**THESIS**

# A STUDY OF THE FLAGELLAR AND SOMATIC AGGLUTININS TO B. TYPHOSUS AND B. PARATYPHOSUS B.

submitted by

J. Wyllie, M.A.,M.B.,B.Sc.,(Glasgow),D.P.H.(Camb.)

for the degree of

Doctor of Medicine.



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

## The Thesis

'A Study of the Flagellar and Somatic Agglutinins to B. typhosus and B. paratyphosus B'

is divided into three parts.

Part I deals with Floccular and Granular Agglutination in normal, inoculated and enteric fever cases;

Part II with the Serological Diagnosis of the Chronic Typhoid Carrier, and

Part III with the Zone Phenomenon in Agglutination Reactions.

PART I

# FLOCCULAR AND GRANULAR AGGLUTINATION IN NORMAL, INOCULATED AND ENTERIC FEVER CASES.

Paper incorporated

Wyllie, J. The Journal of Hygiene, Vol. XXXII, No. 3, July 1932.

Contents

# INTRODUCTION

The main points in the development of the theory of flagellar and somatic antigens may be reviewed briefly.

In 1903, Theobald Smith and Reagh demonstrated two types of agglutination. They performed agglutination tests with immune serum, derived by animal injection of the motile form of B. suipestifer and observed a "large, fluffy precipitate" of the motile strain and a "uniformly fine, compact, granular precipitate" of the non-motile strain in relatively high serum concentrations. On the other hand, both strains were agglutinated in a granular manner and to practically the same titre with an immune serum prepared from the non-motile variant. They deduced that the motile form of B. suipestifer possessed two antigens, flagellar and somatic, while the non-motile form contained only the somatic antigen. The importance of these two types of agglutination, floccular and granular, was not fully appreciated for a number of years afterwards.

Weil and Felix (1917) discovered similar phenomena in their study of B. proteus X strains. They ascertained that in artificial culture media strains of B. proteus X appear in two forms, the one growing in a spreading film (Hauch) and called 'H', the other growing in discrete colonies without a film (Ohne Hauch) and called 'O'. A suspension of the 'O'-type was found to agglutinate in small granular clumps and was thermostable, but suspensions of the 'H'-type agglutinated in coarse flocculi and were thermolabile. Felix explained these results in this way: 'the immune serum of the O-form contains only one agglutinin, which reacts specifically only with the homologous bacilli and agglutinates them small-flakedly: the immune serum of the H-form contains two agglutinins, the specific small-flaking O-agglutinin and a non-specific, large-flaking H-agglutinin, which reacts with the heterologous just as it does with the homologous bacilli. The antigen of the O-forms consists accordingly of one type of receptors

(O-receptors); the antigen of the H-forms, on the other hand, contains two types of receptors (H- and O- receptors).'

Weil and Felix (1918, 1920) were led later to extend their observations to the typhoid-paratyphoid group, in the members of which they found evidence also of a double receptor structure. There is one difference between the structural scheme for B. proteus and that for the members of the typhoid-paratyphoid group. In B. proteus the 'H' receptor is capable on inoculation of giving rise to large-flaking agglutinins which are non-specific or group in character, but in B. typhosus and allied members the labile 'H' receptor is specific while the stable 'O' receptor is responsible for group agglutination.

The recognition that the thermolabile antigen of proteus organisms was associated with the flagella and distinct from the thermostable antigen in the somata is apparently due to Braun and Schäffer (1919) who inhibited the development of flagella by culturing proteus bacilli on phenol agar. The same association for the typhoid group was proved by Feiler (1920).

The studies initiated by Weil and Felix have been confirmed and extended by many investigators, including Orcutt (1924) who prepared flagellar suspensions from the motile strain of B. suipestifer (by shaking and centrifuging) and showed that floccular agglutination is definitely associated with the motile phase and with the flagella. Bruce White (1925) has also carried out elaborate and detailed investigation into the antigenic structure of the Salmonella group. Craigie (1931) made an exhaustive study of the serological reactions of the flagella of B. typhosus. He showed that Orcutt's results in which she claimed to have obtained a pure flagellar agglutinating serum by the inoculation of flagellar suspensions are open to criticism. His own findings

like those of Yokota (1925) and Balteanu (1926) show that flagellar suspensions contain a considerable amount of somatic agglutinogen.

The available evidence is in favour of the view that the 'H' antigen (receptor) of Weil and Felix is associated with the flagella of a motile organism (the flagella may not consist entirely of 'H' antigen) and that this antigen is distinct from the 'O' antigen (receptor) contained in the soma. Although formerly applied to the appearance of the two types of Proteus colony growing on artificial culture media, it is now customary to designate by the initial letters of Hauch and Ohne Hauch (i) the thermolabile and thermostable antigen (ii) the character of the agglutination obtained with these antigens and (iii) the agglutinins present in immune sera.

The term 'qualitative receptor analysis' has been applied by Felix to the study of the 'O'- and 'H'- types of agglutination. In this method of analysis, Felix (1930) prefers the use of living suspensions of special strains of the enterica group. But he has approved the use of bacillary suspensions preserved with phenol or formalin as reagents to detect 'H' agglutinins and an alcoholic suspension as a reagent to demonstrate the presence of 'O' agglutinins.

A trial of the 'qualitative receptor analysis method' has been made in Part II, but in Parts I and III formolised 'H' and 'O' suspensions prepared at the Standards Laboratory, Oxford, have been used in the agglutination tests. This is in accordance with Gardner's view that formolised broth suspensions of the Felix strain B. typhosus $O_{901}$ and of B. aertrycke 'O' are suitable reagents for the detection of somatic agglutinins in the diagnosis of typhoid and paratyphoid B infections. The natural non-motile (O) variant of B. aertrycke is used since the somatic antigens* are identical with those of B. paratyphosus B.

---

* The somatic antigens are designated I and II in B. paratyphosus B and B. aertrycke in Table II of R. Lovell's paper 'The Salmonella Group of Bacteria' (Bull. of Hyg., July, 1932, p. 408).

Macroscopic Agglutination Technique

A slight modification of the macroscopic method recommended by the Standards Laboratory, Oxford, in the use of standardised agglutinable suspensions was employed throughout this investigation. Instead of a total volume of 25 drops in the Dreyer agglutination tubes, the following scheme (learned in Professor C. H. Browning's Laboratory) was used:-

| Reagent | Number of drops | | | | |
|---|---|---|---|---|---|
| Saline | 0 | 4 | 6 | 7 | 8 |
| Serum dilution 1:10 | 8 | 4 | 2 | 1 | 0 |
| Bac.-suspension | 12 | 12 | 12 | 12 | 12 |
| Ultimate serum dilution | 1:25 | 1:50 | 1:100 | 1:200 | Control |

If the end-titre of the serum for a particular organism was not determined by this series of dilutions a further series was set up from 1:250 to 1:2000 and occasionally to 1:5000. The suspensions used were B. typhosus 'H', B. typhosus 'O', B. paratyphosus B 'H' specific, and B. aertrycke 'O'. The agglutination tubes were placed in the water-bath at 50-55°C. for 2 hours for the agglutination of 'H' antigens, and for 4½ hours for the agglutination of 'O' antigens. The tubes were allowed to stand at room temperature for 15 minutes before the results were read in the agglutinoscope with the aid of a lens x10 magnification. Further incubation in the water-bath for 18-20 hours was found beneficial in bringing down completely the granular agglutination.* Examination of the tubes with the lens at frequent intervals during the period of incubation often revealed finely granular

* Final readings of both floccular and granular agglutination titres were made at this stage.

flocculi moving in the convection currents -- a characteristic feature in my experience of 'O' agglutination. This type of agglutination forms and settles slowly in small uniform granules, producing usually after 24 hours a scanty sediment which is difficult to dislodge.

The results are expressed (1) as the highest dilution in which a two plus (++) or three plus (+++) or occasionally four plus (++++) agglutination reaction occurred, and (2) in reduced titres. Usually in floccular agglutination the readings were graded so that an end-titre of two plus reaction was obtained in the range of dilutions. In granular agglutination however, the end-titres were not always of two plus or three plus reaction, for not infrequently the end-titre gave a four plus reading, with the next higher dilution frankly negative.

With a standard technique the observed titre of a serum depends on two factors: (a) the agglutinating power of the serum, and (b) the sensitiveness of the bacterial suspension. The former factor is a fixed quantity, the latter is measured by a standardising process and indicated by a number. The reduced titre (R.T.) is obtained by dividing the observed titre by this number.

During the past 4 years, a large number of blood samples submitted for the Widal test have been examined for the presence of floccular and granular agglutinins using the 'H' and 'O' antigens of B. typhosus and B. paratyphosus B. Three types of sera have been received: (i) normal sera, i.e. sera from cases without history of previous enterica infection or of preventive inoculation, (ii) sera from cases giving a history of previous antityphoid inoculation, subcutaneous or intravenous, and (iii) sera from clinical cases of typhoid fever and paratyphoid fever.

The term 'normal' is applied to sera obtained from cases whose blood samples were sent in for diagnosis, where the physician was anxious presumably to exclude enteric infection.

Agglutinin Absorption Technique

The absorbing emulsions were made from Felix' strains Ty $H_{901}$, Ty $O_{901}$, $HB_2$ and OB. From agar-plated cultures of the stock strains, smooth colonies were selected and the motile organisms - Ty $H_{901}$ and $HB_2$ - grown through semi-solid agar in motility tubes (cf. p. 6, Part II). Roux bottles were inoculated with 18 hour peptone-water cultures of the four strains, the growths washed off with 3 to 5 c.c. of buffer saline and transferred to graduated centrifuge tubes. After centrifuging and washing twice with buffer saline, the bacteria were centrifuged until constant in volume, the supernatant saline being discarded. (The supernatant fluid was at first retained and used as antigen in the agglutination tests, but later the Oxford bacillary suspensions were used.)

To the packed bacterial mass, undiluted serum and saline were added so that the final dilution of serum and saline was 1:10. For optimum absorption the volume of the packed bacterial mass to the final volume of diluted serum should be 1:10. The formulae used by Krumwiede, Cooper and Provost (1925) for this calculation, in which a correction factor is used for the saline included in the bacterial mass are

(1) (Mass - 10%) x dose factor = total volume

(2) $\frac{\text{(Mass - 10\%) x (dose factor - 1)}}{\text{serum dilution factor}}$ = amount undiluted serum required

In an actual experiment,

the packed mass of centrifuged bacteria = 0.9 c.c. (absorbing dose)

10% correction for residual saline $= \frac{0.09 \text{ c.c.}}{0.81 \text{ c.c.}}$

$\therefore$ using formula (1), total volume = 0.81 x 10 = 8.1 c.c., where the dose factor is 1 to 10.

Using formula (2), the amount of undiluted serum required

$$= \frac{0.81 \times 9}{10} = 0.73 \text{ c.c.}$$

Hence for a bacterial mass of 0.9 c.c., it is necessary to add 0.73 c.c. undiluted serum and then saline to a final volume of 8.1 c.c., i.e. 6.47 c.c. saline.

After mixing thoroughly, the bacteria and diluted serum were placed in the incubator at 37°C. for 4 hours with occasional stirring and then in the refrigerator overnight. The bacteria were removed by centrifuging and the supernatant serum, already diluted 1:10, was used for agglutination tests with final serum dilutions of 1:25 to 1:2000 as in Dreyer's procedure.

/. Sera from Normal Cases.

Table 1 presents the agglutination reactions to 'H' and 'O' suspensions of B. typhosus of a group of 146 cases who were without history of past enterica infection or of previous antityphoid inoculation. Twenty of these cases gave a reaction in 1:25 dilution or greater to a suspension of B. typhosus 'H' or of B. typhosus 'O' or to both suspensions.

It is usually considered that the presence of typhoid agglutinins in the sera from presumably normal persons indicates previous infection, clinical or subclinical, or preventive inoculation. Between the typical case of typhoid fever and the infected subject who remains perfectly healthy a variety of more or less severe forms of illness may arise. Hence there exists a proportion of cases in which a reaction occurs with little or no obvious disturbance of health and yet the blood serum acquires a marked agglutinating action for the Bacillus typhosus.

Rosher and Fielden (1922, Table I) examined in London 149 sera from previously inoculated persons in dilutions of 1:20 to 1:640 and found flagellar agglutinins to B. typhosus present in 89 per cent., but in 181 sera from non-inoculated cases only 3 per cent. were positive.

Smith, McVie and Newbold (1930, Table III A. p.58) carried out a similar study in Manchester on 302 specimens of normal sera at a dilution of 1:20 or over and found that 23.3 per cent. for males and 4.7 per cent. for females reacted with B. typhosus. Since Rosher and Fielden's figure for the non-inoculated (3 per cent.) approximates closely to that for females (4.7 per cent.) as given by Smith, McVie and Newbold, we may recollect that during the Great War the majority of the male population had received inoculations of T.A.B. vaccine, so that the decline in the frequency of flagellar agglutinins

from 89 to 23.3 per cent. is accounted for by the lapse of time.

Havens and Mayfield (1931) believe that the occurrence of agglutinins in normal sera should be interpreted as the result of previous exposure to infection. In the laboratories of the Alabama State Board of Health they examined 1136 Wassermann sera for typhoid agglutinins and found 263 or 23 per cent. positive. Information with regard to past infection or inoculation was obtained only in 60 of the 263 positive sera and in 12 of the 60 cases (i.e. 20 per cent.) infection or inoculation had occurred. Even if a deduction of 20 per cent. be applied to the original percentage of positive sera, the incidence is still high for normal individuals.

Giglioli (1933) examined the sera from 350 normal subjects in British Guiana. From his results it is calculated that, in a dilution of 1:20 or more, 24.6 per cent. of the sera agglutinated B. typhosus 'H' suspension and 16.3 per cent. B. typhosus 'O' suspension. His figure for 'H' agglutinins is not much higher than that obtained by Smith, McVie and Newbold but the frequency of 'O' agglutinins is higher. Giglioli's results, like those of Havens and Mayfield suggest that the typhoid agglutinins found in the blood of normal subjects arise, in large part at least, from subclinical infection.

Felix (1930) considers that 'O' agglutinins are present in normal sera and may reach a titre of 1:100. Gardner (1929, Table II) examined 47 normal sera and found negative 'O' agglutination in 1:20 dilution in 24 cases; 22 cases gave titres between 1:20 and 1:50 and 1 case gave a titre between 1:50 and 1:200. More recently Gardner and Stubington (1932) examined a series of 50 normal subjects in England with 'O' suspensions and

found 16 with titres of from 1:25 to 1:50, 2 from 1:50 to 1:100 and 1 from 1:100 to 1:200.

Horgan (1932) failed to detect 'H' agglutinins to B. typhosus in 70 sera from normal persons in the Sudan: only 5 sera (i.e. 7 per cent.) showed the presence of 'O' agglutinins in dilutions of 1:25 to 1:50.

In a series of 28 normal sera Wyllie (1932) found only 2 cases giving 'H' agglutination at titres 1:50 and 1:100 and 5 cases giving 'O' agglutination at titres of 1:25 to 1:50 with B. typhosus. In the present series (Table 1) only 6 cases or 4 per cent. - a figure approximating closely to that of Rosher and Fielden (3 per cent.) and of Smith, McVie and Newbold (4.7 per cent.) - gave 'H' agglutination with B. typhosus at titres of 1:25 to 1:100 while 16 cases or 11 per cent. gave 'O' agglutination at titres of 1:25 to 1:50.

From these results we may conclude that

(i) the frequency of typhoid agglutinins in serum samples from a normal population is a fairly accurate index of the prevalence of this type of infection,

(ii) 'O' agglutinins in low titre - 1:25 to 1:50 - are more common than 'H' agglutinins among the normal non-inoculated population,

(iii) the titre-limit of normal 'O' agglutination for B. typhosus may reasonably be taken as 1:50 in the majority of cases.

Table 1.

showing agglutination titres in a series of normal cases (unvaccinated and without history of enterica).

| Initials or serum number | Date of receipt of blood sample | End-titres | | Reduced titres | |
|---|---|---|---|---|---|
| | | Ty 'H' | Ty 'O' | Ty 'H' | Ty 'O' |
| B6 | Sept. 1931 | 1:100(2) | 1:50 (3) | | 3.1 |
| B17 | " | - | 1:25 (2) | - | 1.6 |
| B18 | " | - | 1:50 (3) | - | 3.1 |
| H.R. | June 1931 | - | 1:50 (2) | - | 3.1 |
| D.Dick | 1931 | - | 1:25 (2) | - | 1.4 |
| Maddock | 1932 | - | 1:25 (2) | - | 1.4 |
| Pendergast | " | 1:50 (2) | 1:25 (3) | 7.1 | 1.4 |
| Bert | " | " | 1:25 (2) | - | 1.4 |
| Luffman | " | 1:50 (2) | - | 7.1 | - |
| Perry | " | - | 1:25 (2) | - | 2.3 |
| Q3 | " | - | 1:25 (2) | - | 2.3 |
| Ferguson | " | 1:50 (2) | - | 5.0 | - |
| Floud | " | - | 1:25 (2) | - | 1.4 |
| Berriault | " | - | 1:50 (4) | - | 2.8 |
| 203 | " | - | 1:25 (3) | - | 1.4 |
| Pettifer | " | 1:25 (2) | - | 3.6 | - |
| 272 | " | - | 1:50 (3) | - | 2.8 |
| Q74 | " | - | 1:25 (3) | - | 2.3 |
| Bell | " | 1:25 (2) | - | 2.5 | - |
| Armstrong | " | - | 1:25 (2) | - | 2.3 |
| 126 samples | 1931 to 1934 | - | - | - | - |

(2), (3), etc. indicate strength of reaction as ++, +++, etc.
- means absence of reaction in 1:25 dilution.

## 2. Floccular and Granular Agglutination following Subcutaneous Inoculation of Typhoid-paratyphoid (T.A.B.) Vaccine in Man.

In a group of 22 cases which had received antityphoid inoculations 6 months or 3 years and 3 months previously, flagellar agglutinins were found present in each serum sample although in 13 cases somatic agglutinins could not be detected in a dilution of 1:25; in the remaining 9 cases, 5 reacted to B. typhosus 'O' suspension in 1:25 dilution, 3 in 1:50 dilution and 1 in 1:100 dilution (Wyllie, 1932).

Owing to recent work on the preparation of an efficient typhoid-paratyphoid (T.A.B.) vaccine* , it was considered desirable to test the sera of a number of inmates in a residential institution after administering 3 doses of a vaccine prepared from freshly isolated or rejuvenated strains instead of from old stock strains.

Table 2 presents the titres against Ty 'H' and Ty 'O' suspensions for a group of 37 young male inmates of an Industrial School one month after the subcutaneous inoculations. In 30 out of 37 cases the Ty 'O' titre was negative in 1:25 dilution, while in the remaining 7 cases the 'O' titre was 1:25 in 3 cases, 1:50 in 3 cases and 1:200 in one case, i.e. the reduced titres† ranged from 2.2 to 18.1. The Ty 'H' titres varied from < 1:25 to 1:5000 or reduced titres < 3.1 to 625.

---

* Referred to on pp. 21 - 24.

† The reduced titre (R.T.) is the observed titre divided by the reduction factor of the suspension.

Table .2.

showing agglutination titres in a group of inmates one month after subcutaneous inoculation.

Dosage of T.A.B. vaccine: 1/4, 1/2 and 1 c.c. at weekly intervals.

Dates of prophylactic inoculation: March 8th, 15th, and 22nd, 1933.

Date of serological test: April 22nd, 1933.

| Number of inmate | End-titres | | Reduced titres | |
|---|---|---|---|---|
| | Ty 'H' | Ty 'O' | Ty 'H' | Ty 'O' |
| 603 | 1:50 (2) | - | 6.2 | - |
| 606 | 1:5000(2) | - | 625 | - |
| 607 | 1:50 (2) | - | 6.2 | - |
| 608 | 1:250 (2) | - | 31.2 | - |
| 609 | 1:100 (2) | | 12.5 | - |
| 612 | 1:100 (2) | - | 12.5 | - |
| 614 | 1:250 (2) | - | 31.2 | - |
| 615 | 1:250 (2) | - | 31.2 | - |
| 616 | 1:2000(2) | - | 250 | - |
| 620 | 1:100 (2) | - | 12.5 | - |
| 623 | 1:250 (2) | - | 31.2 | - |
| 626 | 1:500 (3) | - | 62.5 | - |
| 627 | 1:500 (2) | 1:50 (3) | 62.5 | 4.5 |
| 628 | 1:500 (2) | 1:200 (2) | 62.5 | 18.1 |
| 631 | 1:200 (2) | 1:25 (2) | 25 | 2.2 |
| 633 | 1:50 (2) | - | 6.2 | - |
| 634 | 1:500 (2) | - | 62.5 | - |
| 638 | 1:100 (2) | - | 12,5 | - |
| 640 | 1:500 (2) | 1:25 (2) | 62.5 | 2.2 |
| 642 | 1:200 (2) | 1:50 (2) | 25 | 4.5 |
| 645 | 1:500 (2) | - | 62.5 | - |
| 648 | 1:500 (2) | - | 62.5 | - |
| 650 | 1:500 (2) | - | 62.5 | - |
| 651 | 1:100 (2) | - | 12.5 | - |
| 652 | 1:100 (4) | 1:50 (2) | 12.5 | 4.5 |
| 653 | 1:500 (2) | - | 62.5 | - |
| 654 | - | - | - | - |
| 658 | 1:500 (2) | - | 62.5 | - |
| 664 | 1:1000(2) | - | 125 | - |
| 666 | 1:50 (2) | - | 6.2 | - |
| 667 | 1:100 (2) | - | 12.5 | - |
| 671 | 1:200 (2) | - | 25 | - |
| 672 | 1:100 (2) | - | 12.5 | - |
| 673 | 1:25 (2) | - | 3.1 | - |
| 674 | 1:50 (2) | - | 6.2 | - |
| 676 | 1:100 (2) | - | 12.5 | - |
| 678 | 1:100 (2) | 1:25 (2) | 12.5 | 2.2 |

These results do not agree with those of Gardner (1929) who examined the sera from a group of 11 mental patients inoculated 4 weeks previously with T.A.B. vaccine. His results are reproduced in Table 3.

Table 3 (modified from Gardner's Table I)

| End-titres | No. of cases | Number | Patient | Ty'O' (reduced titre) |
|---|---|---|---|---|
| 1:20-1:50 | 4 | 64 | Belg | 0.5 |
| | | 65 | Bath | 1.0 |
| 1:50-1:200 | 4 | 66 | Robins | 1.0 |
| | | 67 | Money | 2.0 |
| 1:250-1:800 | 3 | 68 | Whit | 3.0 |
| | | 69 | Morris | 3.0 |
| | | 70 | Casey | 4.0 |
| Total | 11 | 71 | Free | 5.0 |
| | | 72 | Craw | 9.0 |
| | | 73 | Reyn | 9.0 |
| | | 74 | Milt | 18.0 |

Gardner's table shows a positive reading in every case but reduced titre* values over 2 occur only in 7 out of 11 patients inoculated <u>one month</u> previously. In Table 2 the 7 reacting sera all show reduced titres exceeding 2. (cf. Table I where only 9 out of 16 cases exceed 2 units)

* The term 'unit' is omitted since theoretical objections have been raised against its use (Gardner, 1930).

In 50 out of 70 cases recorded in Table 4 somatic agglutinins to B. typhosus were not detected in 1:25 dilution 2 months after subcutaneous inoculation. In the remaining 20 cases the Ty 'O' titre was 1:25 in 10 cases, 1:50 in 8 cases, 1:100 and 1:200 in one case each, i.e. the reduced titres varied between 1.4 and 18.1. In 2 cases (Numbers 605 and 647) neither Ty 'H' nor Ty 'O' agglutination was obtained in 1:25 dilution. The Ty 'H' titres ranged from < 1:25 to 1:500 i.e. R.T. < 2.2 to 62.5.

The number of sera reacting with B. typhosus 'O' suspension in Table 2 is 7, i.e. 19 per cent., and if an interval of 2 months after subcutaneous inoculation is considered recent inoculation the number obtained by combining the results of Tables 2 and 4 is 27, i.e. 25.2 per cent. All the reacting sera in Tables 2 and 4 exceed a R.T. = 2 with one exception.

The highest Ty 'O' agglutinin titre was 1:200 and was obtained once in Table 2 one month after subcutaneous inoculation and once in Table 4 two months after subcutaneous inoculation. Gardner found 3 cases in 11 persons inoculated 4 weeks previously with a titre of this order.

Table .4.

showing agglutination titres in a group of inmates two months after subcutaneous inoculation.

Dosage of T.A.B. vaccine: 1/4,1/2 and 1 c.c. at weekly intervals.

Date of prophylactic inoculation: Feb. 10th, 17th and 24th, 1933.

Date of serological test: April 24th, 1933.

| Number of inmate | End-titres | | Reduced titres | |
|---|---|---|---|---|
| | Ty 'H' | Ty 'O' | Ty 'H' | Ty 'O' |
| 405 | 1:100 (2) | 1:25 (2) | 12.5 | 2.2 |
| 406 | 1:200 (2) | - | 25 | - |
| 409 | 1:250 (2) | - | 31.2 | - |
| 410 | 1:250 (2) | - | 31.2 | - |
| 412 | 1:250 (2) | 1:25 (2) | 31.2 | 2.2 |
| 414 | 1:100 (3) | - | 12.5 | - |
| 417 | 1:25 (2) | - | 3.1 | - |
| 418 | 1:100 (2) | - | 12.5 | - |
| 419 | 1:500 (2) | - | 62.5 | - |
| 422 | 1:250 (2) | - | 31.2 | - |
| 423 | 1:500 (2) | 1:50 (2) | 62.5 | 4.5 |
| 424 | 1:100 (2) | - | 12.5 | - |
| 425 | 1:250 (2) | - | 31.2 | - |
| 431 | 1:100 (2) | 1:25 (2) | 12.5 | 2.2 |
| 437 | 1:200 (2) | - | 25 | - |
| 438 | 1:100 (3) | 1:25 (2) | 12.5 | 2.2 |
| 441 | 1:100 (3) | 1:25 (2) | 12.5 | 2.2 |
| 445 | 1:200 (2) | - | 25 | - |
| 446 | 1:100 (3) | - | 12.5 | - |
| 448 | 1:50 (2) | - | 6.2 | - |
| 450 | 1:200 (2) | - | 25 | - |
| 602 | 1:200 (2) | 1:50 | 25 | 4.5 |
| 604 | 1:100 | - | 12.5 | - |
| 605 | - | - | - | - |
| 610 | 1:50 (3) | - | 6.2 | - |
| 611 | 1:25 (3) | - | 3.1 | - |
| 613 | 1:25 (3) | - | 3.1 | - |
| 617 | 1:250 (2) | 1:200 (3) | 31.2 | 18.1 |
| 618 | 1:100 (2) | 1:25 (2) | 12.5 | 2.2 |
| 619 | 1:100 (2) | 1:25 (2) | 12.5 | 2.2 |
| 621 | 1:500 (2) | - | 62.5 | - |
| 622 | 1:250 (2) | 1:25 (2) | 31.2 | 2.2 |
| 624 | 1:50 (2) | - | 6.2 | - |
| 625 | 1:250 (2) | 1:25 (2) | 31.2 | 2.2 |
| 629 | 1:100 (2) | - | 12.5 | - |
| 630 | 1:100 (2) | - | 12.5 | - |
| 632 | 1:500 (3) | 1:50 (2) | 62.5 | 4.5 |
| 635 | 1:25 (2) | - | 3.1 | - |
| 636 | 1:100 (2) | - | 12.5 | - |

Table 4. (continued)

| Number or name | End-titres | | Reduced titres | |
|---|---|---|---|---|
| | Ty 'H' | Ty 'O' | Ty 'H' | Ty 'O' |
| 637 | 1:100 (2) | - | 12.5 | - |
| 639 | 1:100 (2) | - | 12.5 | - |
| 641 | 1:200 (2) | - | 25 | - |
| 643 | 1:200 (2) | - | 25 | - |
| 644 | 1:200 (2) | - | 25 | - |
| 646 | 1:50 (2) | - | 6.2 | - |
| 649 | 1:50 (3) | - | 6.2 | - |
| 656 | 1:500 (3) | - | 62.5 | - |
| 647 | - | - | - | - |
| 655 | 1:250 (2) | - | 31.2 | - |
| 657 | 1:100 (2) | - | 12.5 | - |
| 659 | 1:50 (2) | - | 6.2 | - |
| 660 | 1:200 (2) | - | 25 | - |
| 661 | 1:50 (2) | - | 6.2 | - |
| 662 | 1:50 (2) | - | 6.2 | - |
| 663 | 1:50 (2) | 1:50 (2) | 6.2 | 4.5 |
| 665 | 1:50 (2) | - | 6.2 | - |
| 668 | 1:100 (2) | - | 12.5 | - |
| 669 | 1:50 (2) | - | 6.2 | - |
| 670 | 1:250 (3) | - | 31.2 | - |
| 675 | 1:200 (2) | - | 25 | - |
| 677 | 1:100 (2) | - | 12.5 | - |
| 679 | 1:100 (2) | 1:50 (2) | 12.5 | 4.5 |
| Baker | 1:500 (2) | 1:50 (2) | 71.4 | 2.8 |
| Dickenson | 1:200 (2) | 1:50 (2) | 14.3 | 2.8 |
| Hampton | 1:500 (2) | 1:25 (3) | 71.4 | 1.4 |
| Malkin | 1:1000(3) | 1:100 (2) | 143.0 | 5.6 |
| McCuaig | 1:50 (2) | - | 7.1 | - |
| Park | 1:200 (2) | 1:50 (2) | 28.6 | 2.8 |
| Percival | 1:100 (2) | - | 12.5 | - |
| Rathbun | 1:200 (2) | - | 28.6 | - |

It is apparent from Tables 2 and 4 that subcutaneous inoculation of T.A.B. vaccine induces more frequent and much higher titres of 'H' than of 'O' agglutinins. Not much is known of the rate of disappearance of the 'O' agglutinins when once formed, but the results of agglutination tests, performed 16 months, 22 months and $2\frac{1}{2}$ years after subcutaneous inoculation, on groups of students and nurses have convinced me that the 'O' titre returns to the normal level before the 'H' agglutinins have disappeared. Table 5 is submitted to show that in 17 out of 21 nurses inoculated $2\frac{1}{2}$ years previously the range of the Ty 'H' agglutinin titre is from 1:25 to 1:250 or R.T. 2.5 to 31.2 while in 5 cases the Ty 'O' titre varies from 1:25 to 1:100 or R.T. 2.2 to 9.1.

If the Ty 'O' titre limit is taken as 1:50 as suggested on p. // , after consideration of the range for normal sera, then in the 1st and 2nd months after subcutaneous T.A.B. vaccine, agglutination with B. typhosus 'O' suspension in significant titre (1:50 or more) occurs in 14 out of 107 cases or 13.8 per cent., and after $2\frac{1}{2}$ years in 1 out of 21 cases or 4.7 per cent.

Table .5.

showing agglutination titres in a group of nurses 2 years and 6 months after subcutaneous inoculation.

Dosage of T.A.B. vaccine: 1/4, 1/2 and 1 c.c. at weekly intervals.

Date of prophylactic inoculation: November, 1931.

Date of serological test: May, 1934.

| Name of Nurse | End-titres | | Reduced titres | |
|---|---|---|---|---|
| | Ty 'H' | Ty 'O' | Ty 'H' | Ty 'O' |
| Asselstine | 1:25 | - | 3.1 | - |
| Bell | 1:25 | - | 3.1 | - |
| Crummey | 1:250 | - | 31.2 | - |
| Dickenson | 1:25 | 1:25 | 2.5 | 2.2 |
| Embury | 1:250 | 1:100 | 25 | 9.1 |
| Finnigan | 1:100 | - | 10 | - |
| Hamm | 1:25 | - | 2.5 | - |
| Hampton | 1:25 | - | 2.5 | - |
| Leonard | 1:25 | - | 3.1 | - |
| Malkin | 1:250 | 1:25 | 25 | 2.2 |
| McIntyre | - | - | - | - |
| Palmer | - | - | - | - |
| Park | - | - | - | - |
| Ramsbottom | 1:50 | - | 5 | - |
| Rathbun | 1:50 | - | 5 | - |
| Robertson | 1:50 | - | 6.2 | - |
| Sherman | - | - | - | - |
| Simpson | 1:25 | 1:25 | 3.1 | 2.2 |
| Sylvester | 1:25 | - | 2.5 | - |
| Ward | 1:100 | - | 10 | - |
| Warwick | 1:250 | 1:25 | 25 | 2.2 |

## The Production of 'O' (somatic) Agglutinins by subcutaneous injection of T.A.B. vaccine.

Much controversy has arisen regarding the occurrence of 'O' agglutination following the subcutaneous inoculation of typhoid-paratyphoid vaccine. Felix (1924) asserted that preventive inoculation against typhoid fever produces large-flaking agglutinins exclusively and that, if the polyvalent T.A.B. vaccine is used, large-flaking agglutinins against all three species of bacteria are formed. Stuart and Krikorian (1928) confirmed this view and reported the serological findings, carried out in Palestine in 1926, on six healthy individuals, before and after a course of 2 inoculations with monovalent vaccine (3 cases) and with T.A.B. vaccine (3 cases). All the sera 10 days after inoculation, showed the production of 'H' agglutinins only, in dilutions from 1:100 to 1:1000. Mudd (1932) criticised these results on the ground that both Felix and Stuart and Krikorian used a strain of B. enteritidis Gaertner ($G_3$) as the chief detector for 'O' agglutinins.

It is true that Felix' tests with sera from inoculated persons were made with B. enteritidis Gaertner - a much less sensitive reagent for typhoid 'O' agglutinins - since at this time the 'O' variant of the sensitive strain $Ty_{901}$ had not been isolated. Nevertheless in the interpretation of agglutination results Felix has always insisted that only marked reactions (+++) in a dilution of 1:100 are to be considered positive (J. Immunology (1924), 9, 124; Lancet (1930), 1, 505); and he has stated his preference for the 1:200 dilution to be taken as the limit (J. Hygiene (1929), 28, 440-441).

Mudd used a living suspension of B. typhosus $O_{901}$, an alcohol-treated suspension of B. typhosus and a living suspension of B. enteritidis Gaertner but his technique did not conform to that of Felix nor of Stuart and Krikorian. He added 1 c.c. amounts of antigen, the opacity of which is not stated, to 1 c.c. serial dilutions of serum. Using a vaccine labelled T.A.B. I, he obtained on 19 pupil nurses 10 days after a course of 3 injections, Ty 'O' titres ranging from <1:80 to 1:320 with the alcohol-treated suspension and from <1:40 to 1:640 with the living $O_{901}$ suspension. Of the 17 nurses allowing a comparison between these two suspensions, 11 showed higher end-titres, in general doubled, with the living suspension. With vaccine T.A.B. II, regarded as of inferior quality to I - owing to low bacterial count and failure to prevent small outbreaks of typhoid and paratyphoid fever in a state hospital - he obtained on 17 entrants to an Insane Institution, 10-14 days after the third injection, Ty 'O' titres of from 1:20 to 1:160. The inferiority of vaccine II was proved by its deficient power to stimulate agglutinins.

Mudd's investigation was stimulated by Grinnell's observation (1930) that the bactericidal power of the blood is little increased by the use of a rough vaccine but markedly increased by a smooth vaccine. In a later study, Grinnell (1932) showed that if the maximum protection from anti-typhoid inoculation is to be obtained, it is necessary to substitute virulent, smooth cultures for the old Rawlings' strain of B. typhosus, commonly used in the preparation of typhoid vaccine throughout the World.

It would appear from the clinical observations of Felix and from the experimental researches of Felix and Olitzki (1926) that the development of 'O' agglutinins indicates a degree of immunity, although Grinnell states

that neither flagellar nor somatic agglutinins can be used as a test of the protective power of a vaccine. In Perry, Findlay and Bensted's experiments (March, 1934) however, there seems confirmation of Felix' view that a relationship exists between the 'O' agglutinin titre and the protective power of the serum. Further investigations of these authors (July, 1934) have made it clear that immunizing value is a function of the virulence of the organism; and, since recently isolated 'smooth' strains of typhoid bacilli may vary widely in virulence and protective power, it is necessary, in the selection of a culture for use in a vaccine, to prove its high virulence by animal test and its insensitiveness in the living state to agglutination by an 'O' antiserum (i.e. resistance to the action of 'O' antibody).

The method of preparing efficient T.A.B. vaccine as suggested by Perry, Findlay and Bensted consists in culturing a carefully selected smooth colony of B. typhosus in broth, testing its virulence by intraperitoneal injection in mice, and finally culturing on pea-flour tryptic-digest agar. The dense emulsion obtained is heated for 1 hour at 53°C. and phenol added in a concentration of 0.5 per cent. The expiry date is calculated as 1 year from the date of preparation.

From the experimental work of Felix (1924, 1926), Topley (1929), Schütze (1930) and others it is clear that the 'O' (somatic) antigen of the smooth type of organism is the most important factor in preventive inoculation. It seemed advisable therefore to summarise the Ty 'O' agglutination results of various authors who have investigated the agglutinin response in man to T.A.B. vaccine.

Table 6 relates to sera examined at intervals of from 1 week to 6 months and is regarded as recent inoculation. Table 7 comprises sera examined after 1 year or later and is referred to as late inoculation. From Table 6 it appears that T.A.B. vaccine prepared in a variety of ways and from different strains of organisms is capable of stimulating the production of 'O' agglutinins in considerable amounts. Prompt response yielding titres of 1:320, 1:640, 1:1250, and 1:2000 may be obtained within a period of 2 weeks from the last injection, and titres of 1:320, 1:500 and 1:800 may even persist for 4 to 6 weeks after inoculation.

Giglioli and Dennis and Berberian used multiple strains, isolated locally, in the composition of their vaccines, but the titres obtained by Dennis and Berberian are higher than those of Giglioli. The adverse effect of phenol on the 'O' agglutinin-producing power of a vaccine as shown by Stuart and Krikorian's results is confirmed by Dennis and Berberian who found lower titres after inoculation of phenolised vaccine than after formolised vaccine or vaccine killed by heating to 70°C. for 30 mins. (cf. titres at foot of Table 6 ). Giglioli's lower titres may therefore be due to the use of a phenolised vaccine.

Table 6

Showing range of Ty '0' titres after subcutaneous vaccine obtained by different investigators: recent inoculation

| Author | # Number of sera | Range of Ty '0' titres | Interval after inoculation | Type of vaccine and dosage |
|---|---|---|---|---|
| Gardner (1929) | 11 | 1:20 - 1:800 | 4 weeks | Parke-Davis T.A.B.;2 doses |
| Horgan (1932) | 20 | 1:50 - 1:1250 | 1 week | Stock T.A.B.; 2 doses |
| | 14 (16) | 1:50 - 1:500 | 6 weeks | " " " |
| Giglioli (1933) | 10 (17) | 1:20 - 1:320 | 4 weeks | T.A.B.C. and T.C. prepared from 5 local strains of each species, killed by heating at 56°C. for 2 hrs., and 0.5% phenol added. 2 doses |
| | 11 (21) | 1:20 - 1:160 | 2-3 months | |
| | 11 (29) | 1:20 - 1:160 | 4-6 months | |
| Mudd (1932) | 14 (19) | 1:80 - 1:320 | 10 days | Stock T.A.B. (Philadelphia Health Board) consisting of 1 strain of T and 2 strains each of A and B. 3 doses |
| | 16 (19) | 1:40 - 1:640 | 10 days | |
| Dulaney, et al. (1933) | 3 | 1:80 | 2 weeks | Not stated |
| Stuart and Krikorian (1934) | 7 | 1:200 - 1:2000 | 2 weeks | Typhoid (Rawlings): killed by heating to 60°C.: non-phenolised. 2 doses |
| | 7 | 1:200 - 1:1000 | 3 months | |
| | 7 | 1:100 - 1:200 | 2 weeks | Typhoid (as above) with 1% phenol added. 2 doses. |
| | 4 (7) | 1:100 | 3 months | |
| Wyllie (1932) | 5 (13) | 1:25 - 1:50 | 6 months | Ontario Dept. of Health, Stock T.A.B. with 0.5% phenol. 3 doses. |
| Dennis and Berberian (1934) | 19 | 1:600 (average) | 18-50 days with average of 1 month | Formolised (0.25%) — T.A.B. made from 24 strains of T, 4 of A and 7 of B. (All local strains) |
| | 27 | 1:500 " | | Killed by heat (70°C. for 30 min.) |
| | 29 | 1:400 " | | Phenolised (0.5%) |

# The numbers within brackets indicate the total number of sera examined; the numbers preceding the brackets indicate the number of sera reacting within the range.

The titres of Table 7 are lower than those in Table 6 . The majority of the sera fail to react in the lower dilutions 3 years or more after inoculation although occasional high titres e.g. 1:200 and 1:400 may be found. According to Dennis and Berberian approximately 25 per cent. of inoculated individuals lose all trace of 'O' agglutinins within a year.

Table 7

Showing range of Ty 'O' titres after subcutaneous vaccine obtained by different investigators: late inoculation

| Author | # Number of sera | Range of Ty 'O' titres | Interval after inoculation |
|---|---|---|---|
| Horgan (1932) | 14 (18) | 1:25 - 1:125 | 1 year |
| Giglioli (1933) | 5 (17) | 1:20 - 1:80 | 3 years |
| Wyllie (1932) | 4 (9) | 1:25 - 1:100 | 3 years & 3 months |
| Smith (1932) | 2 (17) | 1:50 - 1:200 | 9 years; 3 years |
| Gardner (1929) | 4 (6) | 1:20 - 1:400 | within 13 years |

# The numbers within brackets indicate the total number of sera examined; the numbers preceding the brackets indicate the number of sera reacting within the range.

## 3. Flocculer and Granular Agglutination following Intravenous Inoculation of Typhoid-paratyphoid (T.A.B.) Vaccine in Man

In a study of 24 cases in June to August 1931 it was shown by Wyllie (1932) that typhoid 'O' agglutinins were formed in the sera of patients who had received intravenous injections of typhoid-paratyphoid (T.A.B.) vaccine. After an interval, varying between 9 and 22 months following the injections, the patients' sera showed 'O' agglutinins to B. typhosus in titres of from 1:50 to 1:250 (R.T. 4.5 to 22.7) in 22 of the cases while 2 cases gave negative results in a dilution of 1:25. When this study had been completed and received for publication (14.1.32) by the Journal of Hygiene, Dr. A. Felix, (Lister Institute, London) in a personal communication, cited a note of S. H. Zia (Peiping, China) in the Proceedings of the Society for Experimental Biology and Medicine (Dec. 1931. 29. 253). In this brief note Zia presents the charts of two illustrative cases from a group of patients undergoing treatment for various conditions and all receiving standard triple typhoid vaccine intravenously with varying dosage. This author regards it as significant that, after intravenous injection of typhoid vaccine, 'O' agglutinins were produced in titres approaching fairly close to that of the 'H' agglutinins. In Case I, 14 days after treatment both the Ty 'H' and Ty 'O' titres were 1:250, and in Case II, 24 days after treatment, the titres were Ty 'H' 1:1280 and Ty 'O' 1:640. The conclusion that 'O' agglutinins are readily induced by intravenous inoculation has been noted by Topley (An Outline of Immunity, London, 1933, p. 305) in which he refers to Wyllie's work and also by A. Fleming in Recent Advances in Vaccine and Serum Therapy, 1934, p. 277. Fleming states that some of his observations agree with Wyllie's results, for he was able to detect 'O' agglutinins in the sera of four patients

within a week after protein shock had been induced by intravenous inoculation of 50 millions of digested typhoid bacilli. Six months after inoculation 'O' agglutinins were still present up to a titre of 1:640.

The complete dosage of typhoid vaccine administered for therapeutic purposes is not detailed by Zia (1931). He states 'the first dose was usually 25 million organisms, and the usual increase was 25 million with each subsequent injection. All patients showed a systemic reaction with fever and chills.'

With regard to the cases shown in Table 8, the technique of non-specific protein therapy (1) for cases of general paralysis (G.P.I.), tabes dorsalis and dementia praecox was carried out as follows:

T.A.B. vaccine containing 1000 million B. typhosus and 500 million each of B. paratyphosus A and B per cc. is diluted with normal saline to eight times its volume. Of this dilution 0.1 cc. containing 25 million bacilli is injected intravenously with sterile precautions and the patient immediately put to bed between blankets. In from 1 - 3 hours, the patient has a rigor lasting generally 10 - 30 mins. The blood pressure is decreased as much as 30 mms. of mercury, the face looks anxious, the patient complains of feeling cold and trembles violently. These symptoms are accompanied at times by pains in the back and legs, headache and general malaise; vomiting rarely occurs. As these symptoms pass the temperature rises, reaching its acme in from 2 - 6 hours; the blood pressure rises to normal, the face becomes flushed and the patient expresses a feeling of well-being. Then the temperature falls rapidly and, if the patient is otherwise sufficiently well, he is up and about his usual pursuits the next day.

---

(1) The author is indebted to Dr. D.R. Fletcher, Medical Superintendent, Ontario Hospital, Whitby, Ontario, for this outline of the technique.

For convenience the treatment is given on Monday, Wednesday and Friday of each week. If the fastigium is less than 104° F, as taken by rectal thermometer, the second dose is usually 50 million bacilli. If this dose, in turn, gives satisfactory results, the third dose is usually about 100 million bacilli with an increment of from 50 to 100 million bacilli for each subsequent dose. If the fastigium is above 105° F after any injection, the same dose will generally suffice for the next injection.

Individual cases vary in their response and hence no rule can be laid down for dosage applicable in every case. In some cases the course lasted for 7 weeks and a total of 21 injections were administered; in others 3 injections only were given.

Table 8 in which is incorporated Table III Group B (Wyllie, 1932) shows a list of 42 patients whose blood sera have been examined for flagellar and somatic agglutinins to B. typhosus. Intravenous injections of T.A.B. vaccine were given between Sept. 1930 and March 1933 and the period between the date of therapeutic inoculation and the date of serological test is recorded in months for each patient. Out of 42 cases 34 showed Ty 'H' titres and 30 showed Ty 'O' titres after periods varying from 9 to 28 months. A titre of 1:100 and over for 'O' agglutinins occurred 18 times in this series, i.e., in 43% of the cases. The Ty 'H' values varied from 1:25 to 1:500 (R.T. 2.5 to 62.5)*, with an average R.T. = 11.6; the Ty 'O' values varied from 1:25 to 1:250 (R.T. 1.5 to 22.7 ), the average R.T. being 6.2.

---

* The word 'unit' is purposely omitted as theoretical objections have been raised (Gardner (1930)).

Table .8.

Showing agglutination titres after intravenous injections of T.A.B. vaccine.

| Name or Initials | Date of Therapeutic Inoculation (Intravenous Injection) | Time interval in months between intravenous injection and serological test | End-titres | | Reduced titres | |
|---|---|---|---|---|---|---|
| | | | Ty 'H' | Ty 'O' | Ty 'H' | Ty 'O' |
| Hope | Oct. 1930 | 9 | - | - | - | - |
| S. C. | Sept. 1930 | 9 | 1:100(3) | - | 12.5 | - |
| A. S. | Sept. 1930 | 9 | 1:500(3) | - | 62.5 | - |
| Carson | Oct. 1930 | 9 | 1:100(2) | 1:50 (3) | 12.5 | 4.5 |
| W. B. | Sept. 1930 | 9 | 1:200(4) | 1:50 (4) | 25 | 4.5 |
| T. W. | Sept. 1930 | 9 | 1:50 (3) | 1:100(2) | 6 | 9.1 |
| Parks | Sept. 1930 | 9 | 1:250(2) | 1:100(3) | 31 | 9.1 |
| Caplan | Sept. 1930 | 9 | 1:100(3) | 1:200(2) | 12.5 | 18.2 |
| Spence | Sept. 1930 | 10 | - | - | - | - |
| Bullen | Sept. 1930 | 10 | - | 1:50 (3) | - | 4.5 |
| Penley | Aug. 1930 | 10 | 1:250(2) | 1:100(2) | 31 | 9.1 |
| Legge | Sept. 1930 | 10 | 1:250(3) | 1:100(2) | 31 | 9.1 |
| P. L. | Aug. 1930 | 10 | 1:100(3) | 1:200(2) | 12.5 | 18.2 |
| F. J. | June 1930 | 12 | 1:500(3) | 1:200(3) | 62.5 | 18.2 |
| Haines,E.A.C. | Mar. 1933 | 16 | 1:50 (3) | 1:200(2) | 5 | 11.8 |
| Williams | Jan. 1930 | 17 | 1:250(3) | 1:100(3) | 31 | 9.1 |
| Arthurs, S. | Dec. 1932 | 19 | 1:50 (2) | 1:25 (4) | 5 | 2.3 |
| Carmody, J.T. | Nov. 1932 | 20 | 1:100(2) | 1:50 (2) | 10 | 4.5 |
| A. D. | Oct. 1929 | 21 | 1:25 (3) | - | 3 | - |
| Cram, W.H. | Oct. 1932 | 21 | 1:100(2) | 1:25 | 10 | 2.3 |

Table .8. (continued)

| Name or Initials | Date of Therapeutic Inoculation (Intravenous Injection) | Time interval in months between intravenous injection and serological test | End-titres | | Reduced titres | |
|---|---|---|---|---|---|---|
| | | | Ty 'H' | Ty 'O' | Ty 'H' | Ty 'O' |
| Durham | Oct. 1929 | 21 | 1:50 (2) | 1:50 (2) | 6 | 4.5 |
| C. O. | Oct. 1929 | 21 | - | 1:50 (3) | - | 4.5 |
| Nadeau, A. | Oct. 1932 | 21 | 1:50 (3) | 1:100(3) | 5 | 5.9 |
| Ellis | Oct. 1929 | 21 | 1:25 (2) | 1:100(2) | 3 | 9.1 |
| Hague | Oct. 1929 | 21 | 1:25 (2) | 1:100(2) | 3 | 9.1 |
| Willis | Oct. 1929 | 21 | 1:25 (2) | 1:100(3) | 3 | 9.1 |
| Cundell, W. | Oct. 1932 | 21 | 1:100(2) | 1:100(3) | 10 | 9.1 |
| W. M. | Oct. 1929 | 21 | 1:50 (2) | 1:250(2) | 6 | 22.7 |
| X. | Oct. 1929 | 21 | 1:50 (3) | 1:250(3) | 6 | 22.7 |
| Monk, G. | Sept. 1932 | 22 | 1:200(3) | - | 20 | - |
| Vaidnoff | Oct. 1929 | 22 | 1:25 (2) | 1:50 (2) | 4 | 2.9 |
| McCormick,M.A. | Sept. 1932 | 22 | - | 1:200(2) | - | 11.8 |
| Hill, W. | Apr. 1932 | 27 | - | - | - | - |
| McRae, A. | Apr. 1932 | 27 | 1:25 (3) | - | 2.5 | - |
| Damfousse, A. | Apr. 1932 | 27 | 1:50 (3) | 1:50 (3) | 5 | 4.5 |
| Chartrand,J.O. | Mar. 1932 | 28 | - | - | - | - |
| Livingstone, O. | Mar. 1932 | 28 | 1:25 (2) | - | 2.5 | - |
| Chartrand, J. | Mar. 1932 | 28 | 1:50 (3) | - | 5 | - |
| Knudson, H.O. | Mar. 1932 | 28 | 1:200(2) | - | 20 | - |
| Evaire, J.A. | Mar. 1932 | 28 | - | 1:25 (3) | - | 1.5 |
| Esdale, G.C. | Mar. 1932 | 28 | 1:25 (3) | 1:50 (2) | 2.5 | 2.9 |
| Desormoux, J. | Mar. 1932 | 28 | 1:200(2) | 1:100(3) | 20 | 5.9 |

The results of Table 8 may be collected into groups according to the number of months elapsing after intravenous inoculation and the average reduced titres for 'H' and 'O' agglutinins calculated.

Table 9

showing the results of table 8 in a summarised form.

| Group | Number of months elapsing after intravenous inoculation | Number of cases | Average reduced titres Ty'H' | Ty'O' |
|---|---|---|---|---|
| 1 | 9-12 | 14 | 21.4 | 7.5 |
| 2 | 16-19 | 3 | 13.7 | 7.7 |
| 3 | 20-22 | 15 | 5.9 | 7.9 |
| 4 | 27-28 | 10 | 5.8 | 1.5 |

In Table 9 it is interesting to note that while the Ty 'H' agglutinin titre diminishes rapidly as the months proceed, the Ty 'O' agglutinin titre tends to persist and only shows a marked decline after the 22nd month.

In each group there is a wide individual variation. The highest Ty 'H' titres occurred in group 1, 2 cases giving a R.T. of 62.5; the highest Ty 'O' titres were in group 3 where 2 cases gave a R.T. of 22.7. Thus even when a course of intravenous injections of T.A.B. vaccine is administered the R.T. for Ty 'O' is seldom above 20 if the inoculation is fairly recent.

The following Table 10 illustrates the individual variation in response to intravenous injections of T.A.B. vaccine in a group of 5 female patients. The titres were determined twelve months after the injections. The first patient, Jones, was given a single injection of 0.1 cc. of the vaccine which is equivalent to 50 million organisms, the second patient two injections with an interval of 5 days apart and the remaining three patients three injections with intervals of 2 or 3 days apart. Of the first three patients, Jones responded with higher titres than Turner or Cutte and even after a single injection. Comparing the responses of Cutte, Maloney and McPhail each of whom received three injections, Cutte gave the lowest titres, although Maloney, and McPhail gave practically similar titres. Only in the cases of Jones and Cutte do the Ty 'H' and Ty 'O' titres approximate.

Table 10.

Date of intravenous injections: June 12th - 17th, 1933.

Date of serological tests: July 6th, 1934.

| Name | Intravenous inoculation of T.A.B. vaccine | | End titres | | | | Reduced titres | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Date 1933 | Dosage c.c. | Ty 'H' | Ty'O' | PB 'H' | Aer'O' | Ty'H' | Ty'O' | PB'H' | Aer'O |
| Jones,C.B. | July 14 | 0.1 | 1:500 (2) | 1:500(2) | 1:500 (3) | 1:500(2) | 50 | 45 | 125 | 100 |
| Turner,D. | " 12 | 0.1 | | | | | | | | |
| | " 17 | 0.1 | 1:200 (2) | 1:100(2) | 1:50 (2) | 1:250(2) | 20 | 9 | 12.5 | 50 |
| Cutte,P. | " 12 | 0.1 | | | | | | | | |
| | " 14 | 0.1 | | | | | | | | |
| | " 16 | 0.1 | 1:100 (2) | 1:100(3) | 1:100 (2) | 1:250(2) | 10 | 9 | 25 | 50 |
| Maloney,I. | " 12 | 0.1 | | | | | | | | |
| | " 14 | 0.1 | | | | | | | | |
| | " 17 | 0.1 | 1:1000(2) | 1:250(2) | 1:1000(2) | 1:250(3) | 100 | 23 | 250 | 50 |
| McPhail,E. | " 12 | 0.1 | | | | | | | | |
| | " 14 | 0.1 | | | | | | | | |
| | " 17 | 0.1 | 1:1000(2) | 1:250(2) | 1:1000(2) | 1:200(2) | 100 | 23 | 250 | 40 |
| | | | | | | Average R.T. | 56 | 21.8 | 132.5 | 58 |

The agglutination titres in 5 female patients one month after a course of intravenous injections are shown in Table 11. The technique of administration and dosage was as follows: the first four doses were given at intervals of 3 days, beginning on June 15th, 1934 and the last 5 doses on successive days, June 26th, 27th, 28th, 29th and 30th; the first injection contained 20 million organisms per c.c., the second 50 million organisms per c.c., the third 85, the fourth 100, the fifth 110, the sixth 150, the seventh 150, and the eighth 200 and the ninth 250.

Zia (1931) was impressed with the close approximation of the end-titres of 'H' and 'O' agglutinins after intravenous injection of typhoid-paratyphoid vaccine but apparently he did not use standardised agglutinable suspensions. In two cases - Carmody and Johnston, the Ty 'H' and Ty 'O' end-titres were exactly similar and in Martel's case the Ty 'O' titre was twice the Ty 'H' titre. When reduced titres are considered however, only in one case - Martel, does the Ty 'O' (R.T.) value exceed that for Ty 'H' (R.T.) The striking fact is the response to the flagellar and somatic antigens of B. paratyphosus B in the vaccine. The para B 'H' (R.T.) values are double those for Ty 'H' (R.T.) and may be as high as 10 times. The Aer. 'O' (R.T.) values are higher than the Ty 'O' values except in the case of McDonell.

Table 11

Date of intravenous injections: June 15th - 30th, 1934.

Date of serological tests: July 31st, 1934.

| Name | Age | End-titres | | | | Reduced titres | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ty 'H' | Ty'O' | PB 'H' | Aer'O' | Ty'H' | Ty'O' | PB'H' | Aer'O' |
| Babcock,A. | 19 | 1:2000(2) | 1:1000(3) | 1:5000(2) | 1:2000(2) | 200 | 59 | 1000 | 400 |
| Carmody,N. | 28 | 1:1000(3) | 1:1000(2) | 1:5000(2) | 1:500 (2) | 100 | 59 | 1000 | 100 |
| Johnston,F.M. | 29 | 1:500 (2) | 1:500 (2) | 1:500 (2) | 1:1000(4) | 50 | 30 | 100 | 200 |
| Martel,E. | 38 | 1:500 (2) | 1:1000(2) | 1:2000(2) | 1:500 (3) | 50 | 59 | 400 | 100 |
| McDonell,M.I. | 25 | 1:2500(3) | 1:1000(2) | 1:2500(3) | 1:250 (3) | 250 | 59 | 500 | 50 |
| | | | | | Average R.T. | 130 | 53.2 | 600 | 170 |

Table 12 presents a list of 50 cases of typhoid fever whose agglutination reactions to the Oxford Standardised Agglutinable Suspensions have been recorded during a period of four years. In the majority of the cases the duration of the disease could be ascertained with reasonable accuracy and advantage has been taken of this information to arrange the series accordingly. The interval elapsing between the onset of the disease and the date of withdrawal of the first blood sample varies from 4 to 23 days. In 12 cases the time interval could not be definitely determined but these samples were also first samples and were taken presumably when suspicion of the disease arose. The end-titres show great variation and bear no apparent relationship to the stage of the disease.

Co-agglutination:

Co-agglutination with Para. B 'H' occurred twice - in CK., male, aged 18 years and V.M., male, aged 23 years in dilutions of 1:50 and 1:25 respectively; with Aer. 'O' forty times and in dilutions from 1:25 to 1:1000. The reduced titre for Ty 'O' exceeded that for Aer. 'O' in only 6 cases (K.H., C.I., A.A.G., F.R., W.G. and Sargent).

Range of agglutination titres:

The range of the Ty 'H' titres was from <1:25 to 1:2500 (R.T.< 3.1 to 833.3).
The range of the Ty 'O' titres was from <1:25 to 1:1000 (R.T.< 1.5 to 90.9).
The range of the Aer.'O' titres was from <1:25 to 1:1000 (R.T.< 4.2 to 200).

Table 12

Cases of Typhoid Fever

| Patient's initials | Interval between onset and 1st sample | End-Titres | | | Reduced Titres | | |
|---|---|---|---|---|---|---|---|
| | | Ty'H' | Ty'O' | Aer'O' | Ty'H' | Ty'O' | Aer'O' |
| I. H. | 4 days | 1:1000(2) | 1:250 (2) | 1:250 (2) | 125 | 15.6 | 41.7 |
| B. C. | 5 days | 1:1000(3) | 1:25 (3) | 1:50 (3) | 100 | 1.5 | 10 |
| G. B. | 6 days | 1:500 (2) | 1:50 (3) | 1:50 (2) | 62.5 | 4.5 | 8.3 |
| R. C. | 6 days | 1:500 (3) | 1:100 (2) | 1:250 (3) | 50 | 5.9 | 50 |
| G. D. | 6 days | <1:25 | 1:200 (2) | 1:200 (2) | <8.3 | 11.8 | 40 |
| W. A. | 7 days | 1:25 (2) | 1:250 (3) | 1:250 (2) | 3.6 | 13.9 | 41.7 |
| W. B. | 7 days | 1:200 (2) | <1:25 | 1:50 (3) | 20 | <1.5 | 10 |
| J. D. | 7 days | 1:200 (2) | 1:500 (2) | 1:250 (2) | 66.7 | 29.4 | 50 |
| D. A. G. | 7 days | 1:2000(2) | 1:100 (2) | <1:25 | 285.7 | 6.3 | <4.2 |
| D. P. | 7 days | 1:250 (2) | <1:25 | 1:100 (2) | 31.3 | <1.6 | 16.7 |
| I. C. | 8 days | <1:25 | 1:500 (2) | 1:500 (2) | <3.1 | 31.3 | 83.3 |
| H. D. | 8 days | 1:50 (2) | 1:250 (2) | 1:500 (2) | 16.7 | 14.7 | 100 |
| R. L. | 8 days | <1:25 | 1:100 (2) | 1:100 (3) | <3.1 | 9.1 | 16.7 |
| G. A. | 9 days | 1:500 (2) | 1:250 (2) | 1:100 (4) | 166.7 | 14.7 | 20 |
| K. H. | 9 days | 1:1000(3) | 1:500 (2) | 1:25 (4) | 250 | 41.7 | 5 |
| C. I. | 9 days | 1:200 (2) | 1:500 (2) | 1:250 (2) | 25 | 45.5 | 41.7 |
| H. B. | 10 days | 1:50 (2) | 1:500 (2) | 1:500 (2) | 6.3 | 45.5 | 100 |
| D. D. | 10 days | 1:250 (2) | 1:1000(2) | 1:1000(3) | 83.3 | 58.8 | 200 |
| C. K. | 10 days | 1:500 (3) | 1:200 (4) | 1:100 (4) | 50 | 11.8 | 20 |
| J. W. M. | 10 days | 1:100 (2) | 1:200 (2) | 1:100 (3) | 25 | 16.7 | 20 |
| C. R. | 10 days | 1:25 (3) | 1:50 (2) | 1:50 (3) | 3.1 | 3.1 | 8.3 |
| A. C. | 11 days | 1:100 (2) | 1:100 (2) | 1:100 (2) | 14.3 | 6.3 | 16.7 |
| A. J. | 11 days | 1:250 (2) | 1:250 (3) | 1:200 (2) | 25 | 14.7 | 40 |
| Malcolm | 11 days | <1:25 | <1:25 | 1:250 (2) | <3.1 | <1.6 | 41.7 |
| J. L. | 12 days | >1:200 (4) | <1:25 | 1:25 (2) | >25 | <1.6 | 4.2 |

Table 12 (continued)

Cases of Typhoid Fever

| Patient's initials | Interval between onset and 1st sample | End-Titres | | | Reduced Titres | | |
|---|---|---|---|---|---|---|---|
| | | Ty'H' | Ty'O' | Aer'O' | Ty'H' | Ty'O' | Aer'O' |
| A. M. | 12 days | 1:250 (2) | 1:200 (2) | 1:200 (3) | 31.3 | 12.5 | 33.3 |
| J. V. | 12 days | 1:100 (3) | 1:1000(2) | 1:1000(2) | 12.5 | 90.9 | 166.7 |
| C. B. | 13 days | 1:100 (3) | 1:50 (2) | <1:25 | 12.5 | 4.5 | <4.2 |
| R. B. | 13 days | 1:500 (2) | <1:25 | 1:100 (2) | 62.5 | <1.6 | 16.7 |
| R. H. Z. | 15 days | 1:25 (3) | 1:25 (3) | 1:25 (3) | 8.3 | 1.5 | 5 |
| A. B. | 18 days | 1:250 (2) | 1:250 (2) | 1:250 (3) | 31.3 | 22.7 | 41.7 |
| J. S. | 18 days | 1:100 (2) | 1:250 (2) | 1:250 (2) | 16.7 | 22.7 | 41.7 |
| V. M. | 19 days | 1:1000(2) | 1:250 (3) | 1:250 (2) | 333.3 | 14.7 | 50 |
| L. C. | 20 days | 1:500 (2) | 1:250 (2) | 1:250 (4) | 83.3 | 22.7 | 41.7 |
| P. M. | 20 days | 1:50 (2) | 1:500 (2) | 1:500 (3) | 8.3 | 45.5 | 83.3 |
| A. A. G. | 21 days | 1:500 (3) | 1:250 (3) | 1:25 (3) | 62.5 | 22.7 | 5 |
| F. R. | 21 days | 1:2000(2) | 1:1000(2) | 1:250 (4) | 250 | 90.9 | 41.7 |
| A. D. | 23 days | 1:2500(2) | 1:200 (3) | 1:250 (2) | 833.3 | 11.8 | 50 |
| J. B. | - | 1:100 (2) | 1:1000(2) | 1:500 (3) | 33.3 | 58.8 | 100 |
| J.Beaton | - | 1:25 (2) | 1:200 (2) | 1:100 (2) | 3.1 | 18.2 | 20 |
| C.Buchanan | - | 1:25 (3) | 1:50 (2) | 1:100 (2) | 3.1 | 4.5 | 20 |
| L.Culbertson | - | 1:500 (2) | 1:100 (3) | 1:200 (3) | 50 | 9.1 | 40 |
| Daly | - | 1:250 (3) | 1:100 (2) | 1:50 (2) | 31.3 | 6.3 | 8.3 |
| E. G. | - | 1:100 (2) | 1:100 (2) | 1:100 (3) | 12.5 | 6.3 | 16.7 |
| H. G. | - | 1:25 (3) | <1:25 | 1:50 (2) | 8.3 | <1.5 | 10 |
| W. G. | - | <1:25 | 1:250 (2) | 1:100 (2) | <3.1 | 22.7 | 20 |
| M. L. | - | <1:25 | 1:200 (3) | <1:25 | <3.1 | 18.2 | <5 |
| G. S. | - | 1:100 (2) | 1:100 (3) | 1:500 (4) | 14.3 | 6.3 | 83.3 |
| Sargent | - | 1:2000(2) | 1:250 (2) | 1:100 (4) | 250 | 22.7 | 16.7 |
| E. W. | - | 1:50 (2) | 1:50 (2) | <1:25 | 6.3 | 4.5 | <5 |

Relative 'H' and 'O' readings:

Five examples occur of a low 'H' (< 1:25) reading associated with a relatively high Ty 'O' reading (from 1:100 to 1:500), viz. G.D., I.C., R.L., W.G. and M.L.)

Four examples of a high 'H' reading with a low Ty 'O' (< 1:25) reading occur, viz. W.B., D.P., J.L. and R.B., but in these cases significant Aer. 'O' reduced titres were obtained with the exception of J.L. This points to the greater sensitiveness of the Aer. 'O' suspension.

Diagnostic Limit:

If we regard a titre of 1:100 as the diagnostic limit the number of cases reacting in this dilution or in greater is for

| | | | | | | |
|---|---|---|---|---|---|---|
| Ty | 'H' | . . . | 34 | . . . | or | . . . 68% |
| Ty | 'O' | . . . | 37 | . . . | or | . . . 74% |
| Aer. | 'O' | . . . | 36 | . . . | or | . . . 72% |

Table 13 gives a list of 20 cases of Paratyphoid B fever collected over a period of four years. The cases have been arranged in order of the duration of the disease when the first blood sample was taken for the agglutination test, i.e. ranging from an interval of 6 days to 4 weeks. Apparently the specific end-titres give no indication of the duration of the disease, as some samples taken early show high titres while other samples taken later show low titres. In four of the cases the time interval could not be ascertained.

Co-agglutination:

Co-agglutination with Ty 'H' occurred once (G.A.) and in a dilution 1:1000; with Ty 'O' in 9 cases and in dilutions from 1:25 to 1:250. In every case the reduced titre for Aer. 'O' exceeded that for Ty 'O'.

Range of agglutination titres:

The range of the Para. B 'H' titres was from <1:25 to 1:5000 (R.T. < 5 to 1250)
The range of the Aer.(Para.B)'O' titres was from 1:25 to 1:2000 (R.T. 5 to 333.3)
The range of the Ty 'O' titres was from <1:25 to 1:250 (R.T. <1.4 to 22.7)

Relative 'H' and 'O' readings:

One case (A.W.) shows a low 'H' (<1:25)reading associated with a high Aer. 'O' reading (1:1000). There are 3 examples of a high 'H' reading with a relatively low Aer. 'O' reading, viz. K. Coon, J.W. and D.P. In these cases the reduced titres for Aer. 'O' were below the diagnostic limit of 10 and, since the Ty 'O' titres were also low, dependence on on the Para. B. 'H' reading was necessary for the diagnosis.

Table 13

Cases of Paratyphoid B Fever

| Patient's initials | Interval between onset and 1st sample | End-Titres | | | Reduced Titres | | |
|---|---|---|---|---|---|---|---|
| | | PB'H' | Ty'O' | Aer'O' | PB'H' | Ty'O' | Aer'O' |
| V. C. | 6 days | 1:250 (3) | <1:25 | 1:100 (3) | 50 | <1.5 | 20 |
| M. M. | 7 days | 1:200 (2) | 1:25 (2) | 1:100 (3) | 50 | 2.3 | 20 |
| K.Coon | 8 days | 1:500 (2) | <1:25 | 1:50 (3) | 125 | <1.4 | 8.3 |
| J. L. | 8 days | 1:250 (2) | <1:25 | 1:500 (3) | 50 | <1.5 | 100 |
| R. R. | 8 days | 1:50 (2) | 1:25 (2) | 1:100 (2) | 12.5 | 2.3 | 20 |
| C. S. | 8 days | 1:100 (4) | <1:25 | 1:250 (4) | 20 | <1.5 | 50 |
| T. B. | 9 days | 1:200 (2) | 1:50 (4) | 1:100 (4) | 40 | 2.9 | 20 |
| R. L. | 9 days | 1:500 (2) | <1:25 | 1:100 (2) | 125 | <2.3 | 16.7 |
| K. P. | 10 days | 1:5000(2) | 1:50 (2) | 1:500 (4) | 1000 | 2.8 | 83.3 |
| J. W. | 12 days | 1:200 (2) | <1:25 | 1:25 (2) | 40 | <1.5 | 5 |
| T. O. | 13 days | 1:2000(3) | <1:25 | 1:200 (4) | 400 | <1.5 | 40 |
| R.Lawler | 14 days | 1:2500(2) | <1:25 | 1:100 (4) | 625 | <2.3 | 16.7 |
| R. U. | 14 days | 1:500 (4) | 1:200(2) | 1:200 (3) | 100 | 11.1 | 33.3 |
| L. A. | 3 weeks | 1:5000(2) | 1:200(2) | 1:2000(2) | 1250 | 12.5 | 333.3 |
| K.Chick | 24 days | 1:1000(3) | 1:25 (2) | 1:100 (3) | 250 | 2.3 | 20 |
| G. A. | 4 weeks | 1:2000(2) | 1:250(2) | 1:250 (4) | 500 | 22.7 | 41.7 |
| H. B. | - | 1:2500(2) | <1:25 | 1:100 (3) | 500 | <1.5 | 20 |
| A. C. | - | 1:500 (2) | <1:25 | 1:250 (2) | 125 | <1.6 | 41.7 |
| D. P. | - | 1:100 (2) | <1:25 | 1:50 (2) | 25 | <1.6 | 8.3 |
| A. W. | - | <1:25 | 1:250(2) | 1:1000(3) | < 5 | 13.9 | 166.7 |

Diagnostic Limit:

Considering an end-titre of 1:100 as a diagnostic limit the number of cases reacting in this dilution or in greater is for

| | | | | | | |
|---|---|---|---|---|---|---|
| Para. B 'H' | . . . | 18 | . . . | or | . . . | 90% |
| Aer. 'O' | . . . | 17 | . . . | or | . . . | 85% |
| Ty 'O' | . . . | 4 | . . . | or | . . . | 20% |

Value of the "O" agglutination test in typhoid and paratyphoid infections:-

In Tables 12 and 13, cases are recorded in which 'H' agglutinins are absent in the first blood-samples examined. The typhoid fever cases showing this peculiarity have been collected in the first part of Table 14, from which it is seen that in only one case, Lillian Card, was the Ty'O' titre definitely below the diagnostic limit. Four blood-samples taken at weekly intervals throughout this patient's illness showed a gradual rise in the 'O' agglutination titre to 1:500 (R.T. 31) but the 'H' agglutination titre did not exceed a dilution of 1:25 (R.T. 4) in the 4th week. This is an example of the 'H' agglutinins developing late in the disease.

In the case of Ina Caverley, the Ty'O' agglutinins developed early and were present in fairly high titre (1:500) on the 10th day of illness although the Ty'H' agglutinins did not appear till the 4th week of illness and only in a dilution of 1:50 (R.T. 6.3). In contrast to Lillian Card, the Ty'O' titre gradually decreased with the progress of the disease. In both these cases the illness was protracted and severe.

The case of Gladys Woodcock is of interest because appendicectomy had been performed 4 days before suspicion of typhoid fever arose and then a blood-sample was submitted for diagnosis. 'H' agglutinins were absent in 1:25 dilution but developed 12 days later to a titre of 1:100 (R.T. 12.5). 'O' agglutinins rose from 1:100 to 1:500 dilution and afterward decreased.

With George Davis, however, Ty'H' agglutinins were not detected at any time during his illness - which was not severe, although 6 blood samples were examined. The Ty'O' agglutinins ranged from a dilution of 1:200 to 1:500 (R.T. 11.8 to 41.7).

In the remaining 3 cases of this series only first samples were obtainable, so that the course of development of 'H' agglutinins could not be traced.

Cases exhibiting only 'O' agglutination are of considerable interest. Pijper (1930) observed 'a large number of typhoid patients who possessed 'O' agglutinins only'. Gardner, Hobson and Stenhouse (1930) reported a case with persistent negative 'H' and positive 'O' agglutination associated with a non-motile strain of B. typhosus in the blood. Giglioli (1933) states he has noted 4 cases showing Ty'O' agglutinins alone.

Table 14

Showing 'O' titres greater than 'H' titres in sera from typhoid fever cases

| Name or Serum Number | End-titres | | | Reduced titres | | |
|---|---|---|---|---|---|---|
| | Ty 'H' | Ty 'O' | Aer 'O' | Ty 'H' | Ty 'O' | Aer 'O' |
| L. Card | <1:25 | 1:50(2) | | - | 4.5 | |
| G. Woodcock | <1:25 | 1:100(2) | | - | 9.1 | |
| M 80 | <1:25 | 1:100(2) | 1:100(3) | - | 9.1 | 16.7 |
| Mrs.M.Lake | <1:25 | 1:200(3) | <1:25 | - | 18.2 | - |
| G. Davis | <1:25 | 1:200(2) | 1:200(2) | - | 11.8 | 40 |
| W. Gibson | <1:25 | 1:250(2) | 1:100(2) | - | 22.7 | 20 |
| I. Caverley | <1:25 | 1:500(2) | 1:500(2) | - | 31.3 | 83.3 |
| N 11 | 1:25(2) | 1:200(2) | 1:100(2) | 3.1 | 18.2 | 20 |
| W. Ashton | 1:25(2) | 1:250(3) | 1:250(2) | 3.6 | 13.9 | 41.7 |
| M. MacAdoo | 1:50(2) | 1:200(2) | | 7.1 | 11.1 | |
| H. Davis | 1:50(2) | 1:250(2) | 1:500(2) | 16.7 | 14.7 | 100 |
| I 2 | 1:50(2) | 1:500(2) | 1:500(3) | 8.3 | 45.5 | 83.3 |
| H. Bryant | 1:50(2) | 1:500(2) | 1:500(2) | 6.3 | 45.5 | 100 |
| 256 | 1:100(2) | 1:1000(2) | | 14.3 | 55.6 | |
| G. Keegan | 1:100(2) | 1:1000(2) | 1:250(3) | 12.5 | 90.9 | 41.7 |
| J. Birrell | 1:100(2) | 1:1000(2) | 1:500(3) | 33.3 | 58.8 | 100 |
| K 38 | 1:100(3) | 1:1000(2) | 1:1000(2) | 12.5 | 90.9 | 166.7 |
| D. Davis | 1:250(2) | 1:1000(2) | 1:1000(3) | 83.3 | 58.8 | 200 |
| Mrs. Card | 1:500(2) | 1:2000(2) | | 62.5 | 181.8 | |

A blank space under column Aer 'O' means insufficient serum for this test.

Further examination of Table 14 shows that there are 7 examples in which 'H' agglutinins have not developed and 12 examples in which the 'H' titres varied from 1:25 to 1:500 (R.T. 3 to 83.3), but in each case the Ty'O' titre exceeded the Ty'H' titre.

In the 14 cases permitting a comparison between Ty'O' and Aer'O' titres only one case, reacting with the Ty'O' suspension, showed a negative Aer'O' reading. In 9 cases the reduced titre for Aer'O' exceeded that for Ty'O' quite considerably; in 2 cases the reduced titres were approximately equal; and in 2 cases the reduced titre for Ty'O' exceeded that for Aer'O'. The case of George Keegan is exceptional, the reduced titre for Ty'O' being over twice that for Aer'O'.

The cases in this Table were all first samples submitted from proved cases of typhoid fever and exhibit the superior sensitiveness of the Aertrycke 'O' suspension in detecting Ty'O' agglutinins.

Although Ty'O' agglutination may appear alone in cases of typhoid fever, the usual findings are Ty'H' and Ty'O' agglutination, rarely Ty'H' agglutination alone and sometimes Ty'H' and Aer'O' agglutination. The undernoted Table 15 has been compiled from an analysis of the results of different authors.

Table 15

showing number of sera reacting with suspension of

| Author | Ty'H' only | Ty'H' and Ty'O' only | Ty'H' and Aer'O' only | Ty'O' alone or with Aer'O' also | PB'H' only | PB'H' and Aer'O' only | PB'H' and Ty'O' only | Aer'O' alone or with Ty'O' also |
|---|---|---|---|---|---|---|---|---|
| Smith (1932) | 4(28) | 11(28) | 0 | 3(28) | 10(42) | 6(42) | 3(42) | 0 |
| Gardner and Stubington(1932) | 0 | 6(40) | 0 | 3(40) | 1(40) | 4(40) | 1(40) | 0 |
| Horgan (1932) | 0 | 8(17) | 0 | 7(17) | 0 | 1(3) | 0 | 0 |
| Wyllie (Table 12). | 0 | 3(50) | 5(50) | 6(50) | 0 | 11(20) | 0 | 1(20) |

The number within brackets indicates the total number of sera examined, the number preceding the bracket the number of reacting sera.

It has been stated by Smith (1932) that it is sufficient for diagnostic purposes to use suspensions of B. typhosus 'H', B. para. B 'H' and B. typhosus 'O', excluding B. aertrycke (B. paratyphosus B) 'O' suspension. In the list of cases shown in Table *16* , the Ty 'H' titre did not exceed a positive result in a dilution 1:25 and a comparison is made between the corresponding titres obtained with Ty 'O' and Aer. 'O' suspensions. Only the results of first samples of blood are given as these are depended upon for confirmation of the clinical diagnosis. All were proved cases bacteriologically.

The Aer. 'O' reduced titres are distinctly higher (2 to 4 times) than the Ty 'O' titres in 7 cases; in 2 cases the titres are approximately the same; in 2 cases the Ty 'O' titre is absent although the Aer. 'O' reading occurs and in 1 case the Aer. 'O' reading is lacking where the Ty 'O' reading is present.

From these findings it is deduced that the Aer. 'O' suspension is more sensitive than the Ty 'O' suspension as a detector of 'O' agglutinins. It must be borne in mind also that Smith used a suspension of B. aertrycke (B. para. B) 'O', grown on ordinary nutrient agar, since a standardised suspension of B. para. B 'O' was not obtainable in 1930 from the Oxford Standards Laboratory. Consequently he was unable to compare the reduced titres of the Ty 'O' and Aer. 'O' readings in his results.

Table 16

Showing agglutination results of first blood samples from typhoid fever cases with the Ty 'H' reading not exceeding a reaction in 1:25 dilution

| Name or Serum Number | End-titres | | | | Reduced titres | | | |
|---|---|---|---|---|---|---|---|---|
| | Ty 'H' | Ty 'O' | P.B.'H' | Aer 'O' | Ty 'H' | Ty 'O' | P.B.'H' | Aer 'O' |
| Mrs. Malcolm | <1:25 | <1:25 | <1:25 | 1:250 (2) | - | - | - | 41.7 |
| M 80 | <1:25 | 1:100(2) | <1:25 | 1:100 (3) | - | 9.1 | - | 16.7 |
| Mrs.Miles Lake | <1:25 | 1:200(3) | <1:25 | <1:25 | - | 18.2 | - | - |
| George Davis | <1:25 | 1:200(2) | <1:25 | 1:200 (2) | - | 11.8 | - | 40 |
| Warren Gibson | <1:25 | 1:250(2) | <1:25 | 1:100 (2) | - | 22.7 | - | 20 |
| Ina Caverley | <1:25 | 1:500(2) | 1:25 | 1:500 (2) | - | 31.3 | - | 83.3 |
| Hugh Gray | 1:25(3) | <1:25 | <1:25 | 1:50 (2) | 8.3 | - | - | 10 |
| R.H.Zufelt | 1:25(3) | 1:25(3) | <1:25 | 1:25 (3) | 8.3 | 1.5 | - | 5 |
| C.Richardson | 1:25(3) | 1:50(2) | <1:25 | 1:50 (3) | 3.1 | 3.1 | - | 8.3 |
| C.Buchanan | 1:25(3) | 1:50(2) | <1:25 | 1:100(2) | 3.1 | 4.5 | - | 20 |
| N 11 | 1:25(2) | 1:200(2) | <1:25 | 1:100(2) | 3.1 | 18.2 | - | 20 |
| W. Ashton | 1:25(2) | 1:250(3) | <1:25 | 1:250(2) | 3.6 | 13.9 | - | 41.7 |

<1:25 means a negative reaction in 1:25 dilution

(2) and (3) indicate intensity of reaction as ++ and +++

5. Co-agglutination

According to Felix (1929) co-agglutination between B. typhosus, B. paratyphosus A and B is generally due to the small-flaking 'O' group-agglutinins and only exceptionally to the large flaking 'H' heterologous-agglutinins. Apart from its occurrence in the carrier [1] state, co-agglutination has been observed in active enteric infections in persons without history of past enterica infection or T.A.B. inoculation. From my personal records, there appear to be two main types:-

(a) where the phenomenon is present in the first serum-sample tested: example (i) co-agglutination occurring between the 'O' agglutinins of B. typhosus and B. paratyphosus B, with co-existing 'H' agglutinins for B. typhosus (in a case of typhoid fever) or for B. paratyphosus B (in a case of paratyphoid B fever).

This is by far the commonest example met with, and the case of Mrs. A.D., aged 42 years, may be cited as an illustration (Table17). This patient became ill on Oct. 7th, 1934 and was admitted to hospital on Oct. 14th without a diagnosis being made. The original source of the infection could not be traced but it is clear that the mother contracted the disease first and communicated the infection to four of her children and to a neighbouring cousin. About 2 weeks after the mother took sick 4 other members of the family whose ages ranged from 7 to 14, began to show similar symptoms and were admitted to hospital on Oct. 28th. Blood samples were taken for the first time on Oct. 29th when a suspicion of typhoid fever arose. Positive serological results on the 4 children led to a blood sample being taken from Mrs. A.D. on Oct. 30th. Six subsequent blood samples were submitted at approximately weekly intervals and the agglutination titres determined.

(1) Cf. p.26, Part II.

Table 17

showing Ty 'H', Ty 'O' and Aer. 'O' titres in 7 samples taken during the course of typhoid fever.

| Serum Number | Date when blood samples taken in 1934 | End-Titres | | | | Reduced Titres | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ty 'H' | Ty 'O' | PB'H' | Aer 'O' | Ty'H' | Ty'O' | PB'H' | Aer'O' |
| T12 | Oct. 30th | 1:2500(2) | 1:200(3) | - | 1:250(2) | 833.3 | 11.8 | - | 50 |
| T21 | Nov. 7th | 1:2000(2) | 1:200(2) | - | 1:100(4) | 666.7 | 11.8 | - | 20 |
| T33 | Nov. 13th | 1:2000(3) | 1:200(2) | - | 1:100(3) | 666.7 | 11.8 | - | 20 |
| T45 | " 21st | 1:1000(2) | 1:200(2) | - | 1:100(3) | 333.3 | 11.8 | - | 20 |
| T69 | " 27th | 1:2000(2) | 1:250(2) | - | 1:100(4) | 666.7 | 14.7 | - | 20 |
| T75 | Dec. 4th | 1:1000(2) | 1:500(2) | - | 1:100(4) | 250.0 | 29.4 | - | 20 |
| T82 | " 13th | 1:500 (2) | 1:250(3) | - | 1:100(3) | 125.0 | 20.8 | - | 20 |

Table 18

showing only Ty 'O' and Aer. 'O' titres in 6 samples taken during the course of typhoid fever.

| Serum Number | Date when blood samples taken in 1934 | End-Titres | | | | Reduced Titres | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ty 'H' | Ty 'O' | PB 'H' | Aer'O' | Ty 'H' | Ty'O' | PB'H' | Aer'O' |
| T20 | Nov. 7th | - | 1:200(2) | - | 1:200(2) | - | 11.8 | - | 40 |
| T35 | " 13th | - | 1:250(3) | - | 1:500(2) | - | 14.7 | - | 100 |
| T46 | " 21st | - | 1:500(3) | - | 1:500(3) | - | 29.4 | - | 100 |
| T70 | " 27th | - | 1:500(2) | - | 1:500(2) | - | 29.4 | - | 100 |
| T76 | Dec. 4th | - | 1:250(3) | - | 1:250(3) | - | 14.7 | - | 50 |
| T83 | " 13th | - | 1:500(2) | - | 1:250(2) | - | 41.7 | - | 50 |

example (ii) co-agglutination occurring between the 'O' agglutinins of B. typhosus and B. paratyphosus B, 'H' agglutinins for B. typhosus or B. paratyphosus B being absent.

This is not commonly found: only 5 examples have been noted during the past 4 years.

G.D., male, aged 11 years, took sick about Nov. 1st, 1934, 3 weeks after his mother (Mrs. A.D. of example (i)) and was admitted to hospital on Nov. 4th. In all, 6 samples of his blood were examined at weekly intervals but reactions were obtained only with the somatic suspensions of B. typhosus and B. aertrycke. During his stay of 7 weeks in hospital, his temperature did not rise very high: the range was from 99° to 102°F. during the month of November. The illness was not severe and the patient was discharged on Dec. 23rd. His physician regarded him clinically as a case of typhoid fever, although typical rose spots were not observed and the specific organism was not isolated from the blood or faeces. In addition to the agglutination tests recorded in Table 18 his serum was examined with flagellated suspensions of B. paratyphosus A, B. paratyphosus C, B. aertrycke and B. enteritidis (Gaertner) and also with B. dysenteriae (Sonne) with negative results.

example (iii) co-agglutination occurring between both the 'H' and 'O' agglutinins of B. typhosus and B. paratyphosus B. This is a rare occurrence in my experience.

Mrs. G.A., aged 57 years was admitted to hospital on October 26th, 1933 in a jaundiced condition with a history of severe headaches, fever and pain in the right hypochondriac region. These symptoms had persisted for about 4 weeks prior to admission and epistaxis had occurred 5 times. She had suffered from an attack of jaundice two years before. A history of previous enteric infection or of T.A.B. inoculation could not be elicited. She was discharged on November 22nd and readmitted 10 days later. Cholecystotomy was performed on December 4th, several small gall stones removed and the gall bladder drained. During the course of her illness four blood samples were tested as recorded in Table 19. B. paratyphosus B was isolated from the faeces and from the bile after operation.

Table 19

showing co-agglutination between 'H' and 'O' agglutinins in a case of paratyphoid B fever.

| Serum Number | Date when blood samples taken in 1933 | End-Titres | | | | Reduced Titres | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ty 'H' | Ty 'O' | PB 'H' | Aer 'O' | Ty 'H' | Ty 'O' | PB 'H' | Aer 'O' |
| N 4 | Oct. 10th | 1:1000(2) | 1:250(2) | 1:2000(2) | 1:250(4) | 125 | 22.7 | 500 | 41.7 |
| N 7 | Nov. 7th | 1:500 (2) | 1:100(2) | 1:2000(2) | 1:250(2) | 62.5 | 9 | 500 | 41.7 |
| N42 | " 16th | 1:250 (2) | 1:50 (2) | 1:1000(2) | 1:100(2) | 31.3 | 4.5 | 250 | 20. |
| N58 | " 21st | 1:250 (2) | 1:50 (2) | 1:1000(2) | 1:100(2) | 31.3 | 4.5 | 250 | 20. |

(b) where the phenomenon is absent in the first sample of serum tested but develops during the course of the disease:

example (i) co-agglutination between the 'H' agglutinins of B. paratyphosus B and B. typhosus occurring in the 3rd sample tested.

V.C., male, aged 19 years, was admitted to hospital on August 1st, 1934 with a complaint of general malaise and loss of appetite of 8 days' duration. This was followed by pain in the left lower abdomen and across the back, stiffness in the neck and chills. Rose spots were noticed on the abdomen on July 31st. B. paratyphosus B was isolated from the blood and faeces. The titres of four blood samples taken during the patient's illness are given in Table 20.

Table 20

showing development of co-agglutination between 'H' agglutinins in a case of paratyphoid B fever.

| Serum Number | Date when blood samples taken in 1934 | End-Titres | | | | Reduced Titres | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ty'H' | Ty'O' | PB'H' | Aer'O' | Ty'H' | Ty'O' | PB'H' | Aer'O' |
| R85 | July 31st | - | - | 1:250 (3) | 1:100(3) | - | - | 50 | 20 |
| R92 | Aug. 2nd | - | - | 1:1000(3) | 1:100(4) | - | - | 200 | 20 |
| S 8(1) | " 13th | 1:50(3) | - | 1:100,000(2) | 1:100(4) | 5 | - | 25,000 | 20 |
| S62 | " 28th | - | - | 1:10,000 (2) | 1:100(4) | - | - | 2,000 | 20 |

(1) repeated a few days later with the same result.

example (ii) co-agglutination between the 'O' and 'H' agglutinins of B. paratyphosus B and B. typhosus, occurring in the 3rd and 4th samples tested.

T.B., male, aged 21 years, was admitted to hospital on September 8th, 1934 having been ill for 3 days previously. The chief symptoms were drenching sweats, persistent headaches, abdominal cramps and occasional nausea and vomiting. During the first two to three weeks the patient was in a more or less semi-comatose condition. After this time the symptoms gradually diminished in severity. The titres of five blood samples taken during his illness are recorded in Table 21.

Table 21

showing development of co-agglutination between 'O' and 'H' agglutinins in a case of Paratyphoid B fever.

| Serum Number | Date when blood samples taken in 1934 | End-Titres | | | | Reduced Titres | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ty 'H' | Ty 'O' | PB 'H' | Aer 'O' | Ty 'H' | Ty 'O' | PB 'H' | Aer 'O' |
| S67 | Sept. 10th | - | - | - | - | - | - | - | - |
| S68 | " 12th | - | - | 1:25 (3) | 1:50 (2) | - | - | 5 | 10 |
| S71 | " 14th | - | 1:50(4) | 1:200 (2) | 1:100(4) | - | 3 | 40 | 20 |
| S99 | Oct. 3rd | 1:200(2) | 1:50(2) | 1:10,000(3) | 1:250(2) | 66.7 | 3 | 2000 | 50 |
| T 2 | " 12th | 1:50 (2) | <1:25 | 1:5000(2) | 1:100(4) | 16.7 | ... | 1000 | 20 |

The frequency of the occurrence of co-agglutination with the somatic suspensions Ty 'O' and Aer. 'O' (Para B 'O') is evident in the serological results of Tables 17 to 21. This has been a frequent occurrence in the examination of many sera for agglutinins to members of the typhoid - paratyphoid group of organisms. Topley and Wilson (1929) suggest that 'O' suspensions containing the Ø antigen may act in this way. In order to ascertain whether this might be a factor in the frequency of the occurrence, sera were chosen at random and subjected to absorption tests. (1) The following may serve as an example of the results.

A sample of human typhoid serum, giving end-titres Ty 'H' 1:2500 (++), Ty 'O' 1:500 (+++) and Aer. (Para B) 'O' 1:500 (+++) was absorbed with a dense emulsion of B. typhosus $H_{901}$; similar samples of the same serum were also absorbed with B. typhosus $O_{901}$ and with B. paratyphosus B (OB). The results of the agglutination tests of the unabsorbed and absorbed serum are given in Table 22.

Table 22

showing end-titres of serum from H.D., a case of typhoid fever, before and after absorption.

| Oxford Antigen | Unabsorbed | Absorbed by | | |
|---|---|---|---|---|
| | | Ty $H_{901}$ | Ty $O_{901}$ | OB |
| Ty 'H' | 1:2500 | 1:100 | 1:2500 | 1:2500 |
| Ty 'O' | 1:500 | <1:25 | <1:25 | 1:500 |
| Aer. (Para. B) 'O' | 1:500 | <1:25 | <1:25 | <1:25 |
| | | (1) | (2) | (3) |

(1) The agglutinin absorption technique is given on p.7.

Table 22 shows that (1) absorption with a flagellated emulsion of B. typhosus (Ty $H_{901}$) removes the agglutinins for the flagellar and somatic antigens,

(2) absorption with a non-motile emulsion of B. typhosus (Ty $O_{901}$) removes the somatic agglutinins for B. typhosus and B. paratyphosus B but leaves the flagellar agglutinins untouched, and

(3) absorption with a non-motile emulsion of B. paratyphosus B (OB) removes only the secondary agglutinins, i.e. the specific flagellar and somatic agglutinins for B. typhosus remain while the group agglutinins for B. paratyphosus B are absorbed.

If the results of absorption with emulsions Ty $O_{901}$ and Para. B (OB) are alone considered, it is clear that the serum saturated with the homologous organism (in the non-motile phase) loses not only its 'primary' but also its 'secondary' agglutinins. On the other hand, when the serum is saturated with the heterologous organism, for which a co-agglutinin had developed, it loses only that co-agglutinin, leaving the 'primary' agglutinin unaffected. It is concluded therefore that in this serum sample from a case of typhoid fever, co-agglutinins have been developed to Ty 'O'.

A contrast to these results was afforded when a sample of serum from a patient, who had received intravenous injections of T.A.B. vaccine, was subjected to absorption tests using the same bacterial strains and following the same technique as outlined on page 7 , Part I. The end-titres of this serum were Ty 'H' 1:1000 (++), Ty 'O' 1:250 (+++), Para. B. 'H' 1:1000 (++), P.B.'O' 1:500 (++). Table shows the results of the agglutination tests of the unabsorbed and absorbed serum.

Table 23

Showing end-titres of serum from J.D., intravenously inoculated with T.A.B. vaccine.

| Oxford Antigen | Unabsorbed | Absorbed by | | | |
|---|---|---|---|---|---|
| | | Ty $H_{901}$ | Ty $O_{901}$ | H $B_2$ | O.B. |
| Ty 'H' | 1:1000 | <1:25 | 1:1000 | 1:1000 | 1:1000 |
| Ty 'O' | 1:250 | <1:25 | <1:25 | 1:200 | 1:200 |
| Para B 'H' | 1:1000 | 1:1000 | 1:1000 | <1:25 | 1:1000 |
| Aer.(Para B)'O' | 1:500 | 1:500 | 1:500 | <1:25 | <1:25 |
| | | (1) | (2) | (3) | (4) |

From these results it is seen that (a) absorption with a flagellated suspension of B. typhosus or B. paratyphosus B. removes its own flagellar and somatic agglutinins only, cf. (1) and (3) ; (b) absorption with a somatic suspension of B. typhosus or B. paratyphosus B. removes its own somatic agglutinins only, cf.(2) and (4).

From the results of the absorption tests on the human typhoid serum and the human serum resulting from intravenous inoculation, we may deduce that if saturation with the homologous organism (non-motile phase) removes both the primary and secondary agglutinins, we are dealing with coagglutinins in the serum, but if only the specific somatic agglutinins are absorbed, we are dealing with a serum in which specific agglutinins are developed.

It seemed evident from a comparison of the agglutinin absorption tests on a human typhoid serum showing somatic co-agglutinins to Ty 'O' (Table 22, page 57 ) and on a human serum obtained after intravenous inoculation of T.A.B. vaccine (Table 23 page 59 ) that it is possible to differentiate between true co-agglutinins and agglutinins developed in response to individual antigens. To test this hypothesis, T.B's serum (4th and 5th samples, S99 and T2, pooled in Table 21 page 56 ) was absorbed with a dense emulsion of B. paratyphosus B ($HB_2$); similar samples of this serum were also absorbed with B. para. B (OB), B. typhosus $H_{901}$ and B. typhosus $O_{901}$. The results of the agglutination tests of the untreated serum, at the time of the absorption test, and of the absorbed serum are given in Table 24 .

Table 24

showing end-titres of T.B's serum, a case of Paratyphoid B fever, before and after absorption.

| Oxford Antigen | Unabsorbed | Absorbed by | | | |
|---|---|---|---|---|---|
| | | Ty $H_{901}$ | Ty $O_{901}$ | $HB_2$ | OB |
| Ty 'H' | 1:100 | <1:25 | - | <1:25 | - |
| Ty 'O' | 1:50 | <1:25 | <1:25 | <1:25 | <1:25 |
| Para. B 'H' | 1:10,000 | 1:10,000 | - | 1:1000* | 1:10,000 |
| Aer.(Para.B)'O' | 1:100 | 1:100 | 1:100 | <1:25 | <1:25 |
| | | (1) | (2) | (3) | (4) |

A consideration of the 'O' titres after absorption with emulsions of $HB_2$ and OB shows that the serum saturated with the homologous organism (whether in the motile or non-motile phase) loses not only its primary but also its secondary somatic agglutinins (cf. (3) and (4)). On the other hand, when the

* Further saturation with a dense emulsion of $HB_2$ removed the agglutinins to B. para. B 'H'.

serum is saturated with the heterologous organism whether in the motile (Ty $H_{901}$) or non-motile phase (Ty $O_{901}$), it loses only the co-agglutinins which had developed and leaves the primary agglutinins unaffected (cf. (1) and (2)). Therefore in this serum, co-agglutinins to Aer. (Para. B) 'O' have been developed.

An examination of the 'H' titres after absorption with emulsions $HB_2$ and Ty $H_{901}$ shows that the homologous organism (motile phase) removes both the primary and secondary flagellar agglutinins, whereas the heterologous organism (motile phase) removes its own flagellar agglutinins, leaving the primary flagellar agglutinins unaffected. This means that co-agglutinins to B. para. B 'H' have been developed in the serum.

The case of Mrs. E.J.S. aet. 32 years is cited as an example of typhoid fever occurring in a patient who gave a history of previous enteric infection, fourteen years before. This patient had been confined to bed at home for three weeks before admission to hospital on Dec. 19th, 1934. During her residence in hospital, four blood-samples were examined at approximately weekly intervals and all gave reactions to 'H' and 'O' suspensions of B. typhosus and B. paratyphosus B. B. typhosus was isolated by blood culture on Dec. 27th, 1934. The patient stated that she had suffered from an attack of typhoid or paratyphoid fever in 1920 and this statement was confirmed by her physician, but unfortunately a record of the agglutination titre could not be traced.

Samples of this patient's serum were absorbed with dense emulsions of B. typhosus $H_{901}$ and $O_{901}$, B. paratyphosus B ($HB_2$) and OB. The results of the agglutination tests of the untreated and absorbed serum are given in Table 25 .

Table 25

showing end-titres of serum from Mrs. S. a case of typhoid fever, before and after absorption.

| Oxford Antigen | Unabsorbed | Absorbed by | | | |
|---|---|---|---|---|---|
| | | Ty $H_{901}$ | Ty $O_{901}$ | $HB_2$ | OB |
| Ty 'H' | 1:500 | <1:25 | - | 1:250 | - |
| Ty 'O' | 1:100 | <1:25 | <1:25 | 1:100 | 1:100 |
| Para. B 'H' | 1:100 | 1:50 | - | <1:25 | - |
| Aer.(Para.B)'O' | 1:100 | <1:25 | <1:25 | <1:25 | <1:25 |
| | | (1) | (2) | (3) | (4) |

As before, if we consider the 'O' titres after absorption with emulsions of Ty $H_{901}$ and Ty $O_{901}$, the serum when saturated with the homologous organism (whether in the motile or non-motile phase) loses both its primary and secondary agglutinins (cf. (1) and (2)), whereas saturation with the heterologous organism whether in the motile ($HB_2$) or non-motile (OB) phase removes only the co-agglutinins which had developed and leaves the primary agglutinins unaffected.-

If we consider now the 'H' titres after absorption with Ty $H_{901}$ and $HB_2$, we find that the homologous organism (motile phase) removes its own flagellar agglutinins but fails to absorb the heterologous flagellar agglutinins present in low titre (1:100), and similarly with the heterologous organism. We may therefore deduce that the B. paratyphosus B agglutinins are not true co-agglutinins. It is suggested that the patient's illness fourteen years ago was probably due to Paratyphoid B infection and that her blood serum contained residual 'H' agglutinins.

Further evidence of co-agglutination occurring between flagellar as well as somatic agglutinins may be presented from the serological results of other investigators.

Smith (1932) recorded the serological and bacteriological findings of 28 cases of typhoid fever and 42 cases of paratyphoid B fever. From a study of his Table II it is found that co-agglutination occurred between Ty 'O' and Para. B 'O' agglutinins twice, between Ty 'H' and Para. B 'H' agglutinins 4 times and between 'H' and 'O' agglutinins to B. typhosus and B. paratyphosus B 3 times. In 6 of these 9 cases B. typhosus was isolated from the blood or excreta. Similarly from his Table V co-agglutination was found to occur between Para. B 'O' and Ty 'O' agglutinins 8 times, between Para. B 'H' and Ty 'H' agglutinins 7 times and between 'H' and 'O' agglutinins to B. paratyphosus B and B. typhosus 7 times. In 13 out of these 22 cases, B. paratyphosus B was isolated from the blood or excreta.

Smith regards these cases as examples of group agglutination although he does not call attention to the varieties of co-agglutination as shown in his tables nor does he state whether inquiry had been made regarding past enterica infection or previous inoculation.

Horgan (1932) examined the agglutination titres of 14 bacteriologically proved cases of typhoid fever. Case 1 of his Table III shows group agglutination between 'H' and 'O' agglutinins to B. typhosus and B. paratyphosus B, viz. Ty 'H' 1:2500, Ty 'O' 1:500, B. Para. B 'H' 1:125 and B. Para. B 'O' 1:125. Presumably this case was not previously inoculated since 2 cases in the table are noted as having had previous T.A.B. inoculation.

Analogous examples of co-agglutination of type a (i) page 52 , are found in Smith's Tables III and V, 2 typhoid fever cases in the former and 8 paratyphoid B fever cases in the latter. An example of co-agglutination of type a (ii) page 53 , is found in Horgan's Table III (case 2) and examples of type a (iii) page 54 , are seen in 3 typhoid fever cases and in 7 paratyphoid B cases of Smith's tables III and V.

Co-agglutination of type b, page 55 , is seen in Smith's table IV. Cases 13 and 16 are analogous to the case of V.C. in Table 20 where the heterologous 'H' agglutinins subsequently disappear. In case 4 there occurs a development of Para. B 'O' agglutinins and, in case 15, a disappearance of Para. B 'O' agglutinins in the second serum sample. These correspond to Table 21 in which the Ty 'O' and 'H' co-agglutinins gradually develop and then recede.

## Summary and Conclusions.

1 (i) In an unselected group of 146 presumably normal persons, i.e. without history of past enterica infection or antityphoid inoculation 4 per cent. gave serological reactions with B. typhosus 'H' suspension in dilutions 1:25 to 1:100 and 11 per cent. with B. typhosus 'O' suspension in dilutions 1:25 to 1:50.

(ii) The frequency of the 'O' agglutinins is greater than the 'H' agglutinins to B. typhosus among the normal non-inoculated population.

2 (i) A group of 37 young adult males were given three subcutaneous inoculations, at weekly intervals, of a T.A.B. vaccine prepared from freshly isolated or rejuvenated strains of B. typhosus, B. paratyphosus A and B. paratyphosus B. Their blood sera were examined one month later for the presence of agglutinins to B. typhosus 'H' and B. typhosus 'O' suspensions. The experience gained in 1932 was repeated, viz. a good response to the flagellar antigen associated with a poor response to the somatic antigen.

(ii) A second group of 50 young adults, male and female, were similarly inoculated and, after a two months' interval, their 'H' and 'O' titres were determined.

(iii) By combining the results in these two subcutaneously inoculated groups, it was found that only 25 per cent. showed typhoid 'O' agglutinins in dilutions ranging from 1:25 to 1:200 (R.T. 1.4 to 18.1). All but 3 cases showed typhoid 'H' agglutinins, the range being 1:25 to 1:5000 (R.T. 3.1 to 625).

(iv) The results of agglutination tests performed 16 months, 22 months and 2½ years after subcutaneous administration of Typhoid-paratyphoid vaccine show that the typhoid 'O' agglutinins return to the normal threshold before the typhoid 'H' agglutinins have disappeared.

(v) Recent work on the preparation of efficient Typhoid-paratyphoid vaccines suggests the possibility of inducing high titres for somatic agglutinins.

3 (i) In a composite group of 42 psychotic patients agglutination titres were determined from 9 to 28 months after intravenous administration of T.A.B. stock vaccine. Thirty-four of these cases showed the presence of 'H' agglutinins and 30 showed 'O' agglutinins. In 31 cases examined 9 to 22 months after inoculation, the average reduced titre for typhoid 'H' agglutinins was 13.4 and for typhoid 'O' agglutinins 7.7. In 10 cases examined 27 to 28 months after inoculation, the average reduced titre for typhoid 'H' and typhoid 'O' agglutinins was 5.8 and 1.5 respectively.

(ii) After intravenous (therapeutic) inoculations of Typhoid-paratyphoid vaccine, the typhoid 'O' agglutinins persist in fairly high titre (average R.T. = 7.7) for about 2 years and thereafter rapidly diminish.

(iii) In a group of 5 cases, given a constant intravenous dosage and tested 12 months later, the average reduced titre for typhoid 'H' agglutinins was 56 and for typhoid 'O' agglutinins 21.8.
The corresponding reduced titres for paratyphosus B 'H' and aertrycke 'O' are 132.5 and 58 respectively.

(iv) In a group of 5 cases, given increasing intravenous doses and tested one month later the average reduced titres for typhoid 'H' and typhoid 'O' agglutinins were 130 and 53.2 respectively and for paratyphosus B 'H' and aertrycke 'O' 600 and 170 respectively.

4 (i) In a series of 50 typhoid fever cases 15 failed to reach the diagnostic limit, a reduced titre of 10, for B. typhosus 'H' agglutinins, but in a series of 20 paratyphoid B fever cases only one had a reduced titre less than 10. Hence paratyphoid B sera react more constantly with the homologous 'H' antigen than do typhoid sera.

(ii) In the same series of typhoid fever cases 21 had reduced titres less than the diagnostic limit, a reduced titre of 10, while only 3 of the paratyphoid B series had a reduced titre of less than 10 for aertrycke (Para B) 'O' agglutinins. Paratyphoid B sera therefore react more constantly with the homologous 'O' antigen than do typhoid sera.

(iii) In both typhoid and paratyphoid B sera the aertrycke 'O' suspension has been found to be more sensitive than the typhoid 'O' suspension as a detector of somatic agglutinins.

(iv) Cases of enteric fever occur which possess only 'O' agglutinins, others only 'H' agglutinins but generally 'H' and 'O' agglutinins.

(v) In some cases the 'O' agglutinins appear earlier than the 'H' agglutinins and in others the 'H' agglutinins prior to the 'O' agglutinins.

5 (i) Co-agglutination between the somatic agglutinins of B. typhosus and B. paratyphosus B occur more frequently than between the flagellar agglutinins in the sera of typhoid and paratyphoid B fever cases.

(ii) These co-agglutinins may develop early or late in the course of the disease.

(iii) It is suggested that the agglutinin absorption test is capable of distinguishing whether or not true co-agglutinins have been developed to the 'H' and 'O' antigens of B. typhosus or B. paratyphosus B.

## References.


Balteanu, I. (1926). The Receptor Structure of V. cholerae with observations on variations in cholera and cholera-like organisms. J. Path. and Bact. 29. 251.

Braun, H. and Schäffer, H. (1919). Zur Biologie der Fleckfieber-proteus-bazillen; ein Beitrag der Wirkunsweise der Desinfektionsmittel. Ztschr. f. Hyg. u. Infektkr. 89. 339.

Bruce White, P. (1925). M.R.C. Sp. Rep. Ser. No. 91. Part I.

Dennis, E.W. and Berberian, D.A. (1934). A Study of the Production of Somatic and Flagellar Agglutinins in response to Anti-typhoid-paratyphoid Inoculation. Amer. J. Hyg. 20. 469.

Dulaney, A.D., Wikle, W.T. and Trigg, R. (1932). H and O agglutination as an aid to the diagnosis of typhoid fever. A.J.P.H. 22. 1033.

Feiler, M. (1924). Zur Biologie des Typhus-bacillus. Ztschr. f. Immunitätsfor Orig. 29. 303.

Felix, A. (1924). The Qualitative Receptor Analysis in its application to Typhoid Fever. J. Immunol. 9. 115.

Felix, A. (1929). J. Hyg. 28. 418.

Felix, A. (1930). The Qualitative Serum Diagnosis of Enteric Fevers. Lancet, i. 505.

Felix, A. and Olitzki, L. (1926). Bactericidal Serum Action and Qualitative Receptor Analysis. J. Immunol. 11. 31.

Gardner, A.D. (1929). The Small-flaking or 'O' Agglutination of Permanent Standardised "O" Suspensions of B. typhosus by the Serums of Normal, Inoculated and Infected Persons. J. Hyg. 28. 376.

Gardner, A.D. (1930). Directions for the Interpretation of Agglutination Tests done with Standardised Suspensions and Serums.

Gardner, A.D. and Stubington, E.F. (1932). Granular "O" Agglutination in Paratyphoid B. and Typhoid Fevers. J. Hyg. 32. 615.

## References.


Giglioli, G. (1933). Agglutinins for the Typhoid - Paratyphoid Group in a Random Sample of the Population of British Guiana. J. Hyg. 33. 379.

Grinnell, F.B. (1930). A Study of the Comparative Value of Rough and Smooth Strains of B. typhosus in the Preparation of Typhoid Vaccines. J. Immunol. 19. 457.

Havens, L.C. and Mayfield, C.R. (1931). The Significance of Agglutinins in Normal Persons. J. Prev. Med. 5. 295.

Horgan, E.S. (1932). Qualitative or Quantitative Methods in the Serological Diagnosis of Enteric Infections? J. Hyg. 32. 523.

Mudd, S. (1932). The Agglutinin Response to Typhoid-paratyphoid Vaccination. J. Immunol. 23. 81.

Orcutt, M.L. (1924). Flagellar Agglutinins. J. Exp. Med. 40. 43.

Perry, H.M., Findlay, H.T. and Bensted, H.J. (1934). Anti-typhoid Inoculation. R.A.M.C. Journal for March and for July.

Rosher, A.B. and Fielden, H.A. (1922). Agglutinins in Normal Sera for some Micro-organisims of the Paratyphoid Group, Lancet, 1. 1088.

Schütze, H. (1930). The Importance of Somatic Antigen in the Production of Aertrycke and Gärtner Immunity in Mice. Brit. J. Exp. Path. 11. 34.

Smith, J. (1932). The Serological Diagnosis of Typhoid and Paratyphoid Fevers. J. Hyg. 32. 143.

Smith, Theobald and Reagh, A.L. (1903). The Non-identity of Agglutinins acting upon the Flagella and upon the Body of Bacteria. J. Med. Res. 10. 89.

Stuart, G. and Krikorian, K.S. (1928). Serological Diagnosis of Enterica by the method of Qualitative Receptor Analysis. J. Hyg. 28. 105.

Stuart, G. and Krikorian, K.S. (1928). Agglutinin Response to Anti-enterica Vaccination. Lancet, ii, 644.

Topley and Wilson (1929). The Principles of Bacteriology and Immunity. Vol. I. p. 436.

## References.


Topley, W.W.C. (1929). The Natural Acquirement of Immunity. Lancet i. 1337.

Weil, E. and Felix, A. (1917). Weitere Untersuchungen über das Wesen der Fleckfieber-agglutination. Wien. Klin. Wchnschr. 30. 1509.

Weil, E. and Felix, A. (1918). Über die Doppelnatur der Rezeptoren beim Paratyphus B. Wien. Klin. Wchnschr. 31. 986.

Weil, E. and Felix, A. (1920). Über den Doppelnatur der Rezeptoren in der Typhus-Paratyphus-Gruppe. Ztschr. f. Immunitätsf. Orig. 29. 24.

Whitehead, A.T. (1930). The Floccular and Granular Agglutinins in Typhoid Fever. R.A.M.C. Journal. 55. 81.

Wong, D.H. and Zia, S.H. (1933). The Practical Value of "O" Agglutination in the Widal Test. Chinese Med. Jour. 47. 154.

Wyllie, J. (1932). Experiences with Small-flaking or Granular Agglutination in Normal, Inoculated and Enteric Fever Cases. J. Hyg. 32. 375.

Yokota, K. (1925). Neue Untersuchungen Zur Kenntis der Bakteriengeisseln. Centralbl. f. Bakt. I. Orig. 95. 261.

Zia, S.H. (1931) Small-flaking or "O" Agglutinin after Intravenous Injections of Typhoid Vaccine. Proc. Soc. Exp. Biol. and Med. 29. 253.

## PART II

## THE SEROLOGICAL DIAGNOSIS OF THE CHRONIC TYPHOID CARRIER

Paper incorporated

Wyllie, J. American Journal of Hygiene, Vol. XVIII. No. 2. Sept. 1933.

## Contents

## Introduction.

In recent years the major avenues for dissemination of enteric infection have been more or less effectively controlled by the widespread installation of water purification and milk pasteurisation plants, so that typhoid fever of the present day originates practically always from carriers through food contamination. The detection of typhoid carriers therefore assumes importance. In the laboratory, the diagnosis of the carrier condition has been chiefly directed to cultural methods for the isolation of B. typhosus, while comparatively little attention has been given to serological methods. The value of the agglutination test in detecting carriers is variously stated by different writers.

According to Ledingham and Arkwright (1912) the agglutination test has proved to be of great service in the diagnosis of chronic typhoid carriers, about two-thirds to three quarters of whom give a titre of 1 in 100 or at least 1 in 50.

Browning in Applied Bacteriology (1918) says 'The Widal reaction if positive in a suspected carrier strengthens the suspicion materially; on the other hand, carriers may manifest no serological reaction to distinguish them from normal individuals, and there is no procedure known which will replace the extended examination of faeces and urine in searching for carriers.'

Gay in his book on Typhoid Fever (1918) refers to the need of a rapid method for detecting carriers and states that the agglutination test is positive in from 65 to 75 per cent. of individuals who harbour the typhoid bacillus and more frequently in recovered than in healthy carriers. He considers the actual demonstration of the typhoid bacillus in the excreta the only certain method of diagnosis.

Simon (1919) says that the recognition of typhoid carriers of the faecal type is facilitated by the fact that the blood serum of such individuals usually gives the Widal reaction: exceptions occur but they are relatively rare.

Nichols (1922) affirms that over 50 per cent. of typhoid carriers give the agglutination test as an indication of their chronic focus, the serum titre being usually about 1:40. He considers, however, that the test has little value in early convalescents and in those who have recently been vaccinated against the disease.

Paul (1926) states 'The blood of a chronic carrier usually gives a marked agglutination reaction with the type of bacillus he carries; this is presumptive evidence of the existence of the carrier state, if the individual has not been recently inoculated against enteric fever, or if the attack of enteric he had was some years previously. Nevertheless the agglutination reaction alone will not suffice to differentiate enteric carriers. It is said that in those contact carriers who are not actually infected with the organism the agglutination test will be negative. Moreover the reaction is occasionally positive in healthy individuals who have not suffered from enteric fever and who are not carriers.'

Hewlett and McIntosh (1932) assert that three-fourths of the typhoid carrier cases are women and usually the serum of the carrier gives a marked agglutination reaction.

Muir and Ritchie (1932) state that usually speaking, the typhoid carrier gives a positive Widal reaction but this may be absent and may only be obtained with a high concentration of serum. Further it has been shown in chronic carriers that the agglutinating capacity of the serum varies from time to time and sometimes may be absent.

Kristensen and Poulsen (1933) examined the sera of 59 chronic typhoid carriers and of 18 chronic paratyphoid B carriers, in all of whom the carrier state had developed at least 2 years previously. Their purpose in this investigation was to decide upon a suitable technique for the

carrier Widal test so as to limit the group in which faeces and urine examinations are made in searching for carriers in a community. They concluded that it suffices to test the sera in dilutions of 1:5 and 1:10, to determine the end-titre only in the positive reactions, to use a polyvalent typhoid culture and a specific paratyphoid B culture, and that incubation at 37°C. for 18 hours gives approximately the same results as 12 hours at 50°C.

Browning (1933) inclines to the view that the Widal test applied to carrier sera is of limited value and calls attention to (i) the great variation in the agglutinin content of a carrier's serum when tested at different times (ii) the occasional occurrence of low titres for the homologous organism and (iii) the phenomenon of co-agglutination between B. typhosus, B. paratyphosus A and B, using formolised bacterial suspensions ('H' phase).

The investigation of an outbreak of typhoid fever in a ward of the Ontario Hospital, Kingston, Ontario resulted in the detection of two carriers and the serological findings stimulated my interest to examine further samples of serum from carriers obtained by request from widely separated areas.

## Technique

In all, 26 samples of carrier sera were examined for their agglutinating capacity (1) by Dreyer's method using the Oxford standardised agglutinable suspensions and (2) by the Qualitative Receptor Analysis Method of Felix. The former method is described in Part I, p. 5 . To ensure success in the employment of the latter method, attention to details of technique [1] is necessary.

---

(1) Indications regarding procedures are suggested in articles by Felix (1914, 1930) and by Stuart and Krikorian (1928) but the technique personally developed is described in detail because difficulty was experienced in following the original papers.

I. Preparation of media:

a. Agar used for plates and slants.

(1) Fresh beef, freed from fat and fibrous tissue, is minced and mixed well with water in the proportion of 500 gms. mince to 1 litre of distilled water. The mixture is allowed to stand overnight in the cold; in the morning it is filtered through two layers of cheesecloth and the fluid expressed by hand from the meat. If necessary, distilled water is added to make up to the original volume. The filtrate is heated in a boiling water-bath for an hour. The subsequent filtering is greatly facilitated by allowing the albumen to coagulate undisturbed by stirring. While hot the fluid is filtered through two layers of cheesecloth and the albumen expressed to recover as much fluid as possible.

(2) Fifteen gms. agar, 5 gms. bacteriologic peptone (P. D. & Co.) and 5 gms. sodium chloride are now added and dissolved by heating in the Koch (current steam at 100°C.) The reaction is adjusted to pH 7.4. (Felix (1924) prescribes the reaction as litmus-neutral; when thus adjusted the pH was found to be 7.4, using phenol-red indicator solutions). If necessary distilled water is added to make up to 1 litre. Sixty c.cs. distilled water per litre are now added to allow for evaporation during the subsequent boiling. The medium is boiled over a free flame for 10 minutes and filtered through a layer of cotton-wool and lint into Erlenmeyer flasks.

(3) On three successive days, the filtered medium is heated in the Koch for 20 minutes after the agar has been melted. The tops of the flasks are covered to prevent evaporation during storage. It is advisable to keep the agar in small amounts of 250 c.c. in flasks, as repeated melting of the agar before all is used tends to cause deterioration of the medium. Culture tubes, 5 x $\frac{1}{2}$ inch, plugged and sterilized, are used for the agar slants. The medium

contained in a flask is melted in a boiling water-bath and while still hot is poured into tubes. The tubes are immediately plugged with tight-fitting cotton-wool stoppers and sloped. As soon as the agar is set, the tubes are placed in the refrigerator inside a covered container to preserve as much water of condensation as possible. (Should the tubes after 4 or 5 days show little or no water of condensation the agar should be remelted and resloped).

b. Agar for motility tubes.

The beef infusion agar for the motility tubes is made as for the agar plates and slants with the exception of the agar content. To 500 gms. beef and 1 litre of water, 2 gms. agar are added. The procedure is the same as above. This semi-solid agar is now poured into culture tubes containing pieces of quill-tubing open at both ends and projecting at its upper end $\frac{3}{4}$ inch or more above the agar surface. (Diagram, p. 6 ) The central core of agar is inoculated from an agar slant with a straight platinum wire and in 24 hours the growth of B. typhosus or its allied members diffuses through the depth of the agar to the surface of the outer concentric zone of agar. The motility of a drop of the growth from the outer zone is very much greater than that of the central core. A feebly-motile organism, which has been subcultured on ordinary agar slants, can in this way be rendered actively motile. Two-tenths or 0.3 per cent. semi-solid agar has been found most suitable in my experience for this purpose but an equal amount of galatin may also be incorporated. As Craigie (1931) suggests it is advisable to make a few successive stab-inoculations into semi-solid agar to obtain a strain which shows rapid diffusion without showing a denser growth along the line of inoculation. Throughout this investigation cultures $H_{901}$, $HB_2$ and $HA_6$ were subcultured twice weekly in semi-solid agar and thence transferred to agar slants with water of condensation. In this way the proper development of the H-receptors was considered to be maximal.

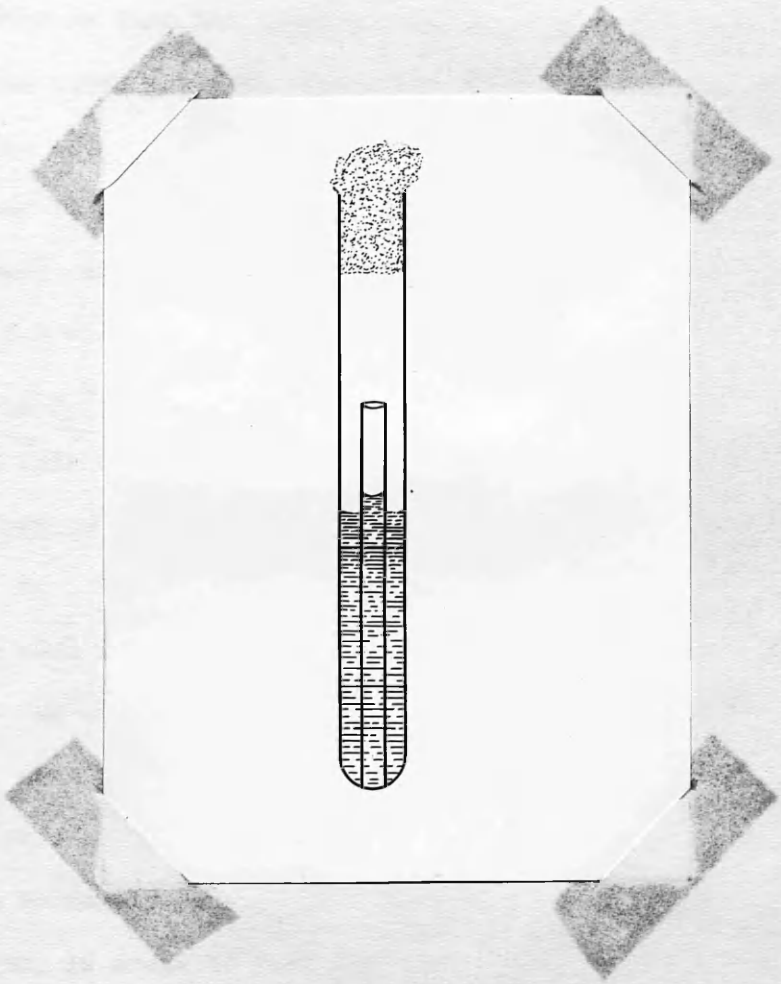


Diagram illustrates test-tube, with inset tube and containing semi-solid agar, used to render organisms actively motile.

II. Scheme of Agglutination Method:

a. Preparation of bacillary suspensions.

Before inoculation, the agar slopes are placed for 15 to 30 minutes in the incubator so that the water of condensation collects at the foot of the tube. These tubes are then inoculated from motility tubes with strains $H_{901}$, $HB_2$ and $HA_6$. The cultures $O_{901}$, OB and $HA_1$ are subcultured from previous slants.

After 18 hours' incubation at 37°C. the growths are emulsified with 0.85 per cent. saline, using 0.5 c.c. to each tube. This is diluted further with 3 or 3.5 c.cs. of saline and the resulting opacity adjusted to tube no. 6 on McFarland's Nephelometer scale (equal to 1800 million organisms per c.c.). I have found this opacity of suspension to be optimum for reading the tests. These living suspensions are freshly prepared on each day of the test.

b. Agglutination Test.

Two rows of tubes, 7.5 x 0.8 cms., are placed in a metal rack, each horizontal row containing the same number of tubes as bacillary suspensions prepared. Here the number is six, for cultures $H_{901}$, $O_{901}$, $HB_2$, OB, $HA_6$ and $HA_1$. (In this country, it is not necessary to employ $HA_6$ and $HA_1$ as paratyphoid A fever is extremely rare.) Into each tube in the front row, 18 drops, and in the back row, 19 drops of 0.85 per cent. sodium chloride solution are placed. A 1 in 10 dilution of the patient's serum is prepared in similar tubes; 2 drops of this are added to each tube in the front row and one drop to each tube in the back row. One drop of the respective bacillary suspensions is added to each front and back row tube. Thus the tubes of the front row contain 1 c.c. of 1 in 100 dilution and those of the back row 1 c.c. of 1 in 200 dilution of the patient's serum, to which one drop of bacillary suspension has been added. The tubes are sharply tapped with the finger to mix and the rack is placed in the incubator at 37°C. for 2 hours. After preliminary reading, they are allowed to stand at

room temperature for 24 hours when final readings are made. For measuring the drops of saline a constant drop is best secured by inserting a 'Mariotte's tube' (1) in an ordinary burette, so that the drops issue at a rate of 1 per second. A Dreyer pipette whose external diameter is exactly similar to the burette tip (determined by a Starrett Drill Gauge) is used for making the serum dilutions and adding the bacillary suspensions.

III. Controls:

a. Saline controls.

For each bacillary suspension, four strengths of sodium chloride solution are used, namely 0.85, 1.7, 3.4, and 6.8 per cent. Into 4 horizontal rows each containing six tubes, are measured 20 drops successively of 0.85, 1.7, 3.4 and 6.8 per cent. saline. One drop of the appropriate suspension is added to each tube in the six serial rows of the four strengths of saline. The tubes are shaken and incubated along with those of the test. By this means it is readily determined whether the cultures have been maintained perfectly 'smooth.' If spontaneous agglutination occurs in these controls, it is an indication that the culture has gone 'rough.' (Schematic representation of Test and Saline controls on page 9.) By replating the culture a smooth colony may be isolated and, before subsequent use, the new culture should be tested to determine its 'antigenic receptor' content.

(1) Diagram, M. R. C. Special Report Series, No. 51, p. 120, fig. 11.

SALINE CONTROLS

| | | $H_{901}$ | $O_{901}$ | $HB_2$ | OB | $HA_6$ | $HA_1$ |
|---|---|---|---|---|---|---|---|
| Saline (6.8%) | 20 drops | | | | | | |
| Bac.suspension | 1 drop | ○ | ○ | ○ | ○ | ○ | ○ |
| Saline (3.4%) | 20 drops | | | | | | |
| Bac.suspension | 1 drop | ○ | ○ | ○ | ○ | ○ | ○ |
| Saline (1.7%) | 20 drops | | | | | | |
| Bac.suspension | 1 drop | ○ | ○ | ○ | ○ | ○ | ○ |
| Saline (0.85%) | 20 drops | | | | | | |
| Bac.suspension | 1 drop | ○ | ○ | ○ | ○ | ○ | ○ |

TEST

| | | $H_{901}$ | $O_{901}$ | $HB_2$ | OB | $HA_6$ | $HA_1$ |
|---|---|---|---|---|---|---|---|
| Saline 0.85% | 19 drops | | | | | | |
| Serum (1 in 10) | 1 drop | ○ | ○ | ○ | ○ | ○ | ○ |
| Final dilution of serum | 1 in 200 | | | | | | |
| Bac.suspension | 1 drop | | | | | | |
| Saline 0.85% | 18 drops | | | | | | |
| Serum (1 in 10) | 2 drops | ○ | ○ | ○ | ○ | ○ | ○ |
| Final dilution of serum | 1 in 100 | | | | | | |
| Bac.suspension | 1 drop | | | | | | |

b. Tests to determine whether correct distribution of 'H' and 'O' antigens has been maintained in the cultures ('antigenic receptor' control.)

To obtain suitable antisera rabbits were inoculated with typhoid and paratyphoid cultures. The bacillary suspensions, the opacity of which corresponded to tube no. 3 on McFarland's Nephelometer scale (equivalent to 900 million bacteria per c.c.) were treated as follows:

| | |
|---|---|
| B. typhosus $H_{901}$ | heated for 1 hour at 60°C. |
| B. typhosus $O_{901}$ | " " " " |
| B. paratyphosus B, $HB_2$ | " " " " |
| B. paratyphosus B, $HB_2$ | " " 2 hours at 100°C. |
| B. paratyphosus A, $HA_6$ | " " 1 hour at 60°C. |
| B. paratyphosus A, $HA_1$ | " " 2 hours at 100°C. |

Tests of these antisera in dilutions of 1 in 500 to 1 in 20,000 were made with the homologous suspensions. The scheme detailed in Table 1 illustrates the proper content of 'H' and 'O' antigens in the suspensions. Felix (1931) suggests that these tests should be done periodically.

c. Serum controls ('agglutinin content' control.)

The 'O' type of agglutination is clearly observed in the use of suspensions of $O_{901}$ and OB. With the 'H' suspensions -- $H_{901}$, $HB_2$, $HA_6$, and $HA_1$ -- difficulty is sometimes encountered in distinguishing between the 'H' and 'O' type of agglutination in the same dilution tube. Hence it is advisable to use, on each test day, serum controls of known 'H' and 'O' agglutinin content, viz. an agglutinating serum containing 'H' and 'O' agglutinins, one containing only 'H' agglutinins and another containing only 'O' agglutinins. Referring to Table 1 , rabbit antiserum 954 shows 'H' and 'O' agglutinins with $H_{901}$ suspension while rabbit antiserum 965 shows 'O' agglutinins exclusively.

By absorbing antiserum 954 with $O_{901}$ emulsion, a pure 'H' agglutinating serum is obtained. (According to Craigie (1931) a pure high-titred flagellar agglutinating serum cannot be obtained by the injection of flagellar suspensions; these also stimulate the production of 'O' agglutinins.)

Owing to lower 'end-titres' of human sera compared with artifically prepared immune sera, it is considered advisable to substitute human sera whose known content of 'H' and 'O' agglutinins has been determined in comparison with the control rabbit antisera. For this purpose it is convenient to use sera from an inoculated case yielding only 'H' and 'O' agglutination and 'O' agglutination exclusively.

IV. Reading of the tests.

Agglutination is of two kinds, large-flaking or floccular and small-flaking or granular. It is frequently easy to distinguish between these two types during the two-hour incubation period. The tubes are examined carefully after two hours with the aid of an agglutinoscope and for observing the small-flaking agglutination a hand lens of 10x magnification (1) is used. The type of agglutination is recorded with the readings. The tubes are allowed to stand at room temperature for 24 hours when final readings and observations are made.

In a purely 'H' agglutination comparatively large, loose, flaky floccules are developed and the supernatant fluid is always hazy. In a purely 'O' and in a mixed 'H' and 'O' agglutination where the readings are recorded 4+, 3+, or even 2+, according to the degree of agglutination, distinct clearing of the supernatant fluid is apparent in the former the granules are uniform in size and slowly settle down in the tube as a compact sediment, in the latter the granules vary in size and are denser than the floccules in a purely 'H' agglutination. These appearances are brought out in the photographs.

(1) A convenient anastigmatic magnifier 10x is supplied by Zeiss.

TABLE I.

| Suspension used for preparation of rabbit antiserum | Type of antibody contained in the serum dilutions tested | Serum dilutions | Agglutination with strain | |
|---|---|---|---|---|
| | | | $H_{901}$ | $O_{901}$ |
| B. typhosus $H_{901}$ heated for 1 hour at 60°C. Rabbit 954. | 'H' and 'O' | 1/500 ..... | ++++ 'H'+'O' | ++++ 'O' |
| | | 1/1000 ..... | ++++ " | ++++ " |
| | | 1/2000 ..... | +++ " | +++ " |
| | | 1/5000 ..... | +++ 'H' | ++ " |
| | | 1/10,000 ..... | ++ " | - |
| | | 1/20,000 ..... | - | - |
| B. typhosus $O_{901}$ heated for 1 hour at 60°C. Rabbit 965. | 'O' | 1/500 ..... | ++++ 'O' | ++++ 'O' |
| | | 1/1000 ..... | +++ " | ++++ " |
| | | 1/2000 ..... | +++ " | ++++ " |
| | | 1/5000 ..... | ++ " | +++ " |
| | | 1/10,000 ..... | + " | + " |
| | | 1/20,000 ..... | ∓ " | ∓ " |
| | | | $HB_2$ | OB |
| B. para B, $HB_2$ heated for 1 hour at 60°C. Rabbit 819. | 'H' and 'O' | 1/500 ..... | ++++ 'H'+'O' | +++ 'O' |
| | | 1/1000 ..... | ++++ " | ++ " |
| | | 1/2000 ..... | +++ " | - |
| | | 1/5000 ..... | +++ 'H' | - |
| | | 1/10,000 ..... | ++ " | - |
| | | 1/20,000 ..... | - " | - |
| B.para B, $HB_2$ heated for 2 hours at 100°C. Rabbit 821. | 'O' | 1/500 ..... | ++++ 'O' | + 'O' |
| | | 1/1000 ..... | ++ " | - |
| | | 1/2000 ..... | + " | - |
| | | 1/5000 ..... | - | - |

TABLE I - (continued)

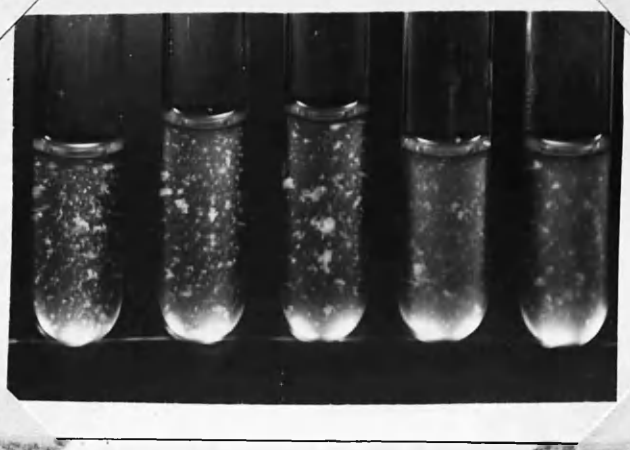
| Suspension used for preparation of rabbit antiserum | Type of antibody contained in the serum dilutions tested | Serum dilutions | Agglutination with strain | |
|---|---|---|---|---|
| | | | $HB_2$ | OB |
| * B.para B, OB heated for 2 hours at 100°C. Rabbit 942. | 'O' | 1/500 ..... | +++ 'O' | +++ 'O' |
| | | 1/1000 ..... | ++ " | ++ " |
| | | 1/2000 ..... | ++ " | + " |
| | | 1/5000 ..... | + " | - |
| | | | $HA_6$ | $HA_1$ |
| B.para A, $HA_6$ heated for 1 hour at 60°C. Rabbit 940. | 'H' and 'O' | 1/500 ..... | ++++ 'H'+'O' | ++++ 'H'+'O' |
| | | 1/1000 ..... | +++ " | ++++ " |
| | | 1/2000 ..... | ++ 'H' | +++ " |
| | | 1/5000 ..... | + " | ++ 'O' |
| | | 1/10,000 ..... | + " | ++ " |
| | | 1/20,000 ..... | - | + " |
| B.para A, $HA_1$ heated for 2 hours at 100°C. Rabbit 930. | 'O' | 1/500 ..... | - | +++ 'O' |
| | | 1/1000 ..... | - | ++ " |
| | | 1/2000 ..... | - | + " |
| | | 1/5000 ..... | - | - |

++++ or +++ denotes complete agglutination
++ denotes definite agglutination
+ denotes slight agglutination
- denotes absence of agglutination

* Inserted to show that higher titres were obtained by injecting OB suspensions than with $HB_2$ suspensions. OB suspensions are extremely toxic to the rabbit and therefore were heated to 100°C. for 2 hours.

954 Rabbit antiserum

Agglutination with $H_{901}$ suspension

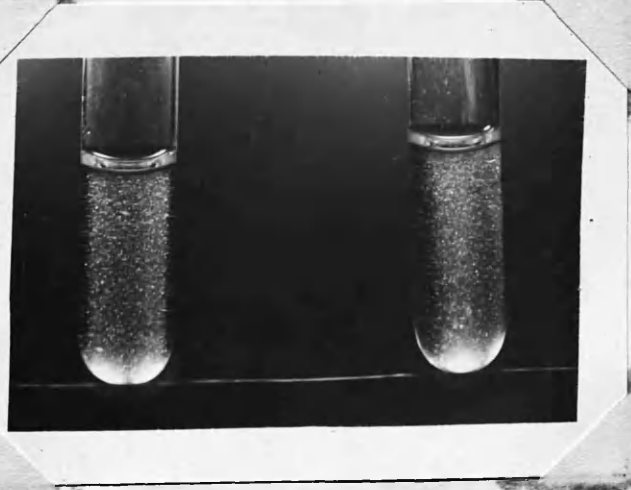


Dilutions 1:500 1:1000 1:2000 1:5000 1:10,000

Readings ++++'H+O' ++++'H+O' +++'H+O' +++'H' ++'H' after 2 hrs. at 37°C.

954 Rabbit antiserum

Agglutination with $O_{901}$ suspension



Dilutions 1:500 1:1000

Readings ++++'O' ++++'O' after 2 hrs. at 37°C.

819 Rabbit antiserum

Agglutination with $HB_2$ suspension

Dilutions 1:500 1:1000 1:2000 1:5000

Readings ++++'H+0' ++++'H+0' +++'H+0' +++'H' after 2 hrs. at 37°C.

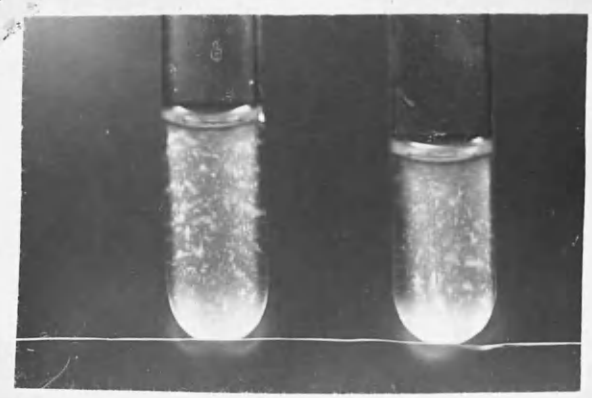
C78 Serum after Subcutaneous Vaccination

Agglutination with $H_{901}$ suspension

Dilution 1:100 +++'H' after 2 hrs. at 37°C.

864 Serum from case of Paratyphoid B fever

Agglutination with $HB_2$ suspension

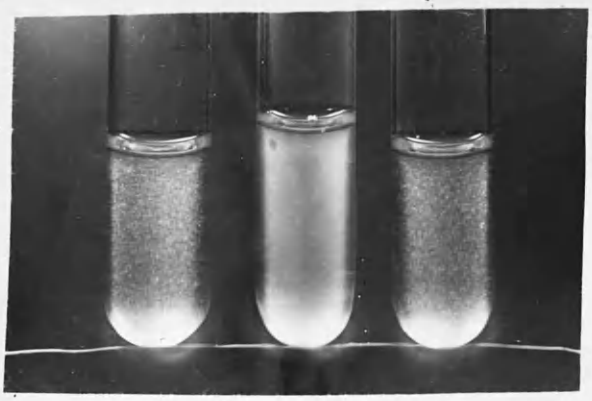


Dilutions 1:100 1:200

Readings +++'H' ++'H' after 2 hrs. at 37°C.

C22 Serum from case of Typhoid fever showing 'O' agglutination only

Agglutination with $O_{901}$ suspension



Dilutions 1:100 1:200

Readings +++'O' control ++'O' after 2 hrs. at 37°C.

## Classification of the Carriers

The typhoid carriers are classified as D, E or F and short histories are given on pp. 19-21.

Class D means a person who has had typhoid fever and the follow-up specimens show that typhoid bacilli continue to be discharged in the stools.

Class E implies a person discovered during an epidemiological study of a sporadic case of typhoid fever.

Class F is brought to light through the examination of food-handlers.

Of the 26 carriers 8 belong to class D, 17 to class E and 1 to class F. In classes E and F a record of having suffered previously from typhoid fever could not be elicited from 6 cases (72, B81, B100, 700, 708 and C16) although 2 cases (504 and 703) appear to have suffered from an illness simulating typhoid fever in early life. Four of the carriers gave a history of immunisation against typhoid fever and the majority had recent positive findings in the stools. (Cf. Table 5, p. 27)

The carriers in classes E and F are assumed to be chronic: the carriers in class D were found to be excreting typhoid bacilli a year or more after convalescence and therefore are to be regarded also as chronic.

In the case of carrier B100, suspicion fell on this patient, from her marked Widal reaction, as being responsible for the occurrence of two cases of typhoid fever in the ward of an insane institution. Four consecutive sttempts over a period of 5 weeks failed to isolate B. typhosus from the faeces by enrichment methods using both Brilliant Green (1)

---

(1) Method adopted from Chapter VI Applied Bacteriology (1918) by C.H. Browning; also outlined in Mackie and McCartney's Practical Bacteriology (1931), p. 280.

peptone water and Müller's medium [2] followed by plating on MacConkey's bile-salt lactose agar. Subsequently at the post-mortem examination, I obtained numerous colonies of B. typhosus in pure culture by direct plating of one loopful of the bile. The gall-bladder contained one fairly large and several smaller gallstones. Although there was no history of previous typhoid fever in the patient the pathological findings indicated a chronic state.

Several of the carriers in class E showed prolonged periods of apparent non-infectivity and are examples where infection was transmitted to numbers of the carrier's family, e.g. 504, 515, 552 and 700.

---

(2) Details of preparation and constituents of Müller's tetrathionate medium are given in Zentralblatt f. Bakt., Parasit. u. Infekt. 1 Abt. Orig. 1929. Bd. 112, p. 397. Experience has shown that this medium is not specific for the typhoid-paratyphoid group of organisms. Frequently non-lactose fermenting colonies on the plate turn out to be B. faecalis alkaligenes, B. proteus, B. morgan No. 1, and B. pyocyaneus i.e. the medium induces the proliferation of various non-lactose fermenters.

| Class | Number | Age | Sex | Source of Infection | Number of Positive Stool Findings |
|---|---|---|---|---|---|
| E | B45 | 71 | F | Ty. fever at age of 47 yrs. Discovered through 2 year old child under her exclusive care developing ty. fever. | Several samples positive in 1932. |
| E | B72 | 60 | M | Detected during examination of contacts from case in his family. | 2 samples positive June and July 1931. |
| D | B80 | 43 | F | Ty. fever 1929. Detected by follow-up specimens after illness. | 5 samples positive from Feb. to Nov. 1930. |
| F | B81 | 61 | F | Detected during routine examination of food-handlers. | Sample positive June 1931; sample negative Aug. 1931. |
| + E | B100 | 51 | F | Discovered Feb. 1st, 1932, through case of ty. fever in same ward. | Faeces positive; pure culture of B. typhosus isolated from gall-bladder at autopsy March 24, 1932. Died from phthisis. |
| D | 325 | 47 | F | Ty. fever, Aug. 1922; source unknown. | 22 samples positive from Jan. 1923 to Nov. 1931; 1 sample negative Oct. 1929. |
| D | 368 | 43 | M | History of ty. fever, 1922. | 22 samples positive from Dec. 1923 to Nov. 1930; 7 samples negative from Mar. 1925 to Nov. 1931. |
| D | 429 | 62 | F | History of exposure to another carrier who was a cook; onset 1924. | 66 samples positive between Jan. 1925 and May 1931; no samples negative. |
| D | 473 | 26 | F | Ty. fever, Aug. 1925. Her husband and sister are said to have suffered from ty. fever at some previous date. | 16 samples positive between Sept. 1925 and June 1930; 1 sample negative June 1931. |
| E | 504 | 68 | F | Believed to have had ty. fever at the age of 18 yrs.; discovered in 1926 when her granddaughter contracted ty. fever. | 8 samples positive between May 1926 and Feb. 1931; no samples negative. |

| Class | Number | Age | Sex | Source of Infection | Number of Positive Stool Findings |
|---|---|---|---|---|---|
| E | 515 | 38 | F | History of ty. fever in Russia during infancy; discovered in 1926 when her two daughters had ty. fever. | 11 samples positive between Oct. 1926 and Oct. 1931; 3 samples negative in Oct. and Nov. 1926. |
| E | 552 | 53 | F | History of ty. fever in 1912; found to be carrier when her son had ty. fever in 1927. Her daughter had ty. fever in 1916. | 10 samples positive between July and Oct. 1928; 3 samples negative Aug. 1929, Nov. 1930 and Nov. 1931. |
| D | 640 | 60 | M | Suffered from ty. fever July, 1929. | 7 samples positive between Sept. 1929 and Dec. 1931; 3 samples negative in Sept. 1929; 6 urine samples positive in Sept. and Oct. 1929. |
| E | 700 | 50 | F | Discovered when her grandson developed ty. fever in 1931. She lived in same household as her grandson but gave no history of ty. fever. | 3 samples positive May, 1931; no samples negative. |
| E | 703 | 71 | F | Discovered through outbreak following wedding celebration at which she helped to prepare chicken salad. She admits having had an illness lasting for 6 weeks and diagnosed as brain fever at age of 6 years. | 3 samples positive May 1931; 1 sample negative Sept. 1931. |
| E | 708 | 35 | F | Discovered when her grandson had attack of ty. fever in 1931. No history of ty. fever in carrier. | 3 samples positive June and July, 1931; no samples negative. |
| E | 713 | 27 | F | History of ty. fever in 1930; found to be a carrier when her daughter developed ty. fever in June, 1931. | 8 samples positive between July and Nov. 1931; no samples negative. |
| E | 726 | 60 | F | History of ty. fever at age of 13 years; discovered through a case in the family where this woman was a boarder. | 9 samples positive between Oct. 1931 and Jan. 1932; no samples negative. |

| Class | Number | Age | Sex | Source of Infection | Number of Positive Stool Findings |
|---|---|---|---|---|---|
| D | C 8 | 42 | F | Ty. fever, Aug. 1921; discovered to be a chronic carrier by examination of release samples. | 6 samples positive between Aug. 1929 and Nov. 1930. |
| E | C10 | 43 | F | Ty. fever at age of 20 years; discovered to be a chronic carrier in 1925 on occurrence of 6th case of ty. fever through personal contact. | Duodenal and faecal samples positive March 1932. |
| E | C11 | 90 | M | Ty. fever in Amer. Civil War, about 1866; discovered to be a carrier through 2 cases of ty. fever in same household, 1931. | 4 samples positive between May and Sept. 1931. |
| + E | C16 | 37 | F | Discovered in Feb. 1932 through case of ty. fever in same ward. | Several samples positive. |
| E | F31 | 53 | M | History of ty. fever in 1903; responsible for cases of ty. fever in 3 homes. | Faeces and urine positive July 1932. Two samples (faeces) positive, Jan. 1933. |
| E | G46 | 49 | M | Ty. fever, 1921; found responsible for 2 localised outbreaks of ty. fever in 1923 and 1931. | Several samples of faeces and urine positive in Sept. 1923 and Sept. 1931. |
| D | G47 | | M | Ty. fever during Montreal epidemic of 1928. | Several samples positive, May 1933. |
| + E | N78 | 62 | M | Ty. fever 1923; discovered through 3 cases occurring in his household. | 3 samples positive Dec. 1933 to July 1934. |

B45 ..... Case history supplied by M.O.H. for Jackson, Mississippi.
B72 to B81 .. Case histories supplied by Provincial Health Dept., Montreal
325 to 726 .. " " " " City of New York Health Dept.
C8 to C11 .. " " " " New York State Health Dept., Albany.
F31, G46 ... " " " " Maryland Health Dept., Baltimore.
G47 ..... Case history supplied by Provincial Health Dept., Toronto.
+ ..... Case histories personally investigated.

Agglutination reactions with carrier sera

(a) In table 2 the results of agglutination tests with sera from 26 typhoid carriers by Dreyer's method using formolised 'H' and 'O' suspensions from the Oxford Standards Laboratory are shown.

In 18 or 70% of the samples, the titre of 'H' agglutinins was at least 1/200 (a reduced titre of 30), in 1 case only was the titre 1/50 (a reduced titre of 7) and in 1 case 'H' agglutinins were absent.

The titre of typhoid 'O' agglutinins seldom exceeded 1/100 (R.T. 5.5), and 17 or 65% of the samples had a titre of 1/50 or 1/100 (R.T. 3.0 or 5.5).

Ashby (1931) determined the titre of 'O' agglutinins in 7 typhoid carriers who had been inoculated with T.A.B. vaccine 4 years previously. He noted that the reduced titre for 'O' agglutinins varied from 6 to 19 for the carriers as compared with a figure of 4 for similarly inoculated non-carriers.

Using 3 monovalent and 2 polyvalent formolised broth suspensions of B. typhosus, Kristensen and Poulsen (1933) found in a group of 14 typhoid carrier sera positive results, generally in dilutions from 1/50 to 1/400, although 3 of these sera reacted only in dilutions from 1/5 to 1/25. A formolised broth suspension of B. ty. $H_{901}$ was as sensitive as a polyvalent suspension comprising 22 typhoid strains. Six of these 14 sera, tested with (a) living, (b) formolised and (c) alcohol-treated [1] suspensions of B. ty. $O_{901}$, gave partly the same and partly a slightly lower titre than the formolised broth cultures of B. ty. $H_{901}$. Kristensen and Poulsen deduced from their results on a total number of 77 chronic typhoid and paratyphoid carriers that the agglutination reactions were weakest when the carrier state had lasted only from 2-5 years, although little evidence in support of this statement is produced in their paper.

---

(1) Gardner, A.D., Jl. of Hyg. 28. 376. (1929)

TABLE 2

Results of agglutination tests with carrier sera by Dreyer's Method

| Number | B.Typhosus 'H' Suspension | | | | B.Typhosus 'O' Suspension | | | | Reduced Titre 'H' | Reduced Titre 'O' |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1/25 | 1/50 | 1/100 | 1/200 | 1/25 | 1/50 | 1/100 | 1/200 | | |
| B. 45 | | | | ++++ | | ++ | | | >30 | 3 |
| B. 72 | | | | +++ | | | ++ | | 30 | 5.5 |
| B. 80 | | | ++ | | | | ++ | | 14 | 5.5 |
| B. 81 | - | | | | | ++ | | | - | 3 |
| B.100 | | | | ++ | | +++ | | | 30 | 3 |
| 325 | | | +++ | | | ++ | | | 14 | 3 |
| 368 | | | | +++ | | | ++ | | 30 | 5.5 |
| 429 | | | ++ | | | | | +++ | 14 | 11 |
| 473 | | | | ++ | | ++ | | | 30 | 3 |
| 504 | | | | ++++* | | ++ | | | >30 | 3 |
| 515 | | | | ++ | +++ | | | | 30 | 1.4 |
| 552 | | +++ | | | +++ | | | | 7 | 1.4 |
| 640 | | | | +++ | | | ++ | | 30 | 5.5 |
| 700 | | | | +++ | | | | ++ | 30 | 11 |
| 703 | | | | +++ | | ++ | | | 30 | 3 |
| 708 | | | ++ | | ++ | | | | 14 | 1.4 |
| 713 | | | | ++++* | | ++++ | | | >30 | 3 |
| 726 | | | | ++ | | +++ | | | 30 | 3 |
| C. 8 | | | | ++ | | | | ++ | 30 | 11 |
| C. 10 | | | +++ | | | | +++ | | 14 | 5.5 |
| C. 11 | | | | ++ | | | ++ | | 30 | 5.5 |
| C. 16 | | | ++ | | ++ | | | | 14 | 1.4 |
| F. 31 | | | | ++ | | +++ | | | 25 | 4.5 |
| G. 46 | | | | ++ | - | | | | 25 | |
| G. 47 | | | | ++++* | | ++ | | | >25 | 4.5 |
| N. 78 | | | | ++++* | - | | | | >25 | |

* End-titres for 'H' agglutinins are not given in these cases.

From the information given in the classification of the carriers on pp. 19-21, table 3 has been compiled to show the number of carriers in the different age periods and corresponding to their end-titres. Persistence of the carrier state is taken to mean the number of years which has elapsed from the occurrence of typhoid fever. All the carriers comprising the 'unknown' group exceeded a duration of 2 years when the serological tests were performed.

Table 3.

| Persistence of carrier state in years | End-titres | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ty H | | | | | Ty O | | | | | Totals |
| | <1/25 | 1/50 | 1/100 | 1/200 | >1/200 | <1/25 | 1/25 | 1/50 | 1/100 | 1/200 | |
| 2-5 | | | 1 | 1 | 2 | | | 2 | 2 | | 4 |
| 5-10 | | | 1 | 1 | 1 | 1 | | 1 | | 1 | 3 |
| 10-20 | | | 1 | 3 | | 1 | | 1 | 1 | 1 | 4 |
| 20-30 | | 1 | 1 | 3 | | | 1 | 2 | 2 | | 5 |
| over 30 | | | | 1 | 1 | | | 2 | | | 2 |
| unknown | 1 | | 2 | 5 | | | 3 | 3 | 1 | 1 | 8 |
| Totals | 1 | 1 | 6 | 14 | 4 | 2 | 4 | 11 | 6 | 3 | 26 |
| | | | | 24 | | | | | 20 | | |

Although table 3 fails to confirm the view of Kristensen and Poulsen it certainly shows that the majority of the sera, 24 out of 26, in the present series, react in at least 1/100 dilution (R.T. 14) with the ty 'H' suspension while with the ty 'O' suspension 20 react in 1/50 dilution (R.T. 3) or greater.

Professor Browning (1933) gives a detailed record of 5 faecal excreters of B. typhosus who were critically studied during a period of 2 or more years. The sera of 3 of these carriers were repeatedly examined and positive agglutination reactions were always obtained with the homologous organism. Although there was a wide range in the reduced titres for B. typhosus i.e. in the number of agglutinin units per c.c. of serum -- for carrier K.O. 9 to 21, for H.T. 9 to 32 and for J.M. 12 to 59 -- a R.T. of 9 occurred <u>once only</u> in carriers K.O. and H.T. The serum of carrier M.M. was tested on three

different occasions and yielded R.T. values of 9, 10 and 5 respectively, while that of carrier R was examined once and gave a R.T. of 9.

Although all the carriers of the present series were faecal excreters of B. typhosus it is interesting to refer to the serological results in urinary excreters which have been noted by different observers. Küster and Günzler (1916) express the view that as a general rule, the Widal reaction in the chronic urinary carrier attains no higher titre than in the bacillary carrier. Moreover typhoid agglutinins are not always present in proved cases of typhoid bacillary excreters, for among 50 patients taken at random in a Military (Garrison) Hospital, they found 6 faecal excreters and 14 urinary excreters without typhoid agglutinins in the blood. Professor Browning (1933) mentions the case of a boy, aged 13 years, who was found to be a urinary excreter of B. typhosus after his discharge from hospital, when 5 members of his family fell ill with typhoid fever. The blood-serum taken approximately 4 months after the onset of his illness failed to agglutinate 4 different suspensions of B. typhosus. On the other hand Bumke (1925) has found that urinary excreters have a remarkably higher titre than faecal excreters. In his table (Tabelle No. 15 s. 488) showing the serological results of 72 serum samples from urinary carriers and classified according to the number of months elapsing since the onset of illness, over 50% of the typhoid and paratyphoid B carriers have a titre exceeding 1:1000 -- 13 out of 24 exceeded a titre of 1:1000 for B. typhosus and 13 out of 25 for B. paratyphosus B. Kristensen and Poulsen (1933) noted 3 male, purely urinary excreters of B. typhosus in their series of 77 carriers: one had been a carrier for 6 years and had a titre of 1:400, another for 7 years with a titre of 1:200, and the third for at least 3 years with a titre of 1:1600.

## Co-agglutination

Professor Browning (1933) has called attention to the occurrence of co-agglutination in the sera of carriers. He employed the Oxford formolised suspensions of B. typhosus and B. paratyphosus A and B according to Dreyer's procedure and recorded the results in agglutinin units, without differentiating between 'H' and 'O' agglutination. His results show that, in general, the titre for the homologous organism is higher than that for the heterologous organism e.g., carriers M.D., B.E., and M.M. In the case of the typhoid carrier K.O., ten serum samples were examined and gave lower reduced titres, with one exception, for B. paratyphosus B than for B. typhosus.

In some 'carrier' sera of the present series co-agglutination was found to occur only between B. typhosus 'O' and B. aertrycke (B. para B) 'O' suspensions. In Table 4 , the titres of four typhoid carriers are grouped together to show this type of co-agglutination. The reduced titre for the homologous somatic agglutinins is not always greater than that for the heterologous somatic agglutinins.

In other 'carrier' sera agglutination was found to occur with the flagellated suspensions of B. typhosus and B. paratyphosus B but in these cases the carriers were known to have been given three injections of T.A.B. vaccine at some time prior to examination of the blood. Table 5 shows the results obtained in vaccinated carriers.

Table 4

showing co-agglutination between the somatic suspensions of B. typhosus and B. aertrycke (B. para B) in four typhoid 'carrier' sera.

| Serum Number | End-Titres | | | Reduced Titres | | |
|---|---|---|---|---|---|---|
| | Ty'H' | Ty'O' | Aer'O' | Ty'H' | Ty'O' | Aer'O' |
| B45 | 1:200(4) | 1:50 (2) | 1:25(2) | 28.6 | 2.8 | 4.2 |
| C 8 | 1:200(2) | 1:200(2) | 1:25(2) | 28.6 | 11.1 | 4.2 |
| C10 | 1:100(2) | 1:100(3) | 1:50(2) | 14.3 | 5.5 | 8.3 |
| C11 | 1:200(2) | 1:100(2) | 1:25(3) | 28.6 | 5.5 | 4.2 |

Table 5

showing agglutination results of certain sera from inoculated typhoid carriers.

Date of test: Jan. 1932.

| Serum Number | End-Titres | | | Reduced Titres | | |
|---|---|---|---|---|---|---|
| | Ty'H' | Ty'O' | PB'H' | Ty'H' | Ty'O' | PB'H' |
| 515 (i) | 1:200(2) | 1:25 (3) | 1:50 (2) | 30 | 1.4 | 10 |
| 700 (ii) | 1:200(3) | 1:200(2) | 1:100(3) | 30 | 11 | 20 |
| 703 (iii) | 1:200(3) | 1:50 (2) | 1:25 (2) | 30 | 3 | 5 |
| 713 (ii) | >1:200(4) | 1:50 (4) | 1:50 (3) | >30 | 3 | 10 |

(i) received 3 injections of T.A.B. vaccine in 1928.
(ii) " " " " " " " 1930.
(iii) " " " " " " " 1931.

(b) In table 6 are shown the results of agglutination tests with 22 (1) sera from typhoid carriers in dilutions of 1:100 and 1:200 using living suspensions of B. typhosus strains $H_{901}$ and $O_{901}$, B. paratyphosus B, strains $HB_2$ and OB, and B. paratyphosus A, strains $HA_6$ and $HA_1$, as employed in the qualitative receptor analysis method of Felix.

The agglutination reactions with $H_{901}$ and $O_{901}$ suspensions are stronger and in higher dilution than those obtained with $HB_2$, OB, $HA_6$ and $HA_1$ suspensions. A marked feature is the occurrence in the majority of the sera of 'O' agglutination both with the $H_{901}$ as well as with the $O_{901}$ suspension. In fact the $H_{901}$ suspension appears in some cases to be a more sensitive agent than the $H_{901}$ suspension for detecting 'O' agglutination. In 13 or 60% of the carriers examined the serum reacted in the higher dilution with the $H_{901}$ suspension and in 11 or 50% of the cases with the $O_{901}$ suspension; in 2 cases the $O_{901}$ suspension failed to react in 1:100 dilution (325 and 504).

In two sera however, 'H' + 'O' agglutination was observed with the $H_{901}$ suspension (B. 45 and 713). These results were confirmed by repeated tests on each serum after the lapse of considerable intervals; in some cases the sera were stored in the ice-chest for several months. Considering the lability of 'O' agglutinins in stored sera, the persistence of 'O' agglutinins in the carrier sera is remarkable.

---

(1) The quantity of serum submitted from carriers F31, G46, G47 and N78 (table 2) was insufficient for the qualitative receptor analysis method.

TABLE 6

Results of agglutination tests with carrier sera by the Qualitative Receptor Analysis Method of Felix

| Number | $H_{901}$ | | $O_{901}$ | | $HB_2$ | | OB | | $HA_6$ | | $HA_1$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/100 | 1/200 | 1/100 | 1/200 | 1/100 | 1/200 | 1/100 | 1/200 | 1/100 | 1/200 | 1/100 | 1/200 |
| B. 45 | | ++'H+O' | | ++ | - | | - | | ++'O' | | ++'O' | |
| B. 72 | | ++'O' | +++ | | - | | - | | - | | - | |
| B. 80 | ++'O' | | +++ | | - | | - | | - | | - | |
| B. 81 | ++'O' | | ++ | | - | | - | | - | | - | |
| B.100 | ++'O' | | + | | - | | - | | - | | +'O' | |
| 325 | +'O' | | - | | - | | - | | - | | - | |
| 368 | | ++'O' | | ++ | - | | - | | ++'O' | | ++'O' | |
| 429 | | +++'O' | | +++ | - | | - | | +'O' | | +'O' | |
| 473 | | ++'O' | | ++ | ++ | | - | | - | | - | |
| 504 | | +++'O' | - | | - | | - | | - | | - | |
| 515 | | ++'O' | | ++ | - | | - | | ++'O' | | ++'O' | |
| 552 | ++'O' | | | ++ | +'O' | | - | | - | | +'O' | |
| 640 | | +++'O' | | ++ | - | | - | | - | | - | |
| 700 | | +++'O' | ++ | | ++'O' | | - | | - | | +'O' | |
| 703 | | ++'O' | + | | ++'O' | | - | | - | | +'O' | |
| 708 | ++'O' | | | ++ | - | | - | | - | | - | |
| 713 | | +++'H+O' | | ++ | ++'O' | | - | | - | | - | |
| 726 | | ++'O' | | ++ | - | | - | | ++'O' | | ++'O' | |
| C. 8 | +'O' | | ++ | | +'O' | | - | | - | | - | |
| C. 10 | ++'O' | | +++ | | ++'O' | | - | | - | | - | |
| C. 11 | | +++'O' | | +++ | | ++'O' | ++ | | ++'O' | | | +++'O' |
| C. 16 | ++'O' | | ++ | | - | | - | | - | | - | |

Pijper (1930) examined the sera from 7 typhoid carriers, of whom 5 excreted B. typhosus in the urine. He noted the occurrence of 'O' agglutination exclusively in 1:100 serum dilutions using different strains of B. typhosus as (a) living, (b) killed (by heat) and phenolised suspensions and (c) the alcoholised suspension of the Oxford Standards Laboratory. The superior sensitiveness of a living suspension of B. typhosus $H_{901}$ to 'O' agglutinins and the inhibitory action of phenol and alcohol on their appearance are indicated in Table V of his paper.

In my series of carriers, some sera examined in 1/25 and 1/50 dilutions by Felix' method showed the presence of low titre 'H' agglutinins, but in the higher dilutions of 1:100 and 1:200 the intensity of the 'O' reaction had completely masked the 'H' reaction. Table 7 shows 5 sera with low titre 'H' agglutinins from 1/25 to 1:50 and 5 sera with 'O' agglutinins exclusively in dilutions from 1/25 to 1/200. These sera, although stored in the ice-chest and maintained sterile for several months, tended to show reduction of the 'O' agglutinins which are labile to storing; for in subsequent tests at varying intervals the appearance of 'H' agglutination was more evident owing to the elimination of the disturbing effect of the 'O' reaction.

It was observed that 'H' agglutination with strain $H_{901}$ using typhoid sera was less typical than that with $HB_2$ using paratyphoid B sera. This is due, in part at least, to the high sensitiveness of strain $H_{901}$ to 'O' agglutinins, so that 'O' agglutination occurring simultaneously with 'H' agglutination has the effect of masking the latter reaction. If a serum contains both 'O' and 'H' agglutinins for B. typhosus the latter are indicated typically only when the 'H' titre is higher than the 'O' titre.

TABLE 7

Results of several carrier sera

in dilutions 1/25, 1/50, 1/100, 1/200.

| Serum Number | H 901 | | | |
|---|---|---|---|---|
| | 1/25 | 1/50 | 1/100 | 1/200 |
| B72 | | +++ 'H & O' | ++++ 'O' | ++ 'O' |
| B100 | +++ 'H & O' | +++ 'O' | ++ 'O' | ± 'O' |
| 504 | | ++ 'H & O' | +++ 'O' | +++ 'O' |
| 552 | +++ 'H & O' | +++ 'O' | ++ 'O' | - |
| C16 | ++ 'H' | ++ 'O' | ++ 'O' | - |
| 368 | | ++++ 'O' | +++ 'O' | ++ 'O' |
| 429 | | ++++ 'O' | ++++ 'O' | +++ 'O' |
| 515 | | ++ 'O' | +++ 'O' | ++ 'O' |
| C10 | | +++ 'O' | ++ 'O' | |
| C11 | ++++ 'O' | ++++ 'O' | ++++ 'O' | +++ 'O' |

The occurrence of 'H' and 'O' agglutination simultaneously with the $HB_2$ suspension in the serum from a paratyphoid B carrier tended to confirm this observation. The reactions are given below:-

| Bacillary Suspension | $H_{901}$ | | $O_{901}$ | | $HB_2$ | | OB | | $HA_6$ | | $HA_1$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum Dilution | 1:100 | 1:200 | 1:100 | 1:200 | 1:100 | 1:200 | 1:100 | 1:200 | 1:100 | 1:200 | 1:100 | 1:200 |
| Results | ++'O' | ++'O' | + | + | +++'H&O' | ++'H&O' | + | + | - | - | +'O' | +'O |

Comparing Tables 2 and 6 it is deduced that (1) a formolised suspension of B. typhosus is an extremely sensitive agent for the demonstration of 'H' agglutinins, and much superior to that of the living strain $H_{901}$, (2) the formolised suspension of B. typhosus as prepared by Gardner's method is a relatively poor agent for detecting 'O' agglutinins and (3) living suspensions of B. typhosus $H_{901}$ and $O_{901}$ constitute very sensitive agents for detecting 'O' agglutinins.

Consequently Felix (1931) approves of formolised 'H' suspensions to replace the living H variants but still recommends the living O variants ($O_{901}$ and OB) for use in a well equipped laboratory, and to test them periodically as outlined in Table 1.

## Diagnosis of a typhoid carrier by the Qualitative Receptor Analysis Method.

In considering the diagnosis of a typhoid carrier from the reactions obtained by Felix' method it is necessary to consider in addition the types of reaction in sera from cases of typhoid fever (Table 8) and from prophylactic inoculation (Table 9).

In a typical case of typhoid fever the serum shows generally well-marked 'H' agglutination with strain $H_{901}$ and 'O' agglutination with strain $O_{901}$ in dilutions of 1:100 and 1:200. In some cases both 'H' and 'O' agglutination are observed simultaneously, e.g. in sera B95, C23, C24, etc. where 'H + O' agglutination occurs with strain $H_{901}$. 'H' agglutination with strain $H_{901}$ and absence of 'O' agglutination may be found as in serum 227 and 'O' agglutination with strain $O_{901}$ and absence of 'H' agglutination may be met with as in sera C22, C26, C28, C40 and 155. In such cases, e.g. sera 227 and C22, symptoms definitely suggestive of enteric infection are usually elicited. The specific organism, B. typhosus, was isolated from the blood or excreta or from both sources in all the cases whose serological reactions are given in Table 8 except numbers 227 and 252.

TABLE 8

Results of agglutination tests with typhoid sera using the Qualitative Receptor Analysis Method of Felix

| Serum Number | Day of Disease | $H_{901}$ | | $O_{901}$ | | $HB_2$ | | OB | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1/100 | 1/200 | 1/100 | 1/200 | 1/100 | 1/200 | 1/100 | 1/200 |
| B92 | 24 days | +++'H' | ++'H' | ++'O' | +'O' | ++'O' | ++'O' | - | - |
| B95 | 33 days | +++'H+O' | ++'H+O' | ++'O' | ++'O' | +'O' | +'O' | - | - |
| B98 | 40 days | +++'H' | ++'H' | +'O' | ±'O' | +++'O' | +++'O' | ++'O' | - |
| B99 | 47 days | ++'H' | ++'H' | +'O' | ±'O' | +++'O' | ++'O' | +'O' | - |
| C 3 | 54 days | +++'H' | ++'H' | +'O' | - | +++'O' | ++'O' | ±'O' | - |
| C14 | 104 days | ++'H' | ++'H' | +'O' | - | ++'O' | ±'O' | - | - |
| C22 | 16 days | ++++'O' | ++++'O' | ++++'O' | ++++'O' | ++++'O' | +++'O' | ++ | + |
| C23 | 23 days | ++++'H+O' | ++++'H+O' | ++++'O' | ++++'O' | | | | |
| C24 | 31 days | ++++'H+O' | +++'H+O' | ++++'O' | ++++'O' | +++'O' | ++'O' | +'O' | - |
| C25 | 37 days | ++++'H+O' | +++'H+O' | ++++'O' | +++'O' | +++'O' | ++'O' | ∓'O' | - |
| C26 | 44 days | ++++'O' | +++'O' | ++++'O' | ++++'O' | ++'O' | ++'O' | - | - |
| C27 | 30 days | +++'H+O' | +++'H+O' | +++'O' | ++'O' | | | | |
| C28 | 52 days | ++++'O' | +++'O' | ++++'O' | +++'O' | ++'O' | +'O' | - | - |
| C29 | 37 days | ++++'O' | ++++'H+O' | ++++'O' | +++'O' | +++'O' | +'O' | - | - |
| C40 | 19 days | ++++'O' | +++'O' | ++++'O' | ++++'O' | +++'O' | ++'O' | ++++'O' | ++'O' |
| C42 | 26 days | ++++'H+O' | ++++'H+O' | ++++'O' | ++++'O' | ++++'O' | +++'O' | ++++'O' | ++++'O' |
| C44 | 32 days | ++++'H' | +++'H' | ++++'O' | ++++'O' | +++'O' | +++'O' | ++++'O' | +++'O' |
| D20 | 44 days | ++++'H+O' | +++'H+O' | ++++'O' | +++'O' | +++'O' | +++'O' | +++'O' | ++'O' |
| 155 | 11 days | +++'O' | +++'O' | +++'O' | +++'O' | +++'O' | ++'O' | ++'O' | - |
| 168 | 15 days | ++++'H+O' | +++'H+O' | ++++'O' | ++++'O' | - | - | - | - |
| 178 | 19 days | ++++'H' | +++'H' | ++++'O' | ++++'O' | ∓'O' | - | - | - |
| 198 | 32 days | ++++'H' | +++'H' | +++'O' | +++'O' | +++'O' | +++'O' | ++'O' | - |
| 227 | —— | ++'H' | ++'H' | - | - | - | - | - | - |
| 251 | 11 days | +++'O' | ++'O' | ++++'O' | ++++'O' | ±'O' | - | +'O' | - |
| 252 | 6 weeks | +++'H+O' | +++'H+O' | ++++'O' | +++'O' | +++'O' | +++'O' | ++++'O' | ++++'O' |
| 283 | 7 days | +++'H' | +++'H' | ++++'O' | +++'O' | ±'O' | - | - | - |

Date of tests: July, August, September, 1932.

In a person giving a history of prophylactic inoculation with triple (T.A.B.) vaccine, the serum shows 'H' agglutination with little evidence of 'O' agglutination by Felix' method as indicated in Table 9. These results were obtained in January and August 1932, by the examination of 21 serum samples from probationer nurses to whom T.A.B. vaccine had been administered. It is apparent that the development of 'O' agglutinins is very poor for in 5 cases only was a single or double plus reading in 1:100 dilution obtained 1 to 4 weeks after the third injection. The vaccine* used was supplied by the Department of Health for Ontario and contained in each cubic centimetre 1000 million typhoid bacilli (strain Rawlings), 500 million paratyphoid A bacilli (strain Kessel) and 500 million paratyphoid B bacilli (strain Rowlands), preserved with 0.5 per cent. phenol. The results recorded in Table 9 are in agreement with the view expressed by Felix (1924) and confirmed by Stuart and Krikorian (1928), viz. that 'H' agglutinins alone occur as the result of typhoid or typhoid-paratyphoid inoculation, whereas 'H' and 'O' agglutinins are generally produced in the sera of enterica patients. Recently, Stuart and Krikorian (1934) have put forward an explanation of the discrepant results of various workers - Gardner (1929, 1930), Whitehead (1930), Smith (1932), Wyllie (1932), Horgan (1932), Mudd (1932) and Dulaney, Wikle and Trigg (1932) - all of whom have noted the occurrence, by different techniques, of 'O' agglutinins in human sera as a result of typhoid or T.A.B. inoculation. Stuart and Krikorian have now shown that variations in the method of vaccine production exert an influence on its agglutinin-producing power. They consider that phenol, so frequently

---

* 3 doses 0.25 c.c., 0.5 c.c. and 1 c.c. were injected subcutaneously at weekly intervals.

TABLE 9

Results of agglutination tests with sera from individuals receiving subcutaneous injections of T.A.B. vaccine.

| Serum Number | Period after subcutaneous vaccination | $H_{901}$ | | $O_{901}$ | | $HB_2$ | | OB | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1/100 | 1/200 | 1/100 | 1/200 | 1/100 | 1/200 | 1/100 | 1/200 |
| B59 | 1 week after 2nd injection | ++'H' | ++'H' | - | - | +'H' | ±'H' | - | - |
| B60 | 1 week after 2nd injection | ++'H' | ++'H' | - | - | ++'H' | ++'H' | - | - |
| B61 | 1 week after 2nd injection | ++'H' | ++'H' | - | - | ++'H' | ++'H' | - | - |
| B63 | 1 week after 2nd injection | +++'H' | ++'H' | - | - | +'H' | - | - | - |
| B64 | 1 week after 2nd injection | +++'H' | ++'H' | + | - | ++'H' | ++'H' | + | - |
| B65 | 1 week after 3rd injection | ++'H' | +'H' | - | - | +'H' | ∓'H' | - | - |
| B66 | 1 week after 3rd injection | ++'H' | ++'H' | - | - | ++'H' | +'H' | - | - |
| B67 | 1 week after 3rd injection | ++'H' | ++'H' | - | - | ++'H' | ++'H' | - | - |
| B68 | 1 week after 3rd injection | +'H' | ±'H' | - | - | ++'H' | +'H' | - | - |
| B69 | 1 week after 3rd injection | +++'H' | ++'H' | - | - | +'H' | - | - | - |
| B70 | 1 week after 3rd injection | ++'H' | ++'H' | + | - | ++'H' | ++'H' | + | - |
| C74 | 1 week after 3rd injection | +++'H' | ++'H' | + | - | +++'H' | +++'H' | - | - |
| C76 | 1 week after 3rd injection | +++'H' | ++'H' | - | - | +++'H' | ++'H' | - | - |
| C77 | 1 week after 3rd injection | +'H' | - | + | - | ++'H' | +'H' | - | - |
| C78 | 1 week after 3rd injection | +++'H' | ++'H' | - | - | +++'H' | ++'H' | - | - |
| C79 | 1 week after 3rd injection | ++'H' | +'H' | ++ | - | ++'H' | +'H' | - | - |
| B75 | 4 weeks after 3rd injection | ++'H' | ++'H' | - | - | ++'H' | ++'H' | - | - |
| B77 | 4 weeks after 3rd injection | ++'H' | ++'H' | - | - | +'H' | +'H' | - | - |
| B78 | 4 weeks after 3rd injection | +++'H' | ++'H' | + | - | ++'H' | +'H' | + | - |
| B84 | 7 weeks after 3rd injection | ++'H' | +'H' | - | - | +'H' | +'H' | - | - |
| B85 | 7 weeks after 3rd injection | ++'H' | ++'H' | - | - | ++'H' | ++'H' | - | - |

Dates of tests: B59 - B70 and B75 - B85, June 1932; C74 - C79, August 1932.

used as a preservative in bacterial vaccines, has an adverse effect on the somatic antigen. Seven normal persons, whose blood sera contained at first no detectable agglutinin were given 2 doses of a vaccine freshly prepared from a Rawlings' strain of B. typhosus and killed by heating in a water bath at 60°C., while to seven similar persons were administered 2 doses of a vaccine prepared at the same time but containing 1 per cent. phenol in addition. The serological results show that (i) 'O' titres may be found 15 days after the second inoculation of the order 1:100 to 1:200 with phenolised vaccine but of the order 1:200 to 1:2000 with non-phenolised vaccine; (ii) while 'O' titres of 1:100 may persist for at least 3 months after the phenolised vaccine, with the non-phenolised vaccine the 'O' titres vary between 1:200 and 1:1000.

It is now realised that the stock vaccine* used in 1932 as supplied by the Ontario Department of Health was deficient in its power to stimulate the formation of somatic agglutinins. With the newer knowledge of the bearing of bacterial variation on vaccine production, it seems possible to prepare a vaccine capable of stimulating somatic agglutinins to as high a degree as may occur in the course of enteric disease. The deleterious effect of 0.5 per cent. phenol on the somatic antigens of a vaccine stored for 3-6 months before issue would, according to Stuart and Krikorian, explain the inconstant and low titres of 'O' agglutinins in the sera of persons after subcutaneous inoculation.

The large majority of the carrier sera examined (Table 6 ) show definite and pronounced 'O' agglutination in titres of 1:100 and 1:200. 'H' agglutinins may be present; if so, they are generally small in amount and their presence is masked by the 'O' agglutinins.

---

* In Part I, pp. 21-24 a fuller statement appears on the preparation of efficient vaccines.

1. In any agglutination method using living bacterial suspensions the pitfalls are due to the S → R variation and to the H → O variation. The former variation is easily detected by the appearance of spontaneous agglutination (granular in type) in increasing strengths of saline controls. A slight granularity in 0.85 per cent. saline is more marked in 1.7 per cent. and more so in 3.4 per cent. and 6.8 per cent. saline. When this occurs the stock culture is replated on agar and a smooth colony picked off. In this way the colony form is taken as an indication of smoothness or roughness and this is afterwards examined for physiological smoothness or roughness.

The latter variation (i.e., H → O) is guarded against by subculturing the H variants on agar slants containing some water of condensation . O variants may be subcultured on dry agar slopes. The agar medium must be carefully prepared as outlined on pages 4 and 5 and the use of motility tubes containing semi-solid agar ensures maximum development of the H-receptors. Felix (1931) recommends however that agar stabs for maintaining stock cultures over long periods should not contain muscle sugar, hence this agar should be prepared from meat extracts instead of fresh meat.

2. In this study of the serological reactions of chronic typhoid carriers by Felix' method, the sera were examined qualitatively with living bacillary suspensions of 'H' and 'O' strains of B. typhosus and B. paratyphosus A and B. In the large majority of these sera 'O' agglutinins were found exclusively. The typhoid 'H' suspension of the Oxford Standards Laboratory, however, revealed the presence of "H" agglutinins in nearly all of these sera.

3. The use of living typhoid and paratyphoid strains necessitated particular care to maintain the cultures in the optimum phase. The results by Felix' method show that sufficient differences in the type and degree of agglutination in the three classes of sera studied -- from chronic typhoid carriers, from typhoid fever cases and from individuals receiving prophylactic inoculation, justify the opinion that an additional serological procedure is available to assist in distinguishing carriers from normal and from inoculated individuals.

## REFERENCES

Ashby, W.R. (1931). J. Path. and Bact., 34, 109.

Browning, C.H. (1918). Applied Bacteriology, p. 44.

" " (1933). Chronic Enteric Carriers and their Treatment. M.R.C. Sp. Rep. Ser. 179.

Bumke, E. (1925). Virchows Arch. 256. 487.

Craigie, J. (1931). Studies on the Serological Reactions of the Flagella of B. typhosus. J. Immunology, 21. 426.

Felix, A. (1924). The Qualitative Receptor Analysis in its Application to Typhoid Fever. J. Immunology, 9. 115.

" " (1930). The Qualitative Serum Diagnosis of Enteric Fevers. Lancet, 1. 505.

" " (1931). Personal communication, 17. 10. 31.

Gardner, A.D. (1929). The Small-flaking or "O" Agglutination of Permanent Standardised "O" Suspensions of B. typhosus by the Serums of Normal, Inoculated and Infected Persons. J. Hyg. 28. 376.

Gay, F.P. (1918). Typhoid Fever, p. 129.

Hewlett and McIntosh. (1932). Manual of Bacteriology, 9th Edition, p. 367.

Kristensen, M. and Poulsen, K.A. (1933). Die Widalreaktion bei chronischen Typhus- und Paratyphusbazillenträgern. Zentralbl. f. Bäkt., Parasit. u. Infekt. Orig. 128. 450.

Küster, E. und Günzler, H. (1916). Zur Behandlung von Typhusbazillen Ausscheidern. Ztschr. f. Hyg. u. Infektkr. 81. 447.

Ledingham and Arkwright. (1912). The Carrier Problem in Infectious Diseases, p. 101.

Mudd, S. (1932). The Agglutinin Response to Typhoid-Paratyphoid Vaccination. J. Immunology, 23, 81.

Muir and Ritchie. (1932). Manual of Bacteriology, 9th Edition, p. 444.

Nichols, H.J. (1922). Carriers in Infectious Diseases, p. 50.

Paul, K.C. (1926). The Carrier Problem, p. 13.

Pijper, A. (1930). Serological Diagnosis of the Enterica. J. Hyg. 29. 380.

Wyllie, J. (1932). Experiences with Small-flaking or Granular Agglutination in Normal, Inoculated and Enteric Fever Cases. J. Hyg. 32. 376.

PART III

# THE ZONE PHENOMENON IN AGGLUTINATION REACTIONS.

Paper Incorporated

Wyllie, J. Transactions of the Royal Society of Canada

Third Series, Section V, Vol. XXVII, 1933.

CONTENTS

## A. INTRODUCTION

It was noted by Neisser and Wechsberg (1901) that an immune body may in the higher dilutions exert its effect with marked activity but exhibit an inhibition in the lower dilutions. A series of graded dilutions are usually made in laboratory tests and the phenomenon of inhibition — commonly referred to as the pre- or pro-zone phenomenon -- appears in the first tubes of the range. This phenomenon is not uncommon in agglutination and precipitation tests and may be made to appear in haemolytic titrations also.

The occurrence of the pre-zone phenomenon in agglutination tests with suspensions of B. typhosus has been observed by Falta and Noeggerath (1929) and many other workers. In agglutination tests for infection due to Br. abortus, the pre-zone phenomenon was observed by Keefer (1924) in the single case he described, by Sensenich and Giordano (1928) in one case out of seven cases recorded, by Kristensen (1928) who frequently noted inhibition of agglutination in the greater serum concentrations (1:25, 1:50 and 1:100) of the agglutination series, by Kling (1929) who records a number of sera exhibiting the pre-zone phenomenon, the extent of which varied from partial to complete inhibition in dilutions of 1:40 to 1:320 and by others. This paradoxical reaction is usually explained as being due to an excess of either the antigen or the antibody. Thus the experimental work of different observers may be divided into two groups (a) where the cause has been attributed to the serum and (b) where the nature of the reaction has been sought in the antigen.

Krumwiede, Cooper and Provost (1925) point out that in the process of absorbing the specific antibodies from an antiserum a pre-zone may be introduced, or, if already present, exaggerated. They express the opinion that this is due mainly to products of autolysis and partly to medium constituents.

Shibley (1929) worked with sera in which inhibition zones were induced by heating to temperatures of 62° to 76°C. for short periods varying from 6 to 10 minutes, and concluded that the pre-zone phenomenon depends on the presence of altered agglutinin or agglutinoid which has a greater affinity for the antigen than the agglutinins.

In the examination of cattle sera for the presence of Br. abortus infection, Detre (1927) found three sera showing mid-zones of inhibition, the first and later tubes of the series showing marked agglutination. He employed a drop method using a micro-pipette and incubated the test-drops in a petri-plate at 37°C., reading the results usually within 30 minutes with the aid of a magnifying lens. He found that by heating a mid-zone serum to 53°C. for 30 minutes or by adding a very small amount of bovine serum nearly complete destruction of the inhibition zone occurred, i.e., agglutination within the zone resulted. He rejects the "agglutinoid" theory and considers that an inhibitory body is the important factor.

Spencer (1930) has found in a mid-zone serum that when the density of the antigen suspension is increased the position of the zone moves towards the lower serum dilutions. By absorption tests he has shown that the agglutinins are active within the zone.

Craigie (1932) noted the inhibitory quality of fresh immune rabbit serum when agglutination tests with suspensions of the elementary bodies of vaccinia were attempted at a temperature of 50 - 55°C. The inhibitory quality was eliminated by previously heating the immune serum to 56°C. for 45 minutes.

In the course of agglutination tests for the detection of 'H' and 'O' agglutinins in sera from suspected cases of enteric fever the pre-zone phenomenon was not infrequently encountered. It was noted that the phenomenon occurred only with non-flagellated (somatic) suspensions - B. typhosus 'O' suspension and B. aertrycke 'O' suspension, although the agglutination tubes were read finally after 22 hours in the water-bath at 50-55°C. This technique was used so that the zones of inhibition might be largely overcome and the slow granular agglutination of the somatic suspensions brought to an end-point. Among a very considerable number of sera examined, the pre-zone phenomenon was not observed with the use of flagellated suspensions of B. typhosus and B. paratyphosus B ('H' suspensions). In some sera showing haemolysis there was an indication of slight inhibition in the earlier tubes but such sera have been excluded from this study as being unsuitable. Table 1 has been compiled from records of serological results over a period of two years, the tests being carried out routinely. It is evident that (i) when compared with the agglutination reactions obtained with 'H' suspensions of B. typhosus and B. paratyphosus B, 'O' suspensions of B. typhosus and B. aertrycke showed reactions which were maximal in dilutions of 1:50, 1:100 and 1:200 but less or minimal in dilutions of 1:25; 1:50 or 1:100 i.e. zonal reactions occurred only when somatic suspensions were employed and (ii) in some cases the zonal reaction tends to recur in succeeding samples of blood-serum from the same person. (Cf. Stencill, Maracle, Hewitt in Table I; in these cases samples were taken at weekly intervals).

TABLE I.

Showing pre-zones with somatic ('O') suspensions.

| Serum No. | B. typhosus 'H' suspension | | | | | | | | | B. typhosus 'O' suspension | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 | 1/2500 | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 |
| 1013 | ++++ | ++++ | ++++ | ++++ | +++ | ++ | - | | | + | + | ++ | ∓ | - | | |
| 1105 | ++++ | ++++ | ++++ | ++++ | +++ | +++ | ++ | + | - | + | + | ++ | ∓ | - | | |
| 1120 | ++++ | ++++ | ++++ | ++++ | +++ | ++ | ∓ | - | | + | + | ++ | ++ | - | | |
| 1152 | ++++ | ++++ | +++ | ∓ | - | | | | | ± | ++ | ++ | + | - | | |
| 1187 | ++++ | ++++ | +++ | ++ | - | | | | | + | + | ++ | + | - | | |
| 1411 | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | ++ | - | + | ++ | ++ | + | - | | |
| 301 (H.Stencill) | ++++ | +++ | ++ | + | + | ± | - | - | - | ± | + | ++ | +++ | ++ | + | - |
| 318 (H.Stencill) | ++++ | ++++ | ++++ | +++ | +++ | + | ± | - | - | ± | + | ++ | +++ | ++ | ++ | + |
| D.72 (A.Maracle) | ++++ | ++++ | +++ | +++ | ++ | ∓ | - | | | - | ∓ | + | ++ | + | - | |
| E.34 (A.Maracle) | ++++ | ++++ | +++ | ++ | - | - | - | | | - | - | ± | + | ++ | - | |
| E.75 (A.Maracle) | ++++ | +++ | ++ | + | ∓ | - | - | | | - | ++ | +++ | ++ | ± | - | |

TABLE I (Continued).

Showing pre-zones with somatic ('O') suspensions.

| Serum No. | B. typhosus 'H' suspension | | | | | | | | | B. typhosus 'O' suspension | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 | 1/2500 | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 |
| D.73 (I.Hewitt) | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | ++ | - | - | ± | ++ | ++ | ++ | ± | - |
| E.33 (I.Hewitt) | ++++ | ++++ | ++++ | ++++ | +++ | +++ | ++ | - | | - | - | ± | + | + | ++ | ± |
| E.35 (D.Peters) | +++ | +++ | +++ | ++ | ++ | + | - | | | - | ± | ++ | ± | - | | |
| D.99 (M.Daly) | ++++ | ++++ | ++++ | +++ | ++ | + | - | | | - | + | ++ | + | + | - | |
| E.24 (M.Caverly) | - | - | - | - | - | - | | | | - | +++ | +++ | +++ | ++ | ++ | - |
| E.30 (R.Browney) | ++++ | ++++ | +++ | ++ | - | | | | | - | + | +++ | ++ | + | - | |
| E.78 (J.Lovelace) | ++++ | ++++ | ++++ | ++++ | +++ | +++ | ++ | - | | + | ++ | +++ | ++ | - | | |

| Serum No. | B. paratyphosus B 'H' suspension | | | | | | | | | B. aertrycke 'O' suspension | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 | 1/2500 | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 |
| 855 | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | + | - | - | ++ | ++++ | ++++ | +++ | +++ | +++ | ++ |
| 1092 | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | ++ | + | ++ | ++++ | ++++ | ++++ | ++++ | +++ | - |

B. RELATION OF THE SOMATIC ANTIGEN TO ZONAL REACTIONS.

The following evidence is put forward in support of the view that the somatic antigen and not the flagellar antigen is concerned in the zone phenomenon.

(a) Comparison of agglutination results using a 'composite' bacillary suspension and separate 'flagellated' ('H') and 'somatic' ('O') suspensions.

The term composite bacillary suspension is here used to designate a suspension of B. typhosus prepared in the ordinary way without strict attention to the maximum development of the flagellated phase. A suspension of this type containing mixed flagellated and non-flagellated bacilli is supplied by the Department of Health of Ontario (1) for routine testing of typhoid sera. In the tests applied to such sera this suspension was diluted to opacity No. 3 of McFarland's Nephelometer Scale ($\doteq 1000 \times 10^6$ organisms per c.c.) and Dreyer's technique used.

The separate flagellated and somatic suspensions used were supplied by the Standards Laboratory, Oxford (2). Their opacities were approximately $500 \times 10^6$ organisms per c.c.

The use of the composite bacillary suspension of B. typhosus resulted not infrequently in the occurrence of floccular agglutination in the lower dilutions and granular agglutination in the higher dilutions of the typhoid sera.

---

(1) This suspension is prepared by growing B. typhosus (Bender) on veal agar, removing the growth with 0.85% NaCl solution and adding formalin to a concentration of 0.1% or more. It is then stored in the ice-chest for 5 days and standardised to an opacity of $2000 \times 10^6$ organisms per c.c. before issue.

(2) The 'H' suspension is prepared by growing a suitable culture of B. typhosus (Rawlings) in veal peptone bouillon for 24 hours at 37°C., diluting with normal saline containing 0.2% formalin to an opacity similar to that of a 'standard' suspension, and comparing the agglutinability with that of a standardised suspension.

The 'O' suspension is prepared by the same method using a natural non-motile variant of B. typhosus. Formolized typhoid 'O' suspensions issued since 1930 have been used in this series of tests.

TABLE 2.

Serum No. 40 (Cox)

| Suspension | Incubation in Water-bath at 50-55°C. for | Serum Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 |
| (a) composite bacillary antigen | 4½ hours | ++ f | ++ f | ± g | ++ g | ++ g | ++ g | + g | ∓ g |
| | 22 hours | ++ f | ++ f | +++ g | ++++ g | ++++ g | ++++ g | +++ g | ++ g |
| (b) B.typhosus 'H' | 22 hours | +++ | +++ | ++ | ± | ∓ | - | | |
| (c) B.typhosus 'O' | 22 hours | + | +++ | ++++ | ++ | ++ | - | | |

Serum No. 301

| Suspension | Incubation in Water-bath at 50-55°C. for | Serum Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 |
| (a) composite bacillary antigen | 22 hours | ++ f | ++ f | +++ fg | ++++ g | ++++ g | ++++ g | ++++ g | ++ g |
| (b) B.typhosus 'H' | 22 hours | ++++ | +++ | ++ | + | + | ± | - | - |
| (c) B.typhosus 'O' | 22 hours | ± | + | ++ | +++ | ++ | + | - | - |

At this dilution the type of agglutination was distinctly granular with suspension (a).
f = floccular agglutination, g = granular agglutination.
++++ = complete agglutination; +++, ++, +, etc. lesser degrees of agglutination.
The difference in the end-titres obtained with suspensions (a) and (b) is due to the relative agglutinability of the strains used in the preparation of the suspensions.

Two examples of this type are given in Table 2 where serum 40 (Cox) and serum 301 showed slight but distinct pre-zones as far as the 1:100 dilution, when the results were read after 22 hours at 50-55°C. and a composite bacillary suspension of B. typhosus used. The suggestion is therefore that when inhibition zones are definitely found with a composite bacillary antigen, floccular agglutination does not extend very far in the series of serum dilutions and may be negligible in some cases. The type of agglutination in dilutions 1:25 and 1:50 was distinctly floccular, while in the 1:100 and as far as the 1:2000 dilution the agglutination was definitely granular. The granular agglutination was observable after $4\frac{1}{2}$ hours' incubation and constituted a marked contrast to the floccular agglutination in the early dilutions. With the flagellated suspension (b) and somatic suspension (c) the same sera exhibited the usual type of floccular agglutination which did not exceed the 1:100 dilution, but attained a titre of 1:250 with a marked pre-zone of inhibition in granular agglutination. Thus the slight floccular agglutination in the lower dilutions observed with the composite antigen (a) is associated with low 'H' agglutinins and a relative absence of 'O' agglutinins in these dilutions, while in the higher dilutions the 'O' agglutinins appear in excess of the 'H' agglutinins as shown by suspensions (b) and (c). In a zone serum which is poor in 'H' agglutinins, therefore, there is more likelihood of the appearance of a pre-zone when the composite type of antigen is used.

On the other hand if a serum is rich in 'H' agglutinins the zone tends to be masked i.e. the zone exhibited by a pure somatic suspension is obscured by the floccular agglutination of the composite antigen. G.23 (Ashton) and 318 are examples of sera which show pre-zones with the somatic antigen but which are masked by the composite antigen. (Table 3 ).

TABLE 3.

Serum No.C.23 (Ashton)

| Suspension | Incubation in water-bath at 50-55°C. for | Serum Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1/25 | 1/50 | 1/100* | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 |
| (a) Composite bacillary antigen | 22 hours | +++f | +++f | ++++fg | +++fg | +++fg | ++fg | ++g | +g |
| (b) B.typhosus 'H' | " | ++++ | ++++ | +++ | ++ | ++ | - | - | - |
| (c) B.typhosus 'O' | " | - | ++ | ++++ | ++++ | +++ | ++ | - | - |

Serum No. 318

| Suspension | Incubation in water-bath at 50-55°C. for | Serum Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1/25 | 1/50 | 1/100* | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 |
| (a) Composite bacillary antigen | 22 hours | +++f | +++f | +++fg | +++fg | +++fg | ++fg | ++g | ++g |
| (b) B.typhosus 'H' | " | ++++ | ++++ | ++++ | +++ | +++ | + | ± | - |
| (c) B.typhosus 'O' | " | ± | + | ++ | +++ | ++ | ++ | + | - |

* At this dilution mixed floccular and granular agglutination was apparent after 4½ hours' incubation.

(b) The Occurrence of the Zone phenomenon in antisera to Brucella abortus, a non-flagellated organism.

During the past few years considerable attention has been given to the serological diagnosis of Br. abortus infection in man and in animals. It is noteworthy that zonal reactions have been found by various workers more frequently in abortus antisera than in typhoid antisera and this fact may be correlated with the non-flagellated state of Br. abortus. Kling (1929) records a case in whose serum positive agglutination only commenced at a dilution of 1:640 and extended to 1:2560. Spencer (1930) in a series of 179 positive undulant fever sera found 30 per cent.* showing zonal reactions. In one example, No. 18 of his series, the pre-zone extended to and included the dilution of 1:160, five showed pre-zones up to and including 1:80, thirteen up to and including the 1:40 dilution and twenty-one as far as the 1:20 dilution.

Two cases of undulant fever whose sera showed pre-zones are noted in Table 4; one as far as the 1:50 dilution, the other up to and including the 1:100 dilution. Serum 146 was from a butcher employed in a large institution, serum 153 was from a farmer operated upon for appendicitis and only two weeks later when the patient's temperature was still above normal was a sample of blood taken for serological tests. Both serum samples were stored in the ice-chest and re-tested after several months. Ageing of the sera had removed the pre-zones.

---

* In a recent article by Jones and Orcutt (J. Immunology, 27, 215) the figure is given erroneously as 34 per cent. These authors conducted experiments on two cattle sera which showed the pre-zone phenomenon.

TABLE 4.

Showing two cases of undulant fever with pre-zones and their disappearance through aging.

| Serum Number | Suspension | Period of Incubation | Date of test | Serum Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 |
| 146 (Copeland) | Brucella abortus suspension (Oxford) | 4½ hrs. at 50-55°C. and overnight at room temp. | 19/5/32 | - | ∓ | ++++ | ++++ | ++++ | ++ | - | |
| | | | 29/4/33 | +++ | +++ | + | ∓ | - | | | |
| 153 (Waters) | | | 2/6/32 | - | ∓ | + | ++++ | ++++ | ++++ | +++ | - |
| | | | 25/4/33 | +++ | ++++ | ++++ | ++++ | ++++ | +++ | - | |

In several hundreds of cattle sera examined in this laboratory for evidence of Brucella abortus infection the pre-zone and mid-zone phenomena were repeatedly seen. Seven examples of pre-zones and six examples of mid-zones are shown in Table 5. In general the end-titres of the sera showing mid-zones, e.g. Nos. 6 and 13, are distinctly higher than those of the sera showing pre-zones. In serum 3 complete inhibition was present up to and including the 1:100 dilution, and in serum 80176 inhibition was complete in dilutions of 1:500 and 1:1000. All the tests were incubated in the water-bath at 50-55°C. for 22 hours, using Dreyer's technique and phenolised Br. abortus suspension diluted to an opacity of approximately 750 million organisms per c.c. Br. abortus suspension preserved with 0.5 per cent. phenol instead of 0.2 per cent. formalin revealed less tendency to inhibition zones and gave much clearer readings.

TABLE 5.

Showing 7 pre-zones and 6 mid-zones

| Date Examined | Suspension | Serum No. | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 | 1/2500 | 1/5000 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 27-9-32 | Phenolised Brucella abortus suspension, opacity of 750 x $10^6$ organisms per c.c. | 31 | - | + | ++++ | ++++ | ++++ | ++++ | - | - | | |
| " | | 39 | - | + | +++ | +++ | ++++ | ++++ | + | - | | |
| 24-10-32 | | 6 | ++++ | ++++ | - | + | ++ | ++++ | ++++ | ++ | | |
| " | | 9 | - | ++++ | ++++ | ++++ | ++++ | + | - | - | | |
| " | | 10 | + | ++++ | ++++ | ++++ | ++++ | ± | - | - | | |
| 17-11-32 | | 9a | ++++ | +++ | + | +++ | +++ | ++++ | ++++ | +++ | | |
| " | | 13 | ++++ | ++++ | ++ | - | - | ++ | ++ | +++ | | |
| " | | 25 | ++++ | ++ | + | + | ++ | ++ | - | - | | |
| " | | 27 | ++++ | + | ++ | +++ | ++++ | ++++ | +++ | - | | |
| " | | 37 | + | ++ | ++ | +++ | + | - | - | - | | |
| 31-1-33 | | 3 | - | - | - | ++ | +++ | +++ | - | - | | |
| 13-4-33 | | 93790 | - | + | ++++ | ++++ | ++++ | +++ | +++ | - | | |
| " | | 80176 | ++++ | ++++ | ++++ | +++ | ++ | - | - | ++ | +++ | ++++ |

## C. FACTORS INFLUENCING THE ZONE PHENOMENON

Conflicting statements have been made by different workers regarding the factors influencing the zone phenomenon and, with a view to elucidating its nature, the effect of (a) density of suspension (b) temperature (c) sodium chloride concentration (d) formolised suspensions and (e) addition of a normally agglutinating serum (to titrate the inhibitory property) have been studied.

### (a) The Antigen/Serum dilution ratio.

Hardy and others (1930) have called attention to the necessity of standardising the density of the antigen in agglutination tests on abortus antisera if comparable end-titres are to be obtained. The adoption of a standard technique is important also in zone sera for, as Spencer (1930) and Priestley (1931) have shown, the position of the mid-zone varies with the density of the bacterial suspension. Cow serum 80176 (Table 6) was examined with varying densities of suspension and illustrates the view that the position of the inhibition zone depends on the relative amounts of antigen and immune body. With suspension of opacity = 1000 million organisms per c.c., complete inhibition occurred in dilutions of 1:200 and 1:250; with opacity = 750 million organisms per c.c. complete inhibition occurred in the 1:500 and 1:1000 dilutions; and in dilutions of 1:1000, 1:2000 and 1:2500 with an opacity of 500 million organisms per c.c.

TABLE 6.

Serum 80176

| Density of suspension per c.c. | Serum Dilutions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 | 1/2500 | 1/5000 | 1/10000 | 1/20000 |
| 1000 x $10^6$ | ++++ | ++++ | +++ | - | - | +++ | +++ | ++++ | ++++ | ++++ | ++ | - |
| 750 x $10^6$ | ++++ | ++++ | ++++ | ++++ | +++ | - | - | + | ++ | ++++ | ++++ | - |
| 500 x $10^6$ | ++++ | ++++ | ++++ | ++++ | ++++ | + | - | - | - | + | + | ++ |

(b) The effect of temperature.

According to Detre (1927) a mid-zone is destroyed by inactivation of the serum. Spencer (1930) working with an unusual human serum showing a mid-zone found that inactivation had no effect on the zone if the tests were incubated at 37°C. although incubation at 56°C. removed it. Priestley's experiments (1931) on a cattle-serum with a mid-zone failed to confirm Spencer's results. He found that incubation at 56°C. instead of 37°C. narrowed the zone without removing it, whether the serum was inactivated or not. In the example given by Priestley the serum dilutions begin with 1:10, in my series with 1:25 and thus sera which give a mid-zone with Priestley's technique may be found to give a pre-zone with Dreyer's technique. My results agree with those of Priestley in that incubation at 50-55°C. for 22 hours fails to destroy the zone. Inactivation and subsequent incubation in the water-bath at 50-55°C. for 22 hours did not remove the pre-zone in serum 3 nor the mid-zones in sera 25, 27 and 80176. The reactions obtained by incubation at 37°C. were distinctly weaker than those obtained at 50-55°C. and this finding suggests that a greater density of antigen is necessary to bring out the zone if the lower incubation temperature is employed. Priestley (private communication, 1931) incubated his tests generally at 37°C. using a suspension having a density of approximately 4500 million organisms per c.c. There is thus further evidence of the relation between density of suspension and serum concentration in the production of zonal reactions. Table 7 shows the results for a pre-zone serum (3) and a mid-zone serum (25).

TABLE 7.

Showing the effect of temperature on the pre-zone and mid-zone reactions

| Serum Number | Condition of Serum | Incubation for 22 hrs. | Serum Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 |
| 3 | Unheated | Water-bath 50-55°C. | - | - | ++ | ++++ | ++++ | ++++ | ++ | - |
| | Inactivated at 56°C. for 30 minutes | " | - | - | +++ | ++++ | ++++ | +++ | - | - |
| | Unheated | Incubator 37°C. | + | + | ± | - | - | - | - | - |
| | Inactivated at 56°C. for 30 minutes | " | + | + | + | + | - | - | - | - |
| 25 | Unheated | Water-bath 50-55°C. | ++++ | ++++ | - | - | - | ++ | +++ | - |
| | Inactivated at 56°C. for 30 minutes | " | ++++ | ++ | - | - | - | ++ | - | - |
| | Unheated | Incubator 37°C. | ++ | ++ | + | - | - | - | - | - |
| | Inactivated at 56°C. for 30 minutes | " | +++ | ++ | + | + | - | - | - | - |

(c) Effect of variations in the salt concentration.

Sera 37 and 3 (pre-zones) and sera 25, 27 and 80176 (mid-zones) were tested in serial dilutions using NaCl solutions varying in concentration from 3.4 to 0.43 per cent. All the results showed disappearance of the zones in concentrations greater than 0.85 per cent. and widening of the zones in a concentration of 0.43 per cent. Table 8 shows the results for a pre-zone serum (3) and a mid-zone serum (80176).

TABLE 8.

Showing the effect of variations in the salt concentration on zone sera.

| Serum Number | Incubation | Salt concentration as a percentage | Serum Dilutions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 | 1/2500 | 1/5000 | 1/10000 | 1/20000 |
| 3 | Water-bath at 50-55°C. for 22 hours | 3.4 | ++++ | ++++ | ++++ | ++++ | ++++ | ++ | - | - | | | | |
| | | 1.7 | ++ | ++++ | ++++ | ++++ | ++++ | +++ | - | - | | | | |
| | | 0.85 | - | - | ++ | ++++ | ++++ | ++++ | - | - | | | | |
| | 28/4/33 | 0.43 | - | - | - | ++ | +++ | +++ | - | - | | | | |
| 80176 | Water-bath at 50-55°C. for 22 hours | 3.4 | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | + |
| | | 1.7 | ++++ | ++++ | ++++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ |
| | | 0.85 | ++++ | ++++ | ++++ | +++ | ++ | - | - | +++ | +++ | ++++ | +++ | ++ |
| | 28/4/33 | 0.43 | ++++ | ++++ | ++++ | +++ | +++ | ++ | - | - | - | ++++ | +++ | ++ |

For each strength of NaCl solution, the same solution was used for making the serum dilutions as for diluting the stock antigen. The opacity of the suspensions corresponded to 750 x $10^6$ organisms per c.c.

In order to ascertain whether the globulin fraction removed the inhibitory property from the serum the following experiment was carried out.

An equal volume of serum 80176 was mixed with a saturated solution of ammonium sulphate, placed in the water-bath at 56°C. for 15 minutes to hasten precipitation of globulin and then centrifuged. The supernatant fluid was withdrawn and preserved, the precipitate was redissolved in as small an amount of 0.85 per cent. NaCl solution as possible. The agglutinative titres of the original serum, globulin solution and supernatant fluid were then examined. The results shown in Table 9 indicate that the inhibitory zone is present in the globulin solution.

TABLE 9.

showing the zone in the globulin solution

| | Dilutions | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/25 | 1/50 | 1/100 | 1/200 | 1/250 | 1/500 | 1/1000 | 1/2000 | 1/2500 | 1/5000 | 1/10000 |
| Serum 80176 | ++++ | ++++ | ++++ | +++ | - | - | ++ | +++ | +++ | ++++ | ++ |
| Globulin 80176 | +++ | ++++ | ++++ | - | - | - | ++ | +++ | +++ | ++++ | ++ |
| Supernatant fluid | ++++ | ++++ | ++++ | ++++ | +++ | + | - | - | - | - | - |

Density of Brucella abortus suspension 500 million organisms per c.c.

Date of test: 4/5/33.

Incubation: Water-bath at 50-55°C. for 22 hours.

(d) Effect of a formolised somatic ('O') antigen in eliciting pre-zones. According to Felix and Olitzki (1928) formalin inhibits 'O' agglutination so strongly that formalin-treated suspensions cannot be used for detecting 'O' agglutinins. They used suspensions prepared with 1.0 per cent. formalin and a temperature of 37°C. for incubation.

Using Dreyer's technique, Gardner (1929) showed that broth suspensions, treated with 0.1 per cent. formalin, such as are used in the ordinary Widal reaction, are capable of detecting 'O' agglutinins but are less suitable for their estimation than alcohol suspensions. In the sheet of instructions issued by the Standards Laboratory (1932), details are given for the preparation of formolised and alcoholised 'O' suspensions. The 'O' suspensions of B. typhosus issued since 1930 by the Standards Laboratory, Oxford, have been prepared by formolising broth suspensions of a non-motile variant.

In an attempt to analyse the mechanism of the action of formalin on agglutination, Mudd and Joffe (1933) treated antisera with neutral formaldehyde solution of varying concentrations -- 9 per cent., 18.5 per cent. and 37 per cent., before and after combination with homologous and heterologous antigens. They concluded that (i) agglutination pre-zones appeared when an antiserum was treated with formaldehyde before combination with antigen and (ii) these agglutination pre-zones are due to changes in the physical properties of the films of specific antibody-globulin on the surface of the bacteria, rather than to failure of the antigen-antibody combination.

To determine whether formalin in a concentration of 0.2 per cent. exercised an inhibitory action on 'O' agglutination, a stock culture of B. typhosus $O_{901}$ was plated on agar, a smooth colony picked off and examined serologically for smoothness. Four Roux bottles were sown with a peptone broth culture, incubated at 37°C. for 18 hours, and the growths washed off in buffer* saline. In this way a dense suspension of B. typhosus $O_{901}$ was prepared. Two c.c. quantities of the suspension were pipetted into a series of four bottles and diluted with buffer saline to an opacity corresponding to No. 3 McFarland's scale (= 900 million organisms per c.c.). Each bottle now contained 100 c.c. of suspension. To A, 0.2 per cent. pure formalin was added: to B, 0.5 per cent. phenol: to C, 0.01 per cent. merthiolate and to D, 5 per cent. chloroform. The bottles were placed in the ice-chest for 2-3 weeks and shaken daily. This procedure was followed so as to exclude all influencing factors except the particular preservative.

A number of human typhoid antisera were examined for their content of 'O' agglutinins with the above suspensions. The results of four sera are embodied in Table 10 to illustrate the general findings.

---

* The composition of the buffer saline is $KH_2PO_4$ 0.2 gm., $Na_2HPO_4$ 1.7 gms., NaCl 7 gms. and aq. dest. 1 litre.

Table 10

showing the relative agglutinability of a bacillary suspension (Ty $O_{901}$) preserved with (a) 0.2 per cent. formalin
(b) 0.5 per cent. phenol
(c) 0.01 per cent. merthiolate
(d) 5 per cent. chloroform

(e) is the Oxford B. typhosus 'O' suspension (formolised).

| Bac. Susp. | Serum D73 1:25 | 1:50 | 1:100 | 1:200 | 1:250 | 1:500 | 1:1000 | Serum F81 1:25 | 1:50 | 1:100 | 1:200 | 1:250 | 1:500 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (a) | - | + | +++ | +++ | +++ | ± | - | ± | ∓ | ∓ | - | - | - |
| (b) | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++ | +++ | ++++ | ++++ | ++ | ++ | ± |
| (c) | +++ | ++++ | ++++ | ++++ | ++++ | +++ | ++ | ++++ | ++++ | +++ | ++ | + | ∓ |
| (d) | +++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++ | ++ | ++++ | ++++ | ++++ | ++ | + |
| (e) | - | + | + | +++ | +++ | ∓ | - | + | +++ | +++ | ++ | ++ | ± |

| Bac. Susp. | Serum F27 1:25 | 1:50 | 1:100 | 1:200 | 1:250 | 1:500 | 1:1000 | Serum E80 1:25 | 1:50 | 1:100 | 1:200 | 1:250 | 1:500 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (a) | + | ++ | ± | - | - | | | - | - | - | - | - | - |
| (b) | + | ++ | +++ | ++ | ∓ | | | + | +++ | +++ | ++ | ++ | ++ |
| (c) | ++ | ++ | ++ | ∓ | ∓ | | | ++ | +++ | ++++ | ++++ | +++ | ++ |
| (d) | ++++ | ++++ | ++++ | +++ | +++ | | | ++ | ++ | +++ | +++ | +++ | ++ |

Two main conclusions may be drawn from this table:-

(1) Agglutination in the highest serum concentrations is consistently inhibited when formolised bacillary suspensions are used, e.g., sera D73 and F27 show pre-zones up to a dilution of 1:50 and 1:25 respectively with Ty. Bovis formolised suspension (a) and as far as 1:100 with the formolised Oxford suspension (e) in serum D73. In some sera, suspension (a) inhibited entirely the occurrence of granular agglutination (e.g. sera F81 and E80).

(2) The end-titre of agglutination is somewhat more regularly reduced when the bacillary suspensions are treated with 0.2 per cent. formalin, instead of 0.5 per cent. phenol, 0.01 per cent. merthiolate or 5 per cent. chloroform. With the three latter preservatives the end-titres are higher than with formalin, (compare sera D73 and E80). Slight pre-zones are present sometimes with the phenolised suspension (e.g. sera F27 and E80), the merthiolated suspension (E80) and occasionally with the chloroform suspension (F81 and E80) but the most marked effects are shown with formolised suspensions.

It is apparent that the pre-zone reactions are brought more sharply into prominence by the use of formolised suspensions than with the same bacillary suspension preserved by other methods -- phenol, merthiolate and chloroform. That such a low concentration of formalin is capable of producing this effect is striking. In Mudd and Joffe's experiments, the concentrations of formaldehyde were much greater than are employed in practice. Their lowest concentration of 9 per cent. formaldehyde exceeds 100 times [1] that used for ordinarily preserving bacillary suspensions, viz. 0.2 per cent. formalin.

---

(1) 0.2 per cent. formalin = 0.2% of 40% H.CHO = 0.08% H.CHO, and 9/0.08 = >100.

Felix and Olitzki's experiments deal with the reactivity of preserved bacillary suspensions and not with the zone phenomenon. They noted that 'O' agglutination was inhibited when suspensions of laboratory strains of B. typhosus or B. paratyphosus A and B were preserved either with 0.5 per cent. phenol or 1 per cent. formalin. In the tubes of the agglutination test the concentration was 0.5 per cent. formalin whether preserved or living suspensions were used. With the modified Dreyer technique employed in the present experiments the concentration of formalin is $\frac{12}{20}$ x 0.2 or 0.12 per cent. i.e. approximately 4 times less than that used by Felix and Olitzki. When freshly prepared suspensions are preserved with 0.2 per cent. formalin [2] and stored in the ice-chest for several weeks, some action on the bacilli presumably takes place. It is suggested that in addition to its preservative action formalin decreases the sensitiveness of the bacilli to agglutination.

---

(2) The presence of formalin in the suspension can be detected by Lebbin's or Mehner's test. (Allen's Commercial Organic Analysis Vol. 1. p. 259).

(e) Titration of the inhibitory factor in a pre-zone serum.

To determine whether or not a pre-zone serum can confer its inhibitory property on a normally agglutinating serum, a method was devised of titrating the pre-zone serum in the presence of a predetermined dilution of the agglutinating serum.

The principle of the titration is a modification of Dreyer's drop method of measurement in agglutination tests.

Serum A, the pre-zone serum, is diluted 1:5 and 1:10 with saline and a varying number of drops added to the series of tubes as indicated in the diagram. Serum B, the normally agglutinating serum, is diluted 1:10, 1:20 or according to the highest dilution in which marked agglutination occurs, and a constant number of drops added to each tube of the series. Saline is added to make an equal volume of 8 drops and then a constant amount of antigen so that each tube has a total volume of 20 drops.

The diagram postulates a case where serum B has a marked agglutination reaction in a dilution of 1:100, 2 drops of a 1:10 dilution being added to each tube. The final dilutions for serum A are the usual range from 1:25 to 1:200 and for serum B a dilution of 1:100 in each tube of the test. Hence the pre-zone serum is titrated in the presence of a dilution of the added serum in which marked agglutination normally occurs. The results are read after incubation in the water bath at 50-55°C. for 22 hours.

| | 1 | 2 | 3 | 4 | Controls | |
|---|---|---|---|---|---|---|
| Serum A (1:5) | 4 | 2 | 1 | 1 (1:10) | 0 | 0 |
| Serum B (1:10) | 2 | 2 | 2 | 2 | 2 | 0 |
| Saline | 2 | 4 | 5 | 5 | 6 | 8 |
| Antigen | 12 | 12 | 12 | 12 | 12 | 12 |
| Final dil. of A | 1:25 | 1:50 | 1:100 | 1:200 | - | - |
| Final dil. of B | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | - |

Experiments were performed to study the effect of a pre-zone serum on sera showing (a) pronounced 'O' agglutination titres and (b) fairly high 'H' agglutination titres. As an illustration of the former effect Table 11 is presented. Serum R83 was obtained after intravenous administration of T.A.B. vaccine, with end-titres Ty. 'H' 1:500 (++), Ty. 'O' 1:250(+++), P.B. 'H' 1:1000 (++) and Aer. 'O' 1:500 (+++). The reactions with B. ty. 'O' suspension in dilutions 1:25 to 1:250 reveal the pre-zonal nature of the serum.

Serum T45 from an active case of typhoid fever gave end-titres Ty. 'H' 1:1000 (++), Ty. 'O' 1:250 (++), PB. 'H' <1:25 and Aer. 'O' 1:100 (+++). The detailed reactions with B. ty. 'O' suspension show a gradually decreasing intensity from 1:25 (++++) to 1:250 (++).

Table 11

showing effect of a pre-zone serum on a normally agglutinating 'O' serum when tested with B. ty. 'O' suspension.

| Serum Number | Nature of serum | Serum Dilutions | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1:25 | 1:50 | 1:100 | 1:200 | 1:250 | 1:500 |
| R83 | Pre-zonal | ∓ | ∓ | +++ | +++ | +++ | - |
| T45 | Normally agglutinating | ++++ | ++++ | +++ | +++* | ++ | - |
| R83 + T45 | Pre-zonal with 1/200 dilution of normally agglutinating serum | - | ∓ | ++ | ++ | | |

* This dilution was chosen as the next lower dilution to the end-titre showing a strong reaction.

Following the scheme presented in the diagram (page 27), serum R83 was titrated in the usual dilutions of 1:25 to 1:200 and to the same tubes serum T45 was added in a constant dilution of 1:200. The results of this titration indicate that the inhibitory property of the pre-zone serum caused complete inhibition of 'O' agglutination in the added serum in dilutions 1:25 and 1:50 and partial inhibition in dilutions 1:100 and 1:200.

To study the effect of a pre-zone serum on a serum showing 'H' agglutination, serum T46 was chosen because it reacted only to B. ty. 'O' suspension, serum R16 because it showed reactions only to B. ty. 'H' suspension, and serum T13 because it reacted only to B. para B 'H' suspension. The results of the agglutination reactions of these sera alone and in combination are shown in Table 12.

Table 12

showing effect of a pre-zone serum on a normally agglutinating 'H' serum.

| Serum Number | Nature of serum | Bac. suspension used | Serum Dilutions | | | |
|---|---|---|---|---|---|---|
| | | | 1:25 | 1:50 | 1:100 | 1:200 |
| (a) T46 | Pre-zonal | Ty 'O' | ++ | ++++ | ++++ | ++++ |
| (b) R16 | Normally agglutinating | Ty 'H' | +++ | +++ | ++ | ± |
| (c) T46 + R16 | Pre-zonal with 1:100 dilution of normally agglutinating serum | Ty 'H' | +++ | +++ | +++ | +++ |
| (d) T46 + R16 | " | Ty 'O' | ± | ++ | +++ | |
| (e) T13 | Normally agglutinating | Para B 'H' | +++ | ++ | ± | - |
| (f) T46 + T13 | Pre-zonal + 1:50 dilution of normally agglutinating serum | Para B 'H' | +++ | +++ | +++ | |

The agglutination reactions of sera T46 and R16 in combination when tested with B. ty. 'H' suspension show that the inhibitory property of the pre-zone serum does not diminish the intensity of the 'H' readings:- in fact, the 'H' titre for serum R16 was increased from 1:100 (++) to 1:200 (+++). (Compare reactions in (b) and (c)). When the combined sera are examined with B. ty. 'O' suspension, the pre-zone is increased both in degree and in extent. (Compare reactions in (a) and (d)). The effect of a pre-zone serum on a serum agglutinating only with B. para B. 'H' suspension is shown in the reactions (e) and (f). The 'H' titre for T13 is increased from 1:50 (++) to 1:100 (+++).

The increase in the end-titre for 'H' agglutination is apparently due to the influence of the additional serum. To confirm this view several sera containing 'H' and 'O' agglutinins were titrated with the addition of a negative serum in a constant dilution of 1:50, 1:100 and 1:200 in each tube. The same scheme as for titration of the inhibitory factor in a pre-zone serum was followed. All the sera showed an increased intensity of reaction and in some cases the end-titre was raised to the next higher dilution. The reactions of sera T58 and P70 singly and in combination illustrate the general result, e.g., serum T58 has an end-titre of 1:500 (++) with B. ty. 'H' suspension and when combined with 1:100 dilution of the negative serum P70 gave an end-titre of 1:1000 (+++). Similar findings were obtained with B. para B 'H' and B. aer. 'O' suspensions.

Table 13

showing increase in intensity of agglutination reactions in a serum from a patient receiving intravenous injections of T.A.B. vaccine by the addition of a negative serum in constant dilution 1:100.

| Serum Number | Nature of serum | Bac. susp. used | Serum Dilutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1:25 | 1:50 | 1:100 | 1:200 | 1:350 | 1:500 | 1:1000 | 1:2000 |
| T58 | positive | Ty'H' | ++++ | ++++ | ++++ | ++++ | +++ | ++ | + | - |
| T58+P70 | pos.with 1:100 dil. of negative serum | " | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | - |
| T58 | positive | Ty'O' | +++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++ | + |
| T58+P70 | | " | +++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | ++ |
| T58 | positive | PB'H' | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | + | - |
| T58+P70 | | " | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | - |
| T58 | positive | Aer'O' | ++++ | ++++ | ++++ | ++ | + | - | | |
| T58+P70 | | " | ++++ | ++++ | ++++ | +++ | +++ | ∓ | | |
| P70 | negative | Ty'H' | - | - | - | - | | | | |
| | | Ty'O' | - | - | - | - | | | | |
| | | PB'H' | - | - | - | - | | | | |
| | | Aer'O' | - | - | - | - | | | | |

The results obtained in Tables 12 and 13 indicate that a serum containing 'H' agglutinins only, when combined with a pre-zone serum, is no more affected as regards its content of 'H' agglutinins than if the 'O' serum contained no agglutinins nor inhibitory factor, since a reacting serum combined with a negative serum shows an increase in the intensity of the reactions to 'H' and 'O' suspensions.

## Discussion

The precise nature of the inhibitory factor is still unknown, nevertheless the following facts are recognised:-

(i) the inhibition zone disappears on ageing of the serum,

(ii) the position of the zone is influenced by the opacity or density of the suspension,

(iii) incubation at 50-55°C. for 22 hours does not remove the zone whether the serum has been previously inactivated or not,

(iv) increasing the salt concentration to 1.7 per cent. and 3.4 per cent. diminishes or removes the zone; decreasing the concentration to 0.43 per cent. widens the zone,

(v) the inhibitory factor is precipitated with the globulin by ammonium sulphate,

(vi) the addition of an inhibitory serum to a normally agglutinating 'O' serum suppresses agglutination.

In his study of Salmonella Agglutination, Bruce White (1931) observed that fresh normal rat serum added to serial dilutions of various anti-salmonella sera exerts very little influence on the course of flagellar agglutination but inhibits specific somatic agglutination. The same selective action was found when fresh rat antiserum was used instead of a mixture of fresh normal rat serum and ageing antiserum. This agglutination-inhibiting property of fresh normal serum disappears on inactivation or on standing at room temperature for 48 hours and behaves exactly like haemolytic complement. A thermostable factor is however also active in the inhibitory effect. Suspensions killed by heat or treated with alcohol were found to be sensitive to the inhibitory factor in fresh serum and the present research indicates that formolised or phenolised somatic suspensions are also sensitive. The view that complement plays an essential part in the production of inhibition zones is supported by Anthadse (1930). According to Detre (1927) there are three zones - normal agglutination,

inhibition and specific agglutination, and he regards complement as counteracting the inhibitory factor of the mid-zone sera. Priestley (1931) suggests on this theory that the mid-zone appears to be merely a pre-zone preceding the zone of specific agglutination and extending beyond the zone of normal agglutination.

## Summary and Conclusions

1. The cause of the irregularities noted by laboratory workers in typhoid agglutination tests can be attributed to the mixed flagellated ('H') and non-flagellated ('O') condition of the suspensions.
2. These irregularities are resolved when separate flagellated and non-flagellated suspensions are used.
3. Zonal reactions have been encountered only with the use of non-flagellated (somatic) suspensions.
4. The more frequent occurrence of pre-zones in agglutination reactions with undulant fever sera than with typhoid sera is apparently due to the natural non-flagellated condition of the Brucella abortus.
5. The addition of formalin as a preservative, in a concentration of 0.2 per cent., to a somatic suspension of B. typhosus tends to decrease the sensitiveness of the bacterial suspension to agglutination.
6. A pre-zone serum can confer its inhibitory property on a normally agglutinating 'O' serum.
7. The inhibitory property in a pre-zone serum exerts no effect on flagellar agglutination.

## References


Anthadse, V. (1930) Action Antagoniste de l'Alexine vis-à-vis du Phénomène de l'Agglutination. Annales de l'Institut Pasteur. 45. 203.

Detre, L. (1927) Ein neuartiges Hemmungsphänomen der Agglutination. Ztschr. f. Immunitätsforschung. 51. 205.

Falta and Noeggerath (1929) Quoted from Handbuch der Pathogenen Mikroorganismen Bd. II. Teil II. 1023.

Felix, A. and Olitzki, L. (1928) The Use of Preserved Bacterial Suspensions for the Agglutination Test. J. Hyg. 28. 55.

Gardner, A. D. (1929) The Small-flaking or "O" Agglutination of Permanent Standardised "O" Suspensions of B. typhosus by the Serums of Normal, Inoculated and Infected Persons. J. Hyg. 28. 376.

Hardy, A. V. et al. (1930) Undulant Fever. U.S. P.H.S. Reports. 45. 2433.

Keefer, C. S. (1924) Bull. Johns Hopkins Hospital, Baltimore, 35.6.

Kling, C. (1929) La Fièvre ondulante èn Suede. Bull. Off. Internation. d'Hyg. Publ. t.XX. 1401 and t.XXI.412.

Kristensen, M. (1928) Untersuchungen über die Rolle des Bangschen Abortbazillus als menschenpathogenen Mikroben. C. f. Bakt. Bd. 108. 89.

Krumwiede, Cooper and Provost (1925). Agglutinin Absorption. J. Immunology 10.55.

Mudd, S. and Joffe, E.W. (1933) The Modification of Antibodies by Formaldehyde. J. Gen. Physiol. 16. 947.

Neisser and Wechsberg (1901) Ueber die Wirkungsart bactericider Sera. Münch. med. Wochenschr. 48. 679.

Priestley, F. W. (1931) The Intermediate Zone Phenomenon encountered in certain Br. abortus agglutinating sera. J. Path. & Bact., 34. 81.

Sensenich and Giordano (1928) Brucella abortus infection in Man. J.A.M.A. 90. 1782.

Shibley, G. S. (1929) Studies in Agglutination. J. Exp. Med. 50. 825.

Spencer, R. R. (1930) A Note on the Zone Phenomenon in Human Sera. Pub. Health Rep. U.S. P.H.S. 45. 2383.
The Zone Phenomenon in Agglutination Tests. J. Inf. Dis. 46. 138.

Bruce White, P. (1931) Observations on Salmonella and related phenomena. J. Path. & Bact. 34. 23.

## ACKNOWLEDGEMENTS

The work involved in this thesis has been personally conducted during the past 4 years. I am indebted to

(1) Dr. A. Felix, Lister Institute, London, for sending me his strains of B. typhosus, B. paratyphosus A and B, and for his advice regarding the Qualitative Receptor Analysis Method (Part II);

(2) Several Health Officers in Ontario and New York chiefly, who have been good enough to send me samples of blood from typhoid carriers and from patients undergoing intravenous injections of T.A.B. vaccine (Parts I and II);

(3) Miss I.F. MacLachlan, my departmental assistant, for technical assistance in preparing media and in checking records of agglutination results;

(4) Dr. W.D. Hay for the photographs in Part II.

The text and tables have been composed solely by myself and the references to the literature were personally consulted and abstracted or translated.

J.H. Wyllie.