THE RELATION BETWEEN ELECTRICAL CHARGE
AND THE AGGLUTINATING ABILITY OF
SALMONELLA PULLORUM1,2

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INTRODUCTION

Attention has been repeatedly called to variations in the agglutinability of strains within a bacterial species. This has been especially true of Eberthella typhi. Some strains of this species have been encountered which were agglutinated so poorly that they could not be used in the Widal test. On the other hand, the extensive study of Ishii (1922b) shows many hyper- or spontaneously agglutinated strains. Zinsser (1923) considers it a common observation that no two strains of a species are exactly similar in their agglutinability. The various reviews of bacterial agglutination published, especially those of Arkwright (1914); Tulloch (1914); Hooker (1916); Buchanan (1919); Ishii (1922a and b); Morishima (1921) and Robinson (1923) make another summary of the literature superfluous. Therefore, reference will be made only to the literature which deals with Salmonella pullorum.

Extreme variations in the agglutinability of strains of Salmonella pullorum, as observed by the senior author, led to the investigation reported in this paper. Recently several investigators have reported similar observations. Mallman (1925) found that

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strains vary in their ability to be agglutinated and questioned the accuracy of the agglutination test unless strains of consistently high titre are used. May and Goodner (1926), after finding considerable variations in agglutinability of strains, concluded that "it seems advisable to select highly agglutinating strains for the routine test for ovarian infection in adult fowls." Gwatkin (1925) has also found that all strains are not agglutinated equally well. It is, therefore, apparent that strains of the species Salmonella pullorum may show as much variation in agglutinability as those of Eberthella typhi.

It is well to call attention to the fact that the environmental conditions under which bacteria are grown and antigens are produced play an important part in their agglutinability. A strain may vary considerably in its agglutinability when grown on media of different hydrogen ion concentration or sugar content (Ishii (1922a)). The kind and concentration of salts in the suspending menstruum play an extremely important rôle (Northrop and DeKruif (1922)). Therefore, before one can conclude that variations exist between strains of a species it must be shown that all environmental conditions are uniform. It seems that the variations reported, both for Eberthella typhi and Salmonella pullorum occurred under uniform environmental conditions. It is true that each group of investigators used a different combination of environmental conditions but consideration must be given to the fact that each supposedly maintained uniformity throughout his investigation.

Bordet's (1899) observation that bacteria are not agglutinated by immune serum unless salts are present placed the phenomenon of bacterial agglutination upon a colloidal basis. He showed that specific bacterial agglutination consists of two phases, first, the specific adsorption of agglutinin by the bacteria, and, second, the agglutination or precipitation of the new complex by the salt. Agglutinin, therefore, itself, does not cause agglutination, but when it is specifically adsorbed by the bacterial cells it forms an unstable complex which is precipitated by the action of the electrolyte of the suspending menstruum. This change of behavior resembles the change from a stable hydrophilic colloid
to a non-stable hydrophobic colloid which is sensitive to electrolytes.

Many investigators have found that colloids, when suspended in water, are electrically charged. It has also been shown that when placed in an electric field they migrate generally toward the anode, that is, that they are negatively charged. The stability of colloidal suspensions is dependent upon two forces, namely, repulsion and attraction. The mutual repulsion of the cells is due to like charges. On the other hand, surface tension which strives to reduce the free surface by bringing the particles together acts as the attractive force. Both forces are constantly at work, except at an absolute isoelectric point. So long as the repelling force is the greater, the particles remain in suspension but if the electric charge is reduced to a point or zone where the attractive force becomes the greater, precipitation or agglutination occurs.

It has been shown in the summaries of Winslow, Falk and Caulfield (1923) and Winslow and Upton (1926) that the relative charge carried by bacteria can be measured by the relative electrophoretic migration and that suspensions of bacteria as ordinarily grown are negatively charged. Bacteria remain in suspension because of the repelling action of the like electric charges which they carry, an action which opposes the attractive force, or, as Northrop and DeKruif (1922) prefer to say, the cohesive force. These investigators measured both the electrophoretic potential and the cohesive force and found that whenever the potential difference between the surface of the bacterium and the suspending menstruum was reduced to about 15 millivolts the bacteria agglutinated, provided that the cohesive force was not affected. If the cohesive force was reduced, the critical potential had to be lowered and if the cohesive force was made very small no agglutination occurred even if the potential was reduced to zero. They found that bacteria sensitized with immune serum had a constant cohesive force and, therefore, it seemed to us that any difference in the relative ability of strains to be agglutinated must be due to differences in the relative electrophoretic potential, practically to differences in relative electrophoretic velocity.
During the progress of our work Falk, Jacobson and Gussin (1925 a, b and c; 1926 a and b) reported upon a study of the relation between electrophoretic migration velocity, virulence, lethality and agglutinability of the groups of pneumococci. Their results suggest that electrophoretic potential is related in some fundamental manner to agglutinability since they found a sequence of decreasing agglutinability accompanied by a sequence of increasing electrophoretic potential.

OBJECTIVES AND GENERAL RESULTS OF THE PRESENT INVESTIGATION

The investigation reported here was begun during the summer of 1925 to determine, first, if strains of Salmonella pullorum vary with respect to their electrophoretic migration velocity, and, second, in case variations were found, to study the quantitative relationship to agglutinability.

Our results show that the species Salmonella pullorum bears a negative charge and it is clearly evident that great variations exist between strains so far as quantity of charge is concerned as evidenced by the differences in the rate of electrophoretic migration velocity. Variations were also found in the agglutinating ability of strains. In general, the sequence of decreasing electrophoretic migration velocity was identical with the sequence of increasing agglutinating ability, therefore, there appears to be a quantitative relation between the electrophoretic velocity and the agglutinating ability of strains of this species. This, we believe, explains at least in part, why some strains of a bacterial species are more easily agglutinated than others.

TECHNIQUE

Preparation of the suspension. Proteose-peptone agar adjusted to approximately pH 7.0 has been successfully and conveniently used for the cultivation of Salmonella pullorum by the senior author during the past several years and, therefore, was considered suitable for this investigation. In preparing this medium Difco proteose-peptone is substituted for ordinary pep-
ELECTRICAL CHARGE OF SALMONELLA PULLORUM

Shibley (1924) found that the charge carried by \textit{Esch. coli} varied until the culture was about eighteen hours old, it was deemed advisable to use a constant incubation period of twenty-four hours. The bacteria were grown upon slants at 37°C. The growth was removed with a small volume of 0.85 per cent NaCl by a gentle rotary movement of the culture tube. The suspension was then shaken with glass beads to break up clumps of bacteria. This suspension was filtered through cotton to remove flakes of agar, centrifuged at about 2500 revolutions per minute and the cells resuspended in 0.87 per cent NaCl. The washing was repeated three times to remove traces of peptone, etc., because DeKruif (1921) found that such impurities affected both the quantity of charge and the agglutinating ability. The final washed suspension was heated for one hour at 56° to 58°C. and standardized to a density which corresponded to approximately 3 cm. by the Gates (1920) method. The suspension thus prepared was used for electrophoretic measurements and agglutination tests, both being made the same day that the suspension was prepared.

\textit{Measurements of the electrophoretic migration.} The apparatus used in this investigation was similar to that devised by Northrop (1922) and of the type now being prepared by the Eimer and Amend Company of New York.

We found that the use of a trace of dye (methylene blue) in the zinc sulphate solution greatly facilitated the filling of the electrode chambers and prevented an excess of zinc sulphate being run into the electrophoresis cell as the colored solution could be seen within the stop-cocks with much greater ease than the colorless one. Before each sample was introduced into the apparatus, the cell was washed with at least one liter of distilled water in order to remove even the slightest amount of zinc sulphate which might have entered when the electrode chambers were filled. During preliminary experiments we found that unless the distilled water was removed completely with NaCl solution of the same concentration as that in which the bacteria were suspended, diffusion currents were produced which prevented the making of readings. Therefore, after the cell was
washed with distilled water it was flushed with about 200 cc. of 0.85 per cent NaCl and finally with about 50 cc. of the bacterial suspension.

A direct current of approximately 60 volts was applied, which gave a potential gradient of approximately 2.1 volts per centimeter. Observations were made with direct illumination and a magnification of 525 diameters. All measurements of electrophoretic velocity were made by timing with a stop-watch the migration of a given bacterium across a distance of 32 micra.

Preliminary readings, made in order to determine the depth of the cell and the level of maximum velocity taken at each one-sixteenth of the depth of the cell, when plotted, reproduced the Smoluchowski (1921) curve. This suggested that four readings be made between six-sixteenths and nine-sixteenths of the depth of the cell, each differing by one-sixteenth, because at these levels the migration was fastest. The advantage to be gained by making these four readings, rather than readings at a single level or at certain specific levels as others have done, lies in the fact that a greater confidence is had in the accuracy of the values obtained, for they can be plotted and it can be observed whether or not the curve obtained passes through a maximum. All velocities reported in this paper actually did this very thing. Readings were made upon 20 cells at each level and an average of the 80 readings are reported. The current was reversed after the fifth, tenth and fifteenth readings at each level. We believe that this system of reversing the current is of distinct advantage because even the slightest amount of drifting can be detected easily by unequal migrations in the two directions. The results of our investigation are expressed in terms of micra per second per volt per centimeter since the Helmholtz-Lamb equation used to convert electrophoretic migration velocity into potential difference is questionable as discussed by Winslow, Falk and Caulfield (1923).

Determination of the agglutinating ability. Antisera from two rabbits immunized with strain X-43 were used. The usual procedure for macroscopic agglutination tests was employed. The NaCl needed for making serum dilutions was taken from
the same lot as that used to prepare the bacterial suspensions. The serum was diluted to each dilution in large tubes and then pipetted out to the agglutination tubes so that all suspensions would be mixed with serum of exactly the same strength. Readings were made after forty-eight hours incubation at 37°C. The results are presented according to the method advocated by Hadley (1917).

Cultures. The 18 strains used were from widely separated sources and dates of isolation. Half of the strains were isolated by the senior author. A-40 and X-61 were from infected ovaries. X-48 was from the heart of a hen. X-57 was from the heart of a three-month-old chick. All other strains were from either the liver or heart of chicks. All strains were transferred daily for three days before they were used in this work.

EXPERIMENTAL RESULTS

Our first experiments, made to determine the experimental error, showed that almost identical average migration velocities could be obtained when several suspensions were prepared from a strain, as indicated in table 1. These suspensions were prepared as described above but from separate lots of agar slants. An inspection of the data shows that the average migration velocity of any suspension did not vary more than approximately 3 per cent from the average of all suspensions of that strain, thus confirming the accuracy of the method as reported by Falk and Reed (1926).

The results of both electrophoretic measurements and agglutination tests made upon 18 strains are summarized in table 2. The strains were grouped according to their agglutinabilities. In general, the greatest dilution of serum at which complete agglutination still occurred (titre) was: for group one, about 100; for group two, 800; for group three, 1600; for group four, 3200 and for the homologous strain X-43, 3200. The average electrophoretic migration velocities were: for group one, -1.578; for group two, -1.069; for group three, -0.955; for group four, -0.722 and for the homologous strain, -1.157. This indicates
TABLE 1

Average migration velocities (in micra per volt per centimeter) of repeated experiments

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>SAMPLE</th>
<th>VELOCITY</th>
<th>AVERAGE OF ALL STRAINS</th>
<th>VARIATIONS FROM THE AVERAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-40</td>
<td>a</td>
<td>-1.123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-40</td>
<td>b</td>
<td>-1.068</td>
<td>-1.095</td>
<td>0.027</td>
</tr>
<tr>
<td>X-43</td>
<td>a</td>
<td>-1.058</td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>X-43</td>
<td>b</td>
<td>-1.078</td>
<td>-1.050</td>
<td>0.028</td>
</tr>
<tr>
<td>X-43</td>
<td>c</td>
<td>-1.015</td>
<td></td>
<td>0.035</td>
</tr>
</tbody>
</table>

TABLE 2

Migration velocities and agglutination tests

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>AVERAGE MIGRATION VELOCITY*</th>
<th>AGGLUTINATION</th>
<th>SOURCE OF CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum dilutions</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9†</td>
<td></td>
</tr>
<tr>
<td>X-48</td>
<td>-1.714</td>
<td>3 1 0 0 0 0 0 0 0</td>
<td>Heart of hen</td>
</tr>
<tr>
<td>1-93</td>
<td>-1.280</td>
<td>4 3 1 0 0 0 0 0 0</td>
<td>Chick</td>
</tr>
<tr>
<td>X-57</td>
<td>-1.740</td>
<td>4 4 4 2 1 0 0 0 0</td>
<td>Heart of three-month-old chick</td>
</tr>
<tr>
<td>A-40</td>
<td>-1.176</td>
<td>4 4 4 4 3 2 1 0 0</td>
<td>Ovary</td>
</tr>
<tr>
<td>A-33</td>
<td>-1.138</td>
<td>4 4 4 4 3 2 0 0 0</td>
<td>Chick</td>
</tr>
<tr>
<td>X-61</td>
<td>-1.100</td>
<td>4 4 4 4 3 1 0 0 0</td>
<td>Ovary</td>
</tr>
<tr>
<td>B-64</td>
<td>-1.019</td>
<td>4 4 4 4 2 1 0 0 0</td>
<td>Heart of hen</td>
</tr>
<tr>
<td>A-24</td>
<td>-0.985</td>
<td>c c 4 4 3 2 1 0 0</td>
<td>Chick</td>
</tr>
<tr>
<td>B-104</td>
<td>-0.995</td>
<td>4 4 4 4 3 2 1 0 0</td>
<td>Chick</td>
</tr>
<tr>
<td>A-12</td>
<td>-0.995</td>
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<td>Chick</td>
</tr>
<tr>
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<td>Chick</td>
</tr>
<tr>
<td>B-71</td>
<td>-1.047</td>
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<td>Chick</td>
</tr>
<tr>
<td>X-49</td>
<td>-0.955</td>
<td>-0.955 4 4 4 4 4 3 1 0 0</td>
<td>Chick</td>
</tr>
<tr>
<td>X-52</td>
<td>-0.993</td>
<td>4 4 c c 4 4 3 0 0</td>
<td>Chick</td>
</tr>
<tr>
<td>B-73</td>
<td>-0.646</td>
<td>c c 4 4 4 4 3 1 0</td>
<td>Chick</td>
</tr>
<tr>
<td>B-74</td>
<td>-0.627</td>
<td>c c c 4 4 3 2 0 0</td>
<td>Chick</td>
</tr>
<tr>
<td>X-43†</td>
<td>-1.157</td>
<td>c c c c 4 4 2 1 0</td>
<td>Chick</td>
</tr>
</tbody>
</table>

* Each average migration velocity represents the average of 80 stop-watch readings.
† Serum dilutions 1:100, 1:200, 1:400, 1:800, etc., represented by 1, 2, 3, 4, etc.
‡ Homologous strain.
very clearly a relationship between the electrophoretic migration velocity and the agglutinability of the species *Sal. pullorum*, since there is a sequence of increasing agglutinability which is accompanied by a sequence of decreasing migration velocity. The apparently abnormal behavior of the homologous strain X-43 will be discussed later.

It is interesting to note that strains isolated from mature birds showed relatively poor agglutinability and a high charge.

**DISCUSSION**

Since electrophoretic migration velocities can be measured to an accuracy of 3 per cent and since a total variation of approximately 63 per cent was obtained, it appears that the variations in electrophoretic results reported are really significant in view of the fact that these variations between strains were observed under uniform environmental conditions, the medium, the 0.85 per cent NaCl, the procedure of making both suspensions and electrophoretic measurements, the pH and the temperature being practically identical in every case.

That the experimental results should reveal relationship between agglutinability and electrophoretic migration velocity was expected as a result of an application of fundamental colloid chemistry. Since bacteria behave as hydrophilic colloids which are changed into hydrophobic colloids by immune serum (Wells (1925)) and since the agglutination of the hydrophobic particles is a question of bringing them into an iso-electric zone (Northrop and DeKruif (1922)), it follows that the charge which is represented by electrophoretic migration velocities must vary in some way with agglutination tendencies. It is, therefore, not surprising, when all experimental conditions except the nature of the organism are kept constant, to find that the series representing the agglutinability and that representing the electrophoretic migration velocities appear to be comparable.

Strain X-43 did not lend itself to a proper placement in the series. In view of the work of Shibley (1924) and the statement made by Falk, Jacobson and Gussin (1926a) namely that "the type specificity of each serum is as plainly evident in the reduc-
tion of electrophoretic potential as in the agglutination of the bacteria," it appears that we should not have expected a placement which would have put agglutinability and electrophoretic migration velocity in the same relative position. The placement that was obtained is just that which is to be expected from the above work, namely, better agglutinability than the charge suggests.

That the readings obtained with strains isolated from mature birds showed relatively poor agglutinability and high charge is interesting in view of the work published by Falk, Jacobson and Gussin (1925a) in which they called attention to the fact that, for the pneumococci, charge and virulence paralleled each other. Such a parallelism is suggested here on the assumption that strains from the adults possess more virulence than those from chicks.

Attention is called to the fact that work is now in progress in which an attempt is being made to vary the charge of the organism by varying the components of the medium upon which it is grown, in order to ascertain whether variations in agglutinability and charge can be given to the organism by the constituents of the growth medium to such a degree that they can be carried over when the organism is suspended in various suspending menstra. This work should throw some light on why different strains should have different agglutinabilities and different charge, and on whether work in this field can be duplicated provided workers do not use exactly the same growth and suspending media.

SUMMARY

1. The use of a dye in the solution used to fill the electrode chambers facilitated the filling of the electrophoresis apparatus.
2. The Smoluchowski curve was obtained.
3. It is suggested that greater confidence in readings is obtained by making measurements which will span the hump of the above curve.
4. Electrophoretic migration velocities were determined with an accuracy of 3 per cent.
5. Eighteen strains of *Sal. pullorum* studied carried a negative charge.

6. Variations of over 60 per cent in the electrophoretic migration velocities and a titre ranging from about 100 to 3200 were obtained.

7. In general, the sequence of decreasing electrophoretic migration velocity followed the sequence of increasing agglutinability.

8. The use of homologous serum produced an agglutinability superior to that suggested by the charge.

9. A relationship between electrophoresis, agglutinability and virulence is suggested.

10. Attention is called to work in progress.

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ARKWRIGHT, J. A. 1914 On the presence in an emulsion of *Bacillus typhosus* of two different substances which are agglutinable by acids and their relation to serum agglutination. Zeitschr. f. Immun., Orig., 22, 396.


