STUDIES OF THE ELECTROPHORETIC MIGRATION

VELOCITY OF VARIOUS MICROORGANISMS

THESIS

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by

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I. INTRODUCTION

Within recent years various workers have shown a certain similarity between bacteria and non-living colloid particles. Thus a bacterium, like a colloid particle, carries on its surface an electric charge, which is influenced by various physical and chemical factors in the same manner as is the charge on the colloid particle. The nature of this charge and the methods for determining its magnitude are of great importance.

Electrophoresis denotes the movement of the colloidal micelle through the dispersion medium when a potential difference is set up between two electrodes dipped into the medium. Following early studies, which concerned themselves chiefly with observations of various types of colloidal micelles and the factors that influenced their migration through the dispersion medium due to an applied E.M.F., attempts have been made to utilize, in the field of bacteriology, the knowledge gained from such observations. Winslow, Falk and Caulfield (86), working with Esch. coli suspensions, were able to demonstrate effects upon the electrophoretic migration of bacteria similar to those Abramson (2) and Briggs (8) had demonstrated with proteins: i.e., that the hydrogen ion concentration of the dispersion medium influences the migration velocity of the micelles. In very acid solutions the surfaces are positively charged. At the isoelectric point the charge becomes zero, then negative and increasing to a maximum in more alkaline solutions, and, finally decreases to a second iso-potential point in very alkaline solutions.

Hydrophilic colloids owe their stability, not only to the charge at the interface, but also to the amount of water which is held there. De Jong (15)(16) has demonstrated that the addition of any substance having a great affinity for water sensitizes the sols and makes them in effect hydrophobic. Northrop and De Kruif (54) have shown that micro-organisms in suspension are both charged and hydrated and that these factors are important for the stability of the suspension. The two factors of stability, hydration and charge, were demonstrated by Mudd, Nugent and Bullock (52) for the colon and dysentery bacilli. They have found that "the factors determining the stability of a suspension of a given type of bacteria are shown to be those determining the stability of a dispersion of colloidal particles of the same surface type". White (90)(91) has noted the hydrophilic nature as well as the hydro-phobic nature of bacterial surfaces.

Powis (57) studying oil emulsions and Desai and Barve working with ferric oxide sols, have found that the addition of small amounts of mono-valent electrolytes increases the mobility of the micelles and that high-er concentrations decrease their mobility. Kruyt (40) and Freundlich and Rona (29) have observed a similar phenomenon for the glass-water interface. Winslow and Fleeson (87) and Winslow and Upton (89) have suggested that the effect of the electrolytes in the suspension medium and the electrophoretic potential are probably the same for silica dust, bacteria, and yeast cells.

It would seem that there is a relationship between those physical characters of the microorganism responsible for the phenomenon of elec-trophoresis and certain biologic characteristics of the microbic cell.

Recent studies indicate that this relationship is of more than academic interest. Attempts have been made to show that the electrophoretic migration velocity is related to the toxigenicity, virulence and agglutinability of various microorganisms. The identification of bacteria by their electrophoretic migration velocity has met with little success. As work continues it is being found, however, that there is an apparent relationship between the migration velocity and certain physical characteristics of the organisms: i.e., capsule formation, Falk, Gussin and Jacobson (28); rough and smooth colony formation (50, 51, 27, 25) and soluble specific substances.

II. PRESENT STATUS OF KNOWLEDGE OF THE SUBJECT

A. Theoretical Consideration

1. The Double Layer

In every stable colloid system a potential exists at the inter-
face between the two different phases. Helmholtz (32) suggested that
this potential is due to the existence of a "double layer" of opposite-
ly charged ions, the positive half belonging to one phase and the nega-
tive half to another. Gouy (30) and Stern (76) have modified this con-
ception so that it is now believed that on the liquid side of the
boundary the electric charge is partly concentrated at the surface,
while the remainder of it is situated in the electrolyte. The density
of the charge decreases asymptotically towards zero as the effective
thickness of the double layer decreases, Gouy (30) and McClendon (48).
A higher electrolyte concentration produces a decrease in thickness of
the double layer and a consequent decrease in electric potential. This
potential double layer is responsible for the phenomenon called electro-
phoresis.

The double layer may result, either from direct ionization of the
molecules composing the surface of the disperse phase, or from the ad-
sorption of ions from the dispersion medium on the surface of the dis-
perse phase. The second phenomenon is usually the cause of the double
layer, but in some cases the first process is also of importance.

The double layer forms a part, and sometimes the whole, of the
thermodynamic potential. If the double layer is on a solid particle,
part of the layer will lie in the liquid which adheres to the solid

while the remainder of the layer will be present in the moveable liquid. The difference of potential between the particle and the moveable liquid is the thermodynamic or epsilon potential. The zeta is the difference in the potential between the immoveable and moveable liquid. The zeta potential may have the sign of, or opposite sign to, the thermo- dynamic potential. Lewis (43) indicates that it is not possible to pre- dict the relationship between the two. The differentiation between the epsilon and the zeta potentials may be shown by their method of measure- ment: epsilon is measured across the potential drop while the zeta is measured at right angles to it.

The measurement of the zeta potential always involves the use of linear velocity. In electrophoresis the linear velocity of the suspend- ed particles, due to an applied E.M.F., is measured. Because of the lack of direct measurement, McBain (47) and Harkins (31) have attacked the validity of the present conception and consider the zeta potential as wholly fictitious.

Briggs (9), Bull and Gortner (10) and Abramson (3) indicate that surface conductance is at least partially dependent upon the absolute mobilities of the positive and negative ions present.

Verwey and Kruyt (82) conclude from their work that the double layer does not cover the particle regularly but collects upon a few points on the surface: an idea which is in conflict with the idea of surface charge.

2. The Equation of Electrophoresis

Considerable controversy has arisen concerning the equation of

electrophoresis. Since the electrophoretic migration is the phenomenon of the migration of a suspended particle in an electrical field, all theories developed so far indicate that velocity V of the particle is given by $V = \dfrac{X\,D}{K\,n}$. X is the strength of the field, D the dielectric constant of the liquid and n the coefficient of viscosity of the liquid. According to this formula the solid particle moves toward the positive pole if its charge is negative. The velocity is proportional to the zeta potential. For quantitive investigations it is necessary to determine the absolute value of K. Various theories lead to different values for this constant.

The equation of Debye and Huckel (16-a) may be reduced to: $U_e = \dfrac{V}{E} = \dfrac{D\,\zeta}{6\,\pi\,n}$. Here U_e is the electrophoretic mobility, V is the velocity of migration, E the impressed electrical field, D the dielectric constant, 6π the potential at the surface, and n the coefficient of viscosity of the liquid. This formula differs from an earlier equation proposed by Helmholtz (32) and improved and extended by Lamb (42) and Somluchowski (72) only be the replacement of a 4π by a 6π in the right hand denominator. This difference is due to the fact that the Debye-Huckel formula holds only when the thickness of the double layer is large in comparison with the radius of the particle.

The formula in which the constant 6π appears was derived for a rigid sphere and the constant equals 4π when the particles are cylindrical. In the Helmholtz-Lamb equation, however, no assumptions were made as to the size and shape of the particles and many workers have considered the electrophoretic mobility to be independent of them. According to Abramson and Michealis (5) and Abramson (2) this is true.

Mooney (49) has found, however, that the electrophoretic mobility of an oil drop in distilled water increases with the diameter of the drop, but that when the oil is suspended in 0.0008 N Cu SO_4 the mobility is constant. Bull and Gortner (11) have shown the zeta potential to increase roughly as the cube root of the diameter of the particle increases from from 4.59 μ to 214 μ. Above 214 μ, however, a constant potential is observed.

In the consideration of electrophoretic phenomena, the properties of the double layer, rather than the properties of the rest of the system, are involved. In the original formula for electrophoresis the coefficient of viscosity and the dielectric constant of a pure dispersion medium were used. It is hardly likely that, in the highly oriented double layer, where the electrolyte concentration is high, the dielectric constant will be that of pure water. Because of the objections to the formulae, and in consideration of the criticism of the theoretical concept of the zeta potential, the writer believes it advisable to express the results in terms of seconds per 0.5 mm. distance at the specified applied potential.·

B. Application of the Theory to Bacteria

    1. Double Layer

A certain similarity between bacteria and non-living colloid particles has been pointed out. Because of this similarity we would expect to encounter a similar state of confusion with regard to electrophoretic phenomena as was found in the true colloid system.

The ionization of the molecules of the disperse phase has been con-

sidered of little importance for the establishment of the double layer
on the colloid. However, as Mudd (50) and Stearn (75) have shown, the
surface of bacteria is made up of amphoteric components which have a
buffering power. The ionization of these amphoteric components would
assist in the formation of the double layer. The part played by the
adsorption of ions is probably similar to the part this plays in the
case of the true colloid.

On a bacterial surface the picture of the formation of the double
layer is complicated by a third factor which may be of greater import-
ance than the two previously mentioned. In each individual bacterium
all of the conditions necessary for the establishment of a Donnan poten-
tial are present. On the inside of the cell membrane are non-diffusible
ions which, together with the diffusible ions present, cause the es-
tablishment of an epsilon potential which must influence the formation
of the double layer. Coulter (14), studying red blood cells, has stated
that in a medium of low electrolytic concentration a thermodynamic poten-
tial difference of considerable size may result from the Donnan equil-
ibrium. This potential will influence the double layer by altering the
orientation of the ions and also by determining the final concentration
of ions surrounding the cell membrane. These changed ionic concentra-
tions will modify surface adsorption, and it is this resultant change in
adsorption which makes the Donnan potential of importance in electro-
phoretic migration studies.

These three factors, ionization, adsorption, and Donnan equilib-
rium, all active at the same time and each modifying the effects of the
others, are instrumental in the formation of a double layer. It is prob-

able that under differing conditions different factors will predominate.

2. Types of Apparatus

Tittsler (80) has given us a brief history of microscopic electrophoresis. Northrop (43), Kunitz (37), Mattson (46), Northrop and Kunitz (55), and Smith and Lisse (70) have described apparatus suitable for the microscopic measurement of electrophoretic velocity. Lortet (45) introduced the first microscopic cell. This cell, as improved by Porvis (57) was used in the very early work. The more recent improvement of this cell by Northrop, Kunitz and Northrop, and Kunitz is well adapted to studies of objects such as bacteria. Dozois (19) gives a complete description of the Von Gyorgyi Szent cell from which some of our modern cells have been devised.

For a number of years the Falk cell (25) has found wide-spread usage. Chapman (13), in his more recent work, finds this cell suitable for general use. As, however, the Falk cell does not give the actual electrophoretic velocities, all results must be referred to some standard.
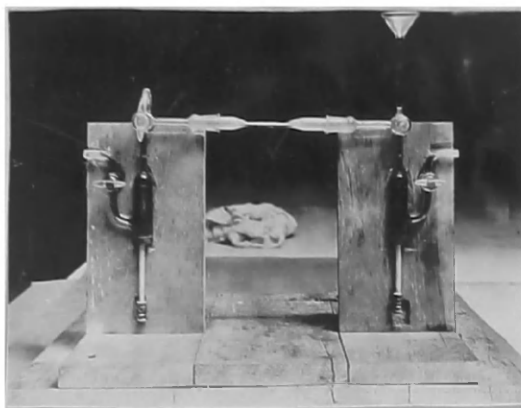
Since the Northrop-Kunitz cell forms the basis on which the more recent cells have been developed, it is worthy of some description. As pictured above, it consists of platinum electrodes extending into the arms of a drawn out flattened tube, the observation cell. On each end of the observation cell, but separated from it by a three way stop-cock, are side arms. The lower chamber of each side arm is filled with saturated zinc sulphate. Zinc electrodes extend into the zinc sulphate. The washing and filling of the apparatus are done through the funnel tube at the right. The observation chamber is filled by turning the three way stop-cocks of the side arms to make connection with the upper chambers. After filling, the stop-cocks are turned to make contact between the cell and the zinc sulphate of the lower chamber. This cell, then, constitutes one of the closed type systems. The distance across the observation chamber is about 28 centimeters and the

liquid thickness is 1.8 mm. The microscope is focused at various depths in the central portion of the cell and the time required for a suspended particle to migrate a given distance at a specific applied E.M.F. is measured. Due to difficulties of manipulation this Northrop-Kunitz cell has been modified. A description of the modified cell will follow later.

In the closed cell apparatus the suspension liquid must return along the axis of the cell, the velocity of the liquid being a parabolic function of the depth. The observed velocity changes rapidly with the depth and this factor may lead to inaccuracies in the results. The actual electrophoretic velocity can be obtained from observations at certain depths.

3. Electrophoresis as a Practical Procedure

A large amount of work has been done upon bacteria in which the electrophoretic technique has been used. The most spectacular has been the attempt to show a correlation between electrophoretic migration and virulence of pathogenic bacteria. Falk, Gussin and Jacobson (27) conclude that a direct relationship exists between the virulence and the electrophoretic migration velocity of pneumococci. Thompson (78) has found no such correlation. With diphtheria bacilli, Jensen and Falk (34) and Thompson (79) have claimed that toxigenic cultures have a lower migration velocity than do non-toxigenic cultures. Stone and Weigel (77) have found that the highest migration rates were obtained with virulent strains and the lowest rates with non-virulent, but that 77 per cent of the strains fell in an intermediate group and could not be class-

ified. Jones (36) and Broom and Brown (10) report no correlation between electrophoretic mobility and the virulence of diphtheria organisms. Rosenow and Jensen (62) report characteristic but different mobilities for arthritic and for encephalitic streptococci but find no correlation between charge and virulence.

The determination of electrophoretic mobility as a means of differentiating among various strains of coli has been proposed by Chapman (13) who has found that each strain has a constant mobility. Rane (59) and Beed and Gardiner (61) report a difference in the electrophoretic mobility of rough and smooth variants of certain organisms and concur in the conclusion that the S type has a greater mobility than the R type.

The conclusion that the practical usefulness of the measurement of electrophoretic mobility of bacteria has not yet been established seems to be justified. The conflicting data which have been reported are doubtlessly in part due to differences in technique and until a simple method of measurement, together with a more nearly standard method of preparing the organism for measurement, is devised, the maximum usefulness of the determinations will not be realized.

4. Action of Various Physical Agents upon the Electrophoretic Migration

Physical agents which cause changes in the migration velocity of the microorganisms are of such a nature that they alter the zeta potential of the organisms. This is brought about either by an alteration of the molecules composing the surface of the dispersed phase,

the adsorption of the ions from the dispersion medium or alteration of the Donnan equilibrium of the cell.

The effect of electrolytes upon the electrophoretic mobility of bacteria has been shown to be a slight increase in mobility in very low concentrations of electrolytes and a marked decrease in mobility with higher electrolytic concentrations. Electrolytes affect the electrophoretic mobility of bacteria and non-living colloid particles in the same qualitative manner. Such changes are, doubtlessly, brought about by a direct alteration of the molecules composing the surface of the suspended organism or by adsorption of ions on this surface. Such alterations are, ordinarily, not sufficient to bring about a lethal effect or even an alteration of the viability of the organism.

Although irradiation does in many instances alter the migration velocity of the bacteria, no definite conclusion has been drawn as to how it is done. The general conception is that irradiations which have a lethal effect or that alter the viability of the organism alter the Donnan equilibrium. Their influence on adsorption and alteration of the surface molecules is not, however, to be overlooked.

Heat and storage may influence the zeta potential in any of three above mentioned ways. Slight changes in temperature and short periods of storage which are sufficient to alter the migration velocity but not sufficient to bring about a lethal effect or a lowering of viability may result in alterations of the molecular arrangement at the outer cell surface. Those effects which destroy the cell perhaps show their greatest action on the Donnan equilibrium.

III. THE PROBLEM

A. Presentation of Topics

Five separate studies which involve changes of the electrophoretic migration velocity of various microorganisms are included in this work. They may be classified as follows:

1. Influence of pH of Growth Medium and Suspension Solution on Electrophoretic Potential of Aerobacter aerogenes.

2. The Influence of Heat and Storage on the Electrophoretic Migration Velocities of Various Microorganisms.

3. The Effect of Radium Irradiation on the Electrophoretic Velocity, Viability and pH of Escherichia coli Suspensions.

4. Variations in the Electrophoretic Mobilities of Escherichia, Aerobacter and "Intermediate" Strains.

5. Relationship between Electrophoretic Migration Velocities, Virulence and the Types of the Diphtheria and Diphtheria-like Bacilli.

B. Factors of Technique

Before studies could be started certain points of technique had to be considered.

1. The Cell

As has been previously mentioned, the Kunitz modification of the Northrop-Kunitz microcataphoresis cell was used. It was preferable to use this cell when the work was begun since it was the only one available which would permit the securing of actual electrophoretic velocities. During the course of the work two cells were used.
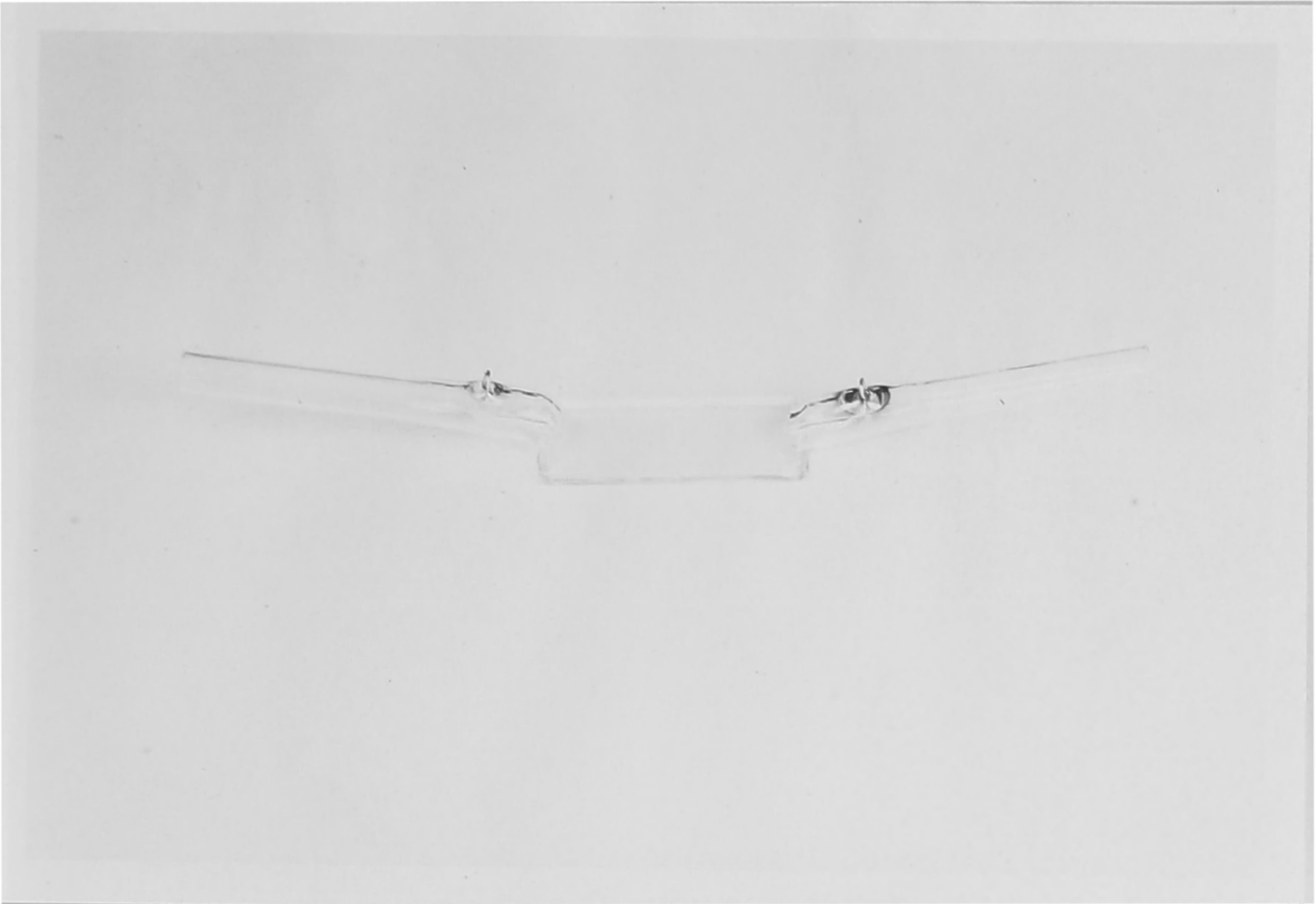
2. The Growth Medium

The medium used depended upon the type of microorganism studied. With the Escherichia, Aerobacter and "intermediate" strains when a solid medium was needed the organisms were grown on proteose-peptone agar slants of an approximate pH of 6.8. Proteose-peptone broth of the pH later indicated was used in the studies of "Influence of pH of Growth Medium on the Migration Velocity". The diphtheria and diphtheria-like bacilli were cultivated on Loeffler's medium, pH 7 to 7.4.

3. Suspension Solution

Since it is not the charge on the particle that determines electrophoretic mobility but the potential which exists between the particle surface and the surrounding liquid, the suspension fluid was kept as near constant as possible for all studies. An exception to this rule appears when studies were made to determine the influence of pH of the suspension fluid on the migration velocity. Distilled water of pH 6.8 was used as the suspension solution for the colon aerogenes "intermediate" cultures, and distilled water of pH 7.0 for the diphtheria and diphtheria-like bacilli.

4. Time of Cultivation

A twenty-four hour period of incubation was chosen because Shibley (66) has shown that the quantity of charge varied until the culture was about eighteen hours old, after which it remained rather constant. Various studies by the writer have confirmed this observation. At 24 hours the migration velocity remained constant.

5. Storage

The effect of storage of the suspension on the migration velocity is a part of the problem discussed. Although, as will be shown later, the suspensions may be stored for a considerable time at icebox temperature without an alteration in the migration velocity, yet unless specified the suspensions were used immediately after preparation.

IV. EXPERIMENTAL

A. Apparatus and Technique

1. The Cell

As previously mentioned the Kunitz modification of the Northrop-Kunitz cell was used. This type of cell, which is often referred to as a closed cell, has frequently been described.
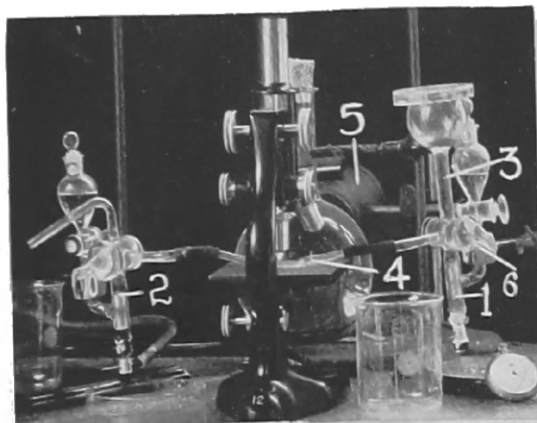
In the use of this cell it is essential that observations be made at those levels at which errors from endosmotic velocity will be reduced to a minimum. Theoretically the cell is symmetrical but actually a considerable difference exists between the upper and lower halves. While the upper surface of both cells used was flat and smooth, the lower surface was slightly curved and contained one or more ridges.

The depth of the cell was determined by means of a calibrated

micrometer scale on the fine adjustment of the microscope. The inside
top and bottom of the cell was found by focusing on a refractive part-
icle on these respective surfaces. By means of numerous observations
on various parts of the cell determinations of the depth of the cell
at several points near the center were obtained. In all the work an
attempt was made to make observations as near as possible in the same
location. Following the technique of Mudd, two stationary levels were
obtained; i.e., 0.21 and 0.79 of the inside depth of the cell. All ob-
servations were made at these two levels, ten with each level, and the
recorded results are the averages obtained.

The electrophoretic apparatus was so set up that by the operation
of a two-way switch the direction of the flow of current could be re-
versed. Ten observations were made, five at each depth indicated; the
current was then reversed and ten more readings were obtained. By av-
eraging these twenty readings several unavoidable errors of technique
were partly eliminated. Although all observations on a single speci-
men were within a limited migration velocity range, only a few were
identical. In instances where great discrepancies in velocity occurred
the cell was thoroughly washed and reset and the experiment repeated.

For the work an applied potential of 118 volts, which gave a drop
of 2.5 volts through the cell, was used. This, with a series resist-
ance of 1100.84 ohms, would duplicate an applied potential of 125 volts
supplied by Radio B batteries.

Due to the arrangement of the apparatus, it could easily be washed
by permitting cleaning solution to flow through it. Following the

cleaning solution the apparatus was rinsed by numerous washings with
tap water, distilled water and suspension solution. Usually 3 or 4
separate experiments were performed, occasionally 6, before it was
found necessary to clean the apparatus.

2. The Microscope

A (F F S 8) Bausch and Lomb, 8 mm., 0.50 n.a. microscope with a
21 X objective and a 10 X eyepiece was used. A micro-lamp was employ-
ed as the source of illumination.

3. Preparation of the Suspension

The organisms were grown on proteose-peptone agar or in proteose-
peptone broth, or, in the case of the diphtheria and diphtheria-like
bacilli, on Loeffler's medium, for 24 hours. With the solid medium
the growth was washed from the surface, the surface washings were hom-
ogenized by gentle shaking, filtered through cotton and centrifugal-
ized at about 2700 r.p.m. for 45 minutes. The supernatant fluid was
removed and the cells re-suspended and re-washed twice. Only distill-
ed water of pH 6.8 was used in the work. After the last washing the
bacterial cells were re-suspended and the suspension standardized so
that there were approximately 5 million organisms per cubic centi-
meter. Electrophoretic migration velocities were measured as soon as
possible after the suspension was prepared. Rarely was the suspension
over an hour old before observations were made, the exception occurring
in the studies of the effect of storage on the migration velocity.
Observations were carried out at room temperature, which varied be-

tween 21° and 27°C.

Colormetric pH determinations, using the "W. A. Taylor Comparator" were made on each sample before and after migration velocities were obtained.

4. Recording of Data

As has been stated, twenty readings on each suspension were obtained, five at each level followed by a reversal of the current. Although non-polarizing electrodes were used, the polarity was reversed in order to overcome possible irregularities in the cell. Three sets of twenty readings each were taken for each organism. In instances where wide variation occurred between these three sets, the work was repeated.

The readings are expressed in terms of seconds required for the organism under observation to travel a distance of 0.5 mm. at an applied potential of 118 volts which gave a potential drop through the cell of 2.5 volts.

B. Presentation and Discussion of Data

1. Preliminary Work

The preliminary work consisted principally in determining the effect of ordinary methods of technique on the migration velocity. The correct manipulation of the electrophoretic apparatus and the determination of the variables that influence the results obtained formed an important part of the introductory work.

After a careful cleaning of the complete apparatus, and its ad-

justment, a suspension of Sudan III was introduced into the observation cell and migration studies were made. Sudan III is negatively charged and is attracted to the positive pole as are the bacteria. By studies of the rate and direction of travel of the dye particles, the technique of manipulation of the apparatus was learned. The particles of dye were also used in the determination of the depth of the observation cell and the location of the position at which future observations were to be made.

Unless the observation cell is clean neither particles of dye, bacteria, nor red blood cells will migrate in a straight line across the field. The even rate of travel is hindered by the introduction of foreign material into the apparatus. Through experience one may easily detect dirt in the apparatus by the irregularities of migration of the suspended particles. When dirt has been introduced it may sometimes be removed by flooding the cell and side arms with cleaning solution and water, but generally it is necessary that the apparatus be taken completely apart.

Observations of migration velocity were made by the use of a stopwatch. It requires considerable practice to time correctly the rate of travel.

The control of the fine adjustment of the microscope, so that the proper depths of the field could be maintained, was gradually acquired.

2. Studies of Washing

The time and number of washings necessary to free the organisms completely from particles of the growth medium depend to a great ex-

tent on the type of medium used and the duration of cultivation.
Using proteose-peptone agar and allowing a 24-hour period of growth,
the migration velocity of the bacteria remained constant after the
first washing. However, three washings were always made so as to be
certain that all foreign material had been removed. The same pre-
cautions are necessary for organisms cultivated in proteose-peptone
broth. In the case of the diphtheria and diphtheria-like bacilli,
which were grown on Loeffler's blood serum, four washings of 45 min-
utes each were made. It had previously been found that two washings
were necessary before the migration velocity would remain constant.
Tittsler, in personal communications, has expressed the opinion that
multiple washings do not influence the migration velocity so long as
the microorganisms are not harmed by the washing, and that at least
three are necessary before the migration velocity remains constant.
The effect on the migration velocity of the high speed centrifugal-
ization during the washing remains to be studied.

3. Time of Cultivation

Corroborating the work of Dozois (21) and Shibley (67), it was
found that after cultivation for 18 hours the migration velocity of
microorganisms will remain constant. Up to 18 hours the migration
velocity gradually increases until it reaches a stationary level. The
velocity then remains constant through a 54 hour period of incubation.
After a longer period considerable fluctuation occurs. In all of the
routine studies the organisms were cultivated for at least 24 hours
but occasionally they were incubated for as long as 36 hours. Chapman,

in private communications, also has expressed the opinion that the
24-hour cultivation time is the most satisfactory for uniform results.

4. Influence of pH of Growth Medium and Suspension Solution on the
   Electrophoretic Potential of Aerobacter aerogenes

It has been suggested that the electrophoretic migration veloc-
ity of bacteria might be affected either by the pH of the growth med-
ium or by that of the solution in which the bacterial cells are sus-
pended. Working with a solid medium, Pedlow and Lisse (56) have con-
cluded that the mobility of the microorganism can be increased or de-
creased by the presence of certain salts in the growth medium.
Winslow, et.al. (86, 89) have reported the effects of electrolytes
upon the migration velocity. In their studies the organisms were
grown, separated from the growth medium, suspended in the electrolyte
solution under investigation, and the migration velocity determined.
They concluded that the presence of low concentrations of electrolytes
increases the mobility, while higher concentrations decrease it.
Analogous reports for non-living particles have been made.

In order to determine the influence of the pH of the growth med-
ium as well as that of the suspension solution, a culture of Aerobacter
aerogenes was used which had been employed in previous work of a simil-
ar nature and found to give uniform electrophoretic migration results.
The cultures were grown for 24 hours in 200 c.c. of proteose-peptone
broth at the pH indicated in the table. The organisms obtained from
the broth culture were washed three times and re-suspended in fiftieth-
molar Walpole's sodium-acetic acid buffer solution of the pH also in-

dicated.

Sufficient N/10 HCl or N/10 Na OH was added to the proteose-peptone broth to obtain the desired pH. As shown in the table, the pH of the medium, within the range of 6.8 to 7.4, had little or no effect on the electrophoretic migration velocity. Bacterial cultures, even though obtained from culture media which differ in pH within the range indicated, if suspended in solutions of uniform pH will give uniform migration results. Studies, not indicated in the table, show that the same results were obtained within the range of 4.6 to 7.8.

Although these results differ from those obtained by Pedlow and Lisse, they do not confirm observations by Tittsler (personal communications) and Verway (personal communication). The work of Pedlow and Lisse may be questioned since they apparently failed to wash the cells sufficiently to remove traces of the electrolytes present in the medium.

A pH determination of the buffered suspension was made just before and immediately following the electrophoretic velocity determinations. As shown in the table, the pH of the bacterial suspension does affect the velocity. As the pH of the bacterial suspension increases from 4.6 to 7.8, there is also a gradual decrease in the velocity. These results have been obtained from numerous experiments carried on over a period of time and represent the average of numerous readings.

It is apparent, then, that any statement concerning electrophoretic migration velocities should also contain information about the pH of the bacterial suspension studied.

Table

pH Susp.	pH Medium	Velocity	pH Medium	Velocity	pH Medium	Velocity	pH Medium	Velocity
4.6	6.8	14.5	7.0	14.2	7.2	13.8	7.4	14.3
4.8	6.8	14.5	7.0	14.5	7.2	13.9	7.4	14.0
5.0	6.8	14.3	7.0	14.7	7.2	14.3	7.4	14.4
5.2	6.8	14.7	7.0	14.7	7.2	14.5	7.4	14.5
5.4	6.8	15.0	7.0	14.4	7.2	14.5	7.4	14.3
5.6	6.8	14.9	7.0	14.8	7.2	14.5	7.4	14.6
5.8	6.8	15.4	7.0	15.3	7.2	14.8	7.4	15.3
6.0	6.8	15.4	7.0	15.6	7.2	15.1	7.4	15.3
6.2	6.8	15.3	7.0	15.3	7.2	15.3	7.4	15.6
6.4	6.8	15.7	7.0	15.6	7.2	15.4	7.4	15.5
6.6	6.8	15.8	7.0	15.6	7.2	15.6	7.4	15.8
6.8	6.8	15.8	7.0	15.8	7.2	15.6	7.4	15.8
7.0	6.8	15.7	7.0	15.7	7.2	16.0	7.4	15.9
7.2	6.8	15.8	7.0	15.8	7.2	16.1	7.4	16.0
7.4	6.8	16.0	7.0	16.0	7.2	15.9	7.4	16.2
7.6	6.8	16.2	7.0	16.2	7.2	16.0	7.4	16.4
7.8	6.8	16.2	7.0	16.3	7.2	16.2	7.4	16.1

5. The Influence of Heat and Storage on the Electrophoretic Migration Velocities of Various Microorganisms

"Although the literature contains numerous references to the effect of heat and storage on the bacterial cell, a careful review fails to reveal any information as to the influence of either on the electrophoretic velocities. Recent workers, in their studies of those physical agents which influence the zeta potential, suggest that heat may play a part, but experimental evidence for this has not, to our knowledge, been published.

In the various studies on the effect of X-rays and radium on the zeta potential (Dozois (23)(24)) the question has arisen as to the part heat may play in the results obtained. In the determination of the zeta potential of pathogenic microorganisms, using the Kunitz modification of the Northrop-Kunitz microcataphoresis cell, it was deemed advisable to determine whether or not microorganisms could first be killed by heating before measurements were made. If heating the microorganisms to a temperature sufficiently high to kill them did not alter their zeta potential or interfere with the final results, then this procedure could be followed."

The cultures of Escherichia coli, Aerobacter aerogenes and Corynebacterium diphtheriae (gravis, mitis, and intermediate) which were used in this investigation had been tested in previous studies and found to give constant migration velocities. The cultures of Esch. coli and Aerobacter aerogenes were grown on proteose-peptone agar slants, pH 6.8, for twenty-four hours. The cultures of Corynebacterium diphtheriae were

grown on Loeffler's medium, pH 7.0 - 7.4, for the same length of time.
The growths of Esch. coli and Aerobacter aerogenes were asehd and sus-
pended in distilled water of a pH 6.8. For Corynebacterium diphtheriae
distilled water of pH 7.0 was used. A pH determination was made on
each suspension before and after heating or storage. In those instances
in which measurements did not follow immediately after treatment, a de-
termination was also made just previous to the electrophoretic measure-
ments. In each case the pH was found to remain unchanged.

TABLE 1

Effect of Storage at 5°C. on Electrophoretic Migration Velocities

Storage at 5°C.	ELECTROPHORETIC MIGRATION VELOCITIES, SECONDS PER 0.5 MM.				
	Bacterial Suspensions				
	E. Coli	A. aerogenes	C. diphtheriae		
			Gravis	Mitis	Intermed.
hours					
Control	13.2	14.6	10.0	8.4	8.8
1	13.3	14.5	10.0	8.3	8.8
2	13.5	14.6	10.0	8.2	8.8
3	13.1	14.5	10.1	8.4	8.9
4	13.5	14.5	10.1	8.4	8.8
5	13.0	14.5	10.1	8.4	8.7
6	13.4	14.5	10.1	8.3	8.8
8	13.3	14.6	10.0	8.4	8.7
10	13.2	14.6	9.9	8.3	8.7
12	13.2	14.6	10.0	8.3	8.7
18	13.5	14.6	10.1	8.4	8.7
24	13.4	14.6	10.0	8.4	8.7
36	13.3	14.6	10.1	8.3	8.8
48	13.3	14.6	10.0	8.4	8.7

TABLE 2

Effect of Storage at Room Temperature on Electrophoretic Migration Velocities

Storage at 27°C.	ELECTROPHORETIC MIGRATION VELOCITIES, SECONDS PER 0.5 MM.				
	Bacterial Suspensions				
	E. Coli	A. aerogenes	C. diphtheriae		
			Gravis	Mitis	Intermed.
hours					
Control	13.3	14.5	10.1	8.3	8.8
1	13.2	14.5	10.2	8.3	8.9
2	13.2	14.6	10.1	8.4	9.0
3	13.4	14.7	10.3	8.4	9.1
4	13.6	14.9	10.4	8.4	9.0
5	13.7	14.8	10.5	8.4	9.1
6	13.8	14.9	10.5	8.5	9.1
8	13.7	14.9	10.6	8.6	9.2

TABLE 3

Effect of Heat on Electrophoretic Migration Velocities

Temperature	ELECTROPHORETIC MIGRATION VELOCITIES, SECONDS PER 0.5 MM.				
	Bacterial Suspensions				
	E. coli	A. aerogenes	C. diphtheriae		
			Gravis	Mitis	Intermed.
degrees					
Control	13.3	14.5	9.7	8.4	8.9
60	13.4	14.5	9.7	8.3	9.1
70	13.4	14.6	9.8	8.5	9.0
75	13.6	14.7	9.9	8.6	9.2
80	13.6	14.9	10.0	8.6	9.2
85	13.7	14.9	9.9	8.7	9.3
90	14.0	15.0	10.1	8.9	9.4
95	13.9	15.1	10.2	8.8	9.6

TABLE 4

Effect of Storage at 27°C. on Electrophoretic Migration Veloc-
ities of Bacterial Suspensions Heated to 60°C. for Thir-
ty Minutes

Storage at 27°C.	ELECTROPHORETIC MIGRATION VELOCITIES, SECONDS PER 0.5 MM.				
	Bacterial Suspensions				
	E. coli	A. aerogenes	C. diphtheriae		
			Gravis	Mitis	Intermed.
hours					
Control*	13.3	14.5	9.7	8.4	8.9
Control+	13.4	14.5	9.7	8.3	9.1
1	13.4	14.6	9.7	8.4	8.9
2	13.5	14.7	9.9	8.5	9.1
3	13.6	14.7	9.9	8.6	9.2
5	13.6	14.8	9.9	8.6	9.3
8	13.7	14.9	10.0	8.7	9.5

*Before heating
+After heating

The bacterial suspensions studied were kept at a temperature of
5°C. for as long as forty-eight hours without significant alteration in
the zeta potential. Suspensions were kept at 27°C. for two hours with-
out a significant change, after which time a measureable but not marked
alteration took place. Bacterial suspensions were heated at a tempera-
ture of 60°C. for thirty minutes with no significant change in the mi-
gration velocity. At temperatures above 60°C. there was a slight tend-
ency toward a decrease in zeta potential. This decrease became more
marked as the temperature was increased. The electrophoretic migra-
tion velocity of bacterial suspensions heated at a temperature of 60°C.
for thirty minutes and then held at 27°C. remained constant for two

hours. After this time a slight but gradual decrease in the electro-phoretic migration velocity occurred."

6. "The Effect of Radium Irradiation on the Electrophoretic Veloc-
   ity, Viability and pH of Escherichia coli Suspensions"

In recent years great interest has been shown in the bacteri-cidal and therapeutic action of light. It has long been known that certain light waves are capable of bringing about changes in a bact-erial cell, even to the extent of killing it. However, it has been only during recent years that an attempt at the practical application of this knowledge has been made.

It has been shown that ultra-violet light is capable of bringing about changes in the cell potential and death of organisms. It re-mains, however, to be demonstrated whether light of other wave lengths has the same effect. Unpublished work by the writer suggests that light of a longer wave length than ultra-violet has, to some degree, the ability to bring about such changes. Laurens (41) has shown that light of shorter wave lengths also possess this property to some ex-tent.

Dozois et.al. (23), using a total radiation from a Coolidge X-ray tube at 30 K.V.$_{R.M.S.}$ (without filters) detected no change in the el-ectrophoretic migration velocity, viability, or pH of an aqueous sus-pension of Esch. coli with exposures as great as 3000 milli-ampere-minutes at 15.2 cm. target-culture distance. Smith, Lisse and Davey (71) confirm these observations but show that much longer radiation, one hundred and twenty minutes exposure of the bacteria to a molybdenum

characteristic K and general radiation, killed all of the organisms but did not alter their migration velocity. From their observations, these later workers feel that a change in the electrophoretic mobility is not a criterion of death of the organism. In instances where death is followed by an alteration in the migration velocity it may be that the death of the bacteria brings about conditions, such as changes in permeability or adsorption, which may result after a time in the decrease in mobility.

Warren has shown that bactericidal rays must be those that are absorbed by the material acted upon. Henri (33) demonstrated that those rays which have the greatest bactericidal power are absorbed by proteins. Rays that are absorbed have a very specific range and wave-lengths either longer or shorter are less active. Although Bovie has shown that Schumann rays (1,230 to 1,850 $\overset{o}{A}$) are more reactive than longer waves, it appears that rays within the range of the ultra-violet light are the most effective.

The purpose of these studies is to determine whether the short waves of radium have a bactericidal effect and an influence on the electrophoretic migration velocity.

"RADIUM APPARATUS AND TECHNIQUE

Four water-tight cells $1\frac{1}{2}$ inches square, inside dimensions, which could be taken apart and washed, were made by cementing together lead-and-aluminum-free glass plates. A rubber tray was made into which two glass cells and the radium-containing aluminum dish could be placed and held in position during the irradiation. The cells were so placed that

the centers of the radium tubes were one-half inch from one inner sur-
face of each cell, and thus one-half inch from the surface of the li-
quid and $1\frac{1}{4}$ inches from the center of the liquid. The suspensions in
each cell received equal amounts of radiation. During the irradiation
the apparatus was placed in a dark ice box at a temperature of 5°C.
The control cells were also kept in the ice box but at least 4 feet away
from the radium-containing tubes - a distance sufficient to avoid being
affected by the radium.

Radium Filtration

Amount of radium element used - 70 milligrams.

Radium tubes of 1 mm. wall of platinum and gold.

Aluminum box wall 1 mm. thick.

Glass wall of cell 3 mm. thick.

Distance from surface of suspension - $\frac{1}{2}$ inch (1.25 cm.).

Distance from center of liquid suspension - $1\frac{1}{4}$ inches (3.1 cm.).

Although it is difficult to state the exact wave length employed in
these experiments, it is usually accepted that the beam coming through
1 mm. of platinum is made up largely of wave lengths varying from 0.06 $\overset{o}{\text{A}}$
down to approximately 0.002 $\overset{o}{\text{A}}$. There is, however, evidence that there
are some wave lengths greater than 0.06 $\overset{o}{\text{A}}$ and some less than 0.002 $\overset{o}{\text{A}}$.
The bulk of the secondary rays from the platinum are absorbed by the
aluminum. The chief aim in this set-up was to use a beam similar to
that employed in our therapeutic applications.

As an erythema dose we give 250 to 300 mg.-hr. per $1\frac{1}{2}$ square inches,

at $\frac{1}{4}$ inch distance from the skin, filtered through 1 mm. of platinum and aluminum foil, the distance being maintained by wax or felt. This amount of radiation causes an erythematous blush and slight desquamation in infants treated for hemangiomata, a rather delicate test. At $\frac{1}{2}$ inch distance, that is, the distance from the center of the radium tubes to the surface of the liquid suspension of bacteria, the erythema dose would be approximately 700 to 800 mg-hr., a dose slightly below that delivered in experiment 12 (Table I, A, B, and C). Of course the total irradiation of the suspension was far below this, as the preparation was $1\frac{1}{2}$ inches square. Thus the bacteria on the far side receive less than those on the proximal side.

The average mean distance from the radium to the suspension is $1\frac{1}{4}$ inches, that is, from the center of the radium tubes to the center of the liquid. At such a distance the erythema dose would be approximately 1,600 mg-hr., a little more than that given in Experiment 21 (Table I, A, B, and C).

Table I, A

ELECTROPHORETIC MIGRATION VELOCITIES OF REPEATED EXPERIMENTS
SHOWING THE EFFECT OF AGE AND RADIUM IRRADIATION

Culture 27F.

Age in hours	Velocity 0.5 mm. in sec. (Control)	Radium doses in mg-hr.	Velocity after irradiation 100 u. per sec.	Percentage deviation from normal
Control	15.33		15.4	0.46
1	15.46	70	15.25	-1.36
2	15.33	140	15.62	1.89
3	15.26	210	15.8	3.54
4	15.43	280	16.6	7.58
5	15.46	350	17.1	10.61
6	15.40	420	17.9	16.23
7	15.40	490	18.75	21.28
12	15.46	840	19.6	27.03
14	15.43	980	20.2	32.89
16	15.20	1,120	20.8	35.68
18	15.33	1,260	21.3	39.22
21	15.4	1,470	21.6	40.26
28	15.4	1,960	22.0	42.86
35	15.46	2,450	23.0	49.42
42	15.30	2,940	24.2	58.17

Table I, B

Culture 27F.

Age in hours	Velocity 0.5 mm. in sec. (Control)	Radium doses in mg-hr.	Velocity after irradiation 100 u. per sec.	Percentage deviation from normal
Control	15.26		15.6	2.23
1	14.93	70	15.55	4.15
2	15.23	140	15.55	2.10
3	15.33	210	16.1	5.02
4	15.34	280	16.50	8.37
5	15.26	350	16.63	8.98
6	15.06	420	17.00	12.88
7	15.23	490	17.40	14.25
12	15.26	840	18.30	19.92
14	15.23	980	19.42	27.51
16	15.26	1,120	20.3	33.03
18	15.4	1,260	21.52	39.74
21	15.33	1,470	22.0	43.51
28	15.3	1,960	23.4	52.94
35	15.4	2,450	23.98	55.32

Table I, C

Culture 2031

Age in hours	Velocity 0.5 mm. in sec. (Control)	Radium doses in mg·hr.	Velocity after irradiation 100 u. per sec.	Percentage deviation from normal
Control				
1	13.9	70	.38	-0.72
2	14.1	140	14.3	1.42
3	13.8	210	14.4	4.35
4	14.2	280		
5	14.0	350	15.3	9.29
6	14.2	420	15.6	9.86
7	13.8	490	15.7	13.77
9	14.3	630		
12	14.1	840	16.6	17.73
14	14.3	980	18.25	27.62
21	14.4	1,470	19.00	31.94
28	14.4	1,960	21.5	49.31
35	14.2	2,450	22.4	57.75

EXPERIMENTAL RESULTS

The results of the measurements of the electrophoretic migration velocities (Table I, A, B, and C) show that the gamma rays of from 0.06 to 0.002Å do influence the zeta potential of the bacteria. As the dose increases the zeta potential decreases, and the length of time, in seconds, which it takes the bacteria to travel over the unit distance (0.5 mm.) under a unit of applied E.M.F. (118 volts) increases. The percentage of decrease in the zeta potential remains fairly constant for the three strains of Esch. coli studied, and the maximum percentage of decrease in the zeta potential is reached with a dosage of 2,940 mg-hr., or approximately 1.84 erythema doses.

Each reading in Table I, A, B, C is the average obtained from three

different experiments carried on over a period of time, conditions being duplicated as near as possible each time.

Table II

RESULTS OF VIABILITY STUDIES

Radiation doses mg-hr.		No. of viable cells per cubic centimeter		Percentage decrease
		Control	Irradiated	
3				
	210	1,950,000	1,880,000	-3.58
7				
	490	2,310,000	1,880,000	-18.13
12				
	840	2,500,000	1,940,000	-22.40
14				
	980	2,120,000	1,480,000	-30.19
18				
	1,260	1,980,000	1,310,000	-33.84
21				
	1,470	2,100,000	1,300,000	-38.09
28				
	1,960	1,920,000	970,000	-50.00

In Table II each figure recorded is the average result of three plate counts. The number of viable cells per cubic centimeter decreases as the dosage increases, and the percentage of decrease reaches a maximum with a dosage of 1,960 mg-hr. or 1.22 erythema doses. These doses were not carried so high as in the experiments to determine the effect upon electrophoretic migration velocities, but it is reasonable to suppose the results would have been similar.

The results of the pH determinations (Table III) show that radium, in the doses studied, does not alter the pH of the suspension of Esch. coli.

Table III

RESULTS OF pH DETERMINATIONS

Irradiation dose		pH	Suspensions	
mg-hr.		Distilled Water	Control	Irradiated
3	210	6.6	6.8	6.8
7	490	6.6	6.7	6.8
12	840	6.6	6.8	6.8
14	980	6.6	6.8	6.7
18	1,260	6.6	6.6	6.6
21	1,470	6.6	6.8	6.8
27	1,890	6.6	6.8	6.8

DISCUSSION

The results in Table I, A, B, C, which show that the gamma rays of from 0.06 to 0.002 $\overset{o}{A}$ do influence the zeta potential of the bacteria is not in accord with the findings of Dorn, Baumann and Valentiner (18). These workers have shown that the lethal action of radium is attributable to the alpha and beta particles, and that the gamma rays are apparently harmless.

Comparing Experiment 21 (Table I, A, B, C) with Experiment 21 (Table II), we find that 1,470 mg-hr. irradiation - a little under the calculated erythema dose of 1,600 mg-hr. - decreases the viability of the bacteria in the preparation by 38.09 per cent. Zinsser and Bayne-Jones (92) show that there is conflicting evidence in the literature on

the effect of radium upon bacteria, but that workers have found that radium emanations kill Esch. coli, Staph. aureus and other pathogenic bacteria after long exposures. Theoretically then, such irradiation should have a beneficial effect upon infections, for example, carbuncles. Such is actually the case, and radium packs do greatly benefit carbuncles and subcutaneous abscesses.

It is generally accepted that slightly less than an erythema dose of roentgen rays, properly filtered, will act in like manner, so that roentgen therapy is rapidly becoming the treatment of choice for carbuncles. Some unknown biophysical phenomenon must come into play during such irradiation of infections, however, for low voltage roentgen rays do not have the same bactericidal action on suspensions (Dozois, Tittsler, Lisse and Davey (23)) as do the gamma rays of radium. Further investigation is necessary to understand more thoroughly the results obtained by irradiation of infections.

Ultraviolet rays, on the other hand, which are much longer waves than gamma rays of radium or roentgen rays, have the greatest bactericidal action of the three, but so far as is known are not used for the treatment of carbuncles. This may be because of their limited penetration, as some more superficial infections are benefited by ultraviolet radiation.

In this connection it is interesting to mention that waves much longer than any of the above have been used in the treatment of superficial and deep infections. Schliephake (63) has studied the effect of radio waves ("ultrashort waves") from 3 to 18 meters long. Heat effects are observed with these waves and it has not been possible, as yet, to

determine to what degree wave length is responsible for the beneficial
therapeutic results. Schliephake (64) says that wave length is not a
very important factor in treating superficial infections, such as fur-
uncles and carbuncles, but that in the deeper ones, better results are
obtained with the shorter waves. This might be explained by the fact
that the shorter waves are more penetrating, but there is a possibility
of specificity of action of the band of waves employed.

The results of the pH determinations (Table III) show that radium,
in the doses studied, does not alter the pH of the suspension of
Esch. coli. The effects of cations upon bacterial viability have been
studied by Winslow and Dolloff (85). Since the hydrogen ion concentra-
tion may have an effect on the bacterial viability, it was important to
note whether an alteration in the pH of the bacterial suspension produc-
ed the change in viability or whether it was due to the radium irradia-
tion. Since there was no change in the pH this did not play a part.
Lisse and Tittsler (44) have found an increase in the pH of suspensions
after lethal doses of ultraviolet radiation, while Dozois, Tittsler,
Lisse and Davey (25) have demonstrated that roentgen rays, of the wave
lengths studied, did not alter the pH."

7. "Variations in the Electrophoretic Mobilities of Escherichia,
Aerobacter and 'Intermediate' Strains"

As mentioned above, attempts to identify various microorganisms
by their electrophoretic velocity have not met with great success.
Smith and Joffe (69) failed to find any variations in the electrophoret-
ic mobilities of the Brucella groups. Birkhaug (6a) found that he could

not use mobility studies in the identification of various strains of Mycobacterium tuberculosis (bovis and hominis). The spectacular work of Rosenow (62) in the identification of various types of streptococci by their zeta potential cannot be taken seriously because of the very obvious errors in technique.

"Since, by cultural methods, it is often difficult to obtain any well defined differentiation between the Escherichia, Aerobacter and "intermediate" organism it was thought that a determination of the zeta potential of the various strains comprising these groups might be of assistance in their identification. Chapman (13) has shown the zeta potential of various strains of Escherichia coli to be constant. Numerous experiments conducted by us show that this is true, not only for Escherichia coli strains, but for Aerobacter aerogenes as well.

One hundred and twenty-seven strains of Escherichia, Aerobacter and "intermediates" were isolated from oysters and oyster water, water, urine, feces, and necropsies, or obtained from the Tittsler collection. They were distributed according to Bergey's Determinative Bacteriology (6) and Tittsler and Sandholzer's (81) review groups as shown in table 2.

Although table 1 is only a partial record of the total results obtained it is sufficient to show that the zeta potential of the various strains of Escherichia, Aerobacter and "intermediates" is a characteristic constant for each strain. By careful control of the period of growth, the pH of the growth medium and suspension solution, and by using the same technique in the operation of the apparatus for determination of the electrophoretic migration velocity, it was found that the

TABLE 1

Tabulation of the electrophoretic velocities of strains of Escherichia,
Aerobacter, and "intermediates"

GENUS AND SPECIES	STRAIN	ELECTROPHORETIC VELOCITY			
		seconds per 0.5 mm.	seconds per 0.5 mm.	seconds per 0.5 mm.	seconds per 0.5 mm.
Escherichia coli	1	10.6	10.4	10.6	10.5
	2	13.0	12.8	12.8	12.9
	3	14.8	14.6	15.0	14.9
	4	13.1	13.5	13.0	13.3
	5	12.2	12.5	12.0	12.3
Escherichia communior.......	1	13.5	13.6	13.2	13.4
	2	11.9	11.9	12.3	12.2
	3	12.9	13.1	13.3	12.8
	4	13.4	13.0	13.0	12.8
	5	13.7	14.1	14.0	13.9
Escherichia neapolitana	1	14.1	13.8	14.1	13.7
	2	13.5	13.3	13.5	13.2
	3	12.3	12.5	12.7	12.4
Aerobacter aerogenes	1	14.5	14.7	14.2	14.6
	2	15.4	14.9	14.3	14.8
	3	11.7	11.7	11.4	11.6
	4	12.8	13.1	13.1	12.6
	5	13.3	13.5	12.9	13.1
Aerobacter liquefaciens	1	12.4	12.4	12.0	11.9
	2	17.2	16.8	16.7	16.9
	3	15.6	15.3	15.3	
	4	14.8	14.7	15.1	15.1
Aerobacter oxytocum	1	11.5	11.8	11.5	11.6
	2	11.4	11.5	11.4	11.6
	3	13.5	13.5	13.6	13.3
	4	14.7	14.4	14.6	14.3
"Intermediates"	1	13.9	14.3	14.1	14.3
	2	11.5	11.0	11.3	11.6
	3	15.3	15.7	15.5	15.5
	4	16.7	16.4	16.4	16.5
	5	14.8	15.3	15.1	15.1
	6	15.3	15.6	15.4	15.4
	7	12.3	12.3	12.3	12.5
	8	13.4	13.6	13.8	13.5
	9	13.9	14.4	14.0	13.8

TABLE 2

Tabulation of the average, minimum electrophoretic velocities of
species of Escherichia, Aerobacter and "intermediates"

CULTURE	Total Number	ELECTROPHORETIC VELOCITIES		
		Average velocity	Minimum velocity	Maximum velocity
		seconds per 0.5 mm.	seconds per 0.5 mm.	seconds per 0.5 mm.
Escherichia	47	13.2	18.9	9.2
coli	10	12.2	15.0	10.6
enterica	9	14.5	18.9	12.3
anaerogenes	6	13.2	16.7	11.0
communior	9	13.6	14.1	11.9
neapolitana	7	13.2	14.1	12.3
communis	8	13.2	18.5	11.9
alba	8	12.3	14.1	9.2
Aerobacter	49	12.3	17.2	11.0
aerogenes	14	12.3	15.4	11.4
oxytocum	10	13.2	14.7	11.4
cloacae	8	12.2	14.7	11.0
liquefaciens	12	13.7	17.2	11.9
levans	5	12.7	13.2	11.4
"Intermediates"	32	13.2	16.7	11.0

zeta potential of the various strains remains constant over a long

series of transplants. Whether the strain was freshly isolated or

carried for a period of time on an artificial growth medium apparently

had little influence on the results. Although the electrophoretic

migration velocity of a single strain remains constant, there is,

nevertheless, a wide variation in velocity between the strains of the

same species. Because of this variation, and since the zeta potential

of one strain of one species may be very near to, or often identical

with, to the potential of a strain of another species it is impossible to identify a strain or a species by its electrophoretic migration velocity.

Although the number of species studied is not large, they represent many of the organisms most commonly isolated from the sources indicated. Had more extensive studies been made, it is hardly to be expected, in view of our observations, that the results would have varied greatly from the recorded determinations. As shown in table 2, the average electrophoretic migration velocity of the 7 species of Escherichia was found to be 13.2 seconds, the minimum 18.9 seconds and the maximum 9.2 seconds. The average migration velocity of the 5 species of Aerobacter was 12.3 seconds, minimum 17.2 seconds, maximum 11.0 seconds; and the average velocity of the 32 strains of the "intermediates" was 13.2 seconds, minimum 16.7 seconds and maximum 11.0 seconds. It is interesting to note that although the range between the minimum and maximum velocity was greater for Escherichia than for the"intermediates" the average migration velocity of the two is the same, which is less than the average found for Aerobacter. All attempts to correlate electrophoretic migration velocities with specific cultural reactions proved unsuccessful."

8. "Relationship Between Electrophoretic Migration Velocities, the Virulence and the Types of the Diphtheria and Diphtheria-like Bacilli"

"These studies were made to ascertain whether the electrophoretic migration velocity measurements made on microorganisms grown on

Loffler's medium for 24 hours might be of value in routine work for determining the virulence of the diphtheria and diphtheria-like-bacilli.

METHOD

The diphtheria strains used were obtained from the collection of the Bureau of Bacteriology of the Maryland State Department of Health through the courtesy of C. A. Perry. The virulence determinations and the typing were made by C. A. Perry (work not published). For the virulence determinations the intracutaneous guinea pig method was employed, while types were determined by colony morphology and carbohydrate reactions. The electrophoretic migration velocity measurements were completed before information as to virulence and type was obtained.

It was/found necessary to convert these results into velocities in microns per second, for the velocities are not constant and depend on various factors of technic. The velocities per unit of time do not represent a basis for comparing these results with those of other workers. Comparisons of the electrophoretic measurements were made with the results of the virulence tests. The results of our observations are comprised in Table I.

DISCUSSION

As shown in Table I, from the standpoint of velocity, 3 zones may be differentiated. The smallest zone (the diphtheroid zone) ranging from 6.51 to 7 seconds includes the more rapidly traveling diphtheroids; the largest zone (the virulent zone), from 7.01 to 9 seconds, comprised the greater part of the virulent diphtheria strains with the medium velocity.

TABLE I

TABULATION OF THE ELECTROPHORETIC VELOCITIES OF STRAINS STUDIED

	Types	Diphther-oid Z one	Virulent Zone				Avirulent Zone			Total
			Seconds per 0.05 mm.							
		6:51-7:00	7:01-7:50 Class 1	7:51-8:00 Class 2	8:01-8:50 Class 3	8:51-9:00 Class 4	9:01-9:50 Class 5	9:51-10:00 Class 6	10:01-10:50 Class 7	Total
Diphtheria	Gravis	0	5	2	3	3 (Avirul)	1 (Virul)	0	1	15
	Intermediate	0	5	0	5	8 (Avirul)	2	1	0	15
	Mitis	0	2	4	10	2 (Avirul)	1 (Virul)	1	4	24
	Total	0	12	6	18	13	4	2	5	60
Diphtheroids	Hofmanni	2	3							
	Xerosis	3	2							
	Total	5	5							
	Grand Total	5	17	6	18	13	4	2	5	70

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The 3rd zone (the avirulent zone) which comprises most of the avirulent strains with the slowest velocity, ranges from 9.01 to 10.50 seconds. The lines between the zones cannot be sharply drawn.

The virulent and avirulent zones are divided into velocity classes. In class 1 are those microorganisms with a velocity between 7.01 and 7.50 seconds, in class 2, those with a velocity between 7.51 and 8.00 seconds, and so on through the 7 classes. The borderline between the virulent and avirulent zone falls between classes 4 and 5. Strains in the velocity classes near the border of the zones often cross that line. Two virulent strains are in class 5, and 3 avirulent strains are in class 4. On the other extreme toward the diphtheroid zone the same phenomenon may be seen. Five diphtheroid strains are found in class 1. The virulent diphtheria strains do not appear in the zone of the diphtheroids.

The 2 virulent strains which appear in the avirulent zone, 3 avirulent strains in the virulent zone, and the 5 diphtheroid strains in the virulent zone would have led to incorrect observations as to virulence had not the fermentation and intracutaneous tests been made. These above strains represent an error of 14 per cent, which is, doubtlessly, a greater error than that found by the intracutaneous test. This error is $3\frac{1}{2}$ times greater than that published by Jensen, Falk, Tonney, and White (35), and approaches the results of Stone and Wiegel (77). The latter have found that there is not a sharp line between the virulent and avirulent strains and that there is also an interchange between them. Randall and Thompson (60) have arrived at the conclusion that because there is no sharply marked line of distinction between

the velocities of the virulent and avirulent strains electrophoretic methods of virulence determinations cannot be used in their present forms as a routine method.

A total of 70 strains were studied which represent all of the known diphtheria bacilli as well as the most common diphtheroids. As shown in the table, about half of the virulent strains appear in the velocity class near the border line which separates the virulent and avirulent organisms. The velocity distribution is sufficiently reliable to assume that at least 50 per cent of the strains of every series would belong to the borderline classes. Although the error found is but 14 per cent, in light of our experience, it may be as great as 50 per cent in other series.

There is another indication that the electrophoretic velocity method is not sufficiently advanced for routine work. Although Falk and his co-workers, and Randall and Thompson report that the avirulent strains migrate more rapidly than do the virulent, our findings are in accord with those of Stone and Weigel that the virulent strains migrate more rapidly than do the avirulent. These differences indicate that the potential difference of the bacteria depends on many and only partly known factors. Falk, etc., and Randall, etc., used 48 hour old broth cultures for their determinations. Stone and Weigel have unfortunately not stated the medium they used. It is possible that the results we obtained were due to the fact that we used a solid medium (Loffler's medium) on which to grow the bacteria. It may be supposed that the electrophoretic velocity is not a constant feature

of the bacteria, but depends upon factors of technic and environment. Although working under the same apparent conditions, a slight unavoidable change in technic might cause an alteration in the measured electrophoretic velocity.

As shown in the table, within each zone there is a certain tendency of the velocities toward the extremes. The gravis type apparently tends toward the extreme of the virulent zone, and the intermediate and the mitis types toward the avirulent zone. This trend suggests that the electrophoretic velocity of the diphtheria strains should be in accord with their virulence and the shift toward one or the other extreme should indicate the degree of their virulence. On the basis of this an attempt was made to determine, by means of the measured electrophoretic velocity, the variation of the virulence inside the three types (gravis, mitis, and intermediate) described by Anderson, Happold, and McLeod (1). The errors in the electrophoretic velocity method were detected by studies of the intracutaneous tests.

In order to determine the variation of the virulence and the differences within each type, as well as the standard deviations and their differences, the mathematical mean of the electrophoretic migration velocity was calculated. The following are the results of these calculations:

1. Mean velocity of the gravis type (and its probable error)

7.94 $\pm$ 0.124 seconds

2. Mean velocity of the intermediate type (and its probable error)

8.16 $\pm$ 0.151 seconds

3. Mean velocity of the mitis type (and its probable error)
8.11 ± 0.107 seconds.

These figures indicate that the mean velocity of the gravis type
is shifted toward the virulent zone; that of the intermediate type
toward the avirulent zone, and that of the mitis type lies between
the two. In other words, the gravis type would contain more strains
with a higher degree of virulence than does the intermediate or the
mitis type. These differences are, however, only apparent. "The
significance test" shows the difference between the means was not
significant (the difference between the gravis and intermediate
0.92 o, between the gravis and mitis 0.65 o, and between the inter-
mediate and mitis 0.22 o). The even scatter of the strains as to
their virulence distribution (migration velocity) was also ascertain-
ed by calculating the standard deviations of the type (standard devia-
tion in order above; 1.33, 1.25, 1.35; differences 0.53 o, 0.18 o,
0.78 o). These calculations permit us to assume that if the velocity
represents a degree of the virulence, the diphtheria strains studied
show a corresponding virulence regardless of the type. Because our
sample is small in regard to type distribution it does not permit us
to draw final conclusions.

The distribution of the avirulent strains into types by means of
colony characteristics and carbohydrate reactions offer figures of
interest: 25 per cent of the avirulent strains belong to the mitis
type, 19.05 per cent to the intermediate, and 13.33 per cent to the
gravis type. These figures offer further evidence for assuming the
possibility of the variation of the virulence inside the diphtheria types."

V. CONCLUSIONS

By means of electrophoretic migration determinations the influence
of various physical and chemical factors on the zeta potential of a
bacterium may be determined. An attempt to show a relationship between
the electrophoretic migration velocity and toxigenicity, virulence, and
agglutinability of various microorganisms has met with little success.
As work continues, however, a relationship between the migration veloc-
ity and certain physical characteristics of the organisms is being
shown.

Since it has been suggested that the electrophoretic migration
velocity of bacteria might be affected either by the pH of the growth
medium or by that of the solution in which the bacterial cells are sus-
pended studies were made to determine the extent of this effect. As
shown, although the organisms may be obtained from media which differ
in pH within the range indicated, yet if they are suspended in a solu-
tion of uniform pH they will give uniform migration results. The pH of
the suspension fluid does, however, affect the velocity. As the pH of
the bacterial suspension increases from 4.6 to 7.8 there is also a grad-
ual decrease in velocity.

Storage and heat, under the conditions observed, do affect the
migration velocity of various microorganisms. Although bacterial sus-
pensions may be held at 5°C. for forty-eight hours without significant
alteration in the zeta potential, if the same organisms are held for
over two hours at 27°C. a measurable change in migration velocity may
be obtained. Bacterial suspensions were heated at a temperature of 60°C.

for thirty minutes with no change in the zeta potential. At temperatures above 60°C. a slight tendency toward a decrease in the zeta potential was observed. This decrease became more marked as the temperature was increased. The electrophoretic migration velocity of bacterial suspensions heated to a temperature of 60°C. for thirty minutes remained constant for two hours at 27°C. After this time a slight but gradual decrease in the migration velocity occurred.

The effects of ultraviolet and low voltage X-rays radiation on the electrophoretic migration velocity of Escherichia coli lead to the study of the effect of radium irradiation on the electrophoretic velocity, viability, and pH of Escherichia coli suspensions. As shown, the electrophoretic migration velocity of suspensions of this organism is reduced by irradiation with the gamma rays of radium. As the dosage increases the velocity decreases. Radium irradiation does influence the viability of the organism and the percentage of decrease in the electrophoretic migration velocity corresponds to the decrease in viability. Radium irradiation has little or no influence on the pH of the suspension.

The electrophoretic migration velocity of the individual strains of Escherichia, Aerobacter and "intermediates" is constant, but a wide range in the velocity does exist between strains of the same species. "Since a strain of one species may have the same migration velocity as a strain of another species, and since the average velocity of one genus may be very near to that of another genus, it has not been possible to differentiate between Escherichia, Aerobacter and the "intermediates"

by a study of their electrophoretic migration velocity."

From a study of the electrophoretic measurements made on 60 diphtheria and 10 diphtheroid strains, grown on Loeffler's medium, it was found that the diphtheroid strains have the greatest velocity, the avirulent strains the least and the virulent strains represent a medium velocity between the two. The line between the diphteroid, avirulent and virulent diphtheria may not be sharply drawn by studies of their zeta potential.

Numerous workers in various institutions are interested in electrophoretic studies of microorganisms. Although much of the work already completed has been verified by further studies there still remains much to be learned. The value of electrophoresis studies in the field of bacteriology has, as yet, not been fully ascertained.

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