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THE SEROLOGICAL DIAGNOSIS OF TYPHOID AND PARATYPHOID FEVERS.

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INTRODUCTION.

RECENT investigations on the antigenic structure of organisms belonging to the Salmonella group have suggested a reconsideration of the various factors which enter into and create difficulty in the serological diagnosis of typhoid and paratyphoid fevers. Dreyer *et al.* (1915, 1916, 1917), with a view to making the results obtained more uniform in character, introduced standardised suspensions; Felix and Mitzenmacher (1918) demonstrated that bacteria of the typhoid-paratyphoid enteriditis group were possessed of the double type ("H" and "O") of receptors; Arkwright (1921) has shown that the antigens of smooth and rough variants are distinct both as regards their corresponding agglutinins and the kind of clumping produced by specific sera; Andrewes (1922) discovered the diphasic phenomenon associated with certain members of the group; and finally, White (1926) has made extensive contributions to our knowledge of the antigenic structure of the Salmonella group as a whole.

In 1920 Weil and Felix discussed the principles of qualitative serum analysis in relation to the antigenic structure of organisms of the typhoid-paratyphoid group, and Felix (1924) suggested the qualitative method for the serological diagnosis of enteric fevers. Further results confirming and amplifying the original work have been published by Felix and Olitzki (1928) and Felix (1929, 1930), while the main principles involved in the method of diagnosis have received support from Burnet (1924), Stuart and Kirkorian (1928), Gardner (1929), Pijper (1930), and Whitehead (1930). On the other hand, Topley and Wilson (1929) deplore the fact that qualitative analysis of "H" and "O" agglutinins has tended to supersede the accurate quantitative estimation of these bodies.

Little work having been published on the practical application of the foregoing methods to the diagnosis of typhoid and paratyphoid fevers in this country, it was resolved to estimate, in all sera submitted for the Widal reaction, the "H" and "O" agglutinins for *B. typhosus* and *B. paratyphosus* B. These two organisms appear to be the main cause of enteric fever in the north of Scotland, since infection due to *B. paratyphosus* C has never been encountered, and infection due to *B. paratyphosus* A only in one instance and that in an Esthonian seaman who had contracted the disease abroad.

METHODS.

Specimens of blood submitted for the Widal reaction were collected by medical practitioners and resident medical officers and sent to the Laboratory for examination. Apart from the specimens taken from patients within the municipal hospitals, the blood samples were mainly collected in Behring

venules so that an ample supply of uncontaminated blood was obtained for the various agglutination tests and for culture of the blood clot. When received, samples of clotted blood were first centrifuged and the serum was then removed with a sterile pipette, and the remaining red blood cells and clot were then transferred to a flask containing 50 c.c. of sterile ox bile, which was placed in the incubator at 37° C. From the bile culture medium samples were from day to day inoculated on to McConkey's bile salt lactose agar plates to determine the presence or absence of typhoid-paratyphoid organisms.

For the agglutination tests the macroscopic method was used. In the first instance, for each bacterial suspension used in the tests, 0·5 c.c. of a dilution of the patient's serum ranging from 1 in 12½ to 1 in 3200 were placed in Dreyer agglutination tubes, and thereafter 0·5 c.c. of the bacterial emulsion was added, the final dilutions thus ranging from 1 in 25 to 1 in 6400. The results were checked by the use of saline and bacterial emulsion, and the tubes were incubated in the water bath at 52° C. for 2 hours before the readings were made of agglutination against "H" antigens, and at the end of a 4-hour period for agglutination against the "O" antigens. If the end-titre of the serum for a particular organism was not determined by the first series of dilutions, then a further series was set up.

Most of the suspensions used in the tests were obtained from the Standards Laboratory, Oxford. Thus, killed suspensions of *B. typhosus* "H" and "O," *B. paratyphosus* A, B, C, *B. aertrycke* and *B. enteriditis* "H" antigens were all obtained from this source. In addition, living cultures of *B. typhosus* "H" (Felix 901) and *B. typhosus* "O" variant (Felix 901), *B. paratyphosus* B "H" (a specific form isolated locally), and *B. aertrycke* ("O" variant of Schutze, 1930) were obtained from the National Collection of Type Cultures, and finally a suspension of *B. paratyphosus* B "O" antigen was prepared by the method described by Gardner (1929). The normal strains of *B. typhosus* and *B. paratyphosus* B were grown on a nutrient agar medium containing 0·5 per cent. agar which had an almost semi-solid consistency, but the "O" variants of *B. typhosus* and *B. aertrycke*—the latter being used as a substitute for *B. paratyphosus* B "O"—were grown on the standard agar, all strains being subcultured daily.

Results of the investigations.

During the past 2 years 264 specimens of blood have been submitted for the Widal reaction, and all these specimens have been examined in detail in regard to their agglutinin content for the "H" and "O" antigens of *B. typhosus* and *B. paratyphosus* B, but were also tested against the "H" antigen of *B. paratyphosus* A and *B. paratyphosus* C. In this paper only the agglutination results with *B. typhosus* and *B. paratyphosus* B will be discussed, since the amount of cross-agglutination which occurred with *B. paratyphosus* A and C was insignificant. The results can be separated into various groups: firstly, those obtained with the sera of definite cases of typhoid fever; secondly, those

obtained with sera from cases of paratyphoid; thirdly, those obtained with sera from inoculated individuals; and, fourthly, results of doubtful significance. It should be noted that for diagnostic purposes agglutination with the "H" antigens in a dilution of the serum of 1 in 50 was regarded as being suspicious of a present or past infection or previous inoculation, and if the blood culture did not show any organism a further specimen of blood was requested, while agglutination in a dilution of 1 in 100 was regarded as definitely diagnostic unless contra-indicated by a history of past infection or by previous prophylactic inoculation. With the "O" antigens agglutination in a dilution of 1 in 100 was considered necessary for diagnostic purposes.

The diagnostic results of these tests are given in Table I, where it will be seen that twenty-eight sera only were obtained from cases of typhoid fever, forty-two from cases of paratyphoid fever, and seventeen from inoculated individuals, while nine sera showing minor amounts of agglutinins were obtained from patients who were later proved to be suffering from other diseases, and finally 168 sera which gave totally negative results.

Table I. *Results of agglutination tests from the City of Aberdeen and the various counties*, 1929-1930.

District	Number of sera from cases of typhoid fever	Number of sera from cases of paratyphoid fever	Number of sera from inoculated patients	Sera giving doubtful results	Number negative
City of Aberdeen	13	19	13	6	101
Banffshire	0	4	3	0	14
Moray and Nairn	3	3	0	0	9
Kincardine	0	0	0	1	4
Ross and Cromarty	0	2	0	0	11
Caithness	3	2	0	0	12
Orkney	0	9	0	1	8
Shetland	9	3	1	1	9
Total...	28	42	17	9	168

AGGLUTINATION TESTS IN 28 CASES OF TYPHOID FEVER.

The results of these tests and of other bacteriological findings in these twenty-eight cases are given in Tables II and III respectively. The end-titres of the serum are given in each case for the "H" and "O" antigens of *B. typhosus* and *B. paratyphosus* B. The number of agglutinin units has also been calculated with the exception of those for *B. paratyphosus* B "O" antigen which was not obtainable from the Standards Laboratory, Oxford. In the majority of cases the agglutinin content of the sera definitely indicated the nature of the infecting organism, and in twenty cases the titre of the sera for the "H" antigen was greater than for the "O" antigen, and was manifest in a dilution of 1 in 100 or higher, while in two cases (Nos. 5 and 15) the actual titres of the sera were the same for both "H" and "O" antigens, but when the number of agglutinin units were determined there was a larger number of units for the "H" antigen. In one case (No. 11) in which a positive blood bile culture was

obtained, the agglutination results were entirely negative. In three cases (Nos. 6, 7 and 25) the only agglutination was 1 in 25, 1 in 100 and 1 in 50 against *B. typhosus* "O" antigen. The blood specimens from cases 6 and 7 were just sufficient for the Widal reaction, and were taken on the second and third days of the disease. These cases were clinically diagnosed early on account of

Table II. *Typhoid fever. Agglutination tests with Oxford standard agglutinable suspensions* B. paratyphosus *B "O" antigen excepted.*

	B. typhosus				*B. paratyphosus* B		
	"H" antigen		"O" antigen		"H" antigen		"O" antigen
Case	Titre	Agg. units	Titre	Agg. units	Titre	Agg. units	Titre
1	100	13	0	0	0	0	0
2	3,200	444	400	40	400	14	0
3	800	111	0	0	0	0	0
4	25	3	50	5	0	0	0
5	200	31	200	20	0	0	0
6	0	0	25	2·5	0	0	0
7	0	0	100	10	0	0	0
8	200	31	25	2·5	0	0	0
9	6,400	100	50	5	100	31	0
10	50	7	200	20	0	0	0
11	0	0	0	0	0	0	0
12	200	32	800	100	0	0	0
13	51,200	8255	25	3	100	20	25
14	1,600	228	400	50	25	5	100
15	200	28	200	25	0	0	200
16	1,600	228	400	50	50	10	100
17	100	28	50	6	0	0	0
18	12,800	1828	50	6	0	0	0
19	200	28	100	12	0	0	0
20	200	32	0	0	25	7	0
21	800	129	200	20	0	0	0
22	400	64	200	20	0	0	0
23	800	129	100	10	—	—	—
24	200	31	800	80	0	0	100
25	0	0	50	5	0	0	0
26	6,400	914	0	0	1600	320	50
27	100	14	0	0	0	0	0
28	100	14	0	0	0	0	0

Associated cases bracketed.

Table III. *Typhoid fever. Bacteriological findings.*

	Cultural results				Cultural results		
Case	Blood	Urine	Faeces	Case	Blood	Urine	Faeces
1	+	−	+	15	−	+	+
2	−	−	+	16	+	+	+
3	−	+	+	17	+	0	0
4	+	+	+	18	+	0	0
5	−	−	−	19	+	0	0
6	0	0	0	20	−	0	−
7	0	0	0	21	+	0	0
8	0	0	0	22	+	0	0
9	+	0	0	23	−	0	+
10	+	0	0	24	−	0	0
11	+	0	0	25	+	0	−
12	−	+	+	26	−	0	−
13	+	−	+	27	−	0	−
14	+	−	+	28	−	0	−

Associated cases bracketed. + =positive culture; − =negative culture; 0= no specimen.

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the fact that they were directly associated with a known typhoid carrier, and the subsequent course of the illness, despite the lack of bacteriological findings, left no doubt as to the disease from which the patients suffered. In case 25 the blood sample was also taken on the third day of the illness, and the serum was found to agglutinate only *B. typhosus* "O" in a dilution of 1 in 25, but the blood culture showed the presence of *B. typhosus*. Finally in two cases (Nos. 10 and 12) the titres of the sera were higher for the "O" antigen than for the "H" antigen, and in seven cases there were no "O" agglutinins present at the time of the examination.

In order to examine the fluctuations in the titre of sera, repeated blood samples were obtained from nine cases and the results are given in Table IV.

Table IV. *Typhoid fever. Repeated agglutination tests with sera from patients.*

		B. typhosus				*B. paratyphosus* B		
		"H" antigen		"O" antigen		"H" antigen		"O" antigen
Case	Test	Titre	Agg. units	Titre	Agg. units	Titre	Agg. units	Titre
1	1	100	13	0	0	0	0	0
	2	800	111	400	40	0	0	0
2	1	3,200	444	400	40	400	129	0
	2	3,200	444	400	40	400	129	0
3	1	800	111	0	0	0	0	0
	2	200	30	100	10	0	0	0
4	1	25	3	50	5	0	0	0
	2	100	15	400	40	0	0	50
5	1	200	31	200	20	0	0	0
	2	200	31	200	20	0	0	0
11	1	0	0	0	0	0	0	0
	2	0	0	200	20	0	0	0
	3	800	111	100	10	0	0	0
13	1	51,200	8255	25	3	100	20	25
	2	800	129	50	6	0	0	25
15	1	200	28	200	25	0	0	200
	2	50	7	50	6	0	0	0
16	1	1,600	228	400	50	50	10	100
	2	3,200	457	400	50	0	0	100

As a rule the first specimen was taken directly on admission to hospital, and the subsequent samples during convalescence. Eight cases showed agglutinins for "H" antigen on admission and seven for the "O" antigen, while during convalescence all showed agglutinins for both "H" and "O" antigens. One case, which gave a negative Widal to begin with, developed "O" agglutinins first, but later the titre for the "H" antigen became much higher than for the "O." In Tables II and III are given also the cross agglutination results of the various typhoid sera with the antigen of *B. paratyphosus* B, the results, however, show that no difficulty was encountered in making a diagnosis on account of group agglutinins.

Agglutination reactions in paratyphoid fever.

In all, blood specimens were obtained from forty-two known cases of paratyphoid fever, and the agglutination results are given in Table V. On the

Table V. *Paratyphoid fever. Agglutination tests with Oxford standard agglutinable suspensions* B. paratyphosus *B "O" antigen excepted.*

	B. typhosus				*B. paratyphosus* B		
	"H" antigen		"O" antigen		"H" antigen		"O" antigen
Case	Titre	Agg. units	Titre	Agg. units	Titre	Agg. units	Titre
1	200	27	100	10	25,600	8,256	200
2	100	13	50	5	51,200	16,512	50
3	0	0	50	5	6,400	2,064	0
4	0	0	50	5	12,800	4,128	0
5	0	0	0	0	6,400	2,064	0
6	0	0	0	0	1,600	516	0
7	0	0	0	0	800	258	0
8	0	0	0	0	1,600	516	0
9	0	0	0	0	6,400	2,064	0
10	0	0	0	0	800	258	0
11	0	0	50	6	3,200	1,032	0
12	0	0	50	6	102,400	51,200	100
13	0	0	25	3	400	200	100
14	0	0	0	0	12,800	6,400	400
15	0	0	200	25	102,400	24,480	100
16	0	0	0	0	51,200	12,240	50
17	200	28	100	12	1,600	320	100
18	0	0	0	0	200	40	0
19	0	0	0	0	800	160	100
20	0	0	0	0	0	0	0
21	1600	228	100	12	1,600	320	200
22	100	14	50	6	200	65	0
23	0	0	50	6	6,400	1,280	200
24	100	14	0	0	1,600	320	0
25	400	64	50	6	3,200	1,600	100
26	50	7	0	0	6,400	1,280	25
27	0	0	50	6	800	400	50
28	1600	228	100	12	1,600	320	200
29	0	0	0	0	400	80	0
30	25	4	25	3	12,800	4,000	50
31	0	0	0	0	200	40	0
32	0	0	0	0	1,600	320	100
33	25	3	0	0	6,400	1,280	400
34	0	0	100	12	800	160	200
35	0	0	0	0	800	160	100
36	0	0	25	3	400	80	50
37	0	0	0	0	800	160	50
38	200	28	0	0	3,200	640	25
39	0	0	400	50	51,200	10,220	800
40	50	7	0	0	12,800	2,560	400
41	0	0	0	0	200	0	0
42	100	14	50	6	200	40	0

average, the titres of the sera for "H" antigen of *B. paratyphosus* B were greater than those obtained for the analogous antigen of *B. typhosus* in cases of typhoid. In only one instance (case 20) was a negative agglutination reaction obtained, but in this case the blood culture was positive. In only one case was the titre of the serum for the "H" antigens of *B. typhosus* and *B. paratyphosus* B similar, but when the agglutinins were calculated in units a greater number of units were present for *B. paratyphosus* B than for *B. typhosus*. As regards

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agglutinins for the "O" antigens of *B. paratyphosus* B, the highest titre obtained was 1 in 400, and in no instance was "O" agglutination only obtained. Again, in no instance was the titre for the "O" antigen greater than for the "H," while the sera from eighteen cases showed no agglutinins for the "O" antigen of *B. paratyphosus* B at the time when the test was made.

A summary of the bacteriological findings in these forty-two cases is presented in Table VI. In thirteen or 25 per cent. of cases the infecting organisms were successfully isolated by culturing the blood clot in bile. In fourteen cases actually admitted to the wards of the City Hospital (cases 1 to 5 and 11 to 19 inclusive), it was possible, by repeated examinations of blood, urine, and faeces to obtain the infecting organism from one or more sources in thirteen cases. The remaining specimens were obtained from cases in outlying areas, and naturally the bacteriological investigation of these was not so thorough.

Table VI. *Paratyphoid fever. Bacteriological findings in* 42 *cases.*

Case	Blood	Urine	Faeces	Case	Blood	Urine	Faeces
1	−	+	+	22	+	−	−
2	−	+	+	23	+	−	−
3	+	+	+	24	−	−	−
4	+	+	+	25	−	−	−
5	+	−	+	26	−	0	0
6	−	−	+	27	−	0	0
7	−	−	+	28	+	−	+
8	−	0	0	29	+	−	+
9	−	0	0	30	−	0	+
10	−	0	0	31	−	0	0
11	−	+	+	32	+	0	0
12	+	+	+	33	+	0	0
13	+	+	+	34	−	0	0
14	−	+	+	35	−	0	0
15	−	−	+	36	−	0	0
16	−	+	+	37	−	0	0
17	−	+	+	38	+	0	0
18	−	−	−	39	−	0	0
19	−	+	+	40	+	0	0
20	−	−	−	41	−	0	0
21	−	−	−	42	−	0	0

Associated cases bracketed. + =positive culture; − =negative culture; 0 =no specimen.

Sera from inoculated patients.

During the course of this work a number of specimens were obtained from individuals who had received during previous years some form of prophylactic inoculation against the enterica group of organisms. In Table VII the positive results are presented, and here it will be seen that agglutination was mainly obtained against the "H" antigen of *B. typhosus*, the highest titre recorded being 1 in 400. In only two instances was agglutination obtained against the "O" antigen of *B. typhosus* and in six instances against the "H" antigen of *B. paratyphosus* B and in not a single instance against the "O" antigen of this latter organism.

Cases with minor amounts of agglutinins.

In all, ten cases showed minor amounts of agglutinins for the "H" and "O" antigens of *B. typhosus* and *B. paratyphosus* B (Table VIII), and, although

some of the reactions were suggestive of an infection due to a member of the enterica group, the subsequent history of same outruled this possibility. Thus, cases 1 and 10 were found to be suffering from tuberculous meningitis, in cases 2 and 7 a diagnosis of tuberculosis of the abdominal lymph glands was made, case 3 suffered from puerperal fever, case 4 from undulant fever, while in the remaining cases the clinical diagnosis was not available.

Table VII. *Examination of sera from inoculated patients. Agglutination results with Oxford standard agglutinable suspensions* B. paratyphosus *B "O" antigen excepted.*

		B. typhosus				*B. paratyphosus* B		
		"H" antigen		"O" antigen		"H" antigen		"O" antigen
Case	Date of last inoculation	Titre	Agg. units	Titre	Agg. units	Titre	Agg. units	Titre
1	1915	50	6	0	0	0	0	0
2	1918	200	24	0	0	0	0	0
3	1915	400	48	0	0	0	0	0
4	1928	50	6	0	0	50	22	0
5	1925	50	7	0	0	0	0	0
6	1917	200	31	0	0	0	0	0
7	1928	200	31	200	10	0	0	0
8	1922	100	15	50	5	0	0	0
9	1918	25	6	0	0	50	14	0
10	1917	200	32	0	0	50	4	0
11	1918	100	16	0	0	0	0	0
12	1928	50	7	0	0	50	10	0
13	1926	200	28	0	0	100	20	0
14	1927	100	14	0	0	0	0	0
15	1918	200	28	0	0	50	10	0
16	1929	25	3	0	0	0	0	0
17	1918	50	7	0	0	0	0	0

Table VIII. *Cases showing minor amounts of agglutinins.*

	B. typhosus				*B. paratyphosus* B		
	"H" antigen		"O" antigen		"H" antigen		"O" antigen
Case	Titre	Agg. units	Titre	Agg. units	Titre	Agg. units	Titre
1	0	0	50	6	0	0	0
2	0	0	0	0	50	14	—
3	25	3	25	3	—	—	—
4	0	0	0	0	50	14	50
5	25	3	0	0	25	7	0
6	25	3	0	0	25	7	0
7	0	0	50	5	0	0	25
8	50	7	50	5	50	10	50
9	0	0	100	10	0	0	0
10	50	7	50	50	0	0	0

Comparative values of living and killed antigens.

The sera from eight cases of typhoid fever and nineteen cases of paratyphoid fever were tested comparatively against the "H" and "O" living and killed antigens of *B. typhosus* and *B. paratyphosus* B as already described, and the results of these tests are presented in Table IX. The results obtained show that, provided the living antigens were grown on a favourable culture medium, the

titres obtained against the living and killed organisms were very similar. Regarding the killed Oxford suspensions as standard, the titre for the living organism was frequently the same, but occasionally varied to the extent of 100 per cent. (or one dilution by the method used); in some cases a higher titre was obtained against the killed Oxford suspensions than against the living organism; in others the reverse occurred.

Table IX. *Comparison of agglutination tests on killed and living suspensions.*

		B. typhosus				*B. paratyphosus* B			
		"H" antigen		"O" antigen		"H" antigen		"O" antigen	
Case	Type of case	Killed	Living	Killed	Living	Killed	Living	Killed	Living
13	Typhoid	800	200	0	0	0	0	25	50
14	,,	1,600	1,600	400	800	25	25	100	100
17	,,	100	100	50	100	0	0	0	0
18	,,	12,800	12,800	50	50	0	0	0	0
19	,,	200	200	100	50	0	0	0	0
26	,,	6,400	6,400	0	0	1,600	800	200	200
27	,,	100	100	0	0	0	0	0	0
28	,,	50	50	25	25	0	0	50	100
12	*Para.* B	0	0	50	50	102,400	51,200	200	200
13	,,	0	0	25	25	400	400	100	200
14	,,	0	0	0	0	12,800	12,800	400	1600
15	,,	50	200	100	100	102,400	51,200	100	50
16	,,	0	0	0	0	51,200	25,600	50	25
17	,,	200	50	100	50	1,600	1,600	100	200
18	,,	0	0	0	0	200	100	0	0
21	,,	200	100	0	0	3,200	1,600	0	0
23	,,	0	0	50	50	6,400	3,200	0	0
24	,,	100	100	0	0	1,600	800	0	0
25	,,	400	100	25	0	3,200	3,200	100	100
30	,,	25	50	25	50	12,800	6,400	50	100
32	,,	0	0	0	0	1,600	1,600	100	50
33	,,	25	0	0	0	6,400	3,200	400	400
34	,,	0	0	100	100	800	800	200	400
35	,,	0	0	0	0	800	400	100	50
36	,,	0	0	25	0	400	400	50	200
37	,,	0	0	0	0	800	200	50	50
38	,,	200	0	0	0	3,200	1,600	25	25

Serum reactions in cases suffering from infections due to members of the Salmonella group other than B. typhosus *and* B. paratyphosus *B.*

During the course of the investigation a few blood specimens were obtained from cases in which the clinical history, given by the medical practitioner, suggested the possibility of an infection due to a member of the Salmonella group other than *B. typhosus* or *B. paratyphosus* B, although in each case a Widal reaction only was requested. In the following cases certain difficulties in serological diagnosis are illustrated.

Case 1. From F.M., a male patient aged 11 years who had been ill for 5 weeks, a blood specimen was received with a request for a Widal reaction. The patient's serum failed to agglutinate *B. typhosus* "H" and "O," *B. paratyphosus* A "H," and *B. paratyphosus* B "O," but agglutinated *B. paratyphosus* B "H" (non-specific) to a dilution of 1 in 6400. In addition, the blood clot was cultured

in bile and an organism giving typical cultural and biochemical reactions of the paratyphoid group was obtained, and, in addition, this organism agglutinated to the full titre of a non-specific paratyphoid B serum. Later absorption tests were carried out, using a specific paratyphoid B serum, with the result it was found that the organism was not a strain of *B. paratyphosus* B and further serological investigation showed it to be a *B. aertrycke* strain. Inquiry was then made as to the clinical history of this patient, and from Dr Bower the following particulars were obtained:

The patient became ill with diarrhoea and vomiting, and this was followed 2 days later by fever and general malaise. He was admitted to hospital 1 week after the onset, when his temperature was evidently of that type usually associated with a septicaemia, rising every evening to 103° F. or thereabout. The boy complained of pain in the larger joints, the tongue was moist and clean, the abdomen was distended, the spleen was enlarged and he had marked diarrhoea. The fever continued, the patient showed marked wasting, and in the fifth week of the illness developed generalised lymphadenitis.

Inquiry also showed that two more members of the same family had had symptoms of food poisoning at the same time as this patient became ill, but rapidly recovered from their illness.

Case 2. M.B. A blood specimen was obtained from this patient with the request for a Widal reaction to be done. It was found that the serum agglutinated the "H" antigen of *B. typhosus* 1 in 50 and *B. paratyphosus* B (non-specific) 1 in 800, and showed no agglutination against the "O" antigen. The serological findings suggested infection due to *B. paratyphosus* B, but inquiry showed that the symptoms—vomiting, diarrhoea, and fever—were not those of paratyphoid fever but of food poisoning.

Accordingly a specimen of faeces was obtained and a non-lactose fermenter, which agglutinated with the patient's serum to a dilution of 1 in 400, was isolated. Later, serological examination of the organism by Dr W. M. Scott, of the Ministry of Health Laboratory, showed it to be Salmonella type Thompson in the non-specific form.

Case 3. K.L. This patient gave a history of vomiting, diarrhoea, abdominal pain, and fever which had continued for some time. As a result of serological examination at another laboratory, a diagnosis of paratyphoid fever was made. Further examination of a fresh specimen showed that the serum did not agglutinate the specific "H" antigen of *B. paratyphosus* B, but agglutinated the specific "H" antigen of *B. aertrycke* to 1 in 12,800 and the "O" antigen of *B. typhosus*, *B. paratyphosus* B and *B. aertrycke* to 1 in 400. The conclusion reached, therefore, was that a non-specific "H" antigen of *B. paratyphosus* B had been used in the first instance and hence the diagnosis of paratyphoid fever.

Case 4. P.S., a male aged 17 years; became ill on 11. x. 30, the main initial symptoms being vomiting, severe diarrhoea, and fever. The fever continued, rose spots appeared on the abdomen and back 5 days after the commencement

of the illness, the spleen became palpable, the symptoms then subsided, and the patient's temperature became normal on 20. x. 30. A blood sample sent in on this latter date showed no agglutination with the "H" antigens of *B. typhosus*, *B. paratyphosus* B (specific), A, or C, but agglutinated the "O" antigens of *B. typhosus* 1 in 100 and *B. paratyphosus* B 1 in 200. In view of the history the serum was then tested against the "H" antigens of *B. aertrycke* (specific) and *B. enteriditis*, with the result that the former organism agglutinated with the serum to a titre of 1 in 800. The patient's physician was then asked to send two specimens of urine and faeces, but bacteriological examination gave negative results for any member of the Salmonella group. The serological findings and clinical history indicated, however, that the patient must, most probably, have suffered from a septicaemia due to *B. aertrycke*.

DISCUSSION.

Felix (1930) has maintained that in the majority of cases of infection due to *B. typhosus* and *B. paratyphosus* B it is possible to make a serological diagnosis on the basis of a qualitative, as opposed to a quantitative, analysis of the agglutinins for the "H" and "O" antigens of the various infecting organisms; "H" agglutination being obtained only with the homologous organism, while "O" agglutination indicates simply infection with the "enterica group." He suggests that two dilutions—1 in 100 and 1 in 200—of the serum should be tested against the various antigens. The results presented in this paper confirm his contention. In the sera obtained from twenty-eight cases of typhoid fever only four of these specimens showed floccular agglutination with the "H" antigen of *B. paratyphosus* B in a dilution of 1 in 100 or more. In two cases the sera agglutinated *B. paratyphosus* B "H" to 1 in 100, and in the remaining two to 1 in 400 and 1 in 1600 respectively. Similarly with the sera from forty-two paratyphoid cases, fourteen specimens agglutinated *B. typhosus* B "H" to a dilution of 1 in 100, three to 1 in 200, one to 1 in 400, and finally two to 1 in 1600. In these cases the qualitative method would, therefore, fail to distinguish between infections due to *B. typhosus* and *B. paratyphosus* B, and it would appear to be essential to titrate the sera to their end-points.

According to Felix (1930) also, "O" agglutination with one or two of the various antigens gives only the diagnosis "enterica group," and further, the differentiation between typhoid and paratyphoid B cannot be reached from "O" agglutination even by titrating these agglutinins to their end-titres. In the present investigation the value of testing the sera against the "O" antigen of *B. typhosus* is evident, since in the early stages of typhoid fever the "O" agglutinins appeared first in the sera in some instances. In the paratyphoid cases, however, the advantage derived from using the "O" antigen of *B. paratyphosus* B is not so evident, since in not a single case was a greater amount of agglutinins present for the "O" antigen than for the "H," and it did not appear that these results were due to comparatively insensitive suspension as this "O" antigen agglutinated freely and to full titre of a serum prepared for

B. aertrycke "O" variant. Again, in only one instance was a negative agglutination reaction obtained and, later, a positive blood culture. Furthermore, in typhoid fever there was no marked correlation between the "O" agglutinins for *B. typhosus* and *B. paratyphosus* B "O" antigen, and in paratyphoid fever a similar state of affairs was evident. In certain cases of typhoid fever "O" agglutinins appeared only for *B. typhosus* and in some cases of paratyphoid fever agglutinins appeared only for *B. paratyphosus* B. White (1929), in his investigations on the antigenic structure of the Salmonella group, has shown that the reactions of the somatic antigens of *B. typhosus* and *B. paratyphosus* B are different, but that minor and variable cross-agglutination does occur.

As regards the comparative values of living and killed antigens, no particular advantage is derived from continued use of living organisms. Satisfactory and, to all intents and purposes, identical reactions can be obtained with either type, but the living organisms require special cultural conditions and their characteristics require to be frequently checked, whereas the killed antigens, as obtained from the Standards Laboratory, Oxford, are readily available whenever required.

Again, the results obtained in other Salmonella infections indicate that the specific phase of *B. paratyphosus* B "H" antigen should be used in the serological diagnosis of paratyphoid fever, and that the group agglutination reactions should be checked by using a suspension of such an organism as *Salmonella suipestifer* Type G. Prior to March, 1930, the Standards Laboratory, Oxford, issued the *B. paratyphosus* B "H" antigen in a mixed specific and non-specific form, but since that date it has been issued in the specific form.

Gardner, Hobson, and Stenhouse (1930) have reported the occurrence of the "O" variant of *B. typhosus* in the blood of a case which showed "O" agglutinins. In one case (25) which showed only "O" agglutination, the organism obtained from the blood appeared entirely normal in character, as it was actively motile and agglutinated to the full titre of a typhoid "H" serum, while in another case (13) the typhoid strains, isolated from the blood and faeces and grown in ordinary agar, would only agglutinate in a granular fashion with a pure "O" serum of *B. typhosus* and not with the pure "H" serum of this organism. Nevertheless, the organism in young broth culture was motile, and a rabbit immunised with a culture grown on the semi-solid agar media produced a serum which agglutinated *B. typhosus* "H" in a dilution of 1 in 12,800 and *B. typhosus* "O" to a dilution of 1 in 400.

The shortcomings of the original Widal reaction have been emphasised by many workers (Pijper, 1930; Manifold, 1930), but with the general adoption of a serological test in which the "H" and "O" components of the various organisms are employed, much more satisfactory results should be obtained. In this particular area an ample supply of blood has been demanded for some years so that a macroscopic and extensive serological examination could be made with the various antigens, and so that the blood clot could be cultured in bile. By employing these methods no serious difficulty has been encountered

in the diagnosis of typhoid and paratyphoid fevers, and, since about half the specimens submitted for examination were obtained from cases under personal observation, any discrepancy would soon have been noted.

SUMMARY.

The analysis of the serological results of blood specimens submitted during the course of the past 2 years for the Widal reaction has been made.

The sera from cases of typhoid and paratyphoid fever were examined in particular for their agglutinin content for the "H" and "O" antigens of *B. typhosus* and *B. paratyphosus* B. The results obtained indicate that it is essential to use the "O" antigen of *B. typhosus*, but no particular advantage has been derived, from the point of view of early diagnosis, from the use of *B. paratyphosus* B "O" antigen.

A comparison of the value of suitable living and killed antigens has been made. Either type of antigen appears to be equally effective, but for convenience and stability the suspensions issued by the Standards Laboratory, Oxford, have proved entirely satisfactory.

Cases showing the serological difficulties which arise when a mixed specific and non-specific form of *B. paratyphosus* B "H" antigen is used are described.

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