SOMATIC AND "X" AGGLUTININS TO THE *SALMONELLA* GROUP

By J. C. CRUICKSHANK

From the London School of Hygiene and Tropical Medicine

It has been recognized for some time that there may be cross-relationships between certain members of the *Salmonella* group due to the possession of minor somatic antigenic components which may not be constantly present. The importance of these relations was noted by Felix (1924) during his studies of the H and O agglutinins in enteric fever, in the course of which he observed that the non-specificity is such that somatic agglutinins produced during paratyphoid A or B infection are often more readily detected by means of a suspension of *Bact. typhosum* "O" 901 than by the homologous strain. He also noted that the antibodies formed in an individual infected with a given *Salmonella* strain may be less specific than those arising during the artificial immunization of the rabbit. This was also observed by White (1926) during his detailed work on the antigenic structure of the Salmonellas. He recorded "minor and variable cross-agglutination" between *Bact. typhosum* and *Bact. paratyphosum* B more evident in the sera of human cases than in the sera of rabbits which, he stated, are prone to form specific sera, agglutinins to the minor components of injected bacteria not appearing. Further knowledge of the extent and frequency of these relations would clearly be of value in the interpretation of the diagnostic agglutination test.

The question also arises as to whether the so-called X antigen described by Topley & Ayrton (1924) has any bearing upon this problem. This antigen was first observed in suspensions of *Bact. typhi-murium*, *Bact. newport* and *Bact. enteritidis* which had been grown in broth for some days at 37° C., but has since been found to develop in almost all the Salmonellas. The X antigen is not specific for each type, so that there are obvious possibilities of cross-agglutination where suspensions contain this antigen and sera contain the X agglutinin.

Although we have extensive evidence in regard to the presence of agglutinins acting on the somatic antigen of *Bact. paratyphosum* B in the sera of patients infected with *Bact. typhosum*, and vice versa, and some evidence in regard to the presence of cross-agglutination between *Bact. typhosum*, *Bact. paratyphosum* A and *Bact. paratyphosum* B, we have almost no evidence with regard to the presence in cases of typhoid or paratyphoid fever of somatic agglutinins acting on other *Salmonella* somatic antigens. Nor have we any information with regard to the presence of X agglutinins in normal human

sera, or in sera from typhoid or paratyphoid infection; so that we cannot tell whether, or in what degree, the presence of these agglutinins might account for the cross-reactions that have been observed. The object of the present inquiry has been to obtain evidence on these particular points and on certain other questions that have arisen in the course of the work.

A. SOMATIC AGGLUTININS

(1) *Normal sera*

In Table I are given the results of testing 200 unheated Wassermann sera for agglutinins against O suspensions representative of each of the *Salmonella* somatic groups which had been described when the work was begun. The suspensions were made by the method recommended by Felix & Gardner (1937) and were standardized in respect of agglutinability against the Oxford Standard Suspensions where these were available. The antigenic components given are those appearing in the latest schema of Kauffmann (1937) which brings up to date the knowledge of the group elucidated by himself and others.

Table I. *Agglutinins in* 200 *Wassermann sera. Percentage agglutinating at various titres*

Antigenic formula		*Para* A [I] II	*Para* B [I] IV [V]	*Para* C VI VII	*Typhosum* IX	London III X	Poona XIII	Onderstepoort XIV	X –
Titres	1 : 20	1·0	11·5	0	15·5	3·0	0·5	0·5	25·0
	1 : 40	0·5	3·5	0	4·5	1·0	0	0	15·0
	1 : 80	0	0	0	0·5	0	0	0	2·5
	1 : 160	0	0	0	0	0	0	0	1·0
	1 : 320	0	0	0	0	0	0	0	0

None of the sera contained O agglutinins at a titre of 1 : 20 or over to *Bact. paratyphosum* C, and agglutinins to *Bact. paratyphosum* A, *Bact. poona* and *Bact. onderstepoort* were very rare. *Bact. paratyphosum* B was agglutinated by 11·5% of the sera at a titre of 1 : 20 and by 3·5% at 1 : 40, *Bact. typhosum* (O 901) by 15·5% at 1 : 20, 4·5% at 1 : 40 and 0·5% at 1 : 80. 3·0% of the sera agglutinated *Bact. london* at 1 : 20, 1·0% at 1 : 40. No higher titres were recorded.

The frequency of agglutinins in normal sera for *Bact. typhosum*, *Bact. paratyphosum* A and *Bact. paratyphosum* B has been recorded by workers in various parts of the world. Agglutinins to *Bact. paratyphosum* A O have very rarely been reported in such sera. The figures relating to *Bact. typhosum* O and *Bact. paratyphosum* B O have been summarized in Table II which has been so arranged as to cover the range of serum dilutions used by the various workers up to a titre of 1 : 100. Most of the workers used suspensions which had been standardized against the Oxford Suspensions, but in certain cases it is not clear that this was done. It will be noted that most of the figures refer to countries other than England, and in certain cases, such as the series of Lewin in South Africa and Giglioli in British Guiana, the community is one in which enteric infections are of frequent occurrence. Martin's group (1)

consisted of healthy persons, handlers of food or water, while group (2) comprised 165 enteric contacts in whom no infection was known to be present. Agglutinins to *Bact. paratyphosum* A at a titre of 1 : 25 were found in 0·6% of the first group only. In no case were agglutinins to *Bact. paratyphosum* C observed at that titre in either group.

(2) *Cases of enteric fever*

(*a*) *Recorded findings in diagnostic agglutination tests.*

A number of the records of agglutinins in cases of typhoid and paratyphoid fever have been summarized in Table II. It will be seen that, of a total of 129 cases of typhoid fever, 118 agglutinated a *Bact. typhosum* O suspension and seventy-two agglutinated *Bact. paratyphosum* B O. Out of 101 of these sera about which information is available, eighty-five had a higher titre to the infecting organism, *Bact. typhosum*, and four to *Bact. paratyphosum* B, while in twelve cases agglutinins were equal in titre or absent. Of 119 sera from paratyphoid B cases, ninety-eight agglutinated *Bact. paratyphosum* B O, sixty-nine *Bact. typhosum* O. In sixty-three sera out of 108, the *Bact. paratyphosum* O titre was higher, in twenty the *Bact. typhosum* titre, and in twenty-five cases agglutinins were equal or absent. A few figures for sera from paratyphoid A cases are also available. Ample evidence thus exists of the

Table II. *Recorded frequencies of somatic agglutinins for* Bact. typhosum *and* Bact. paratyphosum *B in normal sera*

	No. of sera	Percentage of sera agglutinating *Bact. typhosum* O at various titres					
		1 : 20	1 : 25	1 : 40	1 : 50	1 : 80	1 : 100
Gardner & Stubington (England, 1932)	50	–	38	–	6	–	2
Horgan (Sudan, 1932)	70	–	7·1	–	–	–	–
Giglioli (Brit. Guiana, 1933)	350	16·3	–	3·7	–	0·9	–
Martin (1) (Malaya, 1933)	223	–	–	–	–	4·9	–
Martin (2) (Malaya, 1933)	165	–	–	–	–	5·5	–
Lewin (S. Africa, 1934)	442	–	45	–	–	–	4·5
Alves (S. Africa, 1936)	300	–	–	–	9·33	–	2·67
Dowdeswell (Kenya, 1937)	400	–	35·8	–	16·8	–	5·0
Gregory & Atkinson (Victoria, 1938)	480	20·8	–	7·7	–	2·3	–
Cruickshank (England, 1938)	200	15·5	–	4·5	–	0·5	–

	No. of sera	Percentage of sera agglutinating *Bact. paratyphosum* B O at various titres					
		1 : 20	1 : 25	1 : 40	1 : 50	1 : 80	1 : 100
Gardner & Stubington (England, 1932)	50	–	12	–	2	–	0
Horgan (Sudan, 1932)	70	–	4·3	–	–	–	–
Giglioli (Brit. Guiana, 1933)	350	20·3	–	5·7	–	2·0	–
Martin (1) (Malaya, 1933)	223	–	1·8	–	0·45	–	0
Martin (2) (Malaya, 1933)	165	–	9·6	–	6·0	–	0
Lewin (S. Africa, 1934)	442	–	–	–	–	–	–
Alves (S. Africa, 1936)	300	–	–	–	–	–	–
Dowdeswell (Kenya, 1937)	400	–	–	–	–	–	–
Gregory & Atkinson (Victoria, 1938)	480	17·3	–	9·4	–	2·3	–
Cruickshank (England, 1938)	200	11·5	–	3·5	–	0	–

– =not recorded.

https://doi.org/10.1017/S0022172400011852 Published online by Cambridge University Press

lack of specificity of O agglutinins in enteric fever so far as these three organisms are concerned.

Table III. *Somatic agglutinins in enteric cases*

Disease and author	No. of cases	Serum agglutinated *typhosum* O	Serum agglutinated *Para* B O	Where titres recorded higher with: Infecting organism	Heterologous organism	Titres equal or both negative	Serum agglutinated *Para* A O
Typhoid*:							
Felix (1924)	28	28	21	—	—	—	10
Horgan (1932)	14†	13	5	13	1	0	—
Smith (1932)	27	21	6	18	1	8	—
Gardner & Stubington (1932)	40	40	33	40	0	0	13
Downie & Fairbrother (1934)	20	16	7	14	2	4	—
Total	129	118	72	85	4	12	—
Paratyphoid B:							
Felix	11	6	11	—	—	—	2
Horgan	3‡	1	3	2	0	1	—
Smith	42	20	25	21	6	15	—
Gardner & Stubington	40	35	38	24	11	5	24
Downie & Fairbrother	23	7	21	16	3	4	—
Total	119	69	98	63	20	25	—
Paratyphoid A:							
Felix	8	6	6	—	—	—	6
Martin (1933)	11	8	—	—	—	—	9

* Martin (1933) also records 100 typhoid cases—sixteen out of seventy-four agglutinating *Bact. typhosum* O at less than 1 : 500 also agglutinated *Bact. paratyphosum* A O; ten of twenty-four agglutinating *Bact. typhosum* O at a titre of more than 1 : 500 also agglutinated *Bact. paratyphosum* A O.

† One case in this series previously inoculated with T.A.B.

‡ Two cases in this series previously inoculated with T.A.B.

(*b*) *Present series.*

In Tables IV–VI are given the results of testing numbers of sera from typhoid and paratyphoid cases for agglutinins against O suspensions of the *Salmonella* group:

(i) *Sera from typhoid cases* (Tables IV and V). These sera, which were collected at various stages of the disease, were obtained mainly from outbreaks in Croydon and Somerset.

Of the thirty-eight sera examined, twenty-nine (76·3%) agglutinated *Bact. typhosum* O at a titre of 1 : 20 or over, thirty-two (84·2%) *Bact. paratyphosum* B or *Bact. typhi-murium* O, twenty-two (57·9%) *Bact. paratyphosum* A O, seven (18·4%) *Bact. paratyphosum* C O, ten (26·3%) *Bact. london* and two (5·3%) *Bact. poona*. Considering only *Bact. typhosum* and *Bact. paratyphosum* B, it will be seen that in twenty-two cases the titre to the infecting organism was higher, while in seven cases it was lower and in nine cases the titres were equal or agglutinins to these organisms were absent.

(ii) *Sera from paratyphoid cases* (Table VI). These sera were mostly from

https://doi.org/10.1017/S0022172400011852 Published online by Cambridge University Press

Table IV. *Sera of typhoid cases.* (1) *Somerset epidemic. O suspensions*

Case no.	Age	*Para* A [I] II	*Para* B [I] IV [V]	*Para* C VI VII	*Typhosum* IX	London III X	Poona XIII	Onderstepoort XIV	"X'
1	27	80	160	40	1280	0	0	0	0
2	16	40	160	80	320	0	0	0	0
3	25	40	80	0	0	0	0	0	20
4	41	0	0	0	0	0	0	0	0
5	18	20	0	40	0	0	40	0	0
6	18	40	320	0	320	0	0	0	0
7	21	40	320	40	160	0	0	0	20
8	20	0	40	0	80	0	0	0	0
9	31	0	0	0	20	0	0	0	0
10	25	20	80	0	160	0	0	0	0
11	62	0	0	0	0	0	0	0	0
12	6	160	160	0	320	40	0	0	0
13	23	0	0	0	640	0	0	0	0
14	28	160	320	0	320	80	0	0	20
15	23	80	80	0	0	0	0	0	0
16	9	0	320	0	640	0	0	0	0
17	27	80	80	0	160	0	0	0	0
18	58	0	20	0	0	0	0	0	0
19	36	0	0	0	0	0	0	0	0
20	52	0	40	0	0	0	0	0	0

Table V. *Sera of typhoid cases.* (2) *Sera from Croydon, Southampton, etc. O suspensions*

Case no.	*Para* A	*Typhimurium* for *Para* B	*Para* C	*Typhosum*	London	Poona	Onderstepoort	"X"
21	20	80	0	640	20	0	0	0
22	80	320	20	640	0	0	0	80
23	20	40	0	40	0	0	0	0
24	640	640	0	640	0	0	0	0
25	40	160	0	1280	40	0	0	0
26	0	80	0	160	0	0	0	0
27	160	160	20	640	80	0	0	0
28	40	80	20	320	0	0	0	0
29	0	320	0	320	0	0	0	0
30	20	40	0	20	0	20	0	0
31	0	320	0	640	80	0	0	0
32	0	320	0	640	40	0	0	0
33	0	160	0	320	40	0	0	0
34	0	40	0	320	40	0	0	0
35	320	640	0	1280	80	0	0	640
36	0	40	0	0	0	0	0	0
37	40	80	0	640	0	0	0	0
38	80	160	0	640	0	0	0	20
Typhoid sera agglutinating at 1 : 20 or over:								
Total	22	32	7	29	10	2	0	6
%	57·9	84·2	18·4	76·3	26·3	5·3	0	15·8

https://doi.org/10.1017/S0022172400011852 Published online by Cambridge University Press

Table VI. *Sera of paratyphoid B cases. O suspensions*

Case no.	*Para* A	*Para* B	*Para* C	*Typhosum*	London	Poona	Onderste-poort	"X"
1	0	0	0	0	0	0	0	20
2	0	80	0	80	0	0	0	0
3	0	160	0	40	0	0	0	0
4	0	640	0	320	0	0	0	0
5	0	80	0	0	0	0	0	0
6	0	160	0	20	0	0	0	0
7	0	320	0	80	0	0	0	40
8	0	40	0	20	0	0	0	0
9	0	160	0	0	0	0	0	0
10	0	160	0	80	0	0	0	0
11	0	80	20	0	0	0	0	40
12	0	160	0	20	0	0	0	0
13	0	80	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	20	0	0	0	0
16	0	80	0	0	0	0	0	0
17	0	160	0	0	0	0	0	640
18	0	40	0	0	0	0	0	0
19	0	20	0	0	0	0	0	0
20	0	80	0	0	0	0	0	80
21	0	160	0	0	0	0	0	0
22	0	160	0	0	0	0	0	0
23	40	320	0	40	0	0	0	20
24	0	640	0	80	0	0	0	0
25	40	320	0	40	0	0	0	0
Sera agglutinating at 1 : 20 or over:								
Total	2	22	1	12	0	0	0	6
%	8	88	4	48	0	0	0	24

Liverpool and Southampton. Of twenty-five sera, twenty-two (88%) agglutinated *Bact. paratyphosum* B O, twelve (48%) *Bact. typhosum* O, two (8%) *Bact. paratyphosum* A O, one (4%) *Bact. paratyphosum* C O, while none agglutinated any of the other three O suspensions. The *Bact. paratyphosum* B titre was higher than that for *Bact. typhosum* in twenty-one cases, lower in one case, and in three the agglutinins were equal or absent.

(3) *Sera from tuberculous cases* (Table VII)

While this inquiry was in progress it seemed of interest to examine the sera of cases of tuberculosis in view particularly of the reports of Madgwick & Partner (1932) and of others that agglutinins for *Bact. typhosum*, *Bact. paratyphosum* A and *Bact. paratyphosum* B are more frequent in the sera of tuberculous than of normal persons. These workers probably referred to H agglutinins, and a later report of Damon (1937), who tested for H and O agglutinins separately, is of more interest from the present standpoint. Damon recorded that the sera of 24·4% of 143 tuberculous patients having no history of previous enteric infection or prophylactic vaccination reacted with the O antigens of one or more of the organisms mentioned, but that the titre never exceeded 1 : 100 and seldom exceeded 1 : 20.

The sera used in the present work were mainly obtained from cases of pulmonary tuberculosis in the London Chest Hospital. The figures obtained have been summarized in Table VII. The number of sera which contained

agglutinins to *Bact. paratyphosum* A or *Bact. paratyphosum* B or *Bact. typhosum* O was seven out of thirty-one (22·2%), a figure which agrees closely with that of Damon, but which was found not to differ significantly from the percentage of normal sera in the present inquiry, agglutinating one or more of the same suspensions—fifty-two out of 200 (26%).

Table VII. *Agglutinins in thirty-one tuberculous sera. Number agglutinating at various titres. O suspensions*

Titres	*Para* A	*Para* B	*Para* C	*Typhosum*	London	Poona	Onderste-poort	"X"
1 : 20	1	5	1	2	2	0	0	6
1 : 40	1	1	0	0	1	0	0	3
1 : 80	1	1	0	0	0	0	0	2
1 : 160	0	0	0	0	0	0	0	0

(4) *O agglutinin response to T.A.B. inoculation* (Tables VIII and IX)

An opportunity arose to examine the sera of a group of fifteen laboratory assistants before and after the inoculation subcutaneously of two doses of typhoid-paratyphoid vaccine. The vaccine contained 1000 million *Bact. typhosum* and 750 million each of *Bact. paratyphosum* A and B per c.c. The doses, 0·5 and 1·0 c.c. were given with an interval of a week and bleedings were made 14 days after the second dose.

From Table IX, which shows the titres after inoculation, the following points may be observed. (*a*) Two boys produced no O agglutinins and another practically none. (*b*) Of the rest, all produced agglutinins to *Bact. typhosum*, mostly over 1 : 100, the maximum being 1 : 640. (*c*) All produced agglutinins to *Bact. paratyphosum* B, mostly just under 1 : 100, the maximum being 1 : 320. (*d*) The response to *Bact. paratyphosum* A was similar to that to *Bact. paratyphosum* B. (*e*) Six of the sera showed low-titre agglutinins to *Bact. london* O. The only other O response was the increase in *Bact. paratyphosum* C agglutinins existing previously in one individual.

Table VIII. *O agglutinins produced by T.A.B. inoculation.* (1) *Titres prior to inoculation. O suspensions*

No.	*Para* A	*Para* B	*Para* C	*Typhosum*	London	Poona	Onderste-poort	"X"
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	320
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	20	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	Tr.	Tr.	80	0	0	0	0	40
12	0	0	0	0	0	0	0	40
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0

https://doi.org/10.1017/S0022172400011852 Published online by Cambridge University Press

Table IX. *O agglutinins produced by T.A.B. inoculation.*
(2) *Titres* 14 *days after second dose. O suspensions*

No.	*Para* A	*Para* B	*Para* C	*Typhosum*	London	Poona	Onderste-poort	"X"
1	80	80	0	640	40	0	0	0
2	0	20	0	160	0	0	0	640
3	0	160	0	80	0	0	0	40
4	40	80	0	40	0	0	0	0
5	20	80	0	160	40	0	0	20
6	80	160	0	160	40	0	0	20
7	40	320	0	320	80	0	0	0
8	40	40	0	160	0	0	0	0
9	160	160	0	40	40	0	0	0
10	0	20	0	0	0	0	0	0
11	80	80	320	160	20	0	0	40
12	0	0	0	0	0	0	0	80
13	0	160	0	160	0	0	0	160
14	80	160	0	320	0	0	0	80
15	0	0	0	0	0	0	0	0

B. "X" AGGLUTININS

Mention has been made of the appearance in old broth cultures of the X antigen which is not specific but may occur in many of the *Salmonella* organisms. The agglutinogen is thermostable, survives steaming for $\frac{1}{2}$ hr. and is unaffected by 0·25% formalin. It appears in both rough and smooth strains. Happold (1928, 1929), who described a precipitinogen in L_2 filtrates of steamed cultures, was of the opinion that X was not a single antigenic factor, but that there was a wide group relation between the X antigens of different Salmonellas.

X agglutination resembles the granular somatic type, but the flakes are usually slightly coarser and tend to lie along the sides of the agglutination tube. They form in 2–4 hr., but in a reading made after overnight incubation one finds that the agglutination has extended to a few higher dilutions of the serum. On shaking, the floccules disperse imperfectly, so that a fine granulation is still visible with the lens.

While suspensions for use in agglutination tests are not usually prepared in the manner above indicated, experience has shown that X antigen may in fact be present in broth cultures grown for the normal length of time or even in suspensions from agar, especially where the surface is moist, as in a Roux bottle. That confusion may in fact arise was brought home during the investigation of an obscure febrile illness occurring in a colleague, the suspension incriminated in this case actually being an alcoholized suspension of an agar growth.

In the case referred to, the serum was tested against a range of H and O suspensions of the *Salmonella* group and a few strains of *Brucella*. The only suspension to be agglutinated to high titre was *Bact. paratyphosum* B O, and the type of agglutination resembled the usual somatic type sufficiently to mislead those unfamiliar with the X type. This suspension was then tested against a serum, prepared against an unrelated organism, containing "X" agglutinins, and against an X serum from which the true somatic agglutinins

had been absorbed, and was shown to contain X antigen. Further, the patient's serum agglutinated strongly X suspensions deliberately prepared from other organisms. The case was subsequently diagnosed as *Brucella* infection, agglutinins developing later, and the cause of the X agglutinins to the very unusual titre of 1 : 5120, which still persists after many months, is quite obscure.

All the O suspensions used in the present work were therefore tested against an X serum, and only those free from X antigen were used.

In view of the finding referred to above, it was clearly of interest to know whether X agglutinins are common in normal and in pathological human sera. An X suspension was therefore included in all tests in the present series, the results being included in Tables I, IV, V, VI, VII, VIII and IX. It is believed on the grounds of numerous experiments that, although different organisms may have X antigens not identical, as evidenced by attempts at cross-absorption, nevertheless a serum containing X agglutinins will agglutinate almost all X suspensions perhaps to somewhat different titres. By the use of a sensitive suspension few sera containing X agglutinins will be missed. A *Bact. typhi-murium* X suspension was usually used along with a *Bact. typhi-murium* O suspension as a check that only X agglutination was being observed. Later a *Bact. newport* X suspension was employed alone or in addition.

(1) *X agglutinins in Wassermann sera* (Table I)

Of 200 sera, fifty (25·0%) contained X agglutinins at a titre of 1 : 20, thirty (15·0%) at 1 : 40, five (2·5%) at 1 : 80 and two (4·0%) at 1 : 160.

(2) *X agglutinins in enteric sera* (Tables IV–VI)

In the total of sixty-three typhoid and paratyphoid sera, twelve (19·0%) showed X agglutinins at a titre of 1 : 20, six (9·5%) at 1 : 40, four (6·3%) at 1 : 80. In two cases (typhoid no. 35 and paratyphoid no. 17) there were X agglutinins to a titre of 1 : 640, which was never approached in any of the Wassermann sera tested. Further, it was of interest to find that the paratyphoid case (the only one of the two in which information was available) was that of a man who had been excreting *Bact. paratyphosum* B over a period of more than 15 weeks.

The frequency of X agglutinins in enteric cases does not thus appear to be greater than that found in normal individuals.

(3) *X agglutinins in tuberculous cases* (Table VII)

Six of the thirty-one cases showed X agglutinins at a titre of 1 : 20, three at 1 : 40 and two at 1 : 80. There is therefore no evidence of increased frequency of X agglutinins in such cases.

(4) *X agglutinins in inoculated persons* (Tables VIII and IX)

In the sera of the fifteen laboratory assistants before inoculation, X agglutinins were found at a titre of 1 : 40 in two and at 1 : 320 in another. It is of

interest to record that these three boys were amongst the few whose work had brought them over a period of months or years in contact with the *Salmonella* organisms, either in the form of growing bacteria in bulk or of dealing with infected animals.

After inoculation it was found that five more of the boys had developed X agglutinins to titres varying from 1 : 20 to 1 : 160, and that there was an increase of titre in two of the three cases in which agglutinins had previously been present. It would appear that a small amount of the X antigen was present in the T.A.B. vaccine used.

DISCUSSION IN RELATION TO THE DIAGNOSTIC AGGLUTINATION TEST

From the observations recorded above it appears that, apart from *Bact. typhosum* and *Bact. paratyphosum* B, agglutinins to the *Salmonella* somatic groups which have been tested—*Bact. paratyphosum* A and C, *Bact. london*, *Bact. onderstepoort* and *Bact. poona*—are rare in normal sera in this country. Some two dozen organisms belonging to these groups have been described up to the present. O agglutinins to *Bact. typhosum* and *Bact. paratyphosum* B are more frequent, but the titres recorded are low, so that a titre of 1 : 100 in a suspected case of enteric fever would rarely be without diagnostic significance. The frequency of O agglutinins to the Salmonellas in normal sera does not appear to vary with the degree of infection in the community in the way that has been noted in regard to the H agglutinins which are of much higher frequency in areas where enteric infection is common. For example, the percentage of sera agglutinating *Bact. typhosum* O is not materially greater in Giglioli's series for British Guiana than that in the present series for England, whereas his recorded frequency of H agglutinins is much greater. It is probable, therefore, as other workers have suggested, that such low-titre agglutinins in normal sera have not arisen in response to infection, any which have so arisen being of short duration only.

In regard to the specificity of the O antigens, the evidence points to a relation between *Bact. typhosum* and *Bact. paratyphosum* B which is so constant that the presence in a serum of agglutinins to an O suspension of these organisms is unlikely to be a reliable guide to the infecting organism. In the present series of enteric cases, if a *Bact. typhosum* suspension only had been used, O agglutinins in five typhoid cases, one at a titre of over 1 : 80, and in eleven paratyphoid B cases, nine over 1 : 80, would have been missed where they would have been detected if both suspensions had been used. If a *Bact. paratyphosum* B suspension had been used alone, the presence of agglutinins in two typhoid cases, one with high titre, and in one paratyphoid B case, with low titre, would have been missed. For this reason it would seem advisable to use both O suspensions in the investigation of suspected cases of enteric fever, although it is recognized that in a routine laboratory it may be difficult to do so.

The typhoid cases very frequently had agglutinins to *Bact. paratyphosum* A, occasionally at titres over 1 : 100, and occasionally to *Bact. paratyphosum* C and *Bact. london*, but to low titre only. In the paratyphoid B cases there was very little appearance of agglutinins to *Bact. paratyphosum* A or C, and no cross-relation with any of the other O antigens tested. The homologous titres of the paratyphoid cases were on the whole, however, lower, perhaps because the blood had been taken earlier in the disease.

The individuals inoculated with T.A.B. vaccine developed O agglutinins to the organisms injected up to titres of even 1 : 640. Like the typhoid cases, they frequently acquired agglutinins to *Bact. london*, but not to any other somatic antigen.

That X antigen is not responsible for the cross-relations between the Salmonellas which have been under inquiry is proved by the fact that these relations exist where the patient's serum has been shown to be free from X agglutinins and the suspensions free from X antigen. It is clear, however, that there is some possibility of errors arising from the presence in a patient's serum of X agglutinins, as in the case recorded above, so that it is essential that *Salmonella* suspensions used for enteric diagnostic work should not contain X antigen. In view of the frequency of X agglutinins in normal sera, the risk is not a negligible one. An X antiserum for testing suspensions can readily be prepared by the inoculation of a rabbit with an X-containing suspension or filtrate of a suitable organism, grown in broth for 2 or 3 days at 37° C. and steamed for ½ hr. The organism selected may be a *Salmonella* unrelated to those to be tested, or the unwanted O agglutinins may be absorbed out with a suspension not containing X antigen to obtain a pure X antiserum.

Through the courtesy of Dr Gardner, specimens of eight H and three O Standard Agglutinable Suspensions were obtained from the Oxford Standards Laboratory. These were tested against an X antiserum and found to be uniformly free from X antigen. In two out of three *Bact. typhosum* O suspensions from another laboratory which had been stored for some time X antigen was present.

The antigenic structure of the Salmonellas

The factors assigned to the somatic antigens of the organisms used in this inquiry and appearing in Table I are those from Kauffmann's latest schema (1937). It will be observed that there is no factor accounting for the marked cross-relation between *Bact. typhosum* and *Bact. paratyphosum* B. In previous schemata a factor [XII] appeared in both groups, but Kauffmann has now omitted it, noting that it may exist, but being of opinion that it is without diagnostic significance. This view is not supported by the evidence under review, and one would prefer the retention of factor [XII] at least in the formula of *Bact. paratyphosum* B, *Bact. typhi-murium*, *Bact. typhosum* and of *Bact. enteritidis* which has frequently been tested in parallel with the other three. Factor [I] would account for any overlap between the *Bact. para-*

typhosum B group and *Bact. paratyphosum* A, although the paratyphoid cases in the present series produced no great evidence for such relation. The increased frequency of *Bact. paratyphosum* A agglutinins in the typhoid cases suggests again the presence of a common minor antigenic factor between that organism and *Bact. typhosum*. Finally, the appearance of *Bact. london* agglutinins in typhoid cases and in persons inoculated with T.A.B. vaccine suggests a relation between that organism and *Bact. typhosum*. Except for the occasional appearance of *Bact. paratyphosum* C agglutinins in typhoid fever, there is no evidence of non-specificity of the other O antigens tested, though this might of course be revealed by examination of the sera of persons infected with these rarer types, since a small amount of a minor antigen might become evident by virtue of its agglutinogenic properties.

Absorption of agglutinins

Certain of the typhoid sera in which there was a high content of both *Bact. typhosum* and *Bact. paratyphosum* B O agglutinins, and certain similar sera from the vaccinated individuals were absorbed with these organisms. The technique consisted in absorption of the serum in a final dilution of 1 : 10 with a thick suspension of the living organisms from agar plates, twice washed in saline. After absorption for 4 hr. at 37° C., then overnight in the ice-chest, the mixtures were centrifuged and the supernatant sera tested against the O suspensions as usual.

As it was thought that there might be differences between strains of *Bact. typhosum* (in, for example, content of antigen XII), two strains were used in most of the experiments, both for absorption and for agglutination, strain "901 O" and strain "Scott", which had shown slight differences in agglutinability. The results obtained were, however, almost identical with the two strains.

The results, shown in Tables X and XI, in which also percentage reduction in titre on absorption is recorded, are in accord with those of Wyllie (1937) and may be interpreted as follows: An individual infected with *Bact. typhosum* develops agglutinins to that organism and, presumably by virtue of the common antigenic component XII, to *Bact. paratyphosum* B. Complete absorption of that person's serum with *Bact. typhosum* will remove agglutinins for both organisms, while absorption with *Bact. paratyphosum* B will remove only the factor XII agglutinins, leaving those for factor IX which will still agglutinate *Bact. typhosum*, perhaps to a somewhat lower titre. It will be noted that in two cases (seras 5 and 9) absorption with *Bact. typhosum* did not remove all agglutinins to *Bact. paratyphosum* B. A possible explanation of this would be that these individuals possessed "normal" agglutinins to *Bact. paratyphosum* B O prior to infection, but there was no means of confirming this. In vaccinated persons, the sera may contain agglutinins to all the antigenic constituents injected, including IV, V, IX and XII, so that absorption with one organism leaves agglutinins acting on the other.

Table X. *Agglutinin-absorption: (i) typhoid sera*

	Serum	Unabsorbed	Abs. with *Typhosum*	% reduction in titre	Abs. with *Para* B	% reduction in titre
1	*Typhosum* O	640	0	100	640	0
	Para B O	640	0	100	0	100
2	*Typhosum* O	320	0	100	320	0
	Para B O	80	0	100	0	100
3	*Typhosum* O	320	0	100	160	50
	Para B O	320	0	100	0	100
4	*Typhosum* O	320	0	100	320	0
	Para B O	160	0	100	0	100
5	*Typhosum* O	320	0	100	320	0
	Para B O	160	40	75	0	100
6	*Typhosum* O	320	0	100	320	0
	Para B O	80	0	100	0	100
7	*Typhosum* O	320	0	100	320	0
	Para B O	160	0	100	0	100
8	*Typhosum* O	640	40	93·75	640	0
	Para B O	320	0	100	0	100
9	*Typhosum* O	160	0	100	160	0
	Para B O	160	80	50	0	100
10	*Typhosum* O	320	0	100	320	0
	Para B O	80	0	100	0	100
11	*Typhosum* O	1280	0	100	1280	0
	Para B O	160	0	100	0	100
12	*Typhosum* O	320	0	100	160	50
	Para B O	160	0	100	0	100

Table XI. *Agglutinin-absorption: (ii) sera of T.A.B. inoculated persons*

	Serum	Unabsorbed	Abs. with *Typhosum*	% reduction in titre	Abs. with *Para* B	% reduction in titre
1	*Typhosum* O	160	0	100	160	0
	Para B O	80	40	50	0	100
2	*Typhosum* O	160	0	100	80	50
	Para B O	160	80	50	0	100
3	*Typhosum* O	160	0	100	160	0
	Para B O	160	40	75	20	87·5
4	*Typhosum* O	320	20	93·1	160	50
	Para B O	80	40	50	0	100
5	*Typhosum* O	160	0	100	80	50
	Para B O	80	80	0	0	100
6	*Typhosum* O	160	0	100	80	50
	Para B O	80	40	50	0	100

Summary

An examination was made of 200 Wassermann sera, thirty-eight sera from typhoid, twenty-five from paratyphoid B and thirty-one from tuberculous cases, for somatic agglutinins to a range of Salmonellas representative of the O groups then available.

In the Wassermann sera O agglutinins, except to *Bact. typhosum* and *Bact. paratyphosum* B, were rare, and the titres against these organisms were low, attaining a titre of 1 : 80 against *Bact. typhosum* in one serum only.

The sera of the enteric cases mostly possessed agglutinins to both *Bact. typhosum* O and *Bact. paratyphosum* B O. Cross-relations with *Bact. paratyphosum* A, *Bact. london* and, to a less extent, *Bact. paratyphosum* C, were common in the typhoid sera. Otherwise there was no evidence of relation amongst the somatic groups tested.

https://doi.org/10.1017/S0022172400011852 Published online by Cambridge University Press

The response to T.A.B. inoculation of fifteen individuals suggested also the relation of some organism in the vaccine to *Bact. london* and its probable lack of relation to the other somatic groups.

There was no evidence of increased frequency of *Salmonella* agglutinins in tuberculous cases.

The cross-relations of the Salmonellas was found to be due to the possession of minor antigenic components and not to possession of X antigen, which was absent from the suspensions used. X agglutinins were not more frequent in the pathological than in the Wassermann sera.

The relation of these findings to the diagnostic agglutination test is discussed, and the possibility of error consequent upon the use of suspensions containing X antigen is pointed out.

ACKNOWLEDGEMENTS. This inquiry was made possible only by the kind co-operation of the following workers, to whom my thanks are due for providing most of the sera: Dr W. M. Scott (Ministry of Health); Prof. H. D. Wright (Liverpool); Dr Roodhouse Gloyne (the London Chest Hospital); Dr J. Thomson (Southampton Isolation Hospital); Dr S. H. Raza (Bridgwater, Somerset); Dr R. Cruickshank (North Western Group Lab., L.C.C.); the M.O.H. of Croydon and his Assistants; Dr Edith Straker (L.C.C. Group Laboratory, Mile End).

REFERENCES

ALVES, W. D. (1936). *S. Afr. med. J.* **10**, 7.
DAMON, S. R. (1937). *Amer. J. Hyg.* **26**, 40.
DOWDESWELL, R. M. (1937). *Trans. R. Soc. trop. Med. Hyg.* **31**, 363.
DOWNIE, A. W. & FAIRBROTHER, R. W. (1934). *Brit. Med. J.* p. 55.
FELIX, A. (1924). *J. Immunol.* **9**, 115.
—— (1930). *Lancet*, **1**, 505.
FELIX, A. & GARDNER, A. D. (1937). *Bull. Hlth Org.* (League of Nations), **6**, 223.
GARDNER, A. D. & STUBINGTON, E. F. (1932). *J. Hyg., Camb.*, **32**, 516.
GIGLIOLI, G. (1933). *J. Hyg., Camb.*, **33**, 379.
GREGORY, T. C. & ATKINSON, NANCY (1938). *J. Hyg., Camb.*, **38**, 566.
HAPPOLD, F. C. (1928). *J. Path. Bact.* **31**, 237.
—— (1929). *Brit. J. exp. Path.* **10**, 263.
HORGAN, E. S. (1932). *J. Hyg., Camb.*, **32**, 523.
KAUFFMANN, F. (1937). *Z. Hyg. InfektKr.* **120**, 177.
LEWIN, W. (1934). *S. Afr. med. J.* **8**, 731.
MADGWICK, J. R. A. & PARTNER, F. (1932). *Lancet*, **1**, 1091.
MARTIN, P. H. (1933). *Ann. Rep. Inst. med. Res. F.M.S.* p. 14.
SMITH, J. (1932). *J. Hyg., Camb.*, **32**, 143.
TOPLEY, W. W. C. & AYRTON, J. (1924). *J. Hyg., Camb.*, **23**, 198.
WHITE, P. B. (1926). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 103.
WYLLIE, J. (1937). *J. Hyg., Camb.*, **37**, 70.

(*MS. received for publication* 22. XII. 38.—Ed.)