AN INVESTIGATION INTO THE AGGLUTINATING POWER OF HUMAN SERA FOR *BACILLUS TYPHOSUS* AND VARIOUS ALLIED ORGANISMS.

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In 1922 Rosher and Fielden examined the sera of a number of normal persons for the presence of agglutinins for *B. typhosus* and certain members of the paratyphoid and Salmonella group. At that time the studies of Andrewes (1922) on diphasic antigenic variation in organisms of the Salmonella group had not yet been recorded; and it is probable that some, at least, of the bacterial suspensions employed contained organisms in both the type and group phase.

The present paper records the results obtained in an investigation into the agglutinating power of sera obtained from various groups of persons during the years 1925–6. The sera were tested against seven different species belonging to the typhoid-paratyphoid group. In the case of diphasic species the suspension employed was in the type phase, and an eighth suspension was added to the series consisting of one of the diphasic species in the group phase, and thus responding to any group agglutinin which might be present. Only the heat labile agglutinins were considered.

The sera examined fall into two main groups. The first group consists of specimens which were sent to the laboratory for routine examination because the donors were suspected either of suffering from enteric fever, or of being contacts. They are referred to hereafter as "not normals." Sera of the second group, the "normals," were chosen at random from those sent in for a Wassermann test. Altogether 847 "not normal" and 302 "normal" sera were examined. An analysis of the total population dealt with is given in Table I.

Table I.

Total population 1149

- *A.* Normals 302
- *B.* Not normals 847
  - Inoculated 49
    - Suspected of enteric 42
    - Contacts 5
    - No information as to enteric 2
  - Not inoculated 489
    - Suspected of enteric 436
    - Contacts 41
    - No information as to enteric 12
  - No information as to inoculation 309
    - Suspected of enteric 117
    - Contacts 146
    - No information as to enteric 46

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Table II A. *Proportions of the various groups who agglutinated the different strains.*

Organism		Normals *A*	Not normals *B*: Inoculated		Not normals *B*: Not inoculated		Not normals *B*: No information		Not normals *B*: Total not normals (all *B*)		Whole population *A* + *B*	
B. typhosus	45/302	14·9 % ± 0·014	36/49	73·4 % ± 0·043	114/489	23·3 % ± 0·013	48/309	15·5 % ± 0·014	198/847	23·3 %	243/1149	21·1 % ± 0·008
B. paratyphosus A	18/302	5·9 % ± 0·009	20/49	40·8 % ± 0·047	17/489	3·5 % ± 0·006	10/309	3·2 % ± 0·007	47/847	5·5 %	65/1149	5·7 % ± 0·005
B. paratyphosus B	18/302	5·9 % ± 0·009	24/49	48·9 % ± 0·048	60/489	12·3 % ± 0·010	39/308	12·7 % ± 0·013	123/846	14·5 %	141/1148	12·3 % ± 0·007
B. paratyphosus C	1/302	0·3 % ± 0·002	2/49	4·1 % ± 0·019	5/483	1·0 % ± 0·003	1/303	0·3 % ± 0·002	8/835	0·9 %	9/1137	0·8 % ± 0·002
B. aertrycke Mutton	16/302	5·3 % ± 0·009	2/49	4·1 % ± 0·019	13/480	2·7 % ± 0·005	12/305	3·9 % ± 0·008	27/834	3·2 %	43/1136	3·8 % ± 0·004
B. aertrycke Newport	0/302	0	1/36	2·8 % ± 0·016	10/396	2·5 % ± 0·005	4/250	1·6 % ± 0·005	15/672	2·2 %	15/984	1·5 % ± 0·002
Group	19/302	6·3 % ± 0·009	16/49	32·7 % ± 0·045	60/485	12·4 % ± 0·010	39/305	12·8 % ± 0·014	115/839	13·7 %	134/1141	11·7 % ± 0·006
B. enteritidis Gaertner	14/302	4·6 % ± 0·008	0/49	0	27/481	5·4 % ± 0·007	20/302	6·6 % ± 0·010	47/832	5·6 %	61/1134	5·4 % ± 0·004

The figures on the right of each column give the probable error.

TECHNIQUE.

Each serum was put up in a series of 10 dilutions from 1 in 20 up to 1 in 5120, and was tested against suspensions of the following eight organisms: *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B (type phase), *B. paratyphosus* C (type), *B. aertrycke* Mutton (type), *B. aertrycke* Newport (type), *B. enteritidis* Gaertner, and an organism in the group phase either *B. aertrycke* Mutton or *B. paratyphosus* B.

The bacterial suspensions were obtained by growing the organism aerobically in broth at 37° C. for 18 hours. After the addition of formalin (to give a final concentration of 0·5 per cent.), they were standardised by the opacity tube method so as to contain approximately 750×10^6 organisms per c.c.

The serum-suspension mixtures were incubated in a water bath at 55° C. for 2 hours, with the water level adjusted to leave about two-thirds of the column of fluid in the tubes uncovered, and examined for agglutination by artificial light against a black background.

Table II A shows the number of sera in both the total population and the main groups which agglutinated the various organisms, at a dilution of 1/20 or over.

It will be seen that typhoid agglutinins have the highest incidence in every group. Only the normals, of course, can be compared with Rosher and Fielden's results. For each of the organisms employed in common the percentage of their sera containing agglutinins was considerably higher than the percentage of ours. Moreover, this difference cannot be ascribed entirely to the fact that some of their antigenic suspensions were probably diphasic since it holds good for the monophasic *B. typhosus* and *B. paratyphosus* A. Also the percentage of *B. paratyphosus* B agglutinating sera found by them exceeds the sum of the *B. paratyphosus* B (type) and group agglutinating sera in our case.

The "not normal" group shows a significantly higher percentage of agglutinating sera than the "normals" against *B. typhosus*, *B. paratyphosus* B and the group suspension. The further analysis of this group brings out the expected fact that sera from inoculated persons have a much higher incidence of agglutinins against those organisms included in the T.A.B. vaccine and also against the group suspension, than those from non-inoculated, and no-information donors.

Table II B gives an analysis of all the "not normal" sera, classified according to the information supplied with the specimen of serum.

As regards the typhoid-paratyphoid organisms and also the group suspension, the percentage figures of agglutinating sera, as one would expect, are definitely higher in the "suspected of enteric" group than in the "contact" and "no information" groups. This does not hold, however, for Aertrycke and Gaertner agglutinins.

Tables III A and III B give the proportion of positive agglutination in the two sexes.

Table II B. *Proportions of the different divisions of the "not normals" agglutinating the various strains.*

Strain	Suspected of enteric	Contacts	No information given
B. typhosus	$\frac{164}{594}=27\cdot6$ %	$\frac{23}{192}=11\cdot9$ %	$\frac{11}{61}=18\cdot0$ %
B. paratyphosus A	$\frac{39}{594}=6\cdot6$ %	$\frac{5}{192}=2\cdot6$ %	$\frac{3}{61}=4\cdot9$ %
B. paratyphosus B	$\frac{\cdot104}{594}=17\cdot5$ %	$\frac{10}{192}=5\cdot2$ %	$\frac{8}{60}=13\cdot3$ %
B. paratyphosus C	$\frac{7}{589}=1\cdot2$ %	$\frac{1}{190}=0\cdot5$ %	$\frac{0}{59}=0$
B. aertrycke Mutton	$\frac{18}{584}=3\cdot1$ %	$\frac{8}{190}=4\cdot2$ %	$\frac{1}{60}=1\cdot7$ %
B. aertrycke Newport	$\frac{12}{493}=2\cdot4$ %	$\frac{1}{138}=0\cdot7$ %	$\frac{2}{51}=3\cdot9$ %
Group	$\frac{95}{588}=16\cdot2$ %	$\frac{13}{192}=6\cdot8$ %	$\frac{7}{59}=11\cdot9$ %
B. enteritidis Gaertner	$\frac{33}{585}=5\cdot6$ %	$\frac{9}{188}=4\cdot8$ %	$\frac{5}{59}=8\cdot5$ %

Table III A. *Percentage of positive agglutinations among males and females in normals.*

	Suspension of								
	B. typhosus	*B. para.* A	*B. para.* B	*B. para.* C	*B. aertrycke*	*B.* Newport	*B. enteritidis*	Group	Total
Males	23·3	11·6	9·7	0	3·9	0	3·2	9·0	154
Females	4·7	0	2·0	0·6	6·8	0	6·1	5·4	146

Table III B. *Percentage of positive agglutinations among males and females of different age groups among the "normals."*

		Age in years					
		–20	20–30	30–40	40–50	50–60	60+
B. typhosus	Male	—	30·0	39·5	11·1	4·3	22·2
	Female	—	7·4	6·8	—	—	—
B. paratyphosus A	Male	—	15·0	20·8	—	4·3	11·1
	Female	—	—	—	—	—	—
B. paratyphosus B	Male	—	12·5	14·5	11·1	—	—
	Female	—	3·7	2·2	—	—	—
B. paratyphosus C	Male	—	—	—	—	—	—
	Female	—	—	—	—	7·6	—
B. aertrycke Mutton	Male	14·28	7·5	4·1	—	—	—
	Female	5·8	7·4	2·2	21·4	—	25·0
B. aertrycke Newport	Male	—	—	—	—	—	—
	Female	—	—	—	—	—	—
B. enteritidis Gaertner	Male	14·28	2·5	2·0	3·7	4·3	—
	Female	5·8	7·4	6·8	—	7·6	—
Group	Male	—	7·5	12·5	3·7	4·3	—
	Female	—	5·5	2·2	7·1	—	50·0
Numbers	Male	7	40	48	27	23	9
	Female	17	54	44	14	13	4

There is a marked difference between the male and female groups with regard to T.A.B. agglutinins. The male sera show higher percentages than the female. It is interesting to compare Rosher and Fielden's results, which were

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published soon after the war, with ours. They record 42 per cent. positive for *B. typhosus* among males and 5 per cent. positive among females. We find a much lower percentage among males, namely, 23 per cent. positive (Table III A), but practically the same percentage, 4·7, among females. Prophylactic inoculation during the war was much commoner among males than females, and it seems reasonable to assume that the females represent a normal population unaffected by inoculation, such inoculation being responsible for the much higher percentage in the male group. This assumption is supported by the fact that the male preponderance is most evident in the age groups 20–30 and 30–40, that is in those groups which, at the time of this investigation, include the bulk of ex-service men inoculated during the war. Furthermore, the drop from Rosher and Fielden's 42 per cent. to our 23 per cent. indicates a gradual approach of the male group with the passage of time to a normal uninoculated population.

An attempt has been made to determine whether the ability of a serum to agglutinate one organism is correlated with its ability to agglutinate any other. Only the two main groups, "normals" and "not normals," have been dealt with because the numbers of the various sub-groups are too small for useful analysis.

Table IV A. *Association between ability to agglutinate one organism and ability to agglutinate another. Number of sera agglutinating various pairs of suspensions.*

Organism	Number	T.	A.	B.	C.	M.	N.	E.	Gp.
				Total "normals" 302.					
B. typhosus	45	—	16	11	—	2	—	3	7
B. paratyphosus A ...	18	16	—	8	—	1	—	1	6
B. paratyphosus B ...	18	11	8	—	—	1	—	—	7
B. paratyphosus C ...	1	—	—	—	—	—	—	—	—
B. aertrycke Mutton...	16	2	1	1	—	—	—	3	1
B. aertrycke Newport	0	—	—	—	—	—	—	—	—
B. enteritidis Gaertner	14	3	1	—	—	3	—	—	—
Group	19	7	6	7	—	1	—	—	—
				Total "not normals" 847.					
B. typhosus	198	—	41	60	—	7	5	21	44
B. paratyphosus A ...	47	41	—	30	—	2	2	5	19
B. paratyphosus B ...	123	60	30	—	—	4	6	7	80
B. paratyphosus C ...	0	—	—	—	—	—	—	—	—
B. aertrycke Mutton...	27	7	2	4	—	—	4	8	7
B. aertrycke Newport	15	5	2	6	—	4	—	2	6
B. enteritidis Gaertner	47	21	5	7	—	8	2	—	8
Group	115	44	19	80	—	7	6	8	—
				Total population ("normals" + "not normals") 1149.					
B. typhosus	243	—	57	71	—	9	5	24	51
B. paratyphosus A ...	65	57	—	38	—	3	2	6	25
B. paratyphosus B ...	141	71	38	—	—	5	6	7	87
B. paratyphosus C ...	0	—	—	—	—	—	—	—	—
B. aertrycke Mutton...	43	9	3	5	—	—	4	11	8
B. aertrycke Newport	15	5	2	6	—	4	—	2	6
B. enteritidis Gaertner	61	24	6	7	—	11	2	—	8
Group	133	51	25	87	—	8	6	8	—

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Table IV B. *Association between ability to agglutinate one organism and ability to agglutinate another.*

Significant association.

Organisms	Normals χ	Normals r	Not normals χ	Not normals r	Whole population χ	Whole population r
B. typhosus and *B. para.* B	5·7	0·67	7·2	0·38	9·1	0·51
B. typhosus and Group	2·8	0·39	4·0	0·25	5·1	0·32
B. para. B and Group	5·9	0·62	18·2	0·84	20·0	0·83
B. para. A and B	7·1	0·69	9·9	0·70	11·7	0·68
B. para. A and Group	4·9	0·55	5·5	0·45	6·9	0·47
B. typhosus and *B. para.* A	9·1	0·94	10·6	0·90	13·5	0·91
B. enteritidis Gaertner and *B. aertrycke* Mutton	2·8	0·40	5·5	0·47	6·0	0·44
B. para. B and *B. aertrycke* Newport	—	—	2·8	0·40	3·4	0·44
B. typhosus and *B. enteritidis* Gaertner	—	—	3·7	0·36	3·7	0·32
B. aertrycke Mutton and *B. aertrycke* Newport	—	—	5·7	0·51	5·0	0·45

Table IV C. *Not significant association.*

Values of χ.

Organisms	Normals	Not normals	Whole population
B. aertrycke Mutton and Group	−0·01	2·08	1·58
B. typhosus and *B. aertrycke* Mutton	−0·28	0·44	0·08
B. typhosus and *B. aertrycke* Newport	—	1·02	1·29
B. para. A and *B. aertrycke* Mutton	0·05	0·47	0·42
B. para. A and *B. aertrycke* Newport	—	1·50	1·40
B. enteritidis Gaertner and *B. para.* B	−0·96	0·12	−0·15
B. enteritidis Gaertner and *B. aertrycke* Newport	—	1·35	1·48
B. enteritidis Gaertner and Group	−0·99	0·81	0·45
B. typhosus and *B. enteritidis* Gaertner ...	0·70	—	—
B. para. A and *B. enteritidis* Gaertner	0·19	1·63	1·50

Note on the test for the statistical significance of an association observed in a sample, of the ability to agglutinate one organism with the ability to agglutinate a second organism, and on the measure of the association.

	A	not $-A$	
B	$Npp' + \delta$	$Nqp' - \delta$	Np'
not $-B$	$Npq' - \delta$	$Nqq' + \delta$	Nq'
	Np	Nq	

If the fraction p of the sample of N is A in some character and the fraction q $(= 1 - p)$ is not $-A$ and if in a second character the fractions p' and q' $(= 1 - p')$ are B and not $-B$, then if A and B are independent characterisations we expect, in the average of a number of samplings, Npp' to be both A and B and Npq' to be both A and not $-B$, etc.

If we find $Npp' + \delta$ are both A and B then $Npq' - \delta$ must be the numbers who are A and not $-B$, etc., and the numbers will fall as in the table. δ is called the excess or error of random sampling, and if we suppose further samples selected so that the marginal numbers are unchanged and remain the same as found in the observed sample the excess δ will vary from sample to sample and

$$\text{mean } \delta = 0, \qquad \sigma_\delta = \sqrt{Npp'qq'}.*$$

To find the probability P of such an excess, or greater, we find the deviate,

$$\chi = \delta/\sigma_\delta,$$

and use any table of the probability integral to find the area beyond $\pm\chi$.

* To be accurate the s.d. of the hypergeometric series requires a divisor $1 - 1/N$, but for our purposes this may be ignored.

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If $\chi = 2{\cdot}5$, we find, using Sheppard's tables, that $P = 2 \times (1 - {\cdot}9938) = 0{\cdot}0124$, that is, the excess will occur by chance alone once in 80 times. By convention we will say that χ must be $> 2{\cdot}5$ to be treated as significant of an association between A and B beyond what may be expected to arise by the operations of chance alone.

If $\chi = 2$, $P = 0{\cdot}0455$ (occurs by chance once in 22 times, not significant);
if $\chi = 3$, $P = 0{\cdot}00270$ (occurs by chance once in 370 times, significant);
if $\chi = 4$, $P = 0{\cdot}00006$ (occurs by chance once in 16,000 times, significant);

and as χ increases beyond 2·5 we regard the significance as more and more assured.

For example we are given in Tables II A and IV A that in a sample of 302 normals, 45 agglutinate typhoid, 19 agglutinate group and 7 agglutinate both typhoid and group. We expect $\frac{45}{302} \times 19$ or 2·83 to agglutinate both as the result of independent chances and 7 shows an excess of 4·17. The standard error of sampling is

$$\sqrt{2{\cdot}83 \times \left(1 - \frac{45}{302}\right)\left(1 - \frac{19}{302}\right)} = 1{\cdot}50.$$

The deviate is therefore $$\chi = \frac{4{\cdot}17}{1{\cdot}50} = 2{\cdot}8$$

and we treat it as just significant.

It must be admitted that, when we say that significance is established on the ground that 7 persons appear in a category when on the average 2·83 only are expected, and that such an event will only be brought about once in 196 times by chance alone it will need but small disregarded circumstances to modify these calculations.

If the excess is significant we measure the degree of the association of a power to agglutinate the one organism with a power to agglutinate the other as a coefficient of correlation or contingency.

In this instance we suppose the ability to agglutinate to be susceptible of gradations in the manner of a normally distributed variate. For this purpose the formula of Pearson*,

$$Q_5 = \sin \frac{\pi}{2}\sqrt{1 + K^2}, \qquad K^2 = \frac{4abcdN^2}{(ad - bc)^2\,(a + d)\,(b + c)},$$

has been taken for the approximate computation of the correlation coefficient r and the values in the column under r are subject to the errors of this formula.

a	b	$a + b$
c	d	$c + d$
$a + c$	$b + d$	N

In the table shown above it was found that

$$Q_5 = 0{\cdot}619,$$
$$r = 0{\cdot}662,$$

the latter value calculated by tetrachoric functions.

7	11	18
12	272	284
19	283	302

With one exception only those associations which might arise from T.A.B. inoculation are significant in both the "normal" and "not normal" groups. In the latter group only however the degree of association between *B. typhosus* and *B. enteritidis* Gaertner, between *B. aertrycke* Mutton and *B. aertrycke* Newport, between *B. para.* B and *B. aertrycke* Newport, and between *B.*

* *Phil. Trans.* A, **195**, 16 (1900).

enteritidis Gaertner and *B. aertrycke* Mutton are statistically significant. The numbers involved in many of these latter instances are, however, too small to justify any conclusions with regard to the possible factors on which such associations might depend. For the moment we can only note that they are not in accordance with the expectations which would arise from the known antigenic relationships within this group, on the assumption that they are produced by a common antigenic stimulus.

Reviewing the evidence presented in this section, as a whole, it is clear that there is a significant association, in each group, between the ability to agglutinate *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B in the type phase, and the suspension containing the group phase of the diphasic series. This association is most readily explained as a result of inoculation with T.A.B. vaccine. One particular association, that between *B. paratyphosus* B and the group suspension would also arise from infection with *B. paratyphosus* B; and it is of interest to note that this correlation is far higher than any other in the "not normal" group, among which both factors, inoculation and infection, will have been operative; while in the "normal" group among which the inoculation factor alone may be assumed to have been operative, it occupies a far less conspicuous position.

In view of the importance attached by some authorities to the anamnestic reaction—the stimulation of the production of antibodies against a bacterium to which the tissues have already responded, as a result of a subsequent infection with some antigenically unrelated organism—it is of interest to note that the data here presented, as far as they go, afford no suggestion of the interference of such a factor. With the single exception already noted, the correlations among the various pairs of suspensions in question are lower among the "not normals" than among the "normals." This would be expected, if the result of enteric infection were to stimulate the production of agglutinins for the infecting species without affecting those acting on other species against which the infected person had previously been immunised. It would not be expected if the result of infection were to stimulate the renewed production of agglutinins acting on such organisms.

With regard to the causation of agglutinins acting on *B. enteritidis*, *B. aertrycke* and the Newport type, our data throw little light. On the assumption that such agglutinins arise as the result of natural infection only, and neglecting the rather remote possibility that infection with one organism implies a risk of infection with another, we should expect an association between *B. aertrycke* and Group, and between Newport and Group, but no association between the pairs *B. enteritidis* and Group, *B. enteritidis* and *B. aertrycke*, *B. enteritidis* and Newport, and *B. aertrycke* and Newport, nor between *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B and any of the three other species. As will be seen from the tables these conditions are not fulfilled. We do not think that any significance can be attached to this fact. Reference to Table II A shows that very few sera agglutinate the Newport suspension, in its type phase, and the numbers agglutinating *B. enteritidis* and *B. aertrycke* are relatively small. For an adequate analysis of this particular problem along statistical lines far larger numbers of sera must be examined.

Table V A presents a percentage classification of the sera in various groups with regard to their titre or end-point of agglutination. Although rigid conclusions are to be deprecated because of the small numbers on which some of

Table V A. *Percentage classification of the sera of the various groups on a basis of their agglutination titre.*

	Dilution of serum										
	20	40	80	160	320	640	1280	2560	5120	10,000 or over	Total
spected of enteric 472											
B. typhosus	1·3	2·5	4·9	5·5	5·5	3·6	4·5	4·7	1·1	1·3	164
B. paratyphosus A ...	1·5	1·5	2·1	1·9	0·4	0·4	0·2	0·2	—	—	39
B. paratyphosus B ...	1·3	1·5	2·9	2·5	1·3	1·3	1·7	3·8	2·1	3·6	104
B. paratyphosus C ...	0·2	0·6	0·4	0·2	—	—	—	—	—	—	7
B. aertrycke Mutton...	1·5	1·1	0·4	0·4	—	—	0·4	—	—	—	18
B. aertrycke Newport	0·2	0·6	0·4	0·6	0·4	—	—	—	0·2	—	12
B. enteritidis Gaertner	0·4	2·5	1·5	0·8	0·2	0·8	0·2	0·4	—	—	33
Group	0·8	2·9	2·5	1·3	2·9	1·1	2·5	1·5	1·9	2·5	95
ntacts 70											
B. typhosus	7·1	2·9	7·1	5·7	5·7	1·4	2·9	—	—	—	23
B. paratyphosus A ...	1·4	4·3	1·4	—	—	—	—	—	—	—	5
B. paratyphosus B ...	1·4	1·4	2·9	1·4	4·3	1·4	—	—	1·4	—	10
B. paratyphosus C ...	1·4	—	—	—	—	—	—	—	—	—	1
B. aertrycke Mutton...	2·9	5·7	1·4	1·4	—	—	—	—	—	—	8
B. aertrycke Newport	—	—	—	1·4	—	—	—	—	—	—	1
B. enteritidis Gaertner	1·4	5·7	2·9	2·9	—	—	—	—	—	—	9
Group	—	2·9	4·3	4·3	2·9	1·4	—	—	2·9	—	13
information 37											
B. typhosus	5·4	—	—	5·4	5·4	—	5·4	8·1	—	—	11
B. paratyphosus A ...	—	2·7	2·7	—	—	—	—	2·7	—	—	3
B. paratyphosus B ...	—	2·7	8·1	2·7	—	2·7	—	2·7	2·7	—	8
B. paratyphosus C ...	—	—	—	—	—	—	—	—	—	—	0
B. aertrycke Mutton...	—	—	2·7	—	—	—	—	—	—	—	1
B. aertrycke Newport	—	2·7	2·7	—	—	—	—	—	—	—	2
B. enteritidis Gaertner	—	8·1	5·4	—	—	—	—	—	—	—	5
Group	—	5·4	2·7	5·4	—	—	—	2·7	—	2·7	7
rmals 129											
B. typhosus	2·3	3·0	2·6	4·6	0·6	1·0	—	—	0·3	—	44
B. paratyphosus A ...	1·3	1·3	2·0	0·6	0·3	0·3	—	—	—	—	18
B. paratyphosus B ...	0·6	2·6	1·0	0·3	1·0	0·3	—	—	—	—	18
B. paratyphosus C ...	—	—	0·3	—	—	—	—	—	—	—	1
B. aertrycke Mutton...	1·6	1·6	2·0	—	—	—	—	—	—	—	16
B. aertrycke Newport	—	—	—	—	—	—	—	—	—	—	0
B. enteritidis Gaertner	2·3	1·3	0·3	—	0·3	0·3	—	—	—	—	14
Group	3·3	1·0	1·0	0·3	0·3	—	—	—	—	—	18

the percentage numbers are based, it is obvious that in the case of *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B, *B. enteritidis* Gaertner, and the group suspension a greater tendency towards high titre is found in those suspected of enteric than in any of the other groups.

Table V B gives the available data with regard to the titres noted among the normal group. Titres of 1/20 and 1/40 are found to much the same extent in both sexes, but higher titres are found among males than among females.

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Among females it is very rare to find a titre above 1/40, while among males, who comprise the inoculated portion of the population, titres up to 1/640 are found. Normal agglutinins for *B. paratyphosus* A are very rare; none are found among females.

Table V B. *Titres and sex in normals.*

		Titres								
		20 %	40 %	80 %	160 %	320 %	640 %	1280 %	2560 %	5120 %
B. typhosus	Male	2·6	3·9	5·2	8·4	1·2	1·9	—	—	0·68
	Female	2·0	2·0	—	0·7	—	—	—	—	—
B. paratyphosus A	Male	2·6	2·6	4·0	1·2	0·6	0·6	—	—	—
	Female	—	—	—	—	—	—	—	—	—
B. paratyphosus B	Male	0·68	3·9	1·9	0·68	1·9	0·68	—	—	—
	Female	0·7	1·3	—	—	—	—	—	—	—
B. paratyphosus C	Male	—	—	—	—	—	—	—	—	—
	Female	—	—	0·7	—	—	—	—	—	—
B. aertrycke Mutton	Male	2·6	1·2	1·9	—	—	—	—	—	—
	Female	0·7	2·0	2·0	—	—	—	—	—	—
B. aertrycke Newport	Male	—	—	—	—	—	—	—	—	—
	Female	—	—	—	—	—	—	—	—	—
B. enteritidis Gaertner	Male	2·6	0·68	—	—	—	—	—	—	—
	Female	2·0	2·0	0·7	—	0·7	0·7	—	—	—
Group	Male	3·9	1·2	1·9	—	—	—	—	—	—
	Female	2·7	0·7	—	0·7	0·7	—	—	—	—

Summary.

1. Three hundred and two samples of sera from normal people, and 847 samples from cases suspected of enteric, or from contacts, have been examined for heat labile agglutinins against *B. typhosus* and allied organisms.

2. Sera from the "not normals" show a higher percentage of agglutinins against nearly all the organisms tested than sera from the "normals."

3. In the contact group of the "not normals" the incidence of agglutinins is practically the same as in the "normal" group.

4. Sera from inoculated persons show a higher percentage of agglutinins against those organisms included in the T.A.B. vaccine used during the late war, than the sera from non-inoculated persons.

5. There is a higher percentage of agglutinins for *B. typhosus, B. paratyphosus* A and *B. paratyphosus* B among males than among females, especially in the age groups 20–30 and 30–40.

6. The proportion of normal males whose sera agglutinate *B. typhosus, B. paratyphosus* A and *B. paratyphosus* B and the group suspension is significantly lower than corresponding proportion noted in an investigation recorded by Rosher and Fielden in 1922. The proportion of agglutinating sera for these strains among normal females is substantially the same in our findings as in theirs.

7. The associations between ability to agglutinate different pairs of organisms are shown to be statistically significant in those cases which might arise from T.A.B. inoculation.

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8. For these reasons it seems likely that the present distribution among the adult male population of agglutinins for *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B and for Salmonella strains allied to *B. paratyphosus* B when in the group phase, was largely determined, in the period 1925–6, by the results of T.A.B. inoculation carried out during the war. It is probable that this effect is slowly disappearing.

9. Statistically significant associations were found between certain other pairs of organisms, which could not be explained as resulting from inoculation with T.A.B. vaccine, or as a result of any common antigenic stimulus. These associations were found mainly in the "not normal" group; and their meaning, at the moment, remains obscure. It may be noted that the number of sera agglutinating these particular pairs of suspensions was, in most cases, small.

10. Our results, so far as they yield any information on this point, do not suggest that infection with any one of the organisms studied by us increases the probability of the patient's serum agglutinating some other bacterium, with which the infecting organism is not antigenically related.

11. High titres were found chiefly in sera from the "suspected of enteric" group. The normal group usually gave low titres.

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