THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Hoff Daniel Good final oral examination for the degree of

Master of Arts

We recommend that the degree of

Master of Arts

be conferred upon the candidate.

W. Larson
Chairman

E. C. Rosenow

J. B. Hogarty

J. F. McClendon

Arthur T. Henrici

Date May 15, -22

10-20 5M

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Hoff Daniel Good for the degree of Master of Arts.
They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

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J. F. McClendon

Date May 15 - '22

10-20 5M

The Electrical Properties of Bacteria

A Thesis submitted to the
Faculty of the Graduate School of the
University of Minnesota

by

Hoff Daniel Good

In partial Fulfillment of the Requirements for the
Degree of Master of Arts

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The Electrical Properties of Bacteria

A number of investigators have observed the movement of bacteria and other cells in an electric field. Little seems to be known of the details of this phenomenon, but it is explained by assuming these cells have an electrical charge and that the movement observed is true cataphoresis. Surface tension has been suggested, also, as an explanation of this phenomenon by assuming that the potential gradient of the electrical field produces a gradient in the surface tension of the film of liquid surrounding the cells and movement results toward the region of lower surface tension. If this cell movement in an electrical field is due to an electrical charge of the bacterial cell, an understanding of this phenomenon would probably lend much to an explanation of many of the reactions in which bacterial cells take part. It is a common observation that bacterial cells can be brought into closer proximity to one another by centrifuging after heating above the thermal death-point than was possible before. The explanation has been offered that a loss or change in the electrical charge is the responsible factor. Likewise an electric charge can be considered of fundamental importance in such reactions as agglutination, or the combining of a bacterium with an antibody. Just what the nature of the electric charge supposedly held by cells really is does not at present seem to be understood. Explanations based upon the difference of dialectric constant, the Helmholtz double layer, adsorption of ions, and ionization from the surface of the bacteria have been offered. The experimental results of the different investigators of the cataphoresis of bacteria do not always agree, and the individual results of many of them have been subject to wide variation. Inasmuch as it is very difficult to determine just what has been actually established at the present time, it has seemed necessary in undertaking work along this line to conduct experiments covering the most fundamental considerations. The types of apparatus used by various investigators have varied greatly in construction and operation as both macroscopic and microscopic methods of observation have been employed. Much of the apparatus used has not been adequately described, and some of the apparatus described as used for this work seems faulty and incapable of giving constant results. Because of this, it has been found necessary to expend much time in the development of an apparatus that gives results that are constant and would seem less liable to gross error.

It has been noted in the work reviewed that all experimental work has been directed toward the vegetative form of bacterial cells. Further, it has been observed* that inconstant results with a given bacterium could be ascribed to the age of the organism in that opposite results were obtained when young and old cultures were used. In order to test this possible variation to a still greater degree, it was thought advisable to carry on some experimental work with a spore former in that such bacteria represent in their vegetative and spore forms the extreme in young and old cultures. It is considered essential here to review the work of some of the investigators in this field whose work has been concerned with bacteria and bacterial products both for the experimental observations which were made and also to consider the types of apparatus used.

Field** and Teague determined the electric charge of bacterial toxin and antitoxin. Their experiment was performed with the apparatus in Fig. 1.

* Proc. Roy. Soc. London B. 1910, 82, 641

**Jour. Exp. Med. Vol. 9, 1907, PP. 86-91

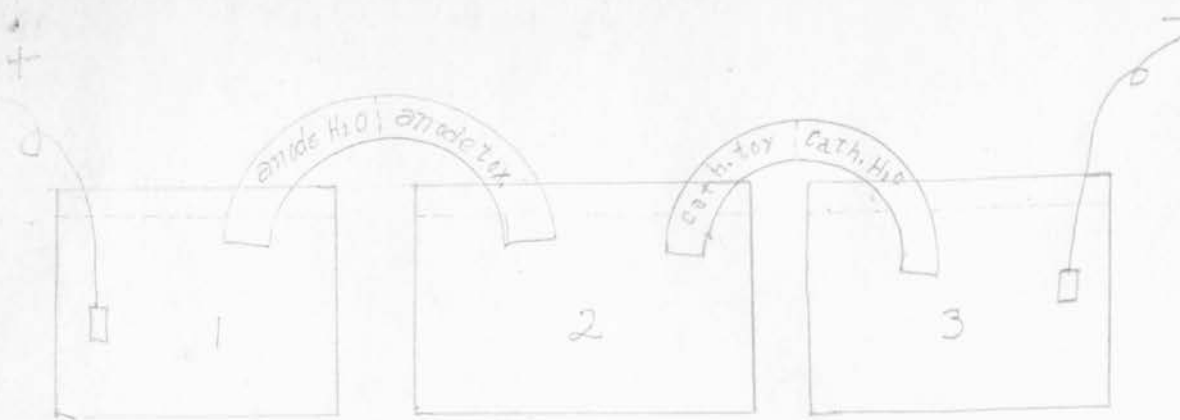


Fig 1.

The two U tubes each 2 cm. in diameter and 20 cm. long were filled with melted agar and cooled. Distilled water was placed in beakers 1 and 3 and antitoxin or toxin in beaker 2. The U tubes were then placed as shown in the diagram. A direct current was passed for four or five hours, the water in beakers 1 and 3 being siphoned off and replaced every thirty minutes to eliminate the effects of the products of electrolysis in the tubes. The tubes were removed and the agar was then pushed out of the ends which were in the electrode compartments. About a quarter of an inch of the portions that dipped into the toxin or antitoxin was discarded and the rest of the mass divided equally. After rinsing each portion in distilled water, it was chopped up and placed in 8 cc. of distilled water for an hour after which the agar was removed by straining through gauze, and the respective filtrates tested for toxic or antitoxic properties. It was found that both toxin and antitoxin moved toward the cathode and that a change in the reaction of the solvent does not cause a reversal of the charge. Since both toxin and antitoxin have a like charge, Field and Teague concluded that the combination of the two is not a true chemical reaction but the adsorption of one by the other.

The technique adopted by Field and Teague was very ingenious and the results seem to be good. In regard to the electrical charges of cells, W. M.

Thornton's work is comprehensive.

Thornton* studied the kataphoresis of organisms in a drop of water rich in pond scum. He placed a drop of the suspension on a glass slide, placed over it a $\frac{3}{4}$ inch cover slip, and studied with a 1/12 inch oil immersion lens. The current was admitted at each side of the cover slip by means of platinum wires connected to a switch so that the polarity could be reversed. A potential gradient of 75 volts per cm. was the highest that could be conveniently used. His chief difficulties were in maintaining contact between the electrodes and the drop, in the cells adhering to the slide and cover slip, and in distinguishing between the movement due to the streaming of the liquid and movement due to the electric force. The diatoms and unicellular algae moved toward the negative pole, and the amoeboid organisms toward the positive pole.

Paramecium as a rule was found to go to the negative pole. When they were found in clusters, they went to the positive pole. Euglena went to the positive pole. These observations, however, were not entirely uniform.

Volvox, a filament of vaucheria, sphoerella, plantagnis, a pleurococcus (chlorophyll green alga), and protococci from moist growth all went to the negative pole.

B. typhosus, B. tuberculosis, B. diphtheria avium, B. prodigiosus, B. lactic acid, B. pyocyaneus, B. coli communis, B. Friedlander, Sarcina aurantiaca, Sarcina lutea, and staphylococcus aireus, spore bearing bacillus, hog cholera, pnsumococcus, when taken from young actively growing cultures and examined at once were found to go, without exception, to the negative pole.** However, old cultures and those which showed poor growth, although subcultured twenty-four hours before examination, almost always moved to the positive pole.

Blood cells and yeasts moved in opposite directions, the yeast going

* Proc. Royal Soc. London B. 1910 Vol. 82 P. 638
**Proc. Royal Soc. London B. 1910 Vol. 82 P. 641

to the negative pole and the blood cells to the positive. In a field they moved past each other and, after collisions, continued in the original direction with velocity unchanged. From this, he concludes that the charges are not on the outside, otherwise they would, at least to some extent, coalesce. Smears of blood cells and yeasts dried for several days show the same charge as fresh cells on being moistened.

From this data, Thornton believes that one can reasonably conclude that fresh animal cells have a negative charge and vegetable cells a positive charge. He states that this might provide a sensitive means of determining whether an organism is vegetable or animal.

Thornton reasoned that since the only mechanical force an electric field can exert is on an electric charge, the opposite movement of cells indicates that they are oppositely charged. Kimball shows that another explanation is possible. A drop of mercury resting on the bottom of a glass tube containing sulphuric acid through which a current is passed moves toward the negative terminal. This, Kimball accounts for by assuming that the surface tension is lowered on the side of the drop toward the cathode. This theory may also be extended to explain why some organisms move in a certain direction.

Thornton admits that he encountered many difficulties, and that his results did not always check. This was probably due to faulty technique. Upon attempting to work with bacteria using his cover slip method, we found it impossible to distinguish definitely between movement due to convection currents and true polar migration. Coward observed that, if the potential difference between the charged particle and the electrode is sufficiently great, the charged particle may even be attracted toward an electrode of like sign. Thus, working with a high potential and having the electrodes close together, Thornton's observations would be extremely liable to error unless they were made exactly midway between the electrodes. He also had trouble with convection currents of liquid which started flowing almost as soon as the current was turned on. These would confuse the observations and naturally we cannot place much confidence in his conclusions.

His results do not check with those of Teague and Buxton* who found that normal B. pyocyaneus, agglutimated B. pyocyaneus, normal B. typhosus and agglutinated B. typhosus moved to the anode and that upon acidifying they moved to the cathode. They used the apparatus shown in the Fig. 2.

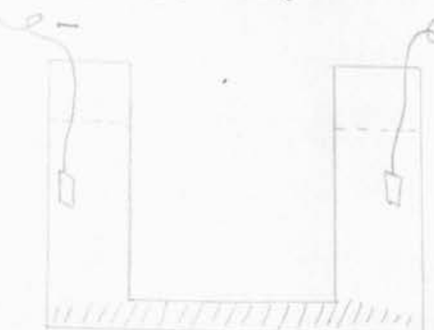


Fig 2.

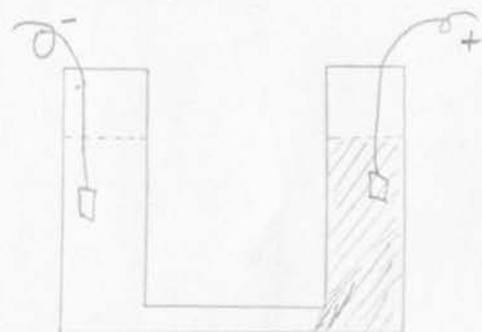


Fig 3.

After the current had passed for a time, the suspension appeared as in Fig. 3.

The results of Cernovodeau and Henri** contradict those of Thornton. They found that B. anthrax, the coli bacillus, B. typhosus, T. B., staph aureus traveled toward the anode, showing that they had a positive charge. B. of dysentery Flexner went toward the negative and therefore had a positive charge. All these bacteria were examined in a watery chamber in the presence of thionine without phenol and acid fuchsin. B. Flexner quickly colored a deep red and all the others a blue. Cernovodeau and Henri believed that these staining reactions of the bacteria were to be explained by their electric charges, since thionine without phenol is a positive colloid and acid fuchsin is a negative

* Zeits Fur Physichalische Chemic 1907, Vol. 5 P. 62

**Compt. Rend Soc. Biol. Paris Vol. 62 PP. 866-904 colloid.

They state that the electric charges of bacteria remain the same after killing by heat and, when acidified, the electric charges become much diminished and may become 0.

Mme. Girard Mangin and M. Victor Henri* found that red cells suspended in physiological salt solution or sucrose solution move toward the anode.

Lillie suspended spermatozoon in isotonic sucrose solution, N/4, and examined them in an electric current under the high power. They traveled toward the anode with their small end first. This, he concludes, shows that there is a difference in potential between the nucleus and cytoplasm. However, if we consider a spermatozoon an insulated conductor, we may offer a different explanation. We may regard a spermatozoon as a conductor because there are certain ions present within it. Then, since it does not lose its charge when suspended in the solution, it behaves like an insulated conductor. In this case, the greatest density of charge would be on the projecting terminal filament. The whole spermatozoon would be attracted toward the same electrode, but the small end would move first because it would be more strongly attracted, due to its greater density of charge.

The determination of whether the spermatozoon and ovum have different charges would be an interesting problem. The head of the spermatozoon penetrates the ovum first. If this penetration is due to a difference in charge between the spermatozoon and ovum, it might indicate that the nucleus of the spermatozoon has a different charge from that of the cytoplasm and be in support of Lillies' theory.

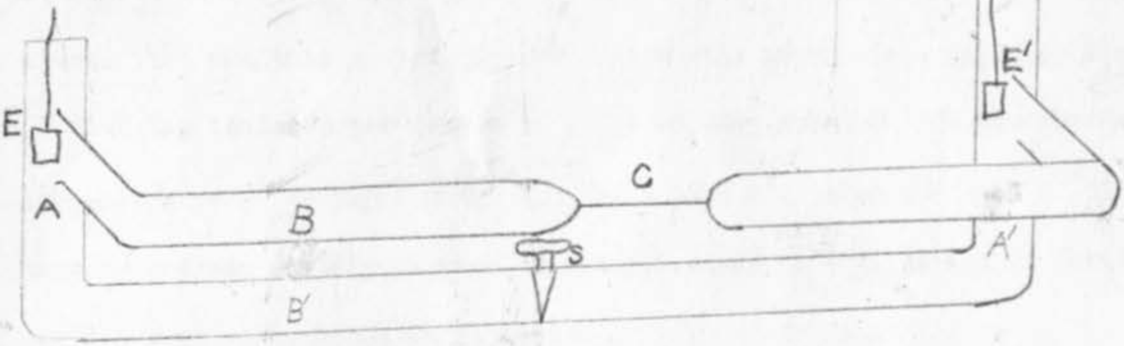
The technical difficulties encountered in carrying out this type of experimentation is attested to not only by the inconstant results obtained by

* Compt. Rend Soc. Biol. Paris Vol. 8 PP. 275, 1903.

the various workers, but they themselves call attention to the difficulties met with. The great source of error is the convection current which is present in most forms of apparatus used, especially those in which observations are made with a microscope on a small portion of a cross section of the electrical field. The causes of these convection currents are probably many, among which may be mentioned the heating effect due to the electric current; changes in the density of the liquid adjacent to the electrodes; the mechanical effect of bubbles forming around the electrode from the electrolysis which always occurs; and changes in the surface tension due to the products of electrolysis, heat and differences of potential.

The products of electrolysis present another great difficulty. In any experiment on the movement of bacteria in an electric field by the microscopic method, the products of electrolysis formed at the electrodes must be completely eliminated from the portion of the field under scrutiny. In a bacterial suspension many ions, among which are Na, Cl, H and OH, are present. The chlorine which is very toxic to the bacteria goes to the anode. The OH ions also go to the anode causing the reaction to become alkaline. The Na and hydrogen go to the cathode. In a physiological salt solution there are more sodium ions than H ions. The sodium unites with water to form NaoH which far overbalances the contribution to acidity by the H ions and, consequently the solution becomes very strongly alkaline around the cathode. If the products of electrolysis are allowed to diffuse through the field, some of the bacteria are agglutinated. Convection currents may carry cells which have been discharged or have received an opposite charge at the electrode back into the field under observation where they may be seen to act differently than formerly.

To avoid evident errors several types of apparatus were built and used. The type finally developed and which has given constant findings is here described in detail in Fig. 4. It is constructed entirely of glass and con-



sists of two upright electrode chambers A and A' connected by two systems of horizontal tubes B and B'. The lower B', has inserted midway between the electrode vessles a stopcock, S. The upper tube, B, has inserted in its central position a fine glass capillary, C. The electrode chambers are 9 cm. high and made of glass of I cm. bore. The horizontal system of tubes was of similar tubing and tube B was 60 cm. in length. The capillary was 4 cm. long and about 75 micra in diameter, this diameter being determined approximately by comparing with human red blood cells. A direct current of 110 volts was used. The fall in potential between the electrodes E and E'is the same through ECE' as through ESE'. Since the capillary is infinitely small in comparison to the rest of the tube, the resistance in C is infinitely great in comparison to the remaining resistance, and we may, therefore, assume that the entire fall of potential comes in the capillary. The potential gradient is then about 27 volts per cm. The distance ECE was about two feet. Thus, cultures could be studied for many hours without the products of electrolysis interfering.

It was found in one type of apparatus in which a capillary connected two electrode vessels that a current of liquid flowed through the capillary when a potential gradient existed. The explanation for this is probably a difference of surface tension in the two electrode vessels which in turn was dependent either upon the products of electrolysis or upon the potential gradient, or this can also be explained upon the basis of the electrolysis of water causing a change of liquid level on the two sides of the capillary. It is believed that the tube B reduces error from this source to a negligible minimum in that any tendency for change of level of the liquid in the two chambers can take place very rapidly through the large tube B'. Also the tube B' allows work to proceed rapidly because the liquid comes to equilibrium of level very rapidly when suspensions are introduced.

The use of a capillary tube has several advantages. It allows a complete cross section of the pathway of moving cells to be under observation at once; it concentrates the potential gradient in the tube B to a limited space; and it removes the potential gradient actually used from the vicinity of the electrodes and products of electrolysis. It appears that electroendosmosis must come into consideration here.

In a capillary containing distilled water, we have a condition resembling that of fine glass particle suspended in water in which the glass becomes charged negatively relative to the water. In a capillary, the glass cannot move, but the water being free to move is displaced through the capillary toward the cathode. Cohen formulated a general rule for the charge which a substance will have when suspended in a liquid. He postulated that the substance with the higher dialectric constant is positive to the other. This rule is only an approximation for pure liquids and breaks down completely for watery solutions. Therefore, since bacteria give out salts when placed in distilled water, this rule cannot, to any extent, be applied to bacterial suspensions. We must, then, rely upon a study of the displacements of the bacteria in the capillary to determine whether there is a movement of the water due to the fact that it becomes charged oppositely to the glass. In the case of a salt solution, there is not much probability that the liquid adjacent to the glass will become positively charged due to the pulling of sodium ions into solution from the glass since there is already present such a large number of sodium ions that a few more would have no great effect. In a capillary as large as 75 microns in diameter, the effect produced by the water next to the glass becoming charged could not be very great. The capillary is very large in comparison to the organisms studied. It is 75 times larger in diameter than a staphylococcus and a staphylococcus is far from molecular size. Thus it seems reasonable to suppose that even in case the electroendomosis of water actually occurs, it can not impair observations in the center of the capillary.

In a capillary with very thin walls the curvature of the outside circumference and the inside circumference are very nearly the same. One can, therefore, see through it almost the same as through a plane piece of glass. If a capillary has relatively thick walls it acts like convexo-concave lens which is thimner in the center than at the sides. One cannot see anything in this capillary unless oil of immersion and a cover slip are used to make the necessary correction.

A capillary is not suitable for the study of bacteria in a strong acid solution on account of the heat developed. The greater the hydrogen ion concentration, the greater is the conductivity of the solution. The heat developed in watts is equal to the product of the current in amperes squared and the resistance or $W=I^2R$. The resistance equals the fall in potential in volts divided by the current in amperes, or $R=E/I$. Thus, while the resistance is decreasing in an arithmetical ratio, the current is increasing in geometrical ratio, and there must besome point as the hydrogen ion concentration is increased where the heat produced cannot be radiated away fast enough and steam will be formed. When a strong acid solution was used bubbles appeared in the capillary almost as soon as the circuit was closed. The circuit was thus broken and no movement of bacteria could be observed. When the current was turned on with the bubbles present they tended to expand and, when the current was turned of, they contracted to their original volume. This is hard to explain. Finally, when the circuit was left closed, the capillary exploded.

In our experiments the bacteria used were always grown on agar medium, the time of growth being 16 hours to a week in different experiments. The bacterial suspension was poured into the apparatus which had been thoroughly cleaned. The bacterial suspension in the capillary was observed under the microscope. and the cells were seen to be stationary. Upon closing the circuit the cells were seen to move, all the cells in the entire cross section being visible. Upon opening the circuit the migration of cells immediately ceased, the movement being entirely dead-beat. Reversal of the current brought about a movement of the cells in the opposite direction. It was possible to study the bacteria in the capillary for 24 hours or more in that the cells were far removed from the products of electrolysis.

Staphylococcus albus was most extensively studied. In suspensions prepared,as described above, these cells were found to move to the anode. This observation was constant in many experiments with this apparatus. In further experiments using both 16-hour cultures and 7-day cultures, movement of the cells toward the anode always took place. There appeared to be no variation in any way in the behavior of young and old cultures of staphylococci. The same organisms when killed by heating at 85 degrees for 5 minutes, moved toward the anode with apparently the same velocity as the same cells when alive. From this it would appear that if these cells have a negative charge, it is the same whether the cells are old or young and is not lost on death. Experiments were likewise performed to determine the effect of the nature of the reaction upon polar migration. A suspension of living staphylococci was acidified to a P H of 2.9 and the cells were seen to migrate constantly to the anode. This movement still occurred after the suspension had stood in the apparatus for 24 hours. A suspension of the same organisms when killed by heat and then acidified to a like degree moved to the anode as did the unheated cell.

In repeated experiments with living suspensions of the vegetative forms of B. subtilis, B. megatherium and B. bienstocki in an electric field, migration of these cells to the anode always occurred. Many experiments were performed with the spore forms of these organisms and in suspensions of pure spores migration to the cathode always took place. The fact that pure cultures of spores and pure cultures or vegetative cells always move in opposite directions seems to indicate that they do have opposite electrical charges. However, if we assume that there is a liquid convection current set up in the capillary when the circuit is closed, due to the liquid becoming charged differently relative to the glass, several conditions are equally possible.

The convection current may flow either toward the cathode or anode. Let us for the convenience of explanation assume that the velocity of this flow is X. Let us first suppose that the direction of the liquid stream is toward the anode. Then, since the vegetative cells move also toward the anode there are three possibilities. First, the vegetative cells may be attracted toward the anode; second, they may not be attracted toward either pole; and, third, they may be attracted toward the cathode at a velocity less than X. At the same time, inasmuch as the spores move toward the cathode, they must be attracted toward the cathode at a velocity greater than X. Now, let us suppose that the direction of the liquid stream is toward the cathode. Since the vegetative cells move toward the anode, they must be attracted toward the anode at a velocity greater than X; while the spores, on the other hand, may be either attracted toward the cathode not attracted toward either pole or they may be attracted toward the anode at a velocity less than X.

In any of these cases, at least either the vegetative cells or the spores must be attracted toward an electrode because the two forms move in opposite directions. Furthermore, if we assume that this polar attraction is due to an electrical charge, then, from the evidence at hand, we are fully warranted in assuming that, if the vegetative cells and spores do not bear opposite charges, they must at least maintain a difference of electrical potential.

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