



The application of the Widal test to a study of non-specific high fever sera and to certain water borne bacteria  
by Kathryn Lyon

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Montana State University  
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Abstract:

Part I 1. Non-specific agglutinins reacting with *Eb. typhosa* do occur in sera from some patients having high fevers which apparently were not due to any organism even remotely related to *Eb. typhosa* 2. Among 27 sera examined only one serum gave a positive Widal reaction with *Eb. typhosa* (flagellar). Two gave positive reactions with *S. schottmuelleri*. and one of these was positive to *S. paratyphi*, and the other positive to *Eb. typhosa* (somatic).

3. Positive agglutination of non-specific high fever sera with *Eb. typhosa* may be an unfavorable prognosis.

Part II.

The results of these experiments indicate that a relationship exists between the typhoid organism, and certain species found in polluted water as indicated by agglutination. That is, these organisms have affinities for a common immune serum, but these affinities exist in different proportions in different species, thus giving agglutination with different degrees of dilution\* 1. The genus *Escherichia* is more closely related to *Eberthella* than to *Aerobacter* according to its agglutination reactions\* 2. Serological reactions may be more important as indicators of phylogenetic relationship than those physiological characters used as a basis for the classification made by Bergey. In any phylogenetic study of these organisms their serological reactions should be considered along with the physiological, morphological, and other reactions.

3. *Es. vesiculiformans*, *Aero. aerogenes*, and *Pro; bombyctis* are not, agglutinated with antityphoid sera.

4. The microscopic and macroscopic tests correlated each other very closely.

5. According to the results of this study the organism described as *Aerobacter levans* should be placed in the genus *Escherichia*, and should be designated as *Escherichia levans* Comb. new.

THE APPLICATION OF THE WIDAL TEST TO A STUDY OF NON-SPECIFIC  
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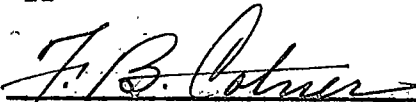
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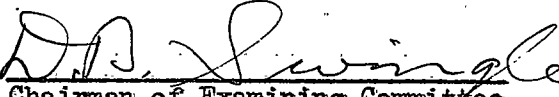
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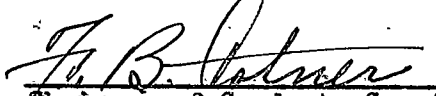
A THESIS

Submitted to the Graduate Committee in partial  
fulfillment of the requirements for the Degree  
of Master of Science in Bacteriology  
at Montana State College.

Approved:

  
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## INTRODUCTION AND HISTORICAL

A study has been made of the occurrence of positive Widal reactions by sera from a number of cases of non-specific high fevers occurring among patients in the Murray Hospital, Butte, Montana. In addition, a rather detailed study was made of the agglutination with anti-typhoid sera of ten organisms isolated from sewage collected in and about Bozeman, Montana.

Studies upon agglutination in the colon-typhoid group of bacilli were reported by Ishii (12) in 1921. All of the important contributions from 1896 to 1921 seemed to have been reviewed by him, and according to his survey of the literature on this subject, there has never been any study of the agglutinating powers of anti-typhoid sera on the particular organisms used by the author.

There are many factors which affect the agglutinating powers. Gouwens (10) has obtained evidence that the acid agglutination of Salmonella paratyphi and S. schottmuelleri might be an aid in differentiation of species of bacteria. However, Stevens (16) claims that it is impossible to identify all strains of a specific organism by means of agglutination. As stated by Ishii (12), acid increases the agglutinating powers.

In Kemp's (14) study of O and H agglutinins, he found that growth-phase organisms selected for O agglutinogen were positively agglutinated later than is usually found in the Widal reaction. Alcoholized Eberthella typhosa was used as a source of O agglutinogen in agglutination tests carried out serially in six cases of typhoid fever. Positive agglutinations were observed earlier than is usually observed in the Widal test.

Formolized growth-phase organisms selected for O agglutinin studies were agglutinated even later than untreated growth-phase selected agglutinin and at much lower titres. Both O and H agglutinins were found in the sera of individuals who had been given typhoid-paratyphoid vaccine. These individuals did not have O agglutinins prior to vaccination. Both O and H agglutinins were found in the sera of individuals suffering with acute exacerbations in chronic arthritis.

This study of certain broad applications of the Widal test may be divided into two definite parts and shall be referred to as Part I and Part II.

Part I. Agglutination of Eberthella typhosa by Non-specific High Fever Sera.

A. Materials and Methods.

1. General Hospital Patients-- The sera used for these studies were obtained from people with high-fevers from various causes, who were patients in the Murray Hospital, Butte, Montana. Every day all of the hospital charts were studied for high-fever patients. Whenever it was permissible blood was taken. Only patients with temperatures over 101.0° F. were regarded. Sometimes a patient might have had a very high temperature, but his condition was such that he could not be disturbed. The patients' initials, temperatures during the height of the fever, duration of fevers over 101.0° F. and diagnosis of each disease are given in Table II. Hereafter, patients will be referred to only by initials.

2. Preparations for Widal Tests-- Approximately 5 cc. of blood was drawn aseptically with a sterile syringe from a vein. After the blood had clotted, it was centrifuged and the serum which had separated was removed. As the sera were placed in sterile tubes and refrigerated, it was possible

to keep them for long periods of time.

Twenty-four hour cultures of rejuvenated organisms were used for the Widal Tests. Eberthella typhosa (Zopf) Weidin (flagellar), Eb. typhosa (New York State Lab.) (somatic), Salmonella paratyphi (Kayser) Bergy et al. and S. schottmulleri (Winslow et al.) Bergy et al. were cultured in standard bouillon.

Hereafter Eberthella typhosa (flagellar), Eb. typhosa (somatic), Salmonella paratyphi and S. schottmulleri shall be referred to in the tables as Eb. typhosa (flagellar), Eb. typhosa (somatic), S. paratyphi, and S. schottmulleri, respectively.

The glass slides used were ordinary microscope slides. Three three-fourths inch rings were placed on each slide. It was necessary to use four slides for each serum. The first row of rings was used for the 1:40 dilution and the second row for the 1:80 dilution. Two rings, one of each dilution, were used for each organism and the controls. To clean the slides they were rubbed with Bon Ami, dried and wiped off with a clean cloth.

3. Technic used for Widal Tests-- A white-blood counting pipette was used for making the dilutions of the sera. The serum was drawn to the mark 0.5 of the pipette; then physiological saline was drawn to the mark 11. This gave a dilution of 1:20. One loopful of the diluted serum mixed with one loopful of a bouillon culture of Eb. typhosa gave a dilution of 1:40. One loopful of the 1:20 diluted serum to three loopfuls of the bacterial cultures gave a dilution of 1:80. These dilutions were

made in the vaselined rings. Cover glasses were placed on these and they were incubated at 37° for one hour before being examined with the high power dry objective of the microscope. A second reading was taken at the end of two hours. This same procedure was used for the Eb. typhosa (somatic), S. paratyphi, S. schottmuelleri, and the controls. The 1:40 control consisted of one loopful of serum to one loopful of physiological saline. The 1:80 control consisted of one loopful of serum to three loopfuls of physiological saline.

The tests were interpreted by the amount of clumping or agglutination that took place. The amount of clumping was estimated as + (complete),  $\frac{+}{2}$  (partial),  $\frac{+}{4}$  (very slight), and 0 (no clumping).

#### B. Experimental Data.

Of the 27 sera tested only that from one case (B. L.D.) gave a positive Widal reaction with Eb. typhosa in the 1:40 dilutions, but not in the 1:80; therefore, this result could not be interpreted as wholly positive. This same serum gave partially positive results with S. schottmuelleri and Eb. typhosa (somatic). There was one serum (9. B.W.) that agglutinated both S. paratyphi and S. schottmuelleri. All of the rest of the sera were entirely negative. The Widal tests are recorded in Table I. The histories corresponding to the numbers and initials of Table I may be found in Table II.

It was planned in each case to take serum and run a Widal test during the height of the fever and after recovery, but both patients that gave

positive tests died before the fever subsided. No patient included in this study gave a history of having had typhoid fever or of having been vaccinated against it.



Table I.

| Time           | Dilution | <i>Eb. typhosa</i><br>(flagellar) | <i>S. para-</i><br><i>typhi</i> | <i>S. schott-</i><br><i>muelleri</i> | <i>Eb. typhosa</i><br>(somatic) | Control |
|----------------|----------|-----------------------------------|---------------------------------|--------------------------------------|---------------------------------|---------|
| 1st Hour       | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour       | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 1. T. N. 105.° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour       | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour       | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2. N. T. 102.° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour       | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour       | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 3. K. G. 103.° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour       | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour       | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |

4. K. G. 106.°

Table I - (continued)

| Time            | Dilution | <i>Eb. typhosa</i><br>(flagellar) | <i>S. para-</i><br><i>typhi</i> | <i>S. schott-</i><br><i>muelleri</i> | <i>Eb. typhosa</i><br>(somatic) | Control |
|-----------------|----------|-----------------------------------|---------------------------------|--------------------------------------|---------------------------------|---------|
| 1st Hour        | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                 | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour        | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                 | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 5. E. T. 103.°  |          |                                   |                                 |                                      |                                 |         |
| 1st Hour        | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                 | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour        | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                 | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 6. G. C. 108.2° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour        | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                 | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour        | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                 | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 7. G. J. 104.°  |          |                                   |                                 |                                      |                                 |         |
| 1st Hour        | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                 | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour        | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                 | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |

9. B. W. 105.°

Table I - (continued)

| Time             | Dilution | <i>Eb. typhosa</i><br>(flagellar) | <i>S. para-</i><br><i>typhi</i> | <i>S. schott-</i><br><i>muelleri</i> | <i>Eb. typhosa</i><br>(somatic) | Control |
|------------------|----------|-----------------------------------|---------------------------------|--------------------------------------|---------------------------------|---------|
| 1st Hour         | 1:40     | $\frac{+}{=}$                     | 0                               | 0                                    | $\frac{+}{=}$                   | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | $\frac{+}{=}$                     | 0                               | $\frac{+}{=}$                        | +                               | 0       |
|                  | 1:80     | 0                                 | 0                               | $\frac{+}{=}$                        | 0                               | 0       |
| 8. L. D. 103.8°  |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 10. E. S. 103.2° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 11. H. P. 104.°  |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |

20. O. J. 101.8°



Table I - (continued)

| Time             | Dilution | <i>Eb. typhosa</i><br>(flagellar) | <i>S. para-</i><br><i>typhi</i> | <i>S. schott-</i><br><i>muelleri</i> | <i>Eb. typhosa</i><br>(somatic) | Control |
|------------------|----------|-----------------------------------|---------------------------------|--------------------------------------|---------------------------------|---------|
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 21. D. C. 103.°  |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 22. J. B. 103.4° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 23. F. B. 102.°  |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |

12. J. S. 105.°

Table I - (continued)

| Time             | Dilution | <i>Eb. typhosa</i><br>(flagellar) | <i>S. para-</i><br><i>typhi</i> | <i>S. schott-</i><br><i>muelleri</i> | <i>Eb. typhosa</i><br>(somatic) | Control |
|------------------|----------|-----------------------------------|---------------------------------|--------------------------------------|---------------------------------|---------|
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 13. O. E. 104.2° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 14. G. D. 102.°  |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 15. J. R. 103.8° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 17. M. B. 105.°  |          |                                   |                                 |                                      |                                 |         |

Table I - (continued)

| Time             | Dilution | <i>Eb. typhosa</i><br>(flagellar) | <i>S. para-</i><br><i>typhi</i> | <i>S. schott-</i><br><i>muelleri</i> | <i>Eb. typhosa</i><br>(somatic) | Control |
|------------------|----------|-----------------------------------|---------------------------------|--------------------------------------|---------------------------------|---------|
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 18. W. S. 102.8° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 16. C. C. 103.2° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 19. R. N. 103.6° |          |                                   |                                 |                                      |                                 |         |
| 1st Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |
| 2nd Hour         | 1:40     | 0                                 | 0                               | 0                                    | 0                               | 0       |
|                  | 1:80     | 0                                 | 0                               | 0                                    | 0                               | 0       |

24. J. R. 102.4°



Table I - (continued)

| Time             | Dilution | Eb. typhosa<br>(flagellar) | S. para-<br>typhi | S. schott-<br>muelleri | Eb. typhosa<br>(somatic) | Control |
|------------------|----------|----------------------------|-------------------|------------------------|--------------------------|---------|
| 1st Hour         | 1:40     | 0                          | 0                 | 0                      | 0                        | 0       |
|                  | 1:80     | 0                          | 0                 | 0                      | 0                        | 0       |
| 2nd Hour         | 1:40     | 0                          | 0                 | 0                      | 0                        | 0       |
|                  | 1:80     | 0                          | 0                 | 0                      | 0                        | 0       |
| 25. C. T. 102.2° |          |                            |                   |                        |                          |         |
| 1st Hour         | 1:40     | 0                          | 0                 | 0                      | 0                        | 0       |
|                  | 1:80     | 0                          | 0                 | 0                      | 0                        | 0       |
| 2nd Hour         | 1:40     | 0                          | 0                 | 0                      | 0                        | 0       |
|                  | 1:80     | 0                          | 0                 | 0                      | 0                        | 0       |
| 26. M. K. 101.°  |          |                            |                   |                        |                          |         |
| 1st Hour         | 1:40     | 0                          | 0                 | 0                      | 0                        | 0       |
|                  | 1:80     | 0                          | 0                 | 0                      | 0                        | 0       |
| 2nd Hour         | 1:40     | 0                          | 0                 | 0                      | 0                        | 0       |
|                  | 1:80     | 0                          | 0                 | 0                      | 0                        | 0       |

27. C. T. 105.°

Legend for Table I

Complete agglutination +  
 Partial agglutination †  
 Very slight agglutination ‡  
 No agglutination 0

Table II.

| Patient | Diagnosis                                | Height of fever | Duration of fever over 101.0° | Outcome of Illness |
|---------|--|-----------------|-------------------------------|--------------------|
| 1.T.N.  | Fractured Skull                          | 105.°           | 3 days                        | Expired            |
| 2.N.T.  | Scarlet Fever                            | 102.°           | 4 days                        | Recovered          |
| 3.K.G.  | Staphylococccic Septicaemia              | 103.°           | 14 days                       | Expired            |
| 4.K.G.  | Staphylococccic Septicaemia              | 106.°           | 14 days                       | Expired            |
| 5.E.T.  | Generalized Peritonitis                  | 103.°           | 3 days                        | Expired            |
| 6.G.C.  | Non-infectious complications             | 108.2°          | 3 days                        | Expired            |
| 7.G.J.  | Streptococccic Septicaemia               | 104.°           | 21 days                       | Recovered          |
| 8.L.D.  | Ruptured Appendix                        | 103.8°          | 2 days                        | Expired            |
| 9.B.W.  | Ruptured Spleen                          | 105.°           | 2 days                        | Expired            |
| 10.E.S. | Erysipelas                               | 103.2°          | 3 days                        | Recovered          |
| 11.H.P. | Streptococccic Infection of Right Hand   | 104.°           | 5 days                        | Recovered          |
| 12.J.S. | Cholecystectomy                          | 105.°           | 2 days                        | Expired            |
| 13.O.E. | Staphylococccic Infection from Carbuncle | 104.2°          | 2 days                        | Expired            |
| 14.G.D. | Influenza                                | 103.°           | Unknown                       | Recovered          |
| 15.J.R. | Fractured Legs                           | 103.8°          | 4 days                        | Recovered          |
| 16.C.C. | Puerperal Infection                      | 103.2°          | 27 days                       | Recovered          |
| 17.M.B. | Pneumonia                                | 105.°           | 3 days                        | Recovered          |
| 18.W.S. | Appendectomy                             | 102.8°          | 14 days                       | Recovered          |
| 19.R.N. | Parturition                              | 103.6°          | 3 days                        | Expired            |
| 20.O.J. | Streptococccic Infection of Arm          | 104.4°          | 14 days                       | Recovered          |
| 21.D.C. | Tuberculosis                             | 103.°           | 6 days                        | Expired            |
| 22.J.B. | Appendectomy, Generalized Peritonitis    | 103.4°          | 4 days                        | Expired            |
| 23.F.B. | Tubal Pregnancy                          | 102.°           | 5 days                        | Recovered          |
| 24.J.R. | Peptic Ulcer                             | 102.4°          | 2 days                        | Recovered          |
| 25.C.T. | Influenza                                | 102.2°          | 2 days                        | Recovered          |
| 26.M.K. | Streptococccic Infection of Right Hand   | 101.°           | 1 day                         | Recovered          |
| 27.C.T. | Diabetic Gangrene                        | 105.°           | 4 days                        | Expired            |



### C. Discussion

Cases often came to the hospital complaining of very little but high fever, in which the clinical diagnosis was not clear. Routine diagnostic tests including agglutination tests for typhoid fever were usually done to either diagnose or rule out certain diseases. It has been found in these cases that the Widal test was very often positive or partially positive in the acute fever stage; but the case proved later to be something other than typhoid fever. The sera from these cases had been checked by the State Hygienic Laboratory, Helena, Montana, and the same results obtained. The question arose as to whether or not the Widal test was specific for Eb. typhosa, S. paratyphi, and S. schottmuelleri.

As may be seen from Table II, the sera were collected from a wide range of diseases. Of all the cases no patient gave a history of having had typhoid fever, or of having been vaccinated against it. This rules out the possibility of the two partially positive results being due to the O agglutinins. However, there is one point concerning which the author had no clue; that is, whether or not the two patients that did have partially positive reactions, would have given positive or negative Widal tests before their last illnesses.

Agglutinins are chemical products of the process of immunization, occurring in the blood serum and causing the homologous cells with which it is brought into contact to coalesce into floccules. These may be stimulated (a) by the ciliated part or motile cells (flagellar) or, (b) from the body of the cells (somatic). Both kinds may coexist in a given serum.

The flagellar types are called H, and the somatic O. Both H and O agglutinins may be found in the sera of individuals who have received typhoid-paratyphoid vaccine. The same individuals may not have had O agglutinins prior to vaccination. It seems apparent that O agglutination can and does take place under conditions other than following typhoid infection. It seems, therefore, that the diagnosis of typhoid fever cannot be made simply upon the demonstration of the presence of O agglutinins in the patients' serum.

Topley (17) holds that a case may be made for this method if a sufficiently higher titre of agglutination can be set as a standard. Such a test would demand; first, the use of a rigidly standardized antigen for both qualitative and quantitative principles, and, second, a titre high enough to rule out completely the "positive" findings of a non-specific origin.

The level which they proposed as diagnostic is 1:100, but from Kemp's (14) study it seems that this would allow the test to be of value only after several weeks of illness. Then, too, several of his students who were vaccinated developed O agglutinins for the growth-phase antigen to even higher titres than 1:100. For this reason the possibility of the serum of a previously vaccinated individual developing a high titre of agglutinins to an infection other than to that of Eb. typhosa must not be overlooked.

Obtaining only two positive sera, only one of which reacted with Eb. typhosa, does not prove very much; but it does show that non-specific agglutinins do occur in patients having high fevers, which apparently were not due to any organism even remotely related to Eb. typhosa. Though the

cases are few, a positive Widal may be a serious prognostic sign, as both patients died.

Part II. Agglutination of Certain Water-borne Organisms with Anti-typhoid Sera.

A. Materials and Methods.

1. Cultures of Organisms

The organisms used were obtained from Miss Lucille Hutchins, who had isolated and identified them from sewage collected in and about Bozeman, Montana. There was one exception. Eberthella typhosa Berg. et al (Bender strain), which was used for making the typhoid vaccine and as a control, was obtained from the Montana State Hygienic Laboratory, at Helena, Montana. The other organisms used were:

Escherichia coli (Migula) Cast. et Chalm.

" paragruenthami Cast. et Chalm.

" pseudocoloides Cast. et Chalm.

" anindilca Berg. et al.

" enterica (Cast.) Weld.

" vesiculiformans (Henrici) Berg. et al.

Aerobacter levans (Wolffin) Berg. et al.

" aerogenes (Kruse) Beijerinck

Proteus bombycis Berg. et al.

These organisms were sub-cultured on lactose agar slants for several days to decrease their auto-agglutinability, and were then mass cultured for

twenty-four hours on large slants in Erlenmeyer flasks. Physiological saline suspensions were then made from these cultures.

## 2. Preparation of Vaccine

Eberthella typhosa (Bender strain) was sub-cultured on agar slants for five days and then mass cultured for twenty-four hours on large slants in Erlenmeyer flasks and harvested. To harvest, 10 cc. of physiological saline solution was added to the first slant with a sterile pipette. This was mixed well until almost all the growth was rubbed off of the slant. The suspension was expelled into a sterile vaccine bottle held with its mouth in the flame. The suspension from the second slant was expelled into the same vaccine bottle. The bottle was shaken well and placed in a water bath for one hour at 65° C. When it was removed from the water bath it was plunged into cold running water. A drop of trikerosol was placed on the stopper and by aid of a sterile needle inserted through the rubber cap, 0.25% of trikerosol was added in order to insure sterility. About 1 cc. was removed with a sterile syringe, placed on an agar slant and incubated at 37° C. for forty-eight hours; but there was no growth. The vaccine was placed in the refrigerator and shaken twice daily for three days. It should be mentioned that clean glass beads were placed in the vaccine bottles before sterilization. These aid in breaking up any clumps of bacteria in the emulsion.

The vaccine was standardized by the haemocytometer method. A red-blood counting pipette was used. The vaccine was drawn up to the 0.5 mark and the diluting fluid to the 101 mark. The diluting fluid was a filtered

1 : 1000 dilution of mercuric chloride, which contained just enough acid fuchsin to make it a cherry red. The fuchsin tinted the organism and made it much easier to count. After the counting chamber was filled it was allowed to settle for ten minutes before counting. It was counted the same as a red-blood count. It was found that there were 7,472,000,000 bacteria per cc. This vaccine was too potent for the first doses, so it was diluted to 750,000,000 per cc. To get this dilution, 1 cc. of vaccine and 9 cc. of sterile physiological saline were added with a sterile syringe to another sterile vaccine bottle.

### 3: Immunization of Rabbits

With the vaccine all prepared, inoculations of rabbits were started October 29th. Four very large rabbits were used. Only one rabbit was inoculated the first day to see if the vaccine was still too strong. The inoculation was intraperitoneally. The first day there were no observable reactions. The second day there seemed to be listlessness, and loss of appetite. On this same day the other three rabbits were inoculated intraperitoneally. These first doses were only 0.1 cc. which was approximately 75,000,000 bacteria.

The following injections were made at five-day intervals. The second inoculation was 0.4 cc. which was approximately 300,000,000 bacteria. There did not seem to be any unfavorable reactions in the rabbits. The third inoculation was 1 cc. (750,000,000 bacteria). The second day, rabbit No. IV became sick and was not used further. The three succeeding inoculations were also of 1 cc. Five days after the last inoculation, blood was

drawn aseptically from each rabbit from the large vein in the ear. Each tube of blood was centrifuged, the supernatant serum pipetted into sterile vials and placed in the refrigerator.

The serum was titrated with the homologous organisms in a dilution of over 1:7,820. This indicated the titre of the serum was high enough to kill the rabbits. They were etherized and bled from the heart and the serum collected.

#### 4. Preparations for Agglutination Reactions

The typhoid organism used for titration of the serum was prepared by making sub-cultures daily for ten days and then making a twenty-four hour mass culture on agar slants. The agar culture was harvested in the same manner as for the vaccine. To the suspension in the vaccine bottle 0.1% formalin was added and shaken well and then placed in the refrigerator for three days.

Dilutions of serum from each rabbit were made up in large sterile test tubes and kept in the refrigerator because these same dilutions were used for all organisms. This necessitated a large number of sterile capillary pipettes for the macroscopic tests. Sterile inoculating loops were used for the microscopic tests.

These dilutions were made by adding 9 cc. of sterile physiological saline solution to 1 cc. of serum. Then 5 cc. of the first tube was added to 5 cc. of the salt in the second tube, and 5 cc. of this mixture to the third, and so on, until the dilutions ranged from 1:10 to 1:10,240.

For the microscopic test, one loopful of the diluted serum and one loopful of a suspension of killed Eb. typhosa were mixed on a cover

glass and inverted over a glycerined hanging drop slide. A drop of water was placed in the concave part of the slide to prevent dessication of the mixture. The slide was set aside at room temperature for twenty minutes before examination with the microscope under a 4 mm. dry objective. If there was only partial or no agglutination in twenty minutes, it was set aside for one hour longer and then examined. There were controls in every instance; that is, one was prepared with bacterial suspension alone and one with bacterial suspension along with the serum which was being tested.

For the macroscopic method small test tubes (1/8" x 3") were used. The dilutions were the same ones used in the microscopic method. The test tubes were set up in series and an equal amount of emulsion of the killed organisms added to each, including the control. Then an equal amount of each dilution of serum was added respectively and shaken. After four hours they were examined for the clearing up of the bacterial suspensions. If the serum in a certain dilution agglutinated, the clumps gravitated to the bottom and the upper part became clear. If there was no agglutination in four hours, they were set aside for eight hours and sixteen hours longer respectively.

#### B. Experimental Date.

The agglutination reactions were carried out both microscopically and macroscopically. All agglutination tests were conducted at room temperature which was about 82° F. Almost all through the tests the microscopic and macroscopic methods exactly agreed with each other. (Table III).

It was found that the serum from rabbit No. I would agglutinate Eberthella typhosa at 1:3840 dilution. The serum from rabbit No. II was still of a higher titre, agglutinating at 1:5120 dilution. The serum of rabbit No. III agglutinated the homologous organisms at 1:7680 dilution.

When these titres were found, the following organisms were sub-cultured daily for ten days, to increase their agglutinability and reduce their auto-agglutinability: Escherichia coli, Es. paratyphosa, Es. pseudocoloides, Es. anindilca, Es. enterice, Es. vesiculiformans, Aerobacter levans, Aero. aerogenes, and Proteus bombycis. They were harvested from twenty-four hour mass cultures with exactly the same procedure as for Eb. typhosa except that lactose agar was used.\* The results of their agglutinability with anti-typhoid sera are seen in Table III.

Es. vesiculiformans agglutinated only after 16 hours in the 1:10 dilution, so it has been omitted from the tables. Aero. aerogenes and Proteus bombycis were entirely negative, so they likewise have been omitted.

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\*Miss Hutchins, who identified the organisms, found them to grow more readily on lactose agar.



Table III  
Serum No. I

| Tube | Dilution        | Eb.typhosa |    |       | Es. coli |    |       | Aero.levans |    |       | Es.paragruen-<br>thali |    |       | Es. anino-<br>dilca |    |       | Es.enterica |    |       | Es. pseudo-<br>coloides |    |       |
|------|-----------------|------------|----|-------|----------|----|-------|-------------|----|-------|------------------------|----|-------|---------------------|----|-------|-------------|----|-------|-------------------------|----|-------|
|      |                 | Micro      |    | Macro | Micro    |    | Macro | Micro       |    | Macro | Micro                  |    | Macro | Micro               |    | Macro | Micro       |    | Macro | Micro                   |    | Macro |
|      |                 | 20         | 60 | 240   | 20       | 60 | 240   | 20          | 60 | 240   | 20                     | 60 | 240   | 20                  | 60 | 240   | 20          | 60 | 240   | 20                      | 60 | 480   |
| 1    | Control<br>1-10 | 0          | 0  | 0     | 0        | 0  | 0     | 0           | 0  | 0     | 0                      | 0  | 0     | 0                   | 0  | 0     | 0           | 0  | 0     | 0                       | 0  | 0     |
|      |                 | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                      | +  | +     | +                   | +  | +     | +           | +  | +     | +                       | +  | +     |
| 2    | 1-20            | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                      | +  | +     | +                   | +  | +     | +           | +  | +     | 0                       | 0  | 0     |
| 3    | 1-40            | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                      | +  | +     | +                   | +  | +     | +           | +  | +     |                         |    |       |
| 4    | 1-80            | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                      | +  | +     | +                   | +  | +     | +           | +  | +     |                         |    |       |
| 5    | 1-160           | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                      | +  | +     | +                   | +  | +     | 0           | +  | +     |                         |    |       |
| 6    | 1-320           | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                      | +  | +     | 0                   | +  | +     | 0           | 0  | +     |                         |    |       |
| 7    | 1-640           | +          | +  | +     | +        | +  | +     | +           | +  | +     | 0                      | +  | +     | 0                   | 0  | +     |             |    | 0     |                         |    |       |
| 8    | 1-1280          | +          | +  | +     | +        | +  | +     | 0           | 0  | 0     | 0                      | 0  | 0     |                     |    |       |             |    |       |                         |    |       |
| 9    | 1-2560          | +          | +  | +     | +        | +  | +     |             |    |       |                        |    |       |                     |    |       |             |    |       |                         |    |       |
| 10   | 1-3840          | +          | +  | +     | 0        | 0  | 0     |             |    |       |                        |    |       |                     |    |       |             |    |       |                         |    |       |
| 11   | 1-5120          | 0          | 0  | 0     | 0        | 0  | 0     |             |    |       |                        |    |       |                     |    |       |             |    |       |                         |    |       |
| 12   | 1-7680          | 0          | 0  | 0     | 0        | 0  | 0     |             |    |       |                        |    |       |                     |    |       |             |    |       |                         |    |       |

Legend: Positive agglutination +  
 Negative agglutination -  
 Partial agglutination ±  
 Very slight agglutination ≡

Table III - (continued)  
Serum No. II

| Tube | Dilution        | Eb.typhosa |       |     | Es. coli |       |     | Aero.levans |       |     | Es. paragru-<br>enthalii |       |     | Es. anino-<br>dilca |       |     | Es. enterica |       |     | Es. pseudo-<br>coloides |       |     |
|------|-----------------|------------|-------|-----|----------|-------|-----|-------------|-------|-----|--------------------------|-------|-----|---------------------|-------|-----|--------------|-------|-----|-------------------------|-------|-----|
|      |                 | Micro      | Macro |     | Micro    | Macro |     | Micro       | Macro |     | Micro                    | Macro |     | Micro               | Macro |     | Micro        | Macro |     | Micro                   | Macro |     |
|      |                 | 20         | 60    | 240 | 20       | 60    | 240 | 20          | 60    | 240 | 20                       | 60    | 240 | 20                  | 60    | 240 | 20           | 60    | 240 | 20                      | 60    | 480 |
| 1    | Control<br>1-10 | 0          | 0     | 0   | 0        | 0     | 0   | 0           | 0     | 0   | 0                        | 0     | 0   | 0                   | 0     | 0   | 0            | 0     | 0   | 0                       | 0     | 0   |
| 2    | 1-20            | +          | +     | +   | +        | +     | +   | +           | +     | +   | +                        | +     | +   | +                   | +     | +   | +            | +     | +   | 0                       | 0     | 0   |
| 3    | 1-40            | +          | +     | +   | +        | +     | +   | +           | +     | +   | +                        | +     | +   | +                   | +     | +   | +            | +     | +   |                         |       |     |
| 4    | 1-80            | +          | +     | +   | +        | +     | +   | +           | +     | +   | +                        | +     | +   | +                   | +     | +   | +            | +     | +   |                         |       |     |
| 5    | 1-160           | +          | +     | +   | +        | +     | +   | +           | +     | +   | +                        | +     | +   | +                   | +     | +   | 0            | +     | +   |                         |       |     |
| 6    | 1-320           | +          | +     | +   | +        | +     | +   | +           | +     | +   | +                        | +     | +   | +                   | +     | +   | 0            | 0     | ±   |                         |       |     |
| 7    | 1-640           | +          | +     | +   | +        | +     | +   | ±           | +     | +   | 0                        | +     | +   | 0                   | +     | +   |              |       | 0   |                         |       |     |
| 8    | 1-1280          | +          | +     | +   | +        | +     | +   | 0           | 0     | 0   | 0                        | 0     | 0   | 0                   | 0     | 0   |              |       |     |                         |       |     |
| 9    | 1-2560          | +          | +     | +   | ±        | +     | +   |             |       |     |                          |       |     |                     |       |     |              |       |     |                         |       |     |
| 10   | 1-3840          | +          | +     | +   | ±        | +     | 0   |             |       |     |                          |       |     |                     |       |     |              |       |     |                         |       |     |
| 11   | 1-5120          | +          | +     | +   | 0        | 0     | 0   |             |       |     |                          |       |     |                     |       |     |              |       |     |                         |       |     |
| 12   | 1-7680          | 0          | ±     | 0   | 0        | 0     | 0   |             |       |     |                          |       |     |                     |       |     |              |       |     |                         |       |     |
| 13   | 1-10,240        | 0          | 0     | 0   | 0        | 0     | 0   |             |       |     |                          |       |     |                     |       |     |              |       |     |                         |       |     |

Legend: Positive agglutination +  
 Negative agglutination -  
 Partial agglutination ±  
 Very slight agglutination =

Table III - (continued)  
Serum No. III

| Tube | Dilution | Eb.typhosa |    |       | Es. coli |    |       | Aero.levans |    |       | Es. paragu-<br>enthalii |    |       | Es. anino-<br>dilca |    |       | Es. enterica |    |       | Es. pseudo-<br>coloides |    |       |
|------|----------|------------|----|-------|----------|----|-------|-------------|----|-------|-------------------------|----|-------|---------------------|----|-------|--------------|----|-------|-------------------------|----|-------|
|      |          | Micro      |    | Macro | Micro    |    | Macro | Micro       |    | Macro | Micro                   |    | Macro | Micro               |    | Macro | Micro        |    | Macro | Micro                   |    | Macro |
|      |          | 20         | 60 | 240   | 20       | 60 | 240   | 20          | 60 | 240   | 20                      | 60 | 240   | 20                  | 60 | 240   | 20           | 60 | 240   | 20                      | 60 | 480   |
|      | Control  | 0          | 0  | 0     | 0        | 0  | 0     | 0           | 0  | 0     | 0                       | 0  | 0     | 0                   | 0  | 0     | 0            | 0  | 0     | 0                       | 0  | 0     |
| 1    | 1-10     | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                       | +  | +     | +                   | +  | +     | +            | +  | +     | 0                       | +  | +     |
| 2    | 1-20     | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                       | +  | +     | +                   | +  | +     | +            | +  | +     | 0                       | 0  | 0     |
| 3    | 1-40     | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                       | +  | +     | +                   | +  | +     | +            | +  | +     |                         |    |       |
| 4    | 1-80     | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                       | +  | +     | +                   | +  | +     | +            | +  | +     |                         |    |       |
| 5    | 1-160    | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                       | +  | +     | +                   | +  | +     | +            | +  | +     |                         |    |       |
| 6    | 1-320    | +          | +  | +     | +        | +  | +     | +           | +  | +     | +                       | +  | +     | +                   | +  | +     | 0            | +  | +     |                         |    |       |
| 7    | 1-640    | +          | +  | +     | +        | +  | +     | +           | +  | +     | 0                       | +  | +     | ±                   | +  | +     | 0            | 0  | 0     |                         |    |       |
| 8    | 1-1280   | +          | +  | +     | +        | +  | +     | 0           | +  | +     | 0                       | 0  | 0     | 0                   | 0  | 0     |              |    |       |                         |    |       |
| 9    | 1-2560   | +          | +  | +     | +        | +  | +     | 0           | 0  | 0     |                         |    |       |                     |    |       |              |    |       |                         |    |       |
| 10   | 1-3840   | +          | +  | +     | +        | +  | +     |             |    |       |                         |    |       |                     |    |       |              |    |       |                         |    |       |
| 11   | 1-5120   | +          | +  | +     | ±        | +  | +     |             |    |       |                         |    |       |                     |    |       |              |    |       |                         |    |       |
| 12   | 1-7680   | 0          | +  | +     | 0        | 0  | 0     |             |    |       |                         |    |       |                     |    |       |              |    |       |                         |    |       |
| 13   | 1-10,240 | 0          | 0  | 0     | 0        | 0  | 0     |             |    |       |                         |    |       |                     |    |       |              |    |       |                         |    |       |

Legend: Positive agglutination +  
 Negative agglutination -  
 Partial agglutination ±  
 Very slight agglutination  $\frac{1}{2}$

### C. Discussion

According to their agglutinability with the anti-typhoid sera, Es. coli and Eb. typhosa seem very closely related. It was found that both agglutinated with all three sera in correspondingly high titres. The potency of the three sera may be seen from Table III. This fact was of some significance in finding the titre for Eb. typhosa, Es. coli, and Aero. levans, as Serum No. III agglutinated at double the dilution of Serum No. I. However, Serum No. III did not show this difference in reaction with the other organisms when compared with Serum No. I.

Es. vesiculiformans, Aero. aerogenes, and Proteus bombycis are not agglutinated with anti Eb. typhosa serum, so they are not mentioned in Table III.

Bergey (1) classified the Escherichia, Aerobacter, and Eberthella according to their ability to form gas. According to Bergey (1) the three genera ferment dextrose with production of acid or acid and gas. Escherichia and Aerobacter form gas from lactose and dextrose but are differentiated from each other only by their ability to form acetyl-methyl-carbinol. According to Bergey's (1) study, the genus Eberthella is not very closely related to Escherichia. Organisms belonging to the genus Eberthella forms acid but no gas from dextrose.

The author has not found any investigations reported on serological studies in relation to the phylogeny of the specific organisms used in this study. Thus the question arises as to whether or not the ability to form gas is as important as Bergey (1) considers it to be. According to

their agglutination reactions Escherichia and Eberthella are more closely related (Table III), than Escherichia and Aerobacter.

Mez, as reported by Gortner (11), made a very extensive study of immunological reactions from the proteins of plant tissues. The studies of Mez represent the phylogenetic relations of plant species and genera, as indicated by serologic reactions. Gortner (11) has found that the phylogenetic relationships shown in Mez's charts are in most instances similar to or identical with those generally accepted by taxonomists. But, in some instances, the serum-diagnostic method of Mez indicates relationships different from those usually accepted. Gortner (11) strongly believes that the charts shown by Mez may well express the true phylogenetic relationships and accordingly it would seem that serum diagnosis may be used in questions of disputed phylogeny.

It seems equally probably that the agglutinability of bacteria with an anti-serum may be just as true a way to differentiate genera as the physiological methods that Bergey (1) has used. Table III indicates that perhaps certain organisms used therein should be placed in different genera from those given them by Bergey. However, the author feels that insufficient research has been done to make any definite conclusions concerning phylogeny of bacteria by serological methods. Every species and genus of the family Bacteriaceae should be tested for their serological reactions with Eb. typhosa and with each other before any complete classification could be made. Even were this done, these methods might be too inconvenient to use for practical differentiations.



According to Ishii (12) Malvoz in 1897 claimed that formalin produced chemical agglutination of Eb. typhosa, but not of Es. coli. But since, as pointed out by Ishii (12), Beco, Remy, Widal, and Noblecourt were unable to confirm Malvoz's observations, the writer used formalin in one-tenth percentage amounts for the killed culture of each organism. This prevents spontaneous agglutination and increases specific agglutination.

Standard agar with a pH 6.8-7.0 was used for the preparation of the vaccine, but lactose agar was used for all the killed cultures. Lactose agar seems to induce a much heavier growth in 24 hours than standard agar. Whether the lactose in the media increased agglutination, the author can not say as no experimental work on this point was done. However, Ishii (12) claims that glucose decreases the agglutinability of Eb. typhosa and shows a tendency toward spontaneous agglutination of Es. coli.

## SUMMARY AND CONCLUSIONS

### Part I.

1. Non-specific agglutinins reacting with Eb. typhosa do occur in sera from some patients having high fevers which apparently were not due to any organism even remotely related to Eb. typhosa.

2. Among 27 sera examined only one serum gave a positive Widal reaction with Eb. typhosa (flagellar). Two gave positive reactions with S. schottmuelleri, and one of these was positive to S. paratyphi, and the other positive to Eb. typhosa (somatic).

3. Positive agglutination of non-specific high fever sera with Eb. typhosa may be an unfavorable prognosis.

### Part II.

The results of these experiments indicate that a relationship exists between the typhoid organism and certain species found in polluted water as indicated by agglutination. That is, these organisms have affinities for a common immune serum, but these affinities exist in different proportions in different species, thus giving agglutination with different degrees of dilution.

1. The genus Escherichia is more closely related to Eberthella than to Aerobacter according to its agglutination reactions.

2. Serological reactions may be more important as indicators of phylogenetic relationship than those physiological characters used as a basis for the classification made by Bergey. In any phylogenetic study

of these organisms their serological reactions should be considered along with the physiological, morphological, and other reactions.

3. Es. vesiculiformans, Aero. aerogenes, and Pro. bombycis are not agglutinated with anti-typhoid sera.

4. The microscopic and macroscopic tests correlated each other very closely.

5. According to the results of this study the organism described as Aerobacter levans should be placed in the genus Escherichia, and should be designated as Escherichia levans comb. new.



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