclusions on the ground that all his streptococci had been modified by their passage through mice, and that each strain had become what he called "un streptocoque de passage."

Conclusions from personal observations.

From what has been said it is plain that when typhoid bacilli circulate in the blood they sometimes undergo a change which manifests itself in diminished agglutinability. The results of my experiments seem to show that the bacilli in the faeces are less changed from the original agglutinable type than those in the blood, which are acted upon to a much greater extent by the body fluids. But the explanation that the bacilli become non-agglutinable by growth in the body of a person whose blood contains immune substances, does not account for the fact that the bacilli isolated from blood were practically all agglutinated by the serum in which they were circulating as well as was the stock typhoid bacillus. It may be that after the organisms have been modified by

the action of the serum, some alteration in the serum itself is called forth by the change in the bacilli, and this might account for the fact that the bacilli grown from faeces were agglutinated rather worse by their respective patient's serum than by the artificial serum.

As has been shown, the bacilli isolated earlier in the disease were agglutinated better than those obtained later, and this, so far as it goes, is in favour of Sacquépée's theory that non-agglutinability is produced by growth in a body containing immune substances. It is to be noted, however, that a bacillus (J.H.) isolated from the blood on the 3rd day of illness was unacted on by the antityphoid serum at 1:25. On the day on which the bacillus was obtained, the patient's serum caused no clumping of the stock bacillus at 1:25. The non-agglutination of this bacillus, therefore, must have been due to some other cause than growth in an agglutinin-containing medium.

SECTION VI.

Agglutination of bacilli by acid solutions (Michaelis)

Another method was employed in the attempt to differentiate the bacilli which had been already tested by antisera.

Michaelis[22] pointed out that many strains of bacteria are agglutinated by acids, and that a fixed degree of acidity corresponds to the maximum of agglutination. This maximum, he said, is characteristic for individual strains of bacteria, and can be used as a help in their identification.

The test is carried through as follows :-

The bacillus to be examined is grown on agar slopes for 24 hours and is then emulsified in distilled water, the emulsion being rather denser than that used for a Widal reaction. The following 6 solutions are required :-

	Normal sodium hydrate.	Normal acetic acid.	Distilled water
1	5 c.c.	7.5 c.c.	87.5 c.c.
2	5 "	10 "	85 "
3	5 "	15 "	80 "
4	5 "	25 "	70 "
5	5 "	45 "	50 "
6	5 "	85 "	10 "

1 c.c. of each of these solutions is put into each of a series of 6 test tubes, and to each tube is added 3 c.c. of the bacterial emulsion. The tubes are then shaken up and

put in the incubator at 37°C. When the first agglutination appears, the row of tubes is taken from the incubator and left at room temperature for some time. In any case, the tubes are not kept at 37°C for more than an hour.

According to Michaelis, with typhoid bacilli agglutination occurs only in tubes 3,4 and 5, as a general rule. It is commonly most marked in tube 3, though occasionally in 4, and in 2 and 5 is much slighter, if it occurs in these at all. Tube 3 is therefore reckoned as the optimum for B. typhosus.

B. paratyphosus has its optimum in tubes 5 and 6, but the A and B strains cannot be distinguished from one another.

B. coli is usually not agglutinated.

Rost[23] applied the test to 8 strains of B.typhosus, a paratyphoid A strain, a paratyphoid B strain, and other organisms. The results he obtained with typhoid bacilli agreed with those of Michaelis; with the paratyphoid B there was marked agglutination in tube 6, and with the paratyphoid A, no agglutination. He concluded that the method is "a valuable addition to our resources for diagnosing typhoid".

A later investigator, Jaffé[24], has criticised the

method. He tested 41 strains of B. coli, 40 of B. typhosus, 11 of B. paratyphosus A, 3 of B. paratyphosus B, 3 of B. typhi murium, with unsatisfactory results. 11 of the B. coli strains showed agglutination#, and the test gave no assistance in the differentiation of the atypical members of the B. coli group. With B. typhosus the results were no more certain. In the 40 strains the optimum occurred in tubes 2 and 3. In 22 agglutination was present only in 1 or 2 tubes, in 5 from tube 2 to tube 6, and in 1, in all 6. (It was found that this last bacillus was agglutinated by distilled water). In the case of 4 of the bacilli no agglutination occurred.

Of the 11 paratyphoid B strains, 2 showed agglutination in tubes 4 and 5, 8 in tubes 4, 5, and 6, and 1 from tube 3 to tube 6. The optimum varied. Of the 3 paratyphoid A strains, 2 were agglutinated in tubes 4, 5, and 6, and in tubes 3 to 6. Here also the optimum varied.

This method was applied to the 46 bacilli tested by means of the antisera, with the exception of W.B. (blood) which had died out; to 2 stock typhoid strains, the laboratory bacillus, and the R.A.M. C. strain; to the 4 paratyphoid strains: and to a strain of B. coli.

By accident, a bacillus (A.W.) was tested twice

on different days, and this was discovered only when the results were tabulated. The figures obtained were precisely the same on each occasion. This points to a constancy of the results obtained by the method.

With regard first to the stock typhoid bacilli, both were agglutinated in tubes 3 to 6, the laboratory strain having its maximum in tube 4, and the R.A.M.C. strain in tubes 3 to 5. With the 4 paratyphoids, the maximum occurred in each case in tube 6, agglutination in the case of Brion-Kayser A being slight, and present only in this tube, while with the 3 others there was some agglutination also in tube 5. B. coli was unaffected.

The agglutination which took place in the case of the 45 bacilli from patients varied in extent and degree, but the maximum was found to occur as follows :-

In tube 1	0	times.
" " 2	0	"
" " 3	23	"
" " 4	3	"
" " 5	4	"
" " 6	5	"
" " 4,5, and 6	1	"
" " 5, and 6	1	"
and no agglutination ..	8	"
Total	45	

That is to say, in half the cases the maximum of agglutination occurred in tube 3,which Michaelis regarded as typical for B. typhosus.

On reference to the table (XX. p.104)it will be seen that the place of occurrence of the maximum was independent of the time of isolation, and also that the only difference in the bacilli from the point of view of their origin was that the bacilli from urine seemed to be less "typical" in reaction than those from blood or faeces.

With regard to the number of tubes showing a reaction in each case, agglutination was found more frequently in the combination of tubes 3 to 5 than in any other. In 4 instances irregular agglutination was present, but in each of these the maximum occurred in tube 3. In 9 cases agglutination was of Michaelis' "paratyphoid type". In 4 of the latter agglutination was present only in tubes 5 and 6. In one case in which it was found in tubes 4-6, and in 4 others with agglutination in tubes 3-6, the maximum occurred in tube 5 or tube 6.

A cross table is given (Table XXII, page 106) to contrast the agglutination of the bacilli with antityphoid serum and with acids. The organisms were classified as before as regards antityphoid serum, and 3 classes were made from the results of the Michaelis' test.-

Class (1) includes those bacilli with which the maximum agglutination occurred in tube 3.

" (2) those with which the maximum occurred elsewhere than in tube 3.

Class (3) those with which no agglutination occurred,

Any correlation which may exist between the results of the 2 tests is slight.

The bacilli showing the 'paratyphoid reaction' with acids were not better agglutinated than the others by the antiparatyphoid sera.

From these results it seems that the test is of interest rather than value in the examination of typhoid bacilli.

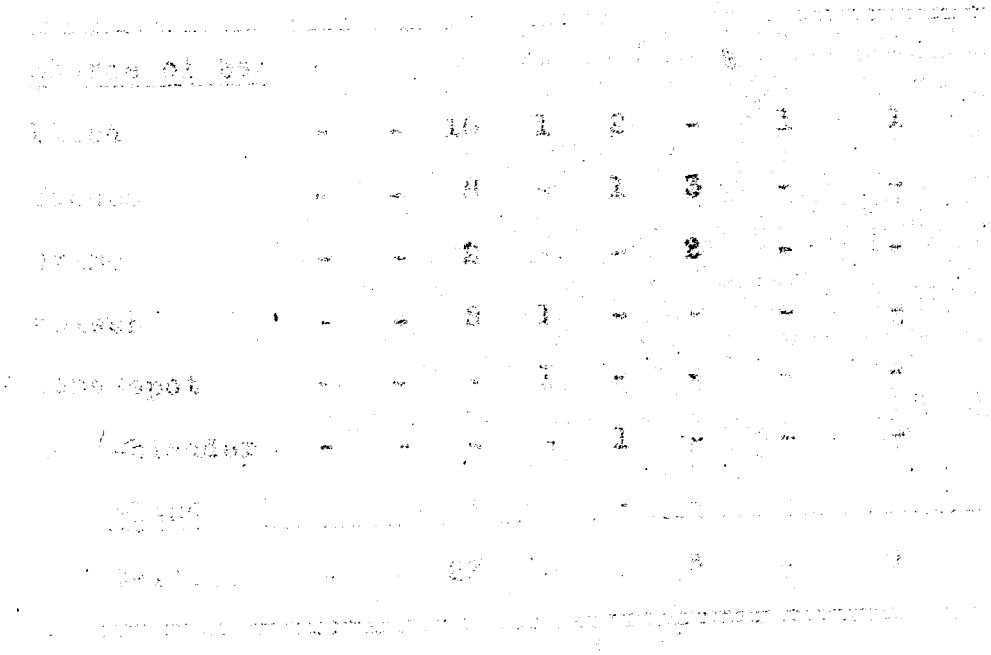

TABLE XX.

Agglutination of bacilli by acid solutions (Michaelis)

Week of isolation of bac.	Maximum agglutination found in tube								
	1	2	3	4	5	6	4-6	5-6	No aggl.
1st	-	-	-	-	1	-	-	-	-
2nd	-	-	9	-	1	3	-	-	3
3rd	-	-	10	3	2	-	-	1	3
4th	-	-	2	-	-	2	1	-	1
5th	-	-	1	-	-	-	-	-	-
.....									
8th	-	-	1	-	-	-	-	-	-
.....									
2nd of conv.	-	-	-	-	-	-	-	-	1
Total	-	-	23	3	4	5	1	1	8
Source of bac.									
blood	-	-	10	1	2	-	1	1	1
faeces	-	-	8	-	1	3	-	-	2
urine	-	-	2	-	-	2	-	-	4
spleen	-	-	2	1	-	-	-	-	1
rose-spot	-	-	-	1	-	-	-	-	-
gall-bladder	-	-	-	-	1	-	-	-	-
mes.gland	-	-	1	-	-	-	-	-	-
Total	-	-	23	3	4	5	-	1	8

TABLE XXI.

Agglutination of bacilli by acid solutions (Michaelis)

Week of isolation of bac.	Agglutination present in tubes								
	3	3 & 4	3-5	3-6	4-6	5 & 6	6	3 & 6	No aggl
1st	-	-	1	-	-	-	-	-	-
2nd	3	1	-	6	-	2	1	-	3
3rd	1	2	2	10	-	-	-	1	3
4th	2	-	-	1	1	1	-	-	1
5th	-	-	-	1	-	-	-	-	-
.....									
8th	-	-	-	1	-	-	-	-	-
.....									
2nd of conv.	-	-	-	-	-	-	-	-	1
Total ...	6	3	3	19	1	3	1	1	8
Source of Bac.									
Blood	2	2	1	8	-	1	-	1	1
faeces	4	1	1	4	-	1	1	-	2
urine	-	-	1	1	1	1	-	-	4
rose-spot	-	-	-	1	-	-	-	-	-
spleen	-	-	-	3	-	-	-	-	1
m. gland	-	-	-	1	-	-	-	-	-
bile	-	-	-	1	-	-	-	-	-
Total ...	6	3	3	19	1	3	1	1	8

Total ..... 45.

TABLE XXII.

Agglutination of bacilli by antityphoid serum and by acids (Michaelis)

(Cross table)

..... Antityphoid serum .....

Agglutination.	Week of isolation of bac.	Agglutination Good	Moderate	Slight or absent
Maximum aggl. in tube 3	1st	0	0	0
	2nd	6	1	2
	3rd	7	3	0
	4th	1	1	0
	5th	0	1	0
	.....			
	8th	0	1	0
	.....			
	2nd of conv.	0	0	0
	Total ...	14	7	2
Maximum aggl. not in tube 3.	1st	0	0	1
	2nd	3	1	0
	3rd	2	2	2
	4th	1	2	0
	5th	0	0	0
	.....			
	8th	0	0	0
	.....			
	2nd of conv.	0	0	0
	Total ...	6	5	3
No aggl.	1st	0	0	0
	2nd	2	1	0
	3rd	0	3	0
	4th	0	0	1
	5th	0	0	0
	.....			
	8th	0	0	0
	.....			
	2nd of conv.	0	0	1
	Total ...	2	4	2

..... Acid solutions .....

TABLE XXII (Contd)

Agglutination of bacilli by antityphoid serum and by acids.(Michaelis)

..... Antityphoid serum .....

Agglutination.	Source of bacillus	Agglutination Good	Moderate	Slight or absent
Maximum aggl. in tube 3.	blood	6	2	2
	faeces	5	3	0
	urine	1	1	0
	rose-spot	0	0	0
	spleen	2	0	0
	gall-bladder	0	0	0
	mesenteric gland	0	1	0
	Total	14	7	2
Maximum aggl. not in tube 3.	blood	0	3	2
	faeces	4	0	0
	urine	1	1	0
	rose-spot	1	0	0
	spleen	0	0	1
	gall-bladder	0	1	0
	mesenteric gland	0	0	0
	Total	6	5	3
No aggl.	blood	0	1	0
	faeces	2	0	0
	urine	0	3	1
	rose-spot	0	0	0
	spleen	0	0	1
	gall-bladder	0	0	0
	mesenteric gland	0	0	0
	Total	2	4	2

..... Acid solutions .....

TABLE XXIII.

Agglutination of bacilli by acid solutions

Complete agglutination is represented by 10.

The bacilli are numbered as in Table XVII (p. 78)

Bacillus	Source	Agglutination in tube 1	2	3	4	5	6
B.typhosus (Stock)		-	-	7	9	2	1
"	R.A.M.C.	-	-	10	10	10	7
1. J.H.	blood	-	-	1	2	4	-
2. J.T.	"	-	-	2	1	-	-
3. E.B.	"	-	-	-	-	6	5
4. M.E.	faeces	-	-	4	2	1	3
5. F.M.	"	-	-	1	-	-	-
6. A.B.	blood	-	-	3	2	1	1
7. I.M.	faeces	-	-	-	-	-	-
8. F.J.	"	-	-	1	3	5	6
9. E.C.	"	-	-	7	4	3	3
10. J.F.	blood	-	-	8	6	6	6
11. C.T.	faeces	-	-	3	-	-	-
12. E.M.	"	-	-	-	-	2	4
13. R.R.	blood	-	-	3	2	1	1
14. M.G.	faeces	-	-	-	-	-	-
15. C.W.	blood	-	-	-	-	-	-
17. F.B.	faeces	-	-	2	-	-	-
18. S.M.	"	-	-	-	-	-	4
19. D.M.	blood	-	-	3	-	-	2

TABLE XXIII (Contd)

Bacillus.	Source	Agglutination in tube 1	2	3	4	5	6
20. A.W.*	faeces	-	-	2	1	-	-
21. J.L.	urine	-	-	-	-	-	-
22. D.R.	blood	-	-	8	5	4	7
23. T.K.	faeces	-	-	3	2	1	-
24. H.M.	urine	-	-	-	-	-	-
25. M.K.	blood	-	-	3	4	2	1
26. M.F.	spleen	-	-	7	4	5	6
27. J.M.	urine	-	-	-	-	-	-
28. P.J.	spleen	-	-	2	6	3	1
29. P.J.	gall-bladder	-	-	2	5	9	8
30. P.J.	mesenteric gland	-	-	8	7	5	1
31. E.H.	blood	-	-	2	4	6	6
32. H.O.	"	-	-	1	-	-	-
33. T.W.	"	-	-	2	1	-	-
34. T.K.	rose-spot	-	-	9	10	8	6
35. J.P.	spleen	-	-	5	2	2	2
36. M.J.	urine	-	-	5	4	3	-
37. M.D.	faeces	-	-	1	2	4	1
38. M.I	blood	-	-	9	10	10	10
39. K.M.	spleen	-	-	-	-	-	-
40. C.W.	urine	-	-	-	-	6	7
41. W.P.	"	-	-	-	1	2	4
42. M.M.	faeces	-	-	1	-	-	-
43. W.M.	blood	-	-	3	-	-	-

* Tested twice with same result.

TABLE XXIII (Contd)

Bacillus	Source	Agglutination in tube 1	2	3	4	5	6
44. W.B.*	urine	-	-	-	-	-	-
45. J.G.	blood	-	-	3	2	1	1
46. R.S.*	urine	-	5	7	8	10	10
47. L.P.	"	-	-	4	3	2	1
48. P.M.*	"	-	-	1	-	-	-
49. C.D.*	"	-	-	-	-	-	-
50. A.R.*	"	-	-	-	-	-	-
51. T.B.	"	-	-	-	-	-	-
52. W.C.*	"	-	-	-	-	-	-
B.paratyphosus A	(Brion-Kayser)	-	-	-	-	-	1
"	(Schottmüller)	-	-	-	-	1	2
B. "	B(Schottmüller)	-	-	-	-	2	3
"	(Achard)	-	-	-	-	7	10
B. coli	urine	-	-	-	-	-	-

* Not B. typhosus.

PART I.

INTRODUCTION.

... e course of the work which has just ...

... it became apparent that an important fa...

... to typhoid bacilluria has hitherto been ove...

... the bacilli which appear in the urine ...

... d bacilli. In 5 out of 17 unselected ...

... from enteric fever, it was found that ...

... members of the typhoid-coli group.

... organism accidentally present as ...

P A R T I I.

... regarded to be typhoid bacilli.

The question of typhoid bacilluria is disc...

... following sections :-

... facts as recorded in the literature ...

... personal experiences.

... occurrence of atypical bacilli in the ...

... teric fever. Discussion of their nat...

... Further experiments on the atypical bac...

... bacilluria in ... patients ...

... teric fever.

PART II.

INTRODUCTION.

In the course of the work which has just been described, it became apparent that an important fact with regard to typhoid bacilluria has hitherto been overlooked, namely, that the bacilli which appear in the urine are not always typhoid bacilli. In 6 out of 17 unselected cases of bacilluria in enteric fever, it was found that the bacilli were atypical members of the typhoid-coli group. With the exception of organisms occasionally present as contaminations, the bacilli in the urine in enteric fever have always been proved or regarded to be typhoid bacilli.

The question of typhoid bacilluria is discussed in the following sections :-

I. The facts as recorded in the literature: and personal experiences.

II. The occurrence of atypical bacilli in the urine in enteric fever. Discussion of their nature.

III. Further experiments on the atypical bacilli.

IV. A note on bacilluria in female patients with enteric fever.

SECTION I.

The facts as recorded in the literature.

In a certain number of typhoid patients (about 25%), large numbers of bacilli suddenly appear in the urine, usually late in the course of pyrexia, or early in convalescence. Their presence is easily recognised by the so-called opalescence or shimmer of the urine, and by the formation of visible waves in the depths of the fluid on shaking. As a general rule they are present in large numbers - 172,000,000 per c.c. (Petruschky [25]); 500,000,000 per c.c. (Gwyn[26]) - but have been found also in a few cases in small numbers in apparently clear urines (Connell[27], Buchan[28]). Bacilluria is commonly unassociated also with the occurrence of pus or albumin in the urine.

The bacilli persist in the urine for a variable period, often for several weeks, and then commonly disappear spontaneously, but in some instances they last for years, 9 years in a case recorded by Liebetrau[29], 5 years in a case mentioned by Gwyn[26], and in cases under the supervision of the Glasgow Public Health Authorities, for 4 to 6 years.

Connell[27] collected the cases from the literature and found, between 1897 and 1909, 150 instances of

bacilluria in 621 cases of enteric fever. In all these cases, the bacilli were proved bacteriologically to be typhoid bacilli. To this list may be added 17 instances of bacilluria in 30 cases (Buchan[28]), and 26 in 100 cases of enteric fever studied by McCall in the City of Glasgow Fever Hospital, Belvidere. These figures show that bacilluria occurred in 25% of 751 cases of enteric fever.

The cause of typhoid bacilluria has been variously regarded. The fact that it comes on comparatively late in the disease, while bacilli are present in the blood in greatest numbers in the early stages, makes it unlikely that they are excreted directly from the blood. Horton Smith[30] found the blood sterile in 4 cases of bacilluria and Connell in 2 cases. Konjajeff[31] held that bacteruria indicated the presence of lymphoid nodules in the kidneys, for in sections of these nodules he had sometimes seen bacilli present. Suppurative foci in the kidney have been described by Flexner[32], and by Brownlee and Chapman[33]. These small kidney abscesses, however, are not very common, for in 289 post-mortem examinations Horton Smith found them only once, though they have been commoner than this in the experience of the City of Glasgow Hospitals. Blumer[7] was of opinion that the urine was infected by direct passage of bacilli from the rectum to the bladder.

That the bacilli are sometimes confined to the bladder was shown by Horton Smith, who at a post-mortem examination of a patient in whom bacilluria had been present, isolated B. typhosus from the bladder and not from the kidney, and by Gwyn[26], who caused bacilluria to terminate in 3 cases, by washing out the bladder with a weak per-chloride of mercury solution.

The commonly accepted view of the aetiology of bacilluria is that the urine becomes infected by a stray bacillus at an early stage in the disease, while organisms are numerous in the circulating blood, and that the bacilli multiply in the urine later when the reaction of the urine becomes favourable. During the earlier part of an attack of enteric fever, the urine is quite acid, and typhoid bacilli grow best in fairly acid media. Later in pyrexia, or in early convalescence, the urine loses its high acidity, and thus becomes a suitable culture medium. The presence of residual urine has been looked on as of importance in harbouring germs.

Park[34], Horton Smith and others made experiments on the growth of typhoid bacilli in urine. Buchan[illegible] noted that the bacilli would not grow if the acidity of the urine rose above $\frac{N}{40}$, but he found them present, though not

multiplying, in urine with acidity as high as $\frac{N}{16}$. In experiments made by Connell, the bacilli grew well in urine of acidity below $\frac{N}{50}$; when the acidity lay between $\frac{N}{50}$ and $\frac{N}{25}$, they were inhibited in varying degrees, and in the highly acid urines, growth was slight. He observed that the degree of inhibition did not bear a constant relation to the degree of acidity, and experimented with urine originally of low acidity, artificially acidified by various substances. He was able to determine that a comparatively high acidity with acid sodium phosphate was required to inhibit growth, while acetic and lactic acids absolutely stopped growth when the acidity reached $\frac{N}{50}$, and even at $\frac{N}{500}$ exercised some inhibition. He concluded that the inhibitory factors normally present were the organic acids of unknown nature which Folin [35] says are the cause of a varying amount, sometimes more than half, of urinary acidity.

It is supposed that bacilluria passes off by the washing of the bacilli out of the bladder by urine, after this has ceased to be a suitable medium for growth.

Bacilli other than B.typhosus have been found in the urine in enteric fever. Blumer[7] mentions 8 cases in which bacilli were present. From 6 a pure culture of B. coli was obtained, from the 7th, B. coli and B. typhosus, and from the 8th, B. typhosus alone. He does not mention

whether or not these patients were females. Connell describes the occurrence in typhoid urines of "colon bacilli, proteus, hay bacilli (?), and mixed bacilli." One of the cases of colon bacilluria in a male was chronic. In every other instance, he says, he was able to satisfy himself that the colon, proteus, and mixed bacilli were contaminations, and that their presence was traceable to previous catheterization. Jacobi[36] obtained B. coli from the urine of several out of 30 cases of enteric fever, but does not say whether the patients were male or female.

Personal experiences of typhoid bacilluria.

My experiences of the phenomena of typhoid bacilluria agreed in the main with those which have been quoted.

58 male patients were observed throughout the course of an attack of enteric fever, and bacilluria visible to the naked eye was found to occur in 17 cases. The bacilli were isolated from the urine according to the method described on page 7, and were subjected to the routine tests, with results which are set forth later.

In every case bacilluria occurred as a casual phenomenon, and was unassociated with symptoms. A rise of temperature to 100°F took place in one patient on the first 2 evenings on which bacilli were present in the urine, but

in no other case was there pyrexia, or other disturbance attributable to this cause. The features of all the cases were the same. Without warning and without apparent cause, bacilli suddenly appeared in the urine, and produced a turbidity corresponding to, roughly, the presence of from 150,000,000 to 500,000,000 per c.c. This turbidity sometimes persisted for days, sometimes disappeared spontaneously, or under treatment, and sometimes reappeared as suddenly as it had originally come, but in no instance became chronic.

The bacilli appeared first in the urine at the following times :-

Time of first appearance of bacilluria

Time	No. of cases	Time	No. of cases
1st week of pyrexia	0	3rd week before apyrexia	2
2nd " " "	1	2nd " " "	3
3rd " " "	3	1st " " "	6
4th " " "	5	1st " of convalesence	2
5th " " "	1	2nd " " "	3
.....		3rd " " "	1
8th week of pyrexia	1	Total :-	17
1st " " convalesence	2		
2nd " " "	3		
3rd " " "	1		
Total :-	17		

The earliest cases occurred on the 10th day of pyrexia and the latest on the 16th day of convalescence.

Pus was present with the bacilli in 2 cases only, and a little albumin in these 2 and in 2 others. In another case, a little pus appeared for a day sometime after the bacilluria was established. The diazo reaction was positive in only 2 instances, the first and the third of the series.

With regard to the duration of bacilluria, some of these cases were notable for the quite short time during which it was present. In 3 cases it lasted for 1 day only, in 3 for 2 days, and in 1 for 3 days. In these 7 patients the condition passed off without treatment, and did not recur. In another case it disappeared on the 2nd day, and recurring on the 3rd day, persisted until urotropin was given on the 10th day. In the other cases the urine cleared up after the administration of from 20 to 50 grains of urotropin by the mouth, within 36 hours of the institution of the treatment, bacilluria having lasted for periods varying from 2 to 15 days. It recurred in one patient for 2 days, in one for 3 days, and in a third for 1 day on 3 occasions, but in all these, disappeared without any drug treatment.

The reaction of the urine was estimated in the case of M.B. from the 30th till the 50th day. (See appendix E, case 1). The primary pyrexia here lasted for 27 days, and the temperature was elevated during a relapse from the 35th till the 55th day. Bacilluria appeared first on the 10th day and persisted till urotropin was given on the 25th. Thereafter it was present on 3 single days, - 30th, 37th, and 46th. It is to be observed that on these days the reaction of the urine* was $\frac{N}{22}$, $\frac{N}{25}$, and $\frac{N}{33}$. On other days when the reaction of the urine was less acid, and presumably more suitable for growth, they did not appear.

It must be said that the explanations of the occurrence of bacilluria offered by the writers on the subject are unconvincing. Where abscesses have formed in the kidney it is reasonable to suppose that the bacilli come from these abscesses, but pus cells also would necessarily be present in the urine. In one of my patients who died, multiple small abscesses, from which B. typhosus was obtained in pure culture, were present in the left kidney, and a small amount of pus, which also gave a pure growth of B. typhosus, had collected in a dilatation of the ureter, just before its entrance into the bladder. Pus

---

* Phenol-phthalein was the indicator used.

was present in the urine in considerable quantity. The ordinary cases of bacilluria, however, in which no pus was present in the urine cannot be explained on the assumption that such a pathological condition exists in the kidney.

As has been pointed out (pages 55-88) the results of agglutination tests by artificial sera are in favour of the idea that the bacilli in the urine are derived from the blood, and do not pass directly from the intestine to the bladder.

The evidence brought forward for infection of the bladder alone, without involvement of the kidney, is scanty, but so far as it goes, it shows that this may sometimes occur.

If, as is supposed, bacilluria disappears by the washing of the bacilli out of the bladder, then the view cannot at the same time be held that the organisms enter the urine early in the disease, and remain there for days without multiplying, until the reaction of the urine becomes suitable for their growth. In such a case, these organisms would be washed out in the same way.

However the infection occurs, it is certain that growth does not take place in the urine in the simple way described. A patient often passes every 4 hours for days, urine of such turbidity from the presence of bacilli as can be obtained by growth in a fluid medium only after 15-18

hours' incubation. It is difficult to obtain a growth of more than 750,000,000 bacilli per c.c. in bouillon or urine at 37°C in 36 hours, and urine with 400,000,000 in each c.c. is often passed in cases of bacilluria.

A more feasible explanation is that the bacilli grow on the wall of the bladder, as they would on a solid culture medium, and that some are constantly washed off into the urine. This conception of their growth on the mucous membrane is compatible with their growing only in contact with urine of a suitable acidity.

SECTION II.

The occurrence of atypical bacilli in the urine in enteric fever

The 17 bacilli isolated from the urine of male patients were examined by the methods of growth and fermentation which were applied to all the bacilli with which experiments were made. Of these 17, 11 showed the following characteristics, and were therefore regarded as typhoid bacilli :-

production of acid, without gas, in glucose, maltose and mannite :

production of slight permanent acidity, without clotting, in litmus milk :

non-fermentation of lactose and saccharose :

non-production of indol in peptone water after 7 days' growth :

non-liquefaction of gelatin :

colourless growth on potato :

presence of motility.

8 of these 11 typhoid bacilli formed a section of the 46 bacilli which were investigated with regard to their agglutinability, and tested by means of acids. The results of this enquiry have already been described and discussed in

Part I.

The remaining 6 bacilli showed the reactions which we give below :-[*]

TABLE XXIV.

Reactions of atypical bacilli (Feb. 1912)

Time of growth 10 days.

	Glucose	Maltose	Mannite	Lactose	Saccharose	Litmus Milk 1 day.	3 days.	15 days.	Lique. of gelatin.	Indol in peptone water.	Motility
B.typhosus	A	A	A	-	-	A	A	A	-	-	+
1. W.B.	A	A	+	-	-	A	A	Alk.	-	-	+
2. W.C.	A	A	A	-	-	A	A	Alk.	-	-	+
3. A.R.	A	A	+	-	-	A	A	Alk.	-	-	+
4. R.S.	A	A	A	-	-	A	A	Alk.	-	-	?
5. C.D.	A	A	+	A	+	A	A	A.C.	-	+	+
6. P.M.	A	A	+	A	A	A.C.	A.C.	A.C.	-	+	+

(all gas formation slight)

A = formation of acid.

\+ = " " " and gas.

C = " " clot.

With the exception of C.D. they all formed an abundant brownish yellow growth on potato. The bacillus C.D. grew on

---

[*] For the fermentation tests, Durham's tubes were used, with litmus as an indicator. 1% of the various sugars (in the case of glucose .5%) was dissolved in peptone water.

potato as a translucent streak, like B. typhosus.

On the modified Endo medium used, they all formed in 24 hours colourless translucent colonies, 1-1.5 m.m. in diameter. In the case of C.D. and P.M. these colonies within 36 hours showed a red centre, and within 48 hours, a red halo was appearing in the surrounding medium.

The 6 bacilli were considered to belong to 2 types

(1) a type not fermenting lactose and saccharose, forming alkali in milk, and showing abundant brownish yellow growth on potato.

(2) A type fermenting lactose, forming acid at least in saccharose, and acid and clot in milk.

The bacilli W.B., W.C., A.R., and R.S. were classified under the first type, the differences among them being slight. W.B. produced a little gas as well as acid, in mannite, and R.S. was of doubtful motility.

The bacilli C.D. and P.M. were attributed to the second type. C.D. produced a bubble of gas in saccharose as well as acid, and took several days to clot milk, while P.M. brought about coagulation of milk within 24 hours. They differed as regards their growth on potato, but this is now usually considered to be an unreliable criterion. (MacConkey[37])

Type 1 resembled most nearly the paracolon bacillus which forms acid and gas in glucose and maltose, and does not ferment lactose and saccharose. The bacilli of Type 2 were thought to be allied to the B. coli A group, which produces acid and gas in glucose, maltose, lactose, and saccharose. None of the 6 bacilli, however, was typical, for gas production was commonly absent, and where present, was slight in amount. It is to be observed that the bacilli of Type 1 showed the same reactions as B. typhosus in tests with glucose, maltose, lactose, saccharose, gelatin and peptone water, while differing markedly in litmus milk and on potato. It is possible that such bacilli in enteric fever urines have previously been overlooked through the application of tests insufficient to ensure identification. The use of litmus milk as a test medium for bacilli from the urine seems advisable.

Agglutination reactions of the atypical bacilli.

The agglutination reactions of these 6 atypical bacilli were investigated by the same methods as the typhoid bacilli isolated -

I. by antisera,

II. by the serum of the patient, and

III. by acid solutions.

I. Agglutination of the atypical bacilli by antisera.

With the 5 antisera agglutination was absent or slight.

(a) With the antityphoid serum, C.D. showed the agglutination limit at 1:80, P.M. at 1:50, and the others were not agglutinated at 1:25.

(b) With the antiparatyphoid A (Brion-Kayser) serum, P.M. was agglutinated to 1:100, W.B. and R.S. to 1:25, and the others were not agglutinated at 1:25.

(c) With the antiparatyphoid A (Schottmüller) serum, W.C. was agglutinated to 1:50, W.B., C.D., and P.M. to 1:25, and the 2 others were unaffected at 1:25.

(d) With the antiparatyphoid B (Schottmüller) serum, P.M. was agglutinated to 1:60, W.B. at 1:25, and the others not at 1:25.

(e) With the antiparatyphoid B (Achard) serum, P.M. was agglutinated to 1:250, C.D. to 1:100, and the others were not affected at 1:25

It will be seen that the extent of agglutination throughout was trifling.

P.M. showed most agglutination of the 6, and was acted on by all the antisera, the limit of 1:250 to which it was agglutinated by the anti-Achard B serum being the highest of any. A.R. was unaffected by any of the sera. R.S. and W.C. were agglutinated each by 1, and W.B. and C.D. by 3. It will be noted that P.M. and C.D., the bacilli which least resembled B. typhosus in the fermentation tests, and the only 2 which formed indol, showed most agglutination.

II. Agglutination of the atypical bacilli by patient's serum.

5 of the bacilli (the exception being C.D.) were tested with the serum of the patient from whose urine they were isolated. The results were very different from those with the artificial sera.

(a) The bacillus W.B. which was unaffected by the anti-typhoid serum at 1:25 was agglutinated by the patient W.B.'s serum to a limit of 1:6400, whereas the stock typhoid bacillus was agglutinated less well (to 1:3000)

(b) The bacillus R.S., which was not agglutinated by the antityphoid serum at 1:25, was agglutinated by the patient R.S.'s serum to 1:600 (limit with stock typhoid bacillus, 1:1800)

(c) The bacillus P.M., which was agglutinated to 1:50 with the antityphoid serum, was agglutinated by P.M.'s serum to 1:200 (limit with stock typhoid bacillus 1:1200)

(d) The bacillus W.C., which was unaffected by the anti-typhoid serum at 1:25, was agglutinated by W.C.'s serum to 1:70 (limit with stock typhoid bacillus 1:500)

(e) The bacillus A.R., which was unaffected by the anti-typhoid serum at 1:25, was agglutinated by A.R.'s serum to 1:50 (limit with stock typhoid bacillus 1:800)

TABLE XXV

Agglutination of the atypical bacilli

Name	Day of test.	Days after iso.of bac. when tested.	Limit with antityphoid serum.	Limit with patient's serum.	Limit of agg.of stock bac with patient's serum.
W.B.	30th	2	No agg.	1:6400	1:3000
R.S.	32nd	2	" "	1:600	1:1800
P.M.	37th of normal temp.	32	1:50	1:200	1:1200
W.C.	34th " "	18	No agg.	1:70	1:500
A.R.	42nd " "	32	" "	1:50	1:800

The agglutination of these bacilli from the urine with the patient's serum and not with the antityphoid serum would seem to point either to some relationship between the bacillus from the urine and the typhoid bacillus which caused the fever, or to a double immunization, both with typhoid and this bacillus. The bacilli, however, were isolated on the first day of their appearance in the urine, and in the 2 instances in which the serum reaction was most active, the test was carried out on the second day after the bacillus first appeared. As agglutininins on organisms are not produced to any extent until the 6th day, it is evident that if a double immunization took place, the bacilli which appeared in the urine must for at least some days previously have been exercising an immunizing influence on the patient.

III. Agglutination of the atypical bacilli by acid solutions (Michaelis)

In Michaelis' acid test 2 of the bacilli showed agglutination.

P.M. was slightly agglutinated in tube 3 ("typhoid" type) and R.S. showed agglutination in tubes 2 to 6, agglutination being complete in 5 and 6. The latter bacillus was the only one tested which gave any reaction in tube 2. The 4 other bacilli were unaffected.

TABLE XXVI.

Agglutination of the atypical bacilli by acid solutions (Michaelis)

(Complete agglutination is represented by 10.)

Name.	Agglutination in tubes. 1	2	3	4	5	6
1. W.B.	-	-	-	-	-	-
2. N.C.	-	-	-	-	-	-
3. N.R..	-	-	-	-	-	-
4. R.S.	-	5	7	8	10	10
5. C.D.	-	-	-	-	-	-
6. P.M.	-	-	1	-	-	-

Remarks on the cases in which the artificial bacilli were found.

The cases in which these bacilli were found in the urine presented no points of difference from the ordinary type of typhoid bacilluria. It is to be noted, however, that the bacilli appeared in the urine rather later in the disease than in the cases of typhoid bacilluria (see Table III "Cultures from urine" page 18).

In the earliest cases (P.M.) the bacilli appeared on the 21st day, but the temperature had then been normal for 5 days. In the others, bacilluria occurred respectively on the 28th day (1st of normal temperature - W.B.), 30th day (last but one of pyrexia - R.S.), 38th day (6th of normal temperature - A.R.), 49th day (16th of normal temperature -

W.C.), and the 57th day (10th of normal temperature - C.D.)

The bacilluria was commonly of short duration, lasting in 3 instances 1 day (W.B., A.R., P.M.), in 1 instance 2 days (C.D.), in one 3 days (W.C.), and in the 6th instance(R.S.) lasting for 10 days with a day's intermission on the 2nd day, until it disappeared permanently after the administration of urotropin.

As the facts tabulated below will show, there was no doubt that the patients from whose urine these 6 bacilli were isolated suffered from enteric fever, although the cultures from blood and faeces were negative, except in the case of the blood of W.B.

Facts to show that the patients from whose urine atypical bacilli were isolated suffered from enteric fever.

1. W.B. Pyrexia lasted 28 days.
Rose-spots present.
Pulse comparatively slow (Temp.103.6° pulse rate 84)
Diarrhoea present.
B. typhosus grown from blood.
Widal reaction + (limit 1:3000)

2. W.C. Pyrexia lasted 33 days.
Rose-spots present.

2. W.C. (Contd.) Pulse comparatively slow (temp.101.6°, pulse rate 84)
Spleen palpable.
Widal reaction + (limit 1:500)

3. A.R. Pyrexia lasted 32 days.
Rose-spots present.
Pulse comparatively slow (temp.103.4°, pulse rate 96)
Diarrhoea present.
Widal reaction + (Limit 1:800)

4. R.S. Pyrexia lasted 33 days.
Rose-spots present.
Pulse comparatively slow (temp.103.6°, pulse rate 92)
Diarrhoea present.
Widal reaction + (limit 1:1800)

5. C.D. Pyrexia lasted 47 days.
Pulse comparatively slow (temp.102.2°, pulse rate 84)
Diarrhoea present with peasoupy motions.
Spleen enlarged.
Widal reaction +

6. P.M. Pyrexia lasted 16 days.
Rose-spots present.
Pulse comparatively slow (temp.103.8°, pulse rate 94)
Widal reaction + (limit 1:1200)

SECTION III.

Further experiments on the atypical bacilli.

The fermentation and other tests to which the atypical bacilli were subjected were carried out after their isolation between November 1911 and February 1912. They were kept in an ice-cupboard for a year on agar slopes at a temperature of from 5° to 7° C. and were sub-cultured at most twice. They were examined again subsequently to February 1913, when the range of tests was extended and the organisms grown in various other fermentable substances. The final results as shown in Table XXVII, together with the results of the tests with B. typhosus in the same media.

The bacilli showed certain features in common. They were all Gram negative; all formed acid in glucose, and acid and gas in maltose, mannite, saccharose, galactose, laevulose, rhamnose, glycerin and inulin. B. typhosus does not ferment saccharose, rhamnose or glycerin, and produces no gas in any of the media employed. All 6 failed to ferment erythrite, adonite, dulcite, and dextrin, none liquefied gelatin, and Vosges and Proskauer's reaction* was negative in each case.

* For this test, the organisms are grown for 3 days in glucose-peptone-water. A solution of caustic potash is added, and the tube allowed to stand at room temperature for 24 hours. If the reaction is positive, a fluorescent appearance is produced, resembling that of a weak alcoholic solution of eosin.

TABLE

Reactions of the

	Glucose	Maltose	Mannite	Lactose	Saccharose	Galactose	Laevulose	Arabinose	Rhamnose	Raffinose	Erythrite	Adonite	Dulcite	Glycerin(1%)	Inulin	Amygdalin	Dextrin
B. typhosus	A	A	A	-	-	A	A	A	-	-	-	-	-	-	A	-	-
W.B.	A	+	+	-	+	+	+	+	+	-	-	-	-	+	+	-	-
W.C.	A	+	+	-	+	+	+	+	+	-	-	-	-	+	+	-	-
A.R.	A	+	+	-	+	+	+	+	+	-	-	-	-	+	+	-	-
R.S.	A	+	+	-	+	+	+	-	+	-	-	-	-	+	+	-	-
C.D.	A	+	+	-	+	+	±	+	+	-	-	-	-	+	+	-	-
P.M.	A	+	+	+	+	+	+	+	+	+	-	-	-	+	+	A	-

A = formation of acid, or in the last column, presence of motility.

\+ = formation of acid and gas.

C = " " " or clot.

Alk. = " " " alkali.

XXVII

atypical bacilli.

Litmus milk.			Indol in peptone water.	Lique. of gelatin.	Vosges & Proskauer's reaction.	Gram's stain.	Motility.
1 day.	3 days.	15 days.					
A	A	A	-	-	-	-	+
A	A	Alk.	-	-	-	-	+
A	A	Alk.	-	-	-	-	+
A	A	Alk	-	-	-	-	+
A	A	Alk	-	-	-	-	-
A	A	Alk.	-	-	-	-	+
A.C.	A.C.	A.C.	+	-	-	-	+

P.M. was distinctly in a class by itself. It alone fermented lactose, raffinose, and amygdalin, forming acid and gas in the 2 former, and acid in the last; and it was the only bacillus to produce indol, and to form acid and clot in litmus milk.

The 5 other bacilli showed that they were closely allied to one another, and 4, W.B., W.C., A.R., and C.D. gave identical results in all the tests used. Perhaps the most characteristic feature of the 5 was the production of alkali in litmus milk after a few days' growth. This sharply distinguished them from the typhoid group, which produces permanent acidity in this medium, and from P.M., which produced acid and clot.

As has been said, W.B., W.C., A.R., and C.D. ultimately gave the same results. In the first culture in inulin, however, C.D. formed acid without gas, but in a sub-culture from this, produced also gas. W. C. at first formed acid without gas in galactose and laevulose, but when a fresh culture was made from the agar tube a month later, gas also was produced. Similarly at first A.R. produced no gas in saccharose, but in a fresh culture after 2 months produced gas as well as acid. R.S. showed a greater divergence. It was non-motile, and did not ferment arabinose, in which all the others produced acid and gas. A subsequent culture a month later yielded the same result.

The result of the tests in 1913 yielded important

important differences from those carried out a year earlier, and showed that the bacilli were in a somewhat unstable condition as regards fermentative powers.

The change in the characters of the bacillus C.D. is difficult of explanation except on the supposition that originally it included 2 strains, one resembling P.M. and the other, the 4 other bacilli, and that the former strain died out. Originally C.D. fermented lactose, formed indol, and produced acid and clot in milk. A year later it did not ferment lactose, did not produce indol, and formed alkali in milk.

The changes in the other bacilli were all in the direction of greater fermentative powers, though they had been cultivated simply on ordinary agar. Whereas at first they all formed acid only in maltose, a year later gas as well as acid resulted from their growth. All now also produced gas as well as acid in mannite, whereas formerly W.C. and R.S. produced only acid. A marked change took place with regard to their action on saccharose.

In the original tests in saccharose (1912) P.M. produced acid, C.D. acid with a bubble of gas, while the other bacilli left the sugar unchanged. When the tests were repeated in 1913, P.M. produced gas as well as acid, and C.D. and R.S. produced acid. Gas production with P.M.

and acid production with R.S. were new characters, and therefore successive sub-cultures were made with all the bacilli to test whether further action on saccharose resulted. A 2nd series of sub-cultures was made from the 1st at the end of 10 days, and a 3rd from the 2nd at the end of a further 10 days.

W.B. and W.C. in the 1st sub-culture were unchanged; in the 2nd they formed acid, and in the 3rd acid and gas.

C.D. and R.S. in the 1st sub-culture formed acid, and in the 2nd acid and gas.

A.R. in the 1st sub-culture was unchanged, and in the 2nd, 3rd, and 4th formed acid, without gas.

The bacillus A.R. was kept for 2 months longer on agar at 5^{0}-6^{0}, and at the end of that time was found to produce gas as well as acid in the first sub-culture.

It was found also that acid tended to be produced more rapidly and gas in greater volume by all the organisms with successive sub-cultures. For instance R.S. in the first sub-culture produced acid at the end of 10 days, in the 2nd acid and some gas at the end of 4 days, and in the 3rd, acid after 2 days, and subsequently a larger volume of gas

These results are shown in the subjoined table:-

TABLE XXVIII.

Reaction of the atypical bacilli in saccharose.

(10 days' growth at 37°C)

	Feb.1912	February, 1913.				April,1913
	1st sub-culture.	1st sub-culture.	2nd sub-culture (after 10 days)	3rd sub-culture (after 10 days)	4th sub-culture (after 10 days)	1st sub-culture
1.W.B.	-	-	A	+		
2.W.C.	-	-	A	+		
3.A.R.	-	-	A	A	A	+
4.R.S.	-	A	+	+b		
5.C.D.	+a	A	+	+b		
6.P.M.	A	+	+b	+b		

A = formation of acid.

\+ = " " " and gas.

a = gas formation very slight.

b = more gas than in previous subculture.

In the tests with glycerin (1% in peptone water) an increase of fermenting power developed in the 2nd sub-culture. At the end of 5 days no change was visible in the colour of the litmus with any of the bacilli. After 10

days, however, all except P.M. showed a tendency to form acid, the litmus having become of a purple tint. In the next subculture (made from the 1st) all except P.M. produced slight acid and a little gas. P.M. showed no tendency to bring about fermentation. In the 3rd subculture, however, P.M. produced slight acid and a little gas at the end of 10 days.

The results of the tests with glycerin are shown in Table XXIX.

TABLE XXIX.

Atypical bacilli in glycerin (1% in peptone water)

(10 days' growth at 37°C)

	1st sub-culture.	2nd sub-culture.	3rd sub-culture
B.typhosus	-	-	-
W.B.	A (slight)	+	+
W.C.	A "	+	+
A.R.	A "	+	+
R.S.	A	+	+
C.D.	A (slight)	+	+
P.M.	-	-	+

As has been mentioned, the bacilli were considered at first to belong to 2 types -

(1) one resembling B.paracoli

(2) one resembling B. coli A.

The power which the bacillus P.M. developed of forming gas as well as acid in saccharose brought it into conformity with the latter type, which, according to Wulff[38], produces acid and gas in lactose, glucose, maltose, and saccharose, and also in galactose, xylose and mannite. He differentiates several subgroups of which 2 do not ferment glycerin, adonite or dulcite. P.M. at first corresponded to these subgroups, but was easily trained to ferment glycerin.

As regard the other bacilli, their development of the power to ferment saccharose made their classification as paracolon bacilli doubtful, as B. paracoli is not a saccharose fermenter. If they were paracolon bacilli, then they were developing new characteristics. The possibility that they were modified typhoid bacilli is unlikely, but their sudden appearance in large number in typhoid urines was in any case a curious phenomenon. If they were not altered typhoid bacilli, then it is practically certain that their origin was the intestine, and the fact that they were agglutinated by the patient's serum, though not by the antityphoid serum, seemed to point to an immunizing action of the bacilli themselves or their toxins on the body.

The question of changes in the character of micro-organisms is an important one, and the work on the subject within the last few years has altered our views with regard

to the distinctions which exist among species of bacteria.

In 1906 Massini[39] obtained a bacillus from a case of enteritis which formed colourless colonies, like B. typhosus, on Endo's medium (lactose-sulphite-fuchsin-agar). On the 3rd day of incubation, however, small red papillae appeared in the originally colourless colonies. Cultures made from these papillae fermented lactose rapidly, like B. coli, while cultures from the unstained parts of the original colonies produced similar unstained colonies, which also on the 3rd day showed red papillae. Two types of bacilli were thus produced, a lactose-fermenting type, which in sub-culture retained this characteristic, and could not be made to lose it, and a non-lactose-fermenting type, which tended constantly in sub-culture to produce both races. Massini called this bacillus B. coli mutabile.

Twort[40] showed that some typhoid-coli organisms acquired the power of fermenting certain sugars by long growth in them. For instance, he trained B. typhosus to ferment lactose and dulcite.

R. Müller[41] showed that B. typhosus behaved towards rhamnose as B. coli mutabile towards lactose, and considered this to be the surest cultural method of recognising B. typhosus.

Thaysen[42] isolated 8 races of typhoid-coli bacilli.- 4 of these fermented dextrose, maltose, and lactose, but not saccharose. They could, however, be trained to ferment saccharose. One fermented dextrose and maltose, not saccharose or lactose, but it came to ferment also the latter, and thus resembled B. coli mutabile. Two others resembled the preceding,but acquired the power of fermenting saccharose. The 8th fermented dextrose, maltose, and saccharose, but not lactose. It was trained to ferment lactose.

Burri[43] isolated a race of organisms which did not ferment lactose or saccharose, but which acquired the power of fermenting the latter. To this mutant he gave the name B. perfectum.

Arkwright[44] isolated a bacillus of the B. acidi lactici group several times from the urine of an old man. This organism he found to exist in 2 varieties,which differed only as regards gas formation, the first forming acid and gas from certain sugars and alcohols, the second only acid. The two varieties gave identical serum reactions, and that which did not produce gas was induced to do so by preliminary growth in a medium containing sodium formate.

It will be seen from these references that bacilli have sometimes been trained to ferment certain sugars by

long growth in them. Twort's experiments were of this nature, and some of the changes which took place in my 6 bacilli. The changes in the latter were of 2 kinds

(1) those which took place as a result of training and

(2) those which occurred spontaneously. Of the former kind were the power of A.R. to form acid and of W.B. and W.C., acid and gas in saccharose; C.D. to form gas in inulin; and of P.M. to form acid and gas in glycerin. But it is to be noted that the production by all of gas in maltose, by W.C. and R.S. of gas in mannite, by P.M. of gas in saccharose, and by R.S. of acid in saccharose were new characteristics which developed during the year the bacilli were stored on agar slopes at a temperature of 5°-7°C, and without any contact with the sugars. The bacillus A.R. also, which failed to produce gas in saccharose in a series of 4 sub-cultures made at intervals of 10 days, developed this power when kept for 2 months longer on agar at a temperature of about 6°C, forming gas as well as acid in the first sub-culture. Similarly W.C. acquired the power of forming gas in addition to acid in galactose and laevulose when left for another month at 6°C.

Reference has been made (see page 95) to the development of agglutinability by a non-agglutinable typhoid bacillus kept without sub-culture for 3 months (Lipschutz[19] and others); but change in fermentative properties has

usually been recorded as occurring after a course of 'training'. Sørensen, however, has described a case of glycosuria in which an organism B. pneumaturiae, was isolated from the urine. This bacillus produced gas in the bladder and also in artificial culture in media containing glucose, lactose and saccharose. After 2 years, gas ceased to be produced in the bladder, and it was found that the organism had lost the power of forming gas in sugar-containing media. A year later the bacillus in culture suddenly re-acquired the power of forming gas, and shortly afterwards the patient began to suffer again from pneumaturia. These changes took place spontaneously.

While it is unlikely that the atypical bacilli which I have described are altered typhoid bacilli, a mutation form of B. typhosus has been described. In 1907 Mandelbaum[46] isolated from the faeces of a typhoid-carrier a bacillus closely allied to B. typhosus, while he called B. metatyphi.. Between 1907 and 1912 he obtained it 50 times from blood and faeces.[47] This organism differed from B. typhosus chiefly in forming acid in the presence of glycerin, instead of alkali, and was considered by Mandelbaum to be a mutation form of B. typhosus produced by growth in the body. He found that on glycerin-agar plates, some colonies of B. metatyphi produced papillae of typical B. typhosus.

Morphological changes were brought about in typhoid bacilli by Almquist[48] who cultivated them in various decaying materials such as watery extracts of dung. Growth was allowed to take place for some weeks at room temperature, and when sub-cultures were made on agar and grown for a week, a production of spore forms was found to have taken place. These spores became typhoid bacilli after 2-6 hours' incubation in a fresh sub-culture. Almquist does not mention whether or not any change occurred in the fermentative powers of the bacilli. This experiment suggests the possibility of alteration in micro-organisms by growth in the faeces.

SECTION IV.

A note on bacilluria in female patients with enteric fever.

The 17 cases of bacilluria which have been described occurred in men. I had afterwards an opportunity of examining the urine of a few women suffering from enteric fever.

Bacilli were found to be present in large numbers in the urine of 7 women, the physical characters of the urine being such as have been described for typhoid bacilluria. In 5 cases bacilli were present on the day in convalescence on which the urine was first examined. In the 6th case bacilluria was known to exist at the onset of the fever, and in the 7th case, (M.A.), the bacilli appeared first in the urine on the 27th day.

In the first 6 instances the bacilli, which were motile, formed acid and gas in glucose, maltose, mannite, and lactose; acid and clot in litmus milk; indol in peptone water, and a yellowish brown growth on potato, They did not ferment saccharose nor liquefy gelatin. These are the reactions of a typical B. coli.

In the 7th instance bacilli appeared first in the urine on the 27th day (6th of normal temperature). This patient was recovering from a severe attack of enteric fever, the Widal reaction was positive, and B. typhosus

had been grown from the blood of her daughter who was ill at the same time. The bacilli were motile, and fulfilled the routine tests which have already been described for B. typhosus. With the bacilli were found cocci which occurred in pairs and short chains. The bacteriuria passed off in 2 days without treatment.

The contrast between the results of cultures from male and from female urines is striking. In 17 cases of bacilluria in men who suffered from enteric fever, B. coli did not occur; in 7 cases in women, the micro-organism isolated in 6 was B. coli.

RECAPITULATION OF RESULTS.

(1) Typhoid bacilli were isolated from a series of patients suffering from enteric fever, chiefly from blood, faeces, urine, and (post-mortem) the spleen. These were identified by fermentative and other reactions, and were then submitted to agglutination tests. In most instances also the blood of the patient from whom a bacillus was obtained was examined for the presence of agglutinins.

(2) The serum of every patient from whom a typhoid bacillus was obtained agglutinated the stock typhoid bacillus well. In the tests with the sera of patients and the stock typhoid and paratyphoid bacilli, group agglutination (that is, agglutination of allied, here paratyphoid, organisms) was found to be present in almost every case: but the serum of a person artificially immunized by inoculation with dead typhoid bacilli did not at first agglutinate any of the paratyphoid organisms. After a few months, slight group agglutination was present. This artificial serum was much more active than any obtained from 50 cases of enteric fever.

(3) In the tests with artificially prepared antityphoid

and antiparatyphoid sera, and typhoid bacilli isolated from the body, the bacilli which were isolated earlier in the disease tended to be agglutinated better than those obtained later. The bacilli isolated from faeces were agglutinated much better by antityphoid, and somewhat better by antiparatyphoid serum than those grown from the blood. The bacilli isolated from urine resembled those grown from blood rather than those from faeces.

(4) In the tests with the bacilli and the serum of the patient from whom each was obtained, no connection could be made out between the time of isolation and the degree of agglutination present. The bacilli from blood were agglutinated very well, and much better than those from faeces. The bacilli from faeces were agglutinated rather less well than by antityphoid serum.

(5) Examination of the bacilli by acid solutions of varying strengths according to the method of Michaelis gave unsatisfactory results, only half of them showing the supposed 'typhoid reaction'. Such uncertain results made the test of little value in the recognition of the typhoid bacillus, and its differentiation from other organisms.

(6) It was found during the investigation of bacilluria in men suffering from enteric fever, that in 6 cases out of 17, the bacilli were not typhoid bacilli, but members of the typhoid coli group, not previously described. It has always been supposed that the organisms present in 'typhoid' bacilluria are typhoid bacilli, or some contaminating organism, such as B. coli. B. coli did not occur in any of the 17 cases. In 7 instances of bacilluria in women with enteric fever, on the other hand, the bacillus present was found in all but one to be B. coli.

(7) These atypical bacilli appeared in the urine on the average rather after the usual time for typhoid bacilluria, and persisted for quite a short time, in 3 instances for 1 day only. Their appearance was not attended by any constitutional disturbance, and in general, the cases resembled the ordinary cases of typhoid bacilluria.

(8) The bacilli, though practically unaffected by the artificial antityphoid serum, were agglutinated in varying degrees by the serum of the patient from whom each was isolated, in one case to a greater extent than was the stock typhoid bacillus by the same serum.

(9) The 6 bacilli were stored on agar slopes at a temperature of about 6°C, and on re-examination a year later were found to have acquired greater fermentative powers with regard to certain sugars. This property has usually been described as developing by continued growth and sub-culture of an organism in a solution of the sugar, but with these bacilli the power, though afterwards increased by sub-culture, developed in many instances spontaneously.

REFERENCES.

1. KUHNAU. quoted by Baumann and Rimpau. Centralbl. f. Bakt. I Abt. Orig. (1908) Vol. XLVII, page 136.

2. CASTELLANI Centralbl. f. Bakt. I Abt. Orig. Vol. XXXI p. 477.

3. CONRADI Münchn, med. Wehnschr. (1906) p.1654.

4. KAYSER quoted by Baumann and Rimpau. ap. loc. cit.

5. KENDALL & DAY. Journal of Medical Research (1911) Vol. 25. p. 95.

6. RUSSELL. Ibid. p.217.

7. BLUMER Johns Hopkins Hosp. Reports (1895) V. p.327

8. PALTAUF in Kolle & Wassermann's 'Handbuch d. path. Mikroorganismen' (1912) Vol. II. p.502 et seq.

9. PORGES & PRANTSCHOFF. Centralbl. f. Bakt. I Abt. Orig. (1906) vol. XLI. p.546.

10. PORGES Zeitschr. f. experiment. Path.(1905) Vol. I. p. 620.

11. PORGES & PRANTSCHOFF. Centralbl. f. Bakt. I. Abt. Orig. (1906) Vol. XLI. p.466.

12. SACQUÉPÉE Ann. Pasteur (1901) Vol. XV. p.249.

13. HORTON SMITH Lancet (1900) I. p. 821.

14. REMY. Ann. Pasteur (1901) Vol. XV. p. 145.

15. SACQUÉPÉE Ibid. p. 249.

16. CAMBIER Revue d' hygiène (1902) p. 64.

17. EMERY Ibid. p.144

18. NICOLLE & TRENEL. Ann. Pasteur (1902) Vol. XVI. p.562.

19. LIPSCHUTZ Centralbl. f. Bakt. I. Abt. Orig.(1904) Vol. XXXV, p. 798.

20. BESREDKA "Bacteriotherapie, Vaccination, Serotherapie" (1909: Paris, Balliere et fils.) p.269.

21. ARONSON Berl. klin. Wehnschr. (1902) pp.979,1006. Deutsch. med. Wehnschr. (1903) p.439.

22. MICHAELIS Deutsch. med. Wchnschr. (1911) Vol. XXXVII page 969.

23. ROST — Centralbl. f. Bakt. I. Abt. Orig.(1911) Vol. LX. p.324.

24. JAFFE — Arch. f. Hyg. Vol. LXXVI. p.1.

25. PETRUSCHKY — Centralbl. f. Bakt. I. Abt. Orig.(1898) Vol. XXIII. p.577

26. GWYN — Johns Hopkins Hosp. Bull.(1899) Vol. X. p. 109.

27. CONNELL — Amer. Journ. of the Med. Scien. (1909) Vol.CXXXVII. p. 637.

28. BUCHAN — Liverpool Medico-Chirurg. Journ.(1908) p. 166.

29. LIEBETRAU — quoted by Connell ap. loc. cit.

30. HORTON SMITH — Lancet (1900) I. p. 910.

31. KONJAJEFF — abstracted in Centralbl. f. Bakt.(1889) Vol. VI. p. 672.

32. FLEXNER — Journ. of Path. and Bact. (1896) Vol. III. p. 202.

33. BROWNLEE & CHAPMAN. — Glasgow Medical Journal (1906) Vol.LXVI. p. 407.

34. PARK — Trans. Assoc. of Amer. Phys.(1901) Vol. XVI. p. 634.

35. FOLIN — American Journ. of Phys.(1903) Vol. IX. p. 265.

36. JACOBI — Deutsch. Arch. f. klin. Med.(1902) Vol. LXXII. p. 442.

37. MACCONKEY — Journal of Hygiene(1909) Vol. IX.p.86

38. WULFF — Centralbl. f. Bakt. I. Abt. Orig.(1912) Vol. LXV. p. 27

39. MASSINI — Arch. f. Hyg. (1906) Vol. LXI. p.250.

40. TWORT — Proc. Royal Soc. B. (1907) Vol. LXXIX p. 329.

41. R. MÜLLER — Centralbl. f. Bakt. I. Abt. Orig.(1911) Vol. LVIII p. 97.

42. THAYSEN — Centralbl. f. Bakt. I. Abt. Orig.(1912) Vol. LXVII. p.1.

43. BURRI — Centralbl. f. Bakt. II. Abt. (1910) Vol. XXVIII. p. 321.

44. ARKWRIGHT — Journal of Hygiene Vol. XIII. p. 68

45. SØRENSEN Centralbl. f. Bakt. I. Abt. Orig. (1912) Vol. LXII. p.582.

46. MANDELBAUM. München. med. Wchnschr.(1907) Vol. LIV. p. 1766.

47. MANDELBAUM Centralbl. f. Bakt. I. Abt. Orig.(1912) Vol. LXIII. p.46.

48. ALMQUIST Centralbl. f. Bakt. I. Abt. Orig. Vol. XLV. p. 491.

INDEX to APPENDICES.

A P P E N D I X A.

Agglutination of B. typhosus (stock) by serum of person inoculated with dead typhoid bacilli.

Widal reactions in person inoculated with B. typhosus.

17/5/12. No agglutination of B. typhosus (Stock) at 1:25.

22/5/12. 500,000,000 bacilli injected subcutaneously.

(36 hours' growth in bouillon, killed at 53°C. 750,000,000 in 1 c.c.)

25/5/12. B.typhosus.	28/5/12 B. typhosus.	1/6/12 B.typhosus
1 : 25	-	+ +	+ + +
1 : 50		+	+ + +
1 : 100		-	+ + +
1 : 200			+ + +
1 : 400			+ + +
1 : 800			+ + +
1 :1600			+ + +
1 :3200			+ + +
1 :6400			+ + +
1 :12800			+ +
1 :25600			+
1 :51200			-

2/6/12. Vaccine 1000,000,000 bacilli.

3/6/12	B.typhosus.	5/6/12 B.typhosus.	8/6/12.B.typhosus.
1 : 25	+ + +	+ + +	+ + +
1 : 50	+ + +	+ + +	+ + +
1 :100	+ + +	+ + +	+ + +
1 :200	+ + +	+ + +	+ + +
1 :400	+ + +	+ + +	+ + +
1 :800	+ + +	+ + +	+ + +
1:1600	+ + +	+ + +	+ + +
1:3200	+ + +	+ + +	+ + +
1:6400	+ + +	+ + +	+ + +
1:12800	+ + +	+ + +	+ + +
1:25600	+ + +	+ +	+ + +
1:51200	+ + +	+	+ +
1:102400	+		+
1:204800			+
1:409600			

12/6/12.	B.typhosus.	B.paratyphosus A Br.-Ka.	B.paratyphosus A Schott.	B.paratyphosus B. Schott.	B.paratyphosus B. Achard.
1 : 25	+ + +	-	-	-	-
1 : 50	+ + +				
1 :100	+ + +				
1 :200	+ + +				
1 :400	+ + +				
1 :800	+ + +				
1:1600	+ + +				
1:3200	+ + +				
1:6400	+ + +				
1:12800	+ + +				
1:25600	+ + +				
1:57200	+ +				
1:102400	+				
1:204800	-				
24/10/12.					
1 : 25	+ + +	+	+	-	+
1 : 50	+ + +	+	-		-
1 :100	+ + +	-			
1 :200	+ +				
1 :400	+ +				
1 :800	+				
1:1600	+				
1:3200	-				

8/5/13.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott	B.paratyphosus B Schott.	B.paratyphosus B Achard
1 : 25	+ + +	+	+	+	+
1 : 50	+ + +	+	-		+
1 : 100	+ + +	-			-
1 : 200	+ +				
1 : 400	+ +				
1 : 800	+				
1 :1600	+				
1 :3200	-				

[illegible] 3.

[illegible] agglutination of

[illegible] and subtenuous bacilli

[illegible] patients' sera.

Bac.
coli from [illegible] B. paratyphosus
(cine) blood. cent. [illegible]

A P P E N D I X B.

Tables showing agglutination of stock bacilli and autogenous bacilli by patients' sera.

1. J.H.

Day of test.	Serum dilution.	B.coli (urine)	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B. paratyphosus B Schott.	B. paratyphosus B Achard
4th	1 : 25			-	-	-	-	-
7th	1 : 25		+ +	+ + +	+ + +	+ + +	+ + +	+ + +
	1 : 50		+ +	+ +	+ + +	+ + +	+ + +	+ + +
	1 :100		+	+	+ + +	+ +	+ +	+ +
	1 :200		+	-	+ +	+	+ +	+
	1 :400		-		+	+	+	-
	1 :800				+	-	-	
	1:1600				+			
	1:3200				-			
8th	1 : 25	+	+ +	+ + +	+ + +	+ + +	+ + +	+ + +
	1 : 50	+	+	+ +	+ + +	+ +	+ + +	+ +
	1 :100	-	+	+	+ + +	+ +	+ +	+
	1 :200		-	-	+ +	+	+	-
	1 :400				+	-	-	
	1 :800				+			
	1:1600				-			
9th	1 : 25			+ + +				
	1 : 50			+ +				
	1 :100			+				
	1 :200			+				
	1 :400			-				

1. J.H. (Contd)

Day of test.	Serum dilution.	B.coli (urine)	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka. Schott.	B.paratyphosus B. Schott.Achard
10th	1:25			+ + +		
	1:50			+ + +		
	1:100			+ +		
	1:200			+		
	1:400			-		
11th	1:25			+ + +		
	1:50			+ + +		
	1:100			+ +		
	1:200			+		
	1:400			+		
	1:800			-		
12th	1:25			+ + +		
	1:50			+ + +		
	1:100			+ +		
	1:200			+		
	1:400			+		
	1:800			-		

2. J.T.

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus Br-Ka.	B.paratyphosus Schott	A.B.paratyphosus Schott.	B. Achard.
8th	1:25		+ + +	+ + +	+ + +	+ + +	+ + +
	1:50		+ + +	+ + +	+	+	+ + +
	1:100		+ + +	+ +	+	-	+ +
	1:200		+ + +	+	+		-
	1:400		+ +	-	-		
	1:800		+ +				
	1:1600		+ +				
	1:3200		+				
	1:6400		-				
23rd	1:25	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	1:50	+ +	+ + +	+ + +	+	+	+ + +
	1:100	+	+ +	+ + +	-	+	+ +
	1:200	+	+	+ +		-	+
	1:400	-	-	+ +			-
	1:800			+			
	1:1600			+			
	1:3200			-			

24th Vaccine 250,000,000 (bacillus from blood)

2. J.T. (Contd)

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B. Schott.Achard
26th	.....	.....	.....			
	1:100	+ +	.....			
	1:200	+	+ +			
	1:400	+	+ +			
	1:800	-	+			
	1:1600		+			
	1:3200		+			
	1:6400		-			
28th (N.1)	.....	.....	.....			
	1:100	+ +	.....			
	1:200	+ +	.....			
	1:400	+	.....			
	1:800	+	+ +			
	1:1600	-	+ +			
	1:3200		+ +			
	1:6400		+			
	1:12800		+			
	1:25600		-			

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br.-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B. Schott.	B.paratyphosus B. Achard
N.42	1:25	+ +	+ + +	+ + +	+ +	-	+ +
	1:50	+	+ + +	+ + +	-		+ +
	1:100	-	+ + +	+ + +			-
	1:200		+ + +	+ +			
	1:400		+ +	+			
	1:800		+	+			
	1:1600		-	+			
	1:3200			+			
	1:6400			-			

3. H.M.

Day of test.	Serum dilution.	Bac. from urine.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B. Schott.	B.paratyphosus B. Achard
10th	1:25		+ + +	+ +	+	-	+ +
	1:50		+ + +	+ +	-		+ +
	1:100		+ +	+ +			+
	1:200		+ +	+			+
	1:400		+	+			-
	1:800		+	+			
	1:1600		-	+			
	1:3200			+			
22nd	1:25	+ + +	+ + +	+ +	+ +	+ +	+ + +
	1:50	+ + +	+ + +	+ +	+	+ +	+ + +
	1:100	+ + +	+ + +	+ +	+	+	+ + +
	1:200	+ +	+ + +	+ +	-	-	+ +
	1:400	+ +	+ + +	+ +			
	1:800	+ +	+ + +	+			
	1:1600	+	+ +	-			
	1:3200	+	+				
	1:6400	+	+				
	1:12800	-	+				
	1:25600		-				

4. I.E.

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B. Schott	B.paratyphosus B. Achard
13th	1:25		+ + +	+ +	+	+ +	+ + +
	1:50		+ + +	+ +	-	+ +	+ + +
	1:100		+ + +	+		+ +	+ +
	1:200		+ + +	+		+	+ +
	1:400		+ + +	-		+	+
	1:800		+ + +			-	-
	1:1600		+ + +				
	1:3200		+ +				
	1:6400		+				
	1:12800		-				

5. M.D.

12th	1:25	-	+ + +	+ +	-	+	+ + +
	1:50		+ + +	+ + +		+	+ + +
	1:100		+ + +	+ + +		-	+
	1:200		+ + +	+ +			+
	1:400		+ + +	+ +			-
	1:800		+	+			
	1:1600		+	-			
	1:3200		-				

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
13th	1:25		+ + +	+	+ +	+ + +	+ +
	1:50		+ + +	+	+	+	+
	1:100		+ + +	-	-	+	+
	1:200		+ + +			+	-
	1:400		+ + +			-	
	1:800		+ +				
	1:1600		+				
	1:3200		+				
	1:6400		-				
25th	1:25	+ + +	+ + +	+	-	+	+ +
	1:50	+ + +	+ + +	-		+	+ +
	1:100	+ + +	+ + +			-	+
	1:200	+ +	+ +				-
	1:400	+	+				
	1:800	-	-				
N.42	1:25	+ + +	+ + +	+	+	+	+
	1:50	+ +	+ + +	+ +	+	+	+
	1:100	+	+ + +	+ + +	-	+	-
	1:200	+	+ + +	+ + +			
	1:400	+	+	+ + +			
	1:800	-	-	+ +			
	1:1600			+ +			
	1:3200			+ +			
	1:6400			-			

7. E.M.

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
14th	1:25	+ + +	+ + +	+	+ +	+ + +	+ + +
	1:50	+ + +	+ + +	+ +	+ +	+ +	+ + +
	1:100	+ + +	+ + +	+ + +	+	+ +	+ + +
	1:200	+ + +	+ + +	+ +	+	+ +	+
	1:400	+ + +	+ + +	+ +	-	+	+
	1:800	+ + +	+ + +	+		-	-
	1:1600	+ + +	+ + +	+			
	1:3200	+	+ +	-			
	1:6400	+	+				
	1:12800	-	-				

8. D.M.

		Bac. from blood.					
14th	.....	.....					
	1:50		+ + +	+	-	-	+ + +
	1:100		+ + +				+ + +
	1:200		+ + +				+ + +

28th	1:25	+ + +	.....	.....			.....
	1:50	+ + +	.....	.....			+ + +
	1:100	+ + +	.....	+			+ + +
	1:200	+ + +	.....	-			+ + +
	1:400	+ +	.....				+ + +
	1:800	+ +	+ + +				+
	1:1600	+ +	+ + +				+
	1:3200	+	+ +				-
	1:6400	-	+				
	1:12800		-				

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
14th	.....						
	1:50		+ + +	+	-	-	+ + +
	1:100		+ + +	-			+ + +
	1:200		+ + +				+ + +
	.....						
28th	1:25	+ + +	.....	.....	.....		.....
	1:50	+ + +	.....	.....			+ + +
	1:100	+ + +	.....	+			+ + +
	1:200	+ + +	.....	-			+ + +
	1:400	+ +	.....				+ + +
	1:800	+ +	+ + +				+
	1:1600	+ +	+ + +				+
	1:3200	+	+ +				-
	1:6400	-	+				
	1:12800		-				
36th	.....	.....	.....				
	1:1600	+	+				
	1:3200	-	+				
	1:6400		-				

36th Vaccine 250,000,000 (bacillus from blood)

8. D.M.(Contd.)

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard
41st (N.4)	.....						
	1:200	+	.....				
	1:400	+	.....				
	1:800		.....				
	1:1600		+ +				
	1:3200		+				
	1:6400		+				
	1:12800		-				
N.7	.....						
	1:200	+	.....				
	1:400	+	.....				
	1:800	-	.....				
	1:1600		+				
	1:3200		-				
N.7	Vaccine 250,000,000 (bacillus from blood)						
N.10	.....						
	1:200	+	.....				
	1:400	+	+ + +				
	1:800	-	+ +				
	1:1600		-				

8. D.M. (Contd)

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard
N.12	.....						
	1:100	.....	.....	+ +	+ +	+	.....
	1:200	+	.....	+ +	+	+	.....
	1:400	+	+ + +	+	-	+	+ +
	1:800	-	+ + +	+		-	-
	1:1600		+ +	-			
	1:3200		+				
	1:6400		+				
	1:12800		-				
N.15	.....						
	1:200	+ +	.....				
	1:400	+	.....				
	1:800	-	.....				
	1:1600		+				
	1:3200		-				
N.19	.....						
	1:200	+ + +	.....				
	1:400	+ +	+ + +				
	1:800	+	+ +				
	1:1600	-	-				
N.39	1:25	+ + +	+ + +	+	+ +	+ +	+ + +
	1:50	+ + +	+ + +	-	+ +	+ +	+ + +
	1:100	+ +	+ + +	+ +	+ +	+ +	+
	1:200	+	+ + +	+	+	+	-
	1:400	+	+ + +	+	-	-	

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
N.39(Contd)							
	1:800	-	+ +	-			
	1:1600		+ +				
	1:3200		-				

9. A.B.

14th	1:25	+ + +	+ + +	+ +	+ + +	+ + +	+ + +
	1:50	+ + +	+ + +	+ + +	+ +	+ +	+ + +
	1:100	+ + +	+ + +	+ +	+	+ +	+ + +
	1:200	+ + +	+ + +	+ +	+	-	+ +
	1:400	+ + +	+ + +	+ +	-		+
	1:800	+ + +	+ + +	+ +			
	1:1600	+ +	+ + +	+ +			
	1:3200	+ +	+ +	-			
	1:6400	+	+				
	1:12800	+	-				
	1:25600	-					

10. A.W.

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
15th	1:25		.....	.....	.....	.....	+ + +
	1:50		+ + +	-	+	-	+
	1:100		.....		-		-
	1:200		.....				
	1:400		.....				
	1:800		+				
	1:1600		-				
23rd (N.2)	.....						
	1:100		.....		-		+ +
	1:200		.....				+
	1:400		+				-
	1:800		-				
N.42	1:25	+ +	+ + +	+	+ +	+ +	+ + +
	1:50	+ +	+ + +	+	+ +	+	+ + +
	1:100	+ +	+ +	+	+	+	+ +
	1:200	+	+	-	-	-	+
	1:400	-	-				-

11. J.R.

Day of test.	Serum dilution.	Bac. from faeces.	B. typhosus.	B. paratyphosus A. Br-Ka.	B. paratyphosus A. Schott.	B. paratyphosus B Schott.	B. paratyphosus B Achard.
15th	1:25		+ + +	+ +	+ + +	+ +	+ +
	1:50		+ + +	+	+	+ +	+
	1:100		+ + +	+	+	+ +	+
	1:200		+ +	+	-	-	-
	1:400		+ +	-			
	1:800		+ +				
	1:1600		+				
	1:3200		+				
	1:6400		-				

12. E.C.

15th	1:25	+ + +	+ + +	+ +	+ +	+ +	+ + +
	1:50	+ + +	+ + +	+ +	+ +	+	+ + +
	1:100	+ + +	+ + +	+ + +	+	+	+
	1:200	+ +	+ + +	+ + +	+	-	-
	1:400	+ +	+ +	+ +	+		
	1:800	+	+	+ +	-		
	1:1600	+	+	+			
	1:3200	-	-	+			
	1:6400			-			

13. M.F.

Day of test.	Serum dilution.	Bac. from spleen.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
15th	1:25	+ +	+ + +	-	+	+	+
	1:50	+ +	+ +	-	-	-	+
	1:100	+ +	+	+	+		-
	1:200	+	+	+	-		
	1:400	+	+	+			
	1:800	+	-	-			
	1:1600	-					

14. W.A.		Bac. (A.W.) faeces.				
15th	.....					
	1:50		+ + +	+	-	-	+
	1:100		.....				
	1:200		.....				
	1:400		.....				
	1:800		+				
	1:1600		-				
23rd	.....						
	1:100		.....	+			
	1:200		.....	-			
	1:400		.....				
	1:800		+				
	1:1600		-				

14. W.A. (Contd)

Day of test.	Serum dilution.	Bac. (A.W.) faeces.	B.typhosus.	B,paratyphosus A. Br-Ka.	B,paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
46th (N.20)	1:25	+ + +	+ + +	+	+	+	+ + +
	1:50	+ + +	+ + +	+	+	+ +	+
	1:100	+ + +	+ + +	+	+	+ +	+
	1:200	+ +	+ +	-	-	+ +	-
	1:400	+	+			+	
	1:800	+	+			+	
	1:1600	-	+			+	
	1:3200		-			-	
N.20	Vaccine 100,000,000 (bacillus from father's faeces)						
N.21	.....						
	1:400	+	+				
	1:800	+	+				
	1:1600	-	-				
N.23	.....						
	1:400	+ + +	+ + +				
	1:800	+	+				
	1:1600	-	-				
N.25	.....						
	1:400	+ +	+ + +				
	1:800	+	+				
	1:1600	-	-				
N. 27	Vaccine 100,000,000 (bacillus from father's faeces)						

Day of test.	Serum dilution.	Bac. (A.W.) faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
N.29	.....						
	1:400	+	+ +				
	1:800	-	+				
	1:1600		+				
	1:3200		-				
N.32	.....						
	1:200	+ +	.....				
	1:400	+	+ +				
	1:800	-	+				
	1:1600		-				
N.38	.....						
	1:200	+ +	.....				
	1:400	+	+ +				
	1:800	-	+				
	1:1600		+				
	1:3200		-				
N.38	Vaccine 100,000,000 (bacillus from father's faeces)						
N.40	.....						
	1:200	+ +	.....				
	1:400	+	+ +				
	1:800	-	+				
	1:1600		-				

14. W.A. (Contd)

Day of test.	Serum dilution.	Bac. (A.W.) faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott	B.paratyphosus B Schott.	B.paratyphosus B Achard.
N.42	.....						
	1:200	+ +	.....				
	1:400	+	+ +				
	1:800	-	+				
	1:1600		+				
	1:3200		-				
N.68	1:25	+ + +	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ + +	+ + +	-	+ + +	+ + +	+ +
	1:100	+ + +	+ + +	+	+ +	+ + +	+ +
	1:200	+ +	+ + +	+	+ +	+ +	-
	1:400	+ +	+ +	-	+ +	+ +	
	1:800	+	+ +		+	+	
	1:1600	-	+		-	-	
	1:3200		+				
	1:6400		-				

15. L.P.

		Bac. from urine.					
56th (N.1)	1:25	+	+ + +	+	-	+	+ + +
	1:50	-	+ +	+		+	+ +
	1:100		+ +	+		+	+
	1:200		+ +	-		-	-
	1:400		+ +				
	1:800		+				
	1:1600		+				
	1:3200		-				

15. L.P. (Contd)

Day of test.	Serum dilution.	Bac. from urine.	B.-typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
N.18	.....						
	1:100		.....	-	+	+	-
	1:200		.....		-	-	-
	1:400		+				+
	1:800		-				-
16. A.R.							
16th	1:25		+ + +	-	+	-	+
	1:50		+ +	+ + +	+		-
	1:100		+ +	+ +	-		
	1:200		+	+ +			
	1:400		-	+			
	1:800			+			
	1:1600			-			
N.42	1:25	+	+ + +	+	+	+	+
	1:50	+	+ + +	+	-	+	+
	1:100	-	+ + +	+ + +		-	-
	1:200		+ +	+			
	1:400		+ +	+ +			
	1:800		+	+			
	1:1600		-	-			

* Not B. typhosus.

17. T.K.

Day of test.	Serum dilution.	Bac. from Rose-spot.	Bac. from Faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B. Schott.	B.paratyphosus B. Achard
16th	1:25			+ + +	+	+	-	+
	1:50			+ + +	-	+		-
	1:100			+ + +		-		
	1:200			+ + +				
	1:400			+ + +				
	1:800			+ + +				
	1:1600			+ +				
	1:3200			+				
	1:6400			-				
24th	1:25	+ + +	+ + +	+ + +				
	1:50	+ + +	+ +	+ + +				
	1:100	+ + +	+ +	+ + +				
	1:200	+ + +	+ +	+ + +				
	1:400	+ + +	+ +	+ + +				
	1:800	+ + +	+	+ + +				
	1:1600	+ + +	-	+ +				
	1:3200	+ +		+ +				
	1:6400	+		+				
	1:12800	-		-				
25th	Vaccine 250,000,000 (bacillus from rose-spot)							

17. T.K. (Contd)

Day of test.	Serum dilution.	Bac. from rose-spot.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.Schott.	B.paratyphosus B Schott.Achard
27th	.....					
	1:400	.....	+	.....		
	1:800	.....	+	.....		
	1:1600	.....	-	.....		
	1:3200	+ +		+		
	1:6400	+		-		
	1:12800	-				
29th	.....					
	1:400	.....	+ +	.....		
	1:800	.....	+ +	.....		
	1:1600	.....	+ +	+ + +		
	1:3200	+	+	+		
	1:6400	+	-	-		
	1:12800	-				
35th	.....					
	1:400	.....	+ +	.....		
	1:800	.....	+	.....		
	1:1600	+ +	-	+ +		
	1:3200	+ +		+ +		
	1:6400	+		+		
	1:12800	-		-		
35th	Vaccine 250,000,000 (bacillus from rose-spot)					

17. T.K. (Contd)

Day of test.	Serum dilution.	Bac from rose-spot.	Bac from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B. Schott.	B.paratyphosus B. Achard
37th	.....							
	1:800	.....	+	.....				
	1:1600	.....	+	.....				
	1:3200	+ +	-	+ +				
	1:6400	+		+				
	1:12800	-		-				
N.2	.....							
	1:800	.....	+	.....				
	1:1600	.....	-	.....				
	1:3200	+ +		+				
	1:6400	+		+				
	1:12800	-		-				
N.42	1:25	+ + +	+ + +	+ + +	+	+	-	-
	1:50	+ + +	+ +	+ + +	+			
	1:100	+ + +	+ +	+ +	-			
	1:200	+ + +	+	+				
	1:400	+ +	-	-				
	1:800	+						
	1:1600	-						

18. B.E.

Day of test.	Serum dilution.	Bac. from urine.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
17th	1:25		-	-	-	-	+ + +
	1:50						+ + +
	1:100						+ + +
	1:200						+ + +
	1:400						+ +
	1:800						+ +
	1:1600						+
	1:3200						+
	1:6400						+
	1:12800						-
27th	.....		-	-	-	-	+ + +
	1:50		-	[illegible]		-	+ + +
	1:100						+ + +
	1:200						+ +
	1:400						+ +
	1:800						+
	1:1600						-

19. J.L.

17th	1:25	+ + +	+ + +	+	-	-	+
	1:50	+ + +	+ + +	+			-
	1:100	+ + +	+ + +	-			
	1:200	+ + +	+ + +				
	1:400	+ +	+ + +				

19. J.L. (Contd)

Day of test.	Serum dilution.	Bac. from urine.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
17th (Contd)							
	1:800	+ +	+ +				
	1:1600	+ +	+				
	1:3200	+	-				
	1:6400	-					
26th (N.3 R.0)	.....						
	1:100	+ +	.....				
	1:200	+	.....				
	1:400	+	.....				
	1:800	-	+				
	1:1600		-				
R.1	Vaccine 100,000,000 (bacillus from urine)						
R.2	.....						
	1.200	+ +	.....				
	1:400	+	+ +				
	1:800	+	+				
	1:1600	-	-				
R.3	.....						
	1:400	+	+ +				
	1:800	+	+				
	1:1600	-	-				
R.4	.....						
	1:400	+	+ +				
	1:800	-	-				

19. J.L. (Contd)

Day of test.	Serum dilution.	Bac. from urine.	B.typhosus.	B.paratyphosus A. Br-Ka. Schott.	B.paratyphosus B Schott. Achard.
R.5.					
	1:400	+	+		
	1:800	-	-		
R.5	Vaccine 100,000,000 (bacillus from urine)				
R.6					
	1:100	+ +	.....		
	1:200	+	.....		
	1:400	-	+ +		
	1:800		-		
R.7					
	1:200	+	.....		
	1:400	-	+		
	1:800		-		
R.8.					
	1:100	+	.....		
	1:200	-	+ +		
	1:400		+		
	1:800		-		
R.9					
	1:100	+ +	.....		
	1:200	+	+ +		
	1:400	-	-		

20. F.B.

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
17th	1:25	+ + +	+ + +	+ +	+ +	+	+ + +
	1:50	+ + +	+ + +	+	+ +	+	+
	1:100	+ + +	+ + +	+ +	+	+	-
	1:200	+ + +	+ + +	+ +	+	+	
	1:400	+ +	+ + +	+	-	-	
	1:800	+ +	+ + +	-			
	1:1600	+ +	+ + +				
	1:3200	+ +	+ + +				
	1:6400	+	+ + +				
	1:12800	+	+				
	1:25600	-	-				
29th (N.O)	.....						
	1:1600	....	+ + +				
	1:3200	....	+ + +				
	1:6400	+ +	+ + +				
	1:12800	+ +	+ +				
	1:25600	+	+				
	1:51200	-	-				

N.O. Vaccine 50,000,000 (bacillus from faeces)

N.1. .....

1:12800	+	+
1:25600	-	-

20. F.B. (Contd)

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
N.2	.....						
	1:6400	+ +	+				
	1:12800	+	+				
	1:25600	-	-				
N.4	.....						
	1:3200	+ +	+ +				
	1:6400	+ +	+ +				
	1:12800	+	+				
	1:25600	+	+				
	1:51200	-	-				
N.4	Vaccine 75,000,000 (bacillus from faeces)						
N.5	.....						
	1:6400	+	+				
	1:12800	-	+				
	1:25600		-				
N.6	.....						
	1:6400	+	+				
	1:12800	+	-				
	1:25600	-					
N.7	.....						
	1:6400	+ +	+ + +				
	1:12800	+	+ +				
	1:25600	-	+				
	1:51200		-				

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard
N.8.							
	1:6400	.....	+				
	1:12800	+	-				
	1:25600	-					
N.8	Vaccine 100,000,000 (bacillus from faeces)						
N.9							
	1:3200	+	+				
	1:6400	-	-				
N.10							
	1:3200	+ +	+				
	1:6400	+	-				
	1:12800	+					
	1:25600	-					
N.11							
	1:3200	+ +	+ +				
	1:6400	+	-				
	1:12800	-					
N.42	1:25	+ + +	+ + +	+ +	+ +	+ +	+ + +
	1:50	+ + +	+ + +	+ +	+ +	+	+
	1:100	+ + +	+ + +	+ + +	-	-	-
	1:200	+ + +	+ + +	+ +			
	1:400	+ + +	+ + +	+ +			
	1:800	+ +	+ + +	+ +			

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
N.42 (Contd)							
	1:1600	+	+ +	+			
	1:3200	+	+	+			
	1:6400	-	+	-			
	1:12800		+				
	1:25600		-				
21. I.M.							
17th (N.O)	1:25	+ +	+ + +	+ +	+ + +	+ + +	+ + +
	1:50	+ +	+ + +	+ +	+ + +	+ +	+ + +
	1:100	+ +	+ + +	+ +	+	+	+ +
	1:200	+ +	+ + +	+ +	+	+	+
	1:400	+ +	+ + +	+	-	-	+
	1:800	+	+ +	+			-
	1:1600	+	-	-			
	1:3200	-					
22. C.T.							
18th	1:25	+ + +	+ + +	+	+	+	+ + +
	1:50	+ + +	+ + +	+	+	-	+
	1:100	+ + +	+ + +	+ +	-		-
	1:200	+ +	+ + +	+ +			
	1:400	+ +	+ +	+			
	1:800	+	+	+			
	1:1600	-	-	-			

23. S.T.

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
19th	1:25		+ + +	+ + +	+	-	+ +
	1:50		+ + +	+ + +	-		-
	1:100		+ + +	+ +			
	1:200		+ + +	+ +			
	1:400		+ +	+ +			
	1:800		+	+			
	1:1600		+	+			
	1:3200		-	-			
24. E.H.							
N.42	1:25	+ + +	+ + +	+ + +	+ +	+ +	+ + +
	1:50	+ + +	+ + +	+ + +	+	+ +	+ +
	1:100	+ +	+ + +	+ + +	+	+	+
	1:200	+ +	+ + +	+ + +	-	+	+
	1:400	+ +	+ + +	+		-	-
	1:800	+	+ + +	-			
	1:1600	+	+				
	1:3200	-	-				
25. M.G.		Bac.fm. faeces.					
19th	1:25	+ + +	+ + +	+ +	+ + +	+ + +	+ + +
	1:50	+ + +	+ + +	+	+ +	+ +	+ + +
	1:100	+ + +	+ + +	+ + +	+ +	+	+ + +
	1:200	+ + +	+ + +	+	+	-	+
	1:400	+ + +	+ + +	+ +	-		-
	1:800	+ +	+ + +	+			
	1:1600	+ +	+ + +	+ +			

25. M.G. (Contd)

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
19th (Contd)							
	1:3200	+ +	+ + +	+			
	1:6400	+	+ + +	+			
	1:12800	-	+	-			
	1:25600		-				
26. J.M.		Bac from urine.					
20th	1:25	+ + +	+ + +	+	+ +	+ +	+ + +
	1:50	+ + +	+ + +	+	+ +	+ +	+ +
	1:100	+ + +	+ + +	+	+	-	-
	1:200	+ + +	+ + +	-	-		
	1:400	+ +	+ +				
	1:800	+	-				
	1:1600	-					
27. D.R.		Bac from blood.					
	1:25	+ + +	+ + +	+ +	+ + +	+ + +	+ + +
	1:50	+ + +	+ + +	+	+ + +	+ + +	+ + +
	1:100	+ + +	+ + +	+ + +	+ +	+ +	+ +
	1:200	+ + +	+ + +	+ + +	+	+	-
	1:400	+ + +	+ + +	+ +	-	+	
	1:800	+ + +	+ + +	+ +		-	
	1:1600	+ +	+ + +	+			
	1:3200	+ +	+ + +	-			
	1:6400	+ +	+ +				
	1:12800	+	+ +				
	1:25600	-	-				

28. M.E.

Day of test.	Serum dilution.	Bac. from faeces.	B. typhosus.	B. paratyphosus A. Br-Ka.	B. paratyphosus A. Schott.	B. paratyphosus B Schott.	B. paratyphosus B Achard.
20th	1:25	+ + +	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ +	+ + +	+	+ +	+ +	+ + +
	1:100	+ +	+ + +	+ +	+	+ +	+ +
	1:200	+ +	+ + +	+ +	+	+	+
	1:400	+	+ + +	+	+	+	-
	1:800	+	+ + +	-	-	-	
	1:1600	+	+ + +				
	1:3200	+	+ +				
	1:6400	-	+ +				
	1:12800		-				

29. A.T.

21st	1:25		+ + +	+	+	+	+ + +
	1:50		+ + +	+	+	+	+ + +
	1:100		+ + +	+	-	-	+ +
	1:200		+ + +	-			+
	1:400		+ + +				-
	1:800		+ + +				
	1:1600		+ +				
	1:3200		-				

30. P.M.

Day of test.	Serum dilution.	Bac. from ✱ urine.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
N.37	1:25	+ +	+ + +	+ + +	+ + +	+ +	+ +
	1:50	+	+ + +	+ + +	+ +	+	+
	1:100	+	+ + +	+ + +	+	+	-
	1:200	+	+ + +	+ + +	-	+ +	
	1:400	-	+ + +	+ + +			
	1:800		+ +	+ +			
	1:1600		-	-			

✱ Not B. typhosus.

31. F.M.		Bac. from faeces.					
21st	1:25	+ + +	+ + +	+ +	+	-	+ +
	1:50	+ + +	+ + +	+ +	-		+
	1:100	+ + +	+ + +	+ +			+
	1:200	+ +	+ + +	+			-
	1:400	+ +	+ +	+			
	1:800	+	+ +	-			
	1:1600	+	+				
	1:3200	-	+				
	1:6400		-				

32. J.B.

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
22nd	1:25		+ +	-	+ +	+	+ +
	1:50		+ +		+	+	+ +
	1:100		+ +		+	-	+
	1:200		+		-		-
	1:400		-				
N.40	1:25		+ + +	-	+	+ +	+ + +
	1:50		+ + +	-	-	+ +	+ + +
	1:100		+ +	+		+	+ +
	1:200		+	+ +		-	+
	1:400		+	+			
	1:800		-	+			
	1:1600			-			
33. J.F.							
23rd	1:25	.....	.....	-	-	-	-
	1:50	+ + +	.....	-			-
	1:100	.....	.....	+			-
	1:200	.....	.....	-			+
	1:400	.....	+ +				+
	1:800	+	+				+
	1:1600	-	-				-

33. J.F. (Contd)

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
N.42	1:25	+ + +	+ + +	+	+	+	+ + +
	1:50	+ + +	+ + +	-	-	-	+
	1:100	+ +	+ + +				+
	1:200	+ +	+ + +				-
	1:400	+ +	+ +				
	1:800	+	+				
	1:1600	-	-				

34. R.R.

N.42	1:25	+ +	+ +	+	-	-	-
	1:50	+	+ +	+			
	1:100	+	+	+			
	1:200	-	-	+			
	1:400			+			
	1:800			-			

35. C.M.

N.42	1:25		+ +	+	+	-	+ + +
	1:50		+	+	-		+ + +
	1:100		+	-			-
	1:200		-				

36. T.H.

Day of test.	Serum dilution.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B. Schott.	B.paratyphosus B. Achard.
24th	1:25	+ + +	-	+	+ +	+ + +
	1:50	+ + +	+	+	+ +	+ + +
	1:100	+ + +	+ +	+	+ +	+ + +
	1:200	+ + +	+ +	+	+	+ + +
	1:400	+ + +	+ +	-	+	+ +
	1:800	+ + +	+ +		+	+
	1:1600	+ + +	+ +		-	-
	1:3200	+ +	+			
	1:6400	+	-			
	1:12800	-				

37. M.H.

25th	1:25	+ + +	+	+	+	+ +
	1:50	+ + +	-	-	+	+
	1:100	+ + +			-	+
	1:200	+ + +				-
	1:400	+ +				
	1:800	+				
	1:1600	+				
	1:3200	+				
	1:6400	-				

38. C.W.

Day of test.	Serum dilution.	Bac.from blood.	Bac.from urine.	B-typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B. Schott.	B.paratyphosus B. Achard
27th	1:25	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +
	1:50	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +
	1:100	+ +	+ +	+ + +	+ +	+	+	+
	1:200	+ +	+ +	+ + +	+	+	+	-
	1:400	+	+ +	+ + +	-	+	-	
	1:800	+	+	+ + +		-		
	1:1600	-	+	+ +				
	1:3200		+	+ +				
	1:6400		-	+				
	1:12800			-				

39. W.C.

28th	1:25			+ + +	+	+ +	+ + +	+ + +
	1:50			+ + +	+	+ +	+ +	+ +
	1:100			+ + +	+	+	+	-
	1:200			+ +	-	-	+	
	1:400			+			-	
	1:800			-				

N.34	1:25		+	+ + +	+	+ +	+ +	+ + +
	1:50		+	+ + +	+ + +	+ +	+ +	+ + +
	1:100		-	+ + +	+ + +	+ +	+ +	+
	1:200			+ +	+ + +	+	+	-
	1:400			+	+ +	-	+	
	1:800			-	+ +		-	
	1:1600				+			
	1:3200				-			

⊠ Not B.typhosus.

Day of test.	Serum dilution.	Bac. from urine.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
	1:25	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	1:50	+ + +	+ + +	+ +	+ +	+ + +	+ + +
	1:100	+ + +	+ + +	+ +	+ +	+ +	+ +
	1:200	+ + +	+ + +	+ +	+	+	+ +
	1:400	+ + +	+ + +	+	+	+	+
	1:800	+ + +	+ + +	-	-	-	+
	1:1600	+ + +	+ + +				-
	1:3200	+ +	+ + +				
	1:6400	+ +	+ + +				
	1:12800	+	+ +				
	1:25600	-	+				
	1:51200		-				
N.42	1:25	+ +	+ + +	+ + +	+ +	+ +	+ +
	1:50	+ +	+ +	+ + +	+ +	+	+
	1:100	+	+	+ + +	+	+	-
	1:200	-	-	+ + +	-	-	
	1:400			+ + +			
	1:800			+ +			
	1:1600			+			
	1:3200			+			
	1:6400			+			
	1:12800			-			

41. J.G.

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
29th	1:25	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	1:50	+ + +	+ + +	+ + +	+ +	+ + +	+ + +
	1:100	+ + +	+ + +	+ + +	+ +	+ +	+ + +
	1:200	+ + +	+ + +	+ + +	-	-	+ +
	1:400	+ + +	+ + +	+ + +			+ +
	1:800	+ + +	+ + +	+ + +			+
	1:1600	+ + +	+ + +	+ + +			-
	1:3200	+ +	+ +	+			
	1:6400	+ +	+	+			
	1:12800	+	+	-			
	1:25600	-	-				

42. G.U.

N.20	1:25		+ + +	+	+ +	+ + +	+ + +
	1:50		+ + +	+	+ +	+ + +	+ +
	1:100		+ + +	+	+ +	+ +	+ +
	1:200		+ +	+	-	+	+
	1:400		+ +	-		-	-
	1:800		+ +				
	1:1600		+				
	1:3200		-				

43. W.B.

Day of test.	Serum dilution.	Bac from blood.	Bac from urine.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard
30th	1:25	+ + +	+ + +	+ + +	+ +	+	+ +	+ + +
	1:50	+ + +	+ + +	+ + +	+ +	-	+ +	+ + +
	1:100	+ + +	+ + +	+ + +	+		+ +	+
	1:200	+ + +	+ +	+ + +	+		+	-
	1:400	+ +	+ +	+ +	-		-	
	1:800	+ +	+	+ +				
	1:1600	+	+	+				
	1:3200	+	+	+				
	1:6400	-	+	-				
	1:12800		-					

* Not B. typhosus.

44. M.M.

Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard
1:25	+ + +	+ + +	+	+	+	+
1:50	+ + +	+ + +	+	-	+	+
1:100	+ +	+ + +	+ +		-	-
1:200	+ +	+ + +	+ + +			
1:400	+ +	+ + +	+ +			
1:800	+ +	+ + +	+ + +			
1:1600	+	+ + +	+ +			
1:3200	+	+ +	+			
1:6400	-	+ +	-			
1:12800		+				
1:25600		-				

45. M.J.

Day of test.	Serum dilution.	Bac. from urine.	B.typhosus.	B.paratyphosus Br-Ka.	B.paratyphosus Schott.	A.B.paratyphosus Schott.	B.paratyphosus Achard.
32nd	1:25	+ +	+ + +	+ + +	+ +	+ +	+ + +
	1:50	+ +	+ + +	+ + +	+	+ +	+ + +
	1:100	-	+ +	+ + +	+	+ +	+ + +
	1:200		+ +	+ + +	+	+	+
	1:400		+ +	+ + +	-	+	+
	1:800		+ +	+ + +		-	-
	1:1600		+	+ + +			
	1:3200		+	+ + +			
	1:6400		-	+ +			
	1:12800			+			
	1:25600			-			

46. R.S.*

32nd	1:25	+ + +	+ + +	+ + +	+ +	+ +	+ + +
	1:50	+ + +	+ + +	+ + +	+ +	+ +	+ +
	1:100	+ + +	+ + +	+ +	+ +	+ +	+
	1:200	+ + +	+ + +	+	+	+ +	-
	1:400	+ +	+ + +	-	-	-	
	1:800	-	+ + +				
	1:1600		+				
	1:3200		-				

* Not B. typhosus.

47. E.H.

Day of test.	Serum dilution.	Bac. from faeces.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott.	B.paratyphosus B Schott.	B.paratyphosus B Achard.
1st.. R29.	1:25		+ + +	+	+	+	+ + +
	1:50		+ + +	+	+	+	+ +
	1:100		+ + +	+	-	-	+
	1:200		+ + +	+			-
	1:400		+ +	+			
	1:800		+	-			
	1:1600		-				

48. J.F.

N.5	1:25	+ + +	+ + +	+ +	+ +	+ +	+ + +
	1:50	+ + +	+ + +	+ +	+	+	+ + +
	1:100	+ + +	+ + +	+ +	+	+	+
	1:200	+	-	-	-	-	-
	1:400	-					

49. T.B.		Bac. from urine.					
N.24	1:25	+ + +	+ + +	+ + +	+ + +	+ + +	+
	1:50	+ + +	+ + +	+ + +	+ + +	+ + +	+
	1:100	+ + +	+ + +	+ + +	+ +	+	-
	1:200	+ + +	+ + +	+ + +	+	-	
	1:400	+ + +	+ + +	+ + +			
	1:800	+ + +	+ +	+			
	1:1600	+	+	+			
	1:3200	+	-	-			
	1:6400	-					

50. M.K.

Day of test.	Serum dilution.	Bac. from blood.	B.typhosus.	B.paratyphosus A. Br-Ka.	B.paratyphosus A. Schott	B.paratyphosus B. Schott.	B.paratyphosus B. Achard.
N.42	1:25	+ + +	+ + +	+	+	-	+
	1:50	+ + +	+ + +	+	+		-
	1:100	+ + +	+ + +	+			
	1:200	+ + +	+ + +	-			
	1:400	+ +	+ +				
	1:800	+	+				
	1:1600	-	-				
N.42	1:25	+ + +	+ + +	+ + +	+ +	+	+ + +
	1:50	+ + +	+ + +	+ + +	+	+	+ +
	1:100	+ + +	+ + +	+ +	-	-	+ +
	1:200	+ + +	+ + +	+ +			+
	1:400	+ + +	+ + +	+			+
	1:800	+ + +	+ + +	+			
	1:1600	+	+	+			
	1:3200	-	-	-			

A P P E N D I X C.

Tables showing agglutination of bacilli by the 5 antisera

	Serum.	Limit of agglutination of its own bacillus.
(1)	Antityphoid	1:80,000
(2)	Antiparatyphoid A (Brion-Kayser)	1:3000
(3)	" A (Schottmüller)	1:200,000
(4)	" B "	1:800,000
(5)	" B (Achard)	1:70,000

B. Typhosus (Stock)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.	Anti A. Sch.	Anti A. Sch. B.	Anti Ach. B
	1:25	+ + +	-	+	+ +	+ + +
	1:50	+ + +		+	+ +	+ +
	1:100	+ + +		-	+	+ +
	1:200	+ + +			-	+
	1:400	+ + +				+
	1:800	+ + +				-
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ + +				
	1:12800	+ +				
	1:25600	+ +				
	1:51200	+ +				
	1:102400	-				

B. paratyphosus Brion-Kayser A.

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
	1:25	+	+ + +	+ + +	+ + +	+ +
	1:50	+	+ + +	+ + +	+ + +	+
	1:100	+ +	+ + +	+ + +	+ + +	+
	1:200	+	+ + +	+ + +	+ + +	+
	1:400	+ +	+ + +	+ + +	+ + +	+
	1:800	+	+ + +	+ + +	+ + +	-
	1:1600	+	+ +	+ + +	+ + +	
	1:3200	-	+	+ +	+ + +	
	1:6400		-	+ +	+ + +	
	1:12800			+ +	+ + +	
	1:25600			+ +	+ +	
	1:51200			+	+ +	
	1:102400			-	+	
	1:204800				-	

B. paratyphosus Schottmüller A.

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
	1:25	-	+ + +	+ + +	+ + +	-
	1:50		+ + +	+ + +	+ + +	
	1:100		+ + +	+ + +	+ + +	
	1:200		+ + +	+ + +	+ + +	
	1:400		+ + +	+ + +	+ + +	
	1:800		+ +	+ + +	+ + +	
	1:1600		+	+ + +	+ + +	
	1:3200		-	+ + +	+ + +	
	1:6400			+ + +	+ + +	
	1:12800			+ + +	+ +	
	1:25600			+ +	+ +	
	1:57200			+ +	+	
	1:102400			+	+	
	1:204800			+	-	
	1:409600			-		

B. paratyphosus Schottmüller B.

Days ill when isolated.	Serum Dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
	1:25	-	+ + +	+ + +	+ + +	-
	1:50		+ + +	+ + +	+ + +	
	1:100		+ + +	+ + +	+ + +	
	1:200		+ + +	+ + +	+ + +	
	1:400		+ + +	+ + +	+ + +	
	1:800		+ +	+ + +	+ + +	
	1:1600		+	+ +	+ + +	
	1:3200		-	+ +	+ + +	
	1:6400			+	+ + +	
	1:12800			+	+ + +	
	1:25600			-	+ + +	
	1:51200				+ +	
	1:102400				+ +	
	1:204800				+	
	1:409600				+	
	1:819200				+	
	1:1638400				-	

B. paratyphosus Achard B.

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
	1:25	+ +	+ +	+ + +	+ + +	+ + +
	1:50	+ +	+	+ + +	+ + +	+ + +
	1:100	+ +	-	+ +	+ +	+ + +
	1:200	+		+ +	+ +	+ + +
	1:400	+		+ +	+	+ + +
	1:800	+		+	-	+ + +
	1:1600	+		+		+ + +
	1:3200	-		+		+ + +
	1:6400					+ + +
	1:12800					+ +
	1:25600					+ +
	1:51200					+
	1:102400					-

B. coli (Faeces)

1:25	-	-	-	+	+
1:50				-	-

1. J.H. (blood)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
3	1:25	-	+	+ + +	+ + +	+ +
	1:50		-	+ +	+ + +	+
	1:100			+	+ +	+
	1:200			-	+	-
	1:400				-	

2. J.T. (blood)

8	1:25	+ + +	-	+ + +	+ + +	+ + +
	1:50	+ + +		+ +	+ + +	+ +
	1:100	+ + +		+	+ +	+
	1:200	+ + +		-	+	+
	1:400	+ + +			-	-
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				

3. E.B. (blood)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
8	1:25	+	+	+ + +	+ +	+ + +
	1:50	+	+	+	+	+ +
	1:100	+	-	+	-	+
	1:200	+		-		-
	1:400	+				
	1:800	+				
	1:1600	+				
	1:3200	+				
	1:6400	-				

4. M.E. (faeces)

8	1:25	+ + +	+	+ +	+ +	+ +
	1:50	+ + +	-	+	+ +	+
	1:100	+ + +		-	+	-
	1:200	+ + +			-	
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+				
	1:25600	+				
	1:51200	+				
	1:102400	-				

5. F.M. (faeces)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
8	1:25	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ + +	-	+ + +	+ + +	+ + +
	1:100	+ + +		+ +	+ +	+
	1:200	+ + +		+	-	+
	1:400	+ + +		-		-
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+				
	1:25600	+				
	1:51200	+				
	1:102400	-				

6. A.B. (blood)

8	1:25	+	-	-	-	+
	1:50	-				-

7. I.M. (faeces)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
9	1:25	+ + +	-	+	+ + +	+ +
	1:50	+ + +		+	+ +	+
	1:100	+ + +		-	+ +	-
	1:200	+ + +			+	
	1:400	+ + +			-	
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				

8. F.J.(faeces)

9	1:25	+ + +	+ + +	+ + +	+ + +	+ + +
	1:50	+ + +	+ +	+ + +	+ + +	+ + +
	1:100	+ + +	+	+ + +	+ +	+ + +
	1:200	+ + +	-	+ +	+	+
	1:400	+ + +		-	+	-
	1:800	+ + +			-	
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+				

8. F.J. (faeces) (Contd)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
9	1:12800	+				
	1:25600	+				
	1:51200	+				
	1:102400	-				

9. E.C. (faeces)

9	1:25	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ + +	-	+ + +	+ +	+ + +
	1:100	+ + +		+ +	+	+ +
	1:200	+ + +		-	-	+
	1:400	+ + +				-
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				

10. J.F. (blood)

9	1:25	-	-	+ +	+ + +	+
	1:50			-	+ +	-
	1:100				+	
	1:200				-	

11. C.T. (faeces)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
10	1:25	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ + +	-	+ +	+ +	+ + +
	1:100	+ + +		+	+ +	+ +
	1:200	+ + +		-	-	+
	1:400	+ + +				-
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				

12. E.M. (faeces)

11	1:25	+ + +	-	+ +	+ +	+ +
	1:50	+ + +		+	+	+ +
	1:100	+ + +		-	-	+
	1:200	+ + +				-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ + +				
	1:12800	+ + +				
	1:25600	+ +				
	1:51200	+				
	1:102400	-				

13. R.R. (blood)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
12	1:25	+ + +	-	+ + +	+ + +	+ +
	1:50	+ + +		+	+ +	+
	1:100	+ + +		-	+	+
	1:200	+ + +			-	-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				

14. M.G.(Faeces)

13	1:25	+ + +	+	+ + +	+ + +	+ +
	1:50	+ + +	-	+ + +	+ +	+
	1:100	+ + +		-	+	+
	1:200	+ + +			-	-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				

15. C.W. (blood)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
14	1:25	+ + +	-	-	+ + +	+ +
	1:50	+ + +			+	+ +
	1:100	+ + +			-	+
	1:200	+ + +				-
	1:400	+ + +				
	1:800	+ +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+				
	1:12800	+				
	1:25600	+				
	1:51200	-				

16. W.B. (blood)

14	1:25	+ + +	-	+	+ +	+ + +
	1:50	+ + +		-	+	+ +
	1:100	+ + +			+	+
	1:200	+ + +			-	-
	1:400	+ + +				
	1:800	+ +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	-				

17. F.B. (faeces)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Soh.A.	Anti Sch.B.	Anti Ach.B
14	1:25	+ +	-	+ +	+ +	+ +
	1:50	+ +		+	+	+
	1:100	+ +		-	+	-
	1:200	+ +			-	
	1:400	+ +				
	1:800	+ +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+				
	1:25600	+				
	1:51200	-				

18. S.M. (faeces)

14	1:25	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ + +	-	+ +	+ +	+ +
	1:100	+ + +		+	+	+
	1:200	+ + +		-	-	-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				

19. D.M. (Blood)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
15	1:25	+ + +	-	+ +	+ +	+ + +
	1:50	+ + +		+	+	+ +
	1:100	+ + +		+	+	+ +
	1:200	+ + +		-	-	+
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ +				
	1:6400	+				
	1:12800	+				
	1:25600	+				
	1:51200	-				

20.A.W. (faeces)

15	1:25	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ + +	-	+ +	+ + +	+ +
	1:100	+ + +		-	+	+
	1:200	+ + +			+	-
	1:400	+ + +			-	
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				

21. J. L. (urine)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
15	1:25	+ + +	-	+ + +	+ + +	+ +
	1:50	+ + +		+ + +	+ +	+
	1:100	+ + +		+ +	+	-
	1:200	+ + +		-	-	
	1:400	+ + +				
	1:800	+ +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+				
	1:12800	+				
	1:25600	+				
	1:51200	-				

22.D.R.(blood)

15	1:25	+ + +	-	+ +	+ + +	+ + +
	1:50	+ + +		+	+ +	+ +
	1:100	+ + +		-	+	+
	1:200	+ + +			-	-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ + +				
	1:12800	+ +				
	1:25600	+ +				
	1:51200	-				

23. T.K. (faeces)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
16	1:25	+ + +	-	+	+	+ +
	1:50	+ +		-	-	-
	1:100	+ +				
	1:200	+ +				
	1:400	+				
	1:800	+				
	1:1600	+				
	1:3200	-				

24. H.M. (urine)

17	1:25	+ +	-	+ +	+ + +	+
	1:50	+ +		+	+ +	+
	1:100	+		-	+	-
	1:200	+			+	
	1:400	+			-	
	1:800	+				
	1:1600	+				
	1:3200	+				
	1:6400	-				

25. M.K. (blood)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
17	1:25	+ + +	-	+	+ + +	+ +
	1:50	+ + +		-	+ +	+
	1:100	+ + +			-	-
	1:200	+ + +				
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ +				
	1:3200	+				
	1:6400	+				
	1:12800	-				

26. M.F. (spleen)

17	1:25	+ + +	-	+ +	+ +	+
	1:50	+ + +		+	+	+
	1:100	+ + +		-	-	-
	1:200	+ + +				
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+ +				
	1:51200	+				
	1:102400	-				

27. J.M. (urine)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka-A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
18	1:25	+ + +	+ +	+ + +	+ + +	+ +
	1:50	+ + +	+	+ + +	+ + +	+ +
	1:100	+ + +	-	+	+	+
	1:200	+ + +		-	-	-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+				
	1:25600	+				
	1:51200	-				

28. P.J. (Spleen)

18	1 :25	-	-	+ + +	+ + +	+ + +
	1:50			+ +	+ + +	+ + +
	1:100			+	+ +	+ +
	1:200			-	-	-

29. P.J. (bile)

Days ill when isolated.	Serum Dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
18	1:25	+ + +	-	+ + +	+ + +	+ +
	1:50	+ + +		+ + +	+ + +	+
	1:100	+ + +		+ +	+	-
	1:200	+ + +		+	-	
	1:400	+ + +		-		
	1:800	+ + +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+				
	1:12800	+				
	1:25600	-				

30. P.J. (mesenteric gland)

18	1:25	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ + +	-	+ + +	+ + +	+ + +
	1:100	+ + +		+ +	+ +	+ + +
	1:200	+ + +		+	+	+
	1:400	+ + +		-	-	+
	1:800	+ + +				-
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+				
	1:25600	+				
	1:51200	-				

31. E.H. (blood)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
19	1:25	+	-	+ +	+ +	+ + +
	1:50	-		+	+	+ + +
	1:100			-	-	+ +
	1:200					+
	1:400					-

32. H.O. (blood)

19	1:25	+	-	-	+ +	+ + +
	1:50	+			+	+ + +
	1:100	+			-	+ +
	1:200	+				+
	1:400	+ +				-
	1:800	+ +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+				
	1:25600	+				
	1:51200	+				
	1:102400	-				

33. T.W. blood.

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Soh.A.	Anti Sch.B.	Anti Ach.B
19	1:25	+ + +	+	+ +	+ + +	+ +
	1:50	+ + +	-	+	+ +	+
	1:100	+ + +		-	+	+
	1:200	+ + +			-	-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				
34. T.K. (rose-spot)						
20	1:25	+ + +	-	+ + +	+ + +	+ + +
	1:50	+ + +		+	+ +	+ + +
	1:100	+ + +		+	+	+
	1:200	+ + +		-	-	+
	1:400	+ + +				+
	1:800	+ + +				-
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ + +				
	1:12800	+ + +				
	1:25600	+ +				
	1:51200	+				
	1:102400	-				

35. J.P.(spleen)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
20	1:25	+ + +	+	+ + +	+ + +	+ + +
	1:50	+ + +	-	+ +	+ +	+ +
	1:100	+ + +		+	+ +	+
	1:200	+ + +		-	+	-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ + +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+ +				
	1:51200	+				
	1:102400	-				
36. M.J. (urine)						
21	1:25	+ + +	-	-	+	+
	1:50	+ + +			-	-
	1:100	+ + +				
	1:200	+ + +				
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1 102400	-				

37. M.D. (faeces)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
21	1:25	+ + +	-	+ +	+ + +	+ +
	1:50	+ + +		+	+ +	+ +
	1:100	+ + +		-	+	+
	1:200	+ + +			-	-
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+ +				
	1:51200	+				
	1:102400	-				

38. M.I. (blood)

22	1:25	+ + +	-	-	+	+
	1:50	+ + +			-	-
	1:100	+ + +				
	1:200	+ + +				
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+				
	1:6400	+				
	1:12800	-				

39. K.M. (spleen)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
23	1:25	+	-	+ +	+	+ +
	1:50	+		+	+	+
	1:100	+		-	-	+
	1:200	-				-
40. C.W. (urine)						
25	1:25	+ + +	-	+	+	+ +
	1:50	+ + +		-	+	+ +
	1:100	+ + +			-	+ +
	1:200	+ + +				+
	1:400	+ + +				-
	1:800	+ + +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +		-		
	1:25600	+				
	1:51200	-				

41. W.P. (urine)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
26	1:25	+ + +	-	+ + +	+ +	+ +
	1:50	+ + +		+ +	+	+
	1:100	+ + +		+	+	-
	1:200	+ + +		+	-	
	1:400	+ + +		-		
	1:800	+ + +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+				
	1:51200	+				
	1:102400	-				
42. M.M. (faeces)						
27	1:25	+	+	+ + +	+ +	+
	1:50	+	-	+	+	+
	1:100	+		+	+	-
	1:200	+		-	-	
	1:400	+				
	1:800	+				
	1:1600	+				
	1:3200	+				
	1:6400	+				
	1:12800	+				
	1:25600	+				
	1:51200	-				

43. W.M. (blood)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B.
28	1:25	+ + +	-	+	+	+
	1:50	+ + +		-	-	-
	1:100	+ + +				
	1:200	+ + +				
	1:400	+ + +				
	1:800	+ + +				
	1:1600	+ + +				
	1:3200	+ +				
	1:6400	+ +				
	1:12800	+ +				
	1:25600	+ +				
	1:51200	+ +				
	1:102400	-				

45. J.G. (blood)

29	1:25	+ + +	-	+ +	+ + +	+ +
	1:50	+ + +		+	+ +	+ +
	1:100	+ + +		-	+ +	+
	1:200	+ +			-	-
	1:400	+ +				
	1:800	+				
	1:1600	+				
	1:3200	-				

47. L.P. (urine)

Days ill when isolated.	Serum dilution.	Antityphoid.	Anti Br-Ka.A.	Anti Sch.A.	Anti Sch.B.	Anti Ach.B
55	1:25	+ + +	-	-	-	+
	1:50	+ + +				-
	1:100	+ + +				
	1:200	+ +				
	1:400	+ +				
	1:800	+ +				
	1:1600	+ +				
	1:3200	+ +				
	1:6400	+				
	1:12800	+				
	1:25600	+				
	1:51200	-				
51. T.B. (urine)						
10 normal.	1:25	+	-	+ + +	+ + +	+ +
	1:50	+		+ +	+ +	+ +
	1:100	-		-	+	+
	1:200				-	-

A P P E N D I X D.

Tables showing agglutination of atypical bacilli isolated from urine.

1. Agglutination of bacillus W.B. by antisera.

Days ill when isolated.	Serum dilution.	antityphoid.	antiparatyphoid A. Br-Ka.	antiparatyphoid A. Schott.	antiparatyphoid B Schott.	antiparatyphoid B Achard
28	1:25	-	+	+	+	-
	1:50	-	-	-	-	-

Agglutination by patient's serum(W.B.)(30th day)

Serum dilution.	B.typhosus (stock)	Bacillus W.B.
1:25	+ + +	+ + +
1:50	+ + +	+ + +
1:100	+ + +	+ + +
1:200	+ + +	+ +
1:400	+ +	+ +
1:800	+ +	+
1:1600	+	+
1:3200	+	+
1:5400		+

2. Agglutination of bacillus W.C. by antisera.

Days ill when isolated.	Serum dilution.	Antityphoid.	anti-paratyphoid A Br-Ka.	anti-paratyphoid A Schott.	anti-paratyphoid B Schott.	anti-paratyphoid B Achard
16th of normal temp.	1:25	-	-	+	-	-
	1:50			+	-	
	1:100			-		

Agglutination by patient's serum (W.C.)

(6 weeks convalescent)

Serum dilution.	B.typhosus (stock)	Bacillus W.C.
1:25	+ + +	+
1:50	+ + +	+
1:100	+ + +	-
1:200	+ +	
1:400	+	
1:800	-	

3. Agglutination of bacillus A.R. by antisera.

Days ill when isolated.	Serum dilution.	Antityphoid.	anti-paratyphoid A Br-Ka.	anti-paratyphoid A Schott.	anti-paratyphoid B Schott.	anti-paratyphoid B Achard
10th of normal temp.	1:25	-	-	-	-	-

Agglutination by patient's serum. (A.R.)

(6 weeks convalescent)

Serum dilution.	B.typhosus (stock)	Bacillus A.R.
1:25	+ + +	+
1:50	+ + +	+
1:100	+ + +	-
1:200	+ +	
1:400	+ +	
1:800	+	
1:1600	-	

4. Agglutination of bacillus R.S. by antisera.

Days ill when isolated.	Serum dilution.	Antityphoid.	anti-paratyphoid A Br-Ka.	anti-paratyphoid A Schott.	anti-paratyphoid B Schott.	anti-paratyphoid B Achard
30	1:25	-	+	-	-	-
	1:50		-			

Agglutination by patient's serum(R.S.) (32nd day)

Serum dilution.	B. typhosus (stock.	Bacillus R.S.
1:25	+ + +	+ + +
1:50	+ + +	+ + +
1:100	+ + +	+ + +
1:200	+ + +	+ + +
1:400	+ + +	+ +
1:800	+ + +	-
1:1600	+	
1:3200	-	

5. Agglutination of bacillus C.D. by antisera.

Days ill when isolated.	Serum dilution.	antityphoid.	anti-paratyphoid A. Br-Ka.	anti-paratyphoid A. Schott.	anti-paratyphoid B Schott.	anti-paratyphoid B Achard
9th of normal temp.	1:25	+ +	-	+	-	+ +
	1:50	+		-		+
	1:100	-				+
	1:200					-

6. Agglutination of bacillus P.M. by antisera.

Days ill when isolated.	Serum dilution.	Antityphoid.	anti-paratyphoid A Br-Ka.	anti-paratyphoid A Schott.	anti-paratyphoid B Schott.	anti-paratyphoid B Achard
5th of normal temp.	1:25	+	+ +	+	+	+ +
	1:50	+	+	-	+	+ +
	1:100	-	+			+
	1:200		-			+
	1:400					-

Agglutination by patient's serum (P.M.)

(37 days convalescent)

Serum dilution.	B.typhosus (stock)	Bacillus P.M.
1:25	+ + +	+ +
1:50	+ + +	+
1:100	+ + +	+
1:200	+ + +	+
1:400	+ + +	-
1:800	+ +	
1:1600	-	

A P P E N D I X E.

Summary of clinical histories of 17 male patients in whom bacilluria occurred.

Case 1. M.B. aged 57.

Admitted to hospital on 6th day.

Pyrexia lasted 27 days: relapse for 20 days from 37th.

Typical and severe attack.

B. typhosus grown from blood on 7th day.

Widal reaction positive.

Urine contained albumin, but no pus: diazo reaction positive.

Bacilluria on 10th day (B. typhosus): no pus.

Day of illness.	State of urine.	Reaction of urine.	Special treatment, if any.
6th-9th	clear		
10th-25th	bacilluria		on 25-26th urotropin gr. 50.
26th-29th	clear		on 29th potass. citrate gr.60 daily begun.
30th	bacilluria	$\frac{N}{22}$	Potass.citrate 60 gr.
31st	clear	$\frac{N}{45}$	"
32nd	"	$\frac{N}{44}$	"
33rd	"	$\frac{N}{104}$	"
34th	pus	$\frac{N}{45}$	"
35th	clear	$\frac{N}{114}$	"
36th	"	$\frac{N}{57}$	"

Case 1 (Contd)

Day of illness.	State of urine.	Reaction of urine.	Special treatment, if any.
37th	bacilluria	$\frac{N}{25}$	Potass.citrate gr.60
38th	clear	$\frac{N}{52}$	"
39th	"	$\frac{N}{67}$	"
40th	"	$\frac{N}{34}$	"
41st	"	$\frac{N}{34}$	"
42nd	"	$\frac{N}{35}$	"
43rd	slight pus	$\frac{N}{26}$	acid.sod.phosph.gr.60
44th	clear	$\frac{N}{26}$	"
45th	"	$\frac{N}{24}$	"
46th	bacilluria	$\frac{N}{33}$	"
47th	clear	$\frac{N}{20}$	"
48th	"	$\frac{N}{45}$	"
49th	"	$\frac{N}{17}$	"
50th	"	$\frac{N}{36}$	
51st-dismissal	"		

Case 2. J.L. aged 36.

Admitted on 8th day.

Pyrexia lasted 23 days: relapse 17 days from 27th.

Severe attack: rose-spots: palpable spleen: diarrhoea: deafness: consolidation at base of left lung: femoral thrombosis. Slow pulse rate (temp.103.2°. P.R. 90)

Urine contained a considerable amount of albumin: diazo reaction positive, but slight.

Widal reaction positive (1:3000)

On 14th day bad abdominal pain and a sudden drop in temperature (? separation of sloughs)

Bacilluria on 10th day (B. typhosus): no pus.

Day of illness.	State of urine.	Reaction of urine.	Special treatment, if any.
8th-14th	clear		None
15th	bacilluria		
16th-dismissal	clear.		

Case 3. H.M. aged 16.

Admitted to hospital on 8th day.

Pyrexia lasted 22 days.

Attack of moderate severity: rose-spots: constipation:

Slow pulse rate (temp. 102°: P.R. 80)

Widal reaction positive (1:6000)

Urine contained no albumin: diazo reaction positive.

Bacilluria on 17th day (B. typhosus): no pus.

Day of illness.	State of urine.	Reaction of urine.	Special treatment, if any.
8th-16th	clear		
17-24th	bacilluria		On 23rd-24th days urotropin gr. 40.
25th-dismissal	clear.		

Case 4. M.J. aged 37.

Admitted to hospital on 21st day.

Was transferred from a general hospital to which he was admitted as a case of acute cystitis and prostatitis.

Pyrexia lasted till he died on 33rd day.

Was very ill on admission, and had consolidation at the bases of both lungs. Became gradually worse till he died.

Widal reaction positive (1:3000)

Urine contained a few pus cells, no albumin, and many bacilli. Diazo reaction negative.

Bacilluria from day of admission (B. typhosus)

Day of illness.	State of urine.	Reaction of urine.	Special treatment, if any.
21st-33rd (day of death)	bacilluria		None.

Case 5. E.B. aged 20.

Admitted to hospital on 7th day.

Pyrexia lasted 42 days: relapse for 9 days from 43rd.

Severe attack: rose-spots: meteorism with diarrhoea: incontinence of urine and faeces: distension of bladder: slow pulse rate (temp. 102°: P.R. 80)

B. typhosus grown from blood (8th day)

Widal reaction positive (1:2000)

Urine contained a trace of albumin: diazo reaction positive.

Bacilluria on 23rd day (B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any.
7th-22nd	clear	
23rd-33rd	bacilluria.	On 32nd-33rd urotropin gr.50
35th-24th of normal temp.	clear	
25th of normal temp.-27th " " "	bacilluria	
28th of normal temp.-dismissal	clear.	

Case 6. C.W. aged 23.

Admitted to hospital on 13th day.

Pyrexia lasted 31 days.

Was very ill with moderate pyrexia: rose-spots: palpable spleen: much bronchial catarrh, and considerable hypostatic congestion of lungs:

Diarrhoea, meteorism incontinence of faeces:

slow pulse rate (temp 102°: P.R. 90)

Widal reaction positive (1:6000)

Urine contained a trace of albumin: diazo reaction markedly positive.

Bacilluria on 25th day (B. typhosus): no pus.

Day of illness.	State of urine.	Reaction of urine.	Special treatment, if any.
13th-24th	clear		
25th-29th	bacilluria		On 28th-29th urotropine gr.50.
30th-dismissal	clear		

Case 7. W.P. aged 23.

Admitted to hospital on 8th day.

Pyrexia lasted 30 days.

Rather severe attack; rose-spots: diarrhoea: palpable spleen: slow pulse rate (temp.104.2°: P.R. 82)

Widal reaction positive (1:25,000)

Urine contained no albumin: diazo reaction faintly positive.

Bacilluria on 26th day (B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any.
8th-25th	clear	
26th-27th	bacilluria.	
28th-dismissal	clear.	

Case 8. B.O aged 35.

Admitted to hospital on 24th day.

Pyrexia lasted till death on 37th day: was very ill throughout.

B. typhosus grown from blood (25th day)

Widal reaction positive.

Urine contained much albumin: diazo reaction negative.

Bacilluria on 26th day (B. typhosus): pus present.

Day of illness.	State of urine.	Special treatment, if any.
24th-25th	pus	
26th-32nd	bacilluria and pus.	On 31st-32nd urotropin gr.50.
33rd-37th(day of death)	pus	

Post-mortem. Right kidney congested: cortex narrow: some fat in pelvis. Pure culture of B. coli from substance.

Left kidney atrophied; evidently an old condition: numerous small abscesses, with pus exuding into the pelvis of the kidney. Both ends of ureter dilated, there being a kind of pocket just before its entrance into the bladder. Mucous membrane of ureters healthy. Pure culture of B. typhosus from pus.

Gall-bladder contained 30 c.c. of bile, with 680,000,000 bacilli per c.c. Pure culture

Case 8 (Contd)

of B. typhosus from bile. Mucous membrane of gall-bladder healthy.

Spleen. Pure culture of B. typhosus.

Cerebro-spinal fluid sterile.

	clear	
	bacilluria	
	clear	

Case 9. W.B. aged 35.

Admitted to hospital on 13th day.

Pyrexia lasted 28 days.

Moderately severe attack: rose-spots: slight diarrhoea: slow pulse rate (temp.103.6°: P.R. 84) deafness; a few fine moist râles at bases of lungs: became very thin.

B. typhosus grown from blood (14th day)

Widal reaction positive (1:3000)

Urine contained albumin: diazo reaction markedly positive.

Bacilluria on 28th day (not B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any
13th-27th	clear	none
28th	bacilluria	
29th-dismissal	clear	

Case 10. R.S. aged 21.

Admitted to hospital on 16th day.

Pyrexia lasted 33 days.

Severe attack: rose-spots: diarrhoea: great meteorism: hypostatic congestion at bases of lungs: cyanosis: delirium: abscess of thigh (staphylococcus grown): slow pulse rate (temp.103.6°: P.R. 92): Erysipelas of face in convalescence.

Widal reaction positive (1:2000)

Urine contained no albumin: diazo reaction positive.

Bacilluria on 30th day (not B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any.
16th-29th	clear	
30th	bacilluria	
31st	clear	
32nd-39th	bacilluria	On 39th-40th urotropin gr. 30.
40th-dismissal	clear.	

Case 11. L.P. aged 25.

Admitted to hospital on 12th day.

Pyrexia lasted 55 days.

Moderately severe attack: rose-spots, erythematous rash, yellow skin, diarrhoea, deficient respiratory murmur: loss of flesh:

Widal reaction positive (1:1600)

Urine contained no albumin: diazo reaction negative.

Bacilluria on 55th day (B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any.
12th-54th	clear	
55th-57th(2nd of normal temp.)	bacilluria	On 2nd-3rd of normal temp. urotropin gr. 50.
3rd-13th of normal temp.	clear	
14th-15th of normal temp.	bacilluria	
16th of normal temp.- dismissal.	clear.	

Case 12. P.M. aged 37.

Admitted to hospital on 9th day.

Pyrexia lasted 16 days.

Mild attack: rose-spots: constipation: a few rhonchi in chest.

Widal reaction positive (1:1000)

Urine contained no albumin: diazo reaction negative.

Bacilluria on 5th day of normal temperature (not B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any
9th-4th of normal temp.	clear	
5th of normal temp.	bacilluria	
6th of normal temp.- dismissal.	clear	

Case 13. A.R. aged 52.

Admitted to hospital on 14th day.

Pyrexia lasted 32 days.

Moderately severe attack: rose-spots: diarrhoea: fine moist râles at bases of lungs: breathing at times slightly cyclical.

Widal reaction positive (1:800)

Urine contained albumin: diazo reaction positive.

Bacilluria on 6th day of normal temperature (not B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any.
14th-5th of normal temp.	clear	none.
6th of normal temp.	bacilluria.	
7th of normal temp.- dismissal.	clear.	

Case 14. T.B. aged 24.

Admitted to hospital about 21st day, just over an attack of enteric fever.

No pyrexia when under observation.

Widal reaction positive (1:3000)

Urine contained no albumin: diazo reaction negative.

Bacilluria on 10th day of normal temperature (B. typhosus)

pus present.

Day of illness.	State of urine.	Special treatment, if any.
1st of normal temp.- 9th " " "	clear	
10th-11th of normal temp.	bacilluria and pus.	On 11th of normal temp. urotropin gr. 20.
12th of normal temp.- dismissal	clear	

Case 15. C.D. aged 35.

Admitted to hospital on 21st day.

Pyrexia lasted 47 days.

Moderately severe attack: diarrhoea: pea-soupy stools: some distension of abdomen; occasional incontinence: slow pulse rate (Temp. 102.2° - P.R. 84)

Widal reaction positive.

Urine contained no albumin: diazo reaction negative.

Bacilluria on 10th day of normal temperature (not B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any.
21st-9th of normal temp.	clear	
10th-11th " " "	bacilluria.	
12th of normal temp. - dismissal.	clear.	

Case 16. J.M. aged 31.

Admitted to hospital on 9th day.

Pyrexia lasted 27 days.

Moderately severe attack: rose-spots: diarrhoea: pleural effusion: femoral thrombosis: slow pulse rate (temp. 102.2°: P.R. 88)

Widal reaction positive (1:800)

Urine contained albumin: diazo reaction markedly positive.

Bacilluria on 18th day (B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any.
9th-17th	clear	
18th-20th	bacilluria.	
21st-13th of normal temp.	clear.	
14th-18th " "	bacilluria	On 18th-19th of normal temp. urotropin gr. 30.
19th of normal temp.-dismissal.	clear.	

Case 17. W.C. aged 19.

Admitted to hospital on 28th day.

Pyrexia lasted 33 days.

Moderately severe attack: rose-spots: erythema and yellow staining of skin: palpable spleen: constipation: a few rhonchi in chest: slow pulse rate (temp 101.6°. P.R. 84)

Widal reaction positive (1:400)

Urine contained albumin: diazo reaction faintly positive.

Bacilluria on 16th day of normal temperature (not B. typhosus): no pus.

Day of illness.	State of urine.	Special treatment, if any
28th-15th of normal temp.	clear	none.
16th-18th " "	bacilluria	
19th of normal temp.- dismissal.	clear.	