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 Robert Gladding Green 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.

W. Parson
Chairman

J. F. McClendon

A. H. Sanford

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May 24 1920

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 Robert Gladding Green final oral examination for the degree of Master of Arts . We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

May 24 1920

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THE PERMEABILITY OF BACTERIA

A Thesis submitted to the
Faculty of the Graduate School of the
University of Minnesota
by
Robert G. Green
In partial fulfilment of the requirements
for the degree of
Master of Arts
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1920

PERMEABILITY OF BACTERIA

The limiting membrane of bacteria has not received much study by bacteriologists. Inasmuch as it is the seat of exchange of all materials concerned in metabolism, and is also a possible vulnerable point of attack for anything capable of destroying bacteria, it appears worthy of much more study than has been given it in the past. The function of the bacterial membrane is to serve as a container for the substances interacting in the process of metabolism and to give entrance and exit to substances concerned, only at the proper time. This complex function has been bound up in the simple expression, permeability.

The permeability of the plasmic membrane was first studied by the production of plasmolysis. This procedure has been supplanted in late years by studying the resistance offered by cells to an alternating current, a phenomenon described by Stewart in 1897 from observations on blood cells. An interpretation of results obtained from a study of the effect of cells on the flow of alternating currents is based upon the observed properties and actions of artificial membranes which can be studied directly. The plasmolysis experiments have shown that most cells are surrounded by some sort of a membrane that is permeable to water and impermeable to colloids. This membrane is penetrated only very slowly by neutral salts of strong acids but more readily by glycerine and other alcohols, aldehydes, ketones, neutral esters of organic and inorganic acids and similar compounds. The presence of certain crystalloids within cells and their retention in hypotonic solutions seems explainable only upon the basis of complete impermeability of the membrane for these substances. A number of membranes similar in action in many

respects to vital membranes have been prepared and studied extensively. Those that are impermeable to colloids, completely permeable to water and variously permeable or impermeable to crystalloids and other molecules of moderate weight are spoken of as semipermeable membranes.

It might be considered that water forms a continuous pathway for molecules passing into the cell from the fact that only substances in solution may pass into the cell. This did form the basis of a theory of permeability as put forth by Traube. He pictured the membrane as a sieve, the size of the molecular interstices being the determining factor as to what molecules could pass thru it. This theory carried with it the assumption that the volume of a molecule depends upon its weight and complexity. Not only have many inconsistencies been proven for this view but other factors appear to be much the more important in many cases.

The absorptive action of the membrane for a given substance plays an important role. A membrane of water allows carbon dioxide to pass thru rapidly while oxygen and nitrogen are held back. There is a flow of alcohol to ether when the two are separated by a membrane of pig's bladder which seems due to a higher rate of absorption of alcohol by the membrane.

The third possibility is a reversible reaction whereby a penetrating substance forms a loose chemical union with the membrane on one side and again breaks free at the opposite side where its tension is lower. Among the artificial membranes this has been given as the probable explanation of the permeability of a membrane of palladium to hydrogen. These actions of artificial membranes at least form a basis upon which one can theorize concerning the permeability of living membranes in that a number of remarkable indiv-

idual similarities do exist.

The semipermeability of a living membrane is more complete than the best artificial membranes which depend upon the size of openings for their properties. Crystalloids may be held inside a cell because the membrane is not permeable to them but their presence demands the assumption that the membrane be permeable to them at some time. In a study of compounds that were able to penetrate plant and animal cells, Overton found that those substances penetrated that were soluble in fatty oils. This led him to the conclusion that the membrane was lipoid in character. Since fatty substances have the property of reducing surface tension and since surface tension depressing substances concentrate in the surface, the idea has been gradually developed that the membrane is a concentration of the lipoid of the cell in the surface layers. It has been observed that if protoplasm is mechanically squeezed out of a paramecium, the protoplasm forms droplets that exhibit plasmolysis and plasmoptysis as did the original membrane of the organism. Ruhland finds that the parallelism of solubility in lipoids with the ability to penetrate vital membranes is not as complete as set forth by Overton. Osterhout finds the membrane permeable to many substances not soluble in lipoids. Clowes assumes the presence of lipoid in the plasma membrane and explains the antagonistic effects of certain electrolytes on cells as demonstrated by Loeb, Osterhout, and others, by their effect upon oil and water emulsions.

In studying permeability by plasmolysis and similar methods, as the precipitation of tannin inside the cell, a passage of substances into the interior of the cell is noted and so only the plasma membrane comes under consideration. When a cell offers resistance to an electric current the resistance may be considered as being offered

only by the membrane, or in part by the structural framework of the cell. In the case of red blood cells which seem to be practically nonconductors, the resistance drops to such an extent when the cells are hemolyzed that the resistance may be considered due entirely to the membrane. Osterhout finds that with the death of Laminaria, the resistance of a solution containing Laminaria is the same as that of the solution alone and concludes that the dead cells offer no resistance and that here also the resistance is due to the membrane. This resistance at the cell membrane is ascribed to an inability of the ions moving in the electric current to pass thru the living semipermeable membrane. It has been pointed out that the inability of only one ion of a compound to penetrate the membrane will result in a penetration of the other ions only so far as the attraction of the ions for each other will permit.

It seems impossible to prove cells absolutely nonconductors as all electrolytes cannot be removed from solutions around them without great probability of a change in the membrane. Attempts have been made to explain the high resistance offered by cells without regarding the semipermeable membrane as the cause. The theory has been advanced that the high resistance might be accounted for by the presence of electrolytically disassociated colloids inside a membrane impermeable only to colloids. Bayliss has shown that if only one ion is in the colloidal state, a current can be carried only in one direction thru a membrane, the direction depending upon the sign of the colloidal ion. This would account for the resistance of cells only if the salts in the cell interior were in a colloidal combination. All salts not in colloidal combination of both the exterior and interior of the cell must then be regarded as freely diffusible. As this is known not to be the case it is evident that

the resistance offered by the cell is not due to the action of colloidal salts alone.

That the property of semipermeability makes a membrane offer resistance is shown by the resistances obtained in preparing copper ferrocyanide membranes electrolytically. In one of the membranes made by Berkley and Hartley, the resistance gradually rose from 2,700 ohms to 300,000 ohms as the interstices were gradually filled up by the deposition of copper ferrpcyanide.

The resistance offered by cells has been measured by having the cells in suspension in a solution carrying a current, as has been done by Stewart with red blood cells and by McClendon with eggs, or by placing moist animal or plant tissue directly between two electrodes. The latter has been used by Osterhout in his extensive investigations with Laminaria. The high resistance of red blood cells observed by Stewart in 1897 was noted to disappear entirely when the cells were hemolyzed. McClendon in 1910 found that eggs of sea urchins in concentrated suspensions have a resistance much higher than sea water alone and he regarded the impermeability of the cell membrane to ions as the cause. He observed a decrease in the resistance offered by these eggs with fertilization. In working with the living cells of Laminaria, Osterhout found that they increased the resistance of sea water several times its actual resistance and concluded the cells took no part in the conduction of the current.

From the fact that diffusible ions have gained entrance to a cell and are held within apparently by impermeability of the surrounding membrane, one must conclude that a functional change in this membrane does occur. A large number of direct observations shows it to be true. Osterhout observed that root hairs of Dianthus barbatus contained no crystals of calcium oxalate when grown in dis-

tilled water but with the addition of calcium salts the crystals soon appeared. Nitella which is impermeable to sodium hydroxide is made permeable by the addition of sodium salts. It was found by Fluri that aluminium sulfate makes spirogyra permeable for most salts and that, if the cells are returned to pure water, they again become normal in their behavior. These changes in permeability have been detected and measured by the resulting change in the resistance to an electric current. As before mentioned, McClendon noted a decrease in the resistance of eggs upon fertilization. Osterhout found that while Laminaria was impermeable to ions of sea water, if the cells were placed in a solution of pure sodium chloride of the same conductivity as sea water, the resistance rapidly drops until they offer practically no resistance, the total being hardly greater than that of sea water alone. The cells are then considered dead and the results seem analogous to those obtained by the laking of red blood cells. It was further found that, if the Laminaria was again immersed in sea water before the change in permeability had become too great, a normal condition of the membrane as regards permeability was then reestablished.

The work of Osterhout seems to be the most complete investigation on permeability by conductivity methods yet made and from his results he has formulated a theory of antagonism of salts as regards their action on the plasmic membrane. His results agree quite generally with those obtained by Loeb and others using different methods of investigation. A review of Osterhout's methods is considered essential here. From 100 to 200 discs 13 mm in diameter were cut from the fronds of Laminaria. These discs are about .5 mm thick and are stiff enough to withstand considerable handling. These discs were packed together like a stack of coins and this cylinder of tis-

sue fitted snugly in a glass rod frame in the shape of a hollow cylinder. Spaces between the rods allowed free access of fluid to the discs when the whole was immersed in a solution. At each end of the cylinder was a block of hard rubber bearing an electrode. This block could be pressed against the ends of the tissue cylinder by means of a screw. The cylinder was placed in sea water or other solutions being used and after time for penetration of the solution, removed, the superfluous liquid being allowed to drain off. Readings were then taken. When changing from one solution to another the electrodes were loosened and moved apart, and each disc was moved separately back and forth in the new solution to wash out traces of the solution used before. His cell in sea water had a resistance of 320 ohms. With a cylinder of Laminaria discs between the electrodes it has a resistance of about 1100 ohms. No change was noted in the reading during twenty-four hours. When the solution was changed from sea water to .52 M. NaCl, which had a conductivity equal to sea water, the resistance gradually dropped from 1000 ohms after 5 minutes to 890 ohms in 10 minutes and thence to 780 ohms after 15 minutes. It was found that if the cells were replaced into sea water before the resistance had dropped over 100 ohms, the resistance again rose to the previous level and remained there. This action of calcium chloride was shown to be opposite to sodium chloride. If the tissue was placed in calcium chloride solution and washed free of the sodium salt, the resistance rose from 1100 ohms to 1700 ohms and remained there for some hours. Osterhout also observed that the presence of calcium prevented the action of sodium in decreasing the resistance. If only a trace of calcium were present in the sodium chloride solution, no change in resistance took place. The antagonism of calcium to the action of other salts similar in their effect

to sodium chloride was also demonstrated.

Gray, working with eggs of Echinus, Strongylocentrotus and Arbacia, found a similar antagonism between trivalent ions as Cerium and Lanthanum and the negative ion, citrate. He suspended the eggs in the solution under consideration and made his measurements after centrifuging the eggs to a mass, by means of removable electrodes. The positive trivalent ions, like Cerium, increased the resistance and were effective in a concentration of .0001 Mol, while the citrate was active in .2 mol.

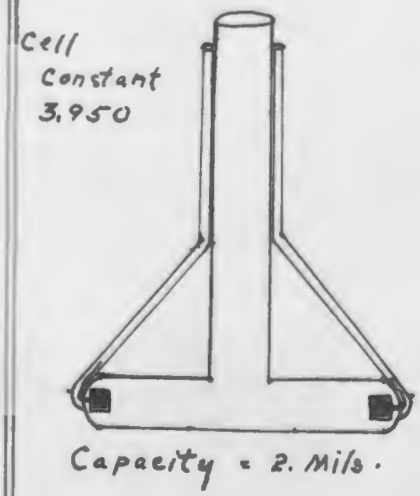
From these investigations and others to which these are similar, these important propositions are to be noted. Echinoderm eggs and sheets of Laminaria fronds do conduct a current or it would not be possible to increase their resistance. This power of conduction is taken to mean permeability, and may be increased or decreased by specific agents. Any change of permeability in either direction over a certain point brings death to the cell. Cell death means the complete loss of the property of offering resistance to a flow of an electric current. Attention is called to the abundance of data given in the literature in proof of all the propositions stated except the last.

It was felt from the beginning of these studies that the investigation of permeability by the conductivity methods would find an ideal application in bacteria. Only one kind of cell is present. Unlike an egg it is a whole organism with a membrane actively "working". Each cell is individual and so its whole extent of membrane is exposed. The surface area is enormous per gram of cells. It forms even suspensions and can be separated from its menstruum. It can be grown under a variety of conditions. The cells will stand considerable treatment without death and, what is very important, it

can be proven easily and positively whether the cells are dead or alive at any time, by a simple culture.

The first experiments showed that a bacterial suspension offered a higher resistance than the liquid in which they were suspended which is in agreement with the behavior of other cells investigated. The experiments were performed at 25°C in a thermostat which maintained a constant temperature within one-twentieth degree. The measurements were made by one of the modifications of the Wheatstone bridge. In the earlier experiments a meter sliding wire resistance was used but this was later replaced by a rotary wire resistance of about seven meters length. An alternating current of 450 oscillations was used and was obtained from a Vreeland oscillator. A variety of electrolytic cells were tried. Fixed electrodes were finally adopted as the most reliable. A vertical cell with electrodes one above the other gave trouble when there was a tendency for agglutination, so a T-shaped cell as pictured was finally devised as most satisfactory. It keeps the material evenly distributed between the electrodes and in the same stratum of temperature in the bath. Altho the cell constant was determined and is given, the gross resistance of the material in the cell is given in this paper.

Figure I.



Bacteria were obtained from growths upon agar in Kolle flasks. About fifteen flasks were used in each experiment and only young twelve-hour growths were used. The bacteria were washed off in a large amount of distilled water and centrifuged. The supernatant liquid was drawn off and the cells then washed once or twice

with the solution in which they were to be finally suspended. As a rule, the final suspension contained an amount of centrifuged cells equal to half the suspension volume. This gave a very opaque milk-white liquid.

It was found that the resistance obtained with the bacteria was not of as large an order as had been obtained with other cells, the increase being 15 to 35% of the resistance of the liquid, depending on the strength of the suspension. Osterhout raised 320 ohms to 1100 or over 300 per cent but he had very little solution present in proportion to the cellular material. Gray raised the resistance of his solutions from 16 ohms to 300 ohms, an increase of 20 times, by centrifuging the eggs to a mass around the electrodes. His results, however, were only of an order of 15 to 25%, while Osterhout's varied over his entire range of resistance. On heating a suspension of B. coli for 30 min. at 65°C, it was found that the resistance decreased. This decrease in resistance was at that time thot to be analogous to the decrease in resistance of blood upon hemolysis and was explained upon the basis of Osterhout's findings to be due to an infinitely increased permeability of the cell membrane due to the death of the cell. The following experiments give the results and the degree of change.

The growth on fifteen Kolle flasks was removed with distilled water, centrifuged, washed again with distilled water and suspended in a .3% potassium chloride solution. This suspension was centrifuged and the supernatant liquid transferred to the conductivity cell and the resistance measured. The menstruum was returned to the centrifuge tube and the bacterial mass worked up to an even suspension by a spiral glass rod. The suspension was placed in the conductivity cell and its resistance measured, after which it was returned

to the centrifuge tube, placed in a water bath and heated at 65°C for 40 minutes. The resistance of the dead suspension was again measured. In one case where the resistance of the fluid removed from the suspension was 850 ohms, addition of the bacteria raised the resistance to 957 ohms. After heating, the resistance was found to be 846 ohms or practically the same as the potassium chloride solution alone. In another experiment the resistance of the solution was 623 ohms which was raised to 720 ohms by the bacteria and the resistance dropped to 617 ohms after heating. It is to be noted in both these cases that the resistance of the suspension after heating is a little lower than was the resistance of the liquid alone before heating. This suggests that not only has the resistance of the cells decreased but some electrolyte has been added to the solution by death of the bacteria.

To determine errors due to experimental manipulation, several test experiments were done. A suspension of B. coli in .5% KCl was centrifuged and resuspended several times with no appreciable variation in readings. There was no change in readings over a period of six hours worthy of note. Readings taken after 30 hours showed a considerable decrease in resistance, so plating was done on a suspension before and after standing as well as the resistance measured. Before standing, dilutions of one, ten, and a hundred millions gave counts of thirty and one half, thirty-three, and thirty billions, respectively. After standing thirty-six hours, dilutions as above gave seventeen, seventeen, and sixteen billions. This shows that the live bacteria decreased to one half in number and a corresponding decrease in resistance was noted.

Due to the resistance of the heated bacterial suspension being lower in many cases than the original solution without bacteria, data

on the actual resistance of the fluid in which bacteria had been killed was sought. Bacterial suspensions were prepared and after washing in distilled water, were suspended in .3% sodium chloride. It should be mentioned that the washing was not sufficient to remove all of the salts carried over from the media so that the concentration given is only approximate, but the salt added was greatly in preponderance. The bacteria were centrifuged down to a mass and the resistance of the supernatant menstruum measured. An even suspension was again made in this menstruum and its resistance measured. To obtain a reliable mixing the suspension was transferred from the centrifuge tube to the electrolytic cell several times to incorporate all residue of the menstruum left in the cell when the supernatant fluid was measured. Mixing was continued until successive readings for the suspension were constant. The suspension again transferred to the centrifuge tube was tightly corked with clean tinfoil and heated in a water bath at a temperature kept constant for the experiment. After heating sufficiently to kill the cells an even suspension was again obtained and its resistance measured. The killed bacteria were then centrifuged down and the supernatant menstruum measured. The bacteria were again suspended in this same liquid and the resistance measured. The results are shown graphically in Figs. II and III.

It is seen in these experiments that by killing the bacteria the resistance of the menstruum surrounding the cells was greatly decreased, dropping from 335 ohms to 295 ohms. It is evident that the increase in conductivity of the liquid is due to an increase in saline concentration. Höber showed by indirect methods, as the damping effect of a solution of an electrolyte upon high frequency currents and the effect of similar solutions in increasing the capacity of a

Figure II

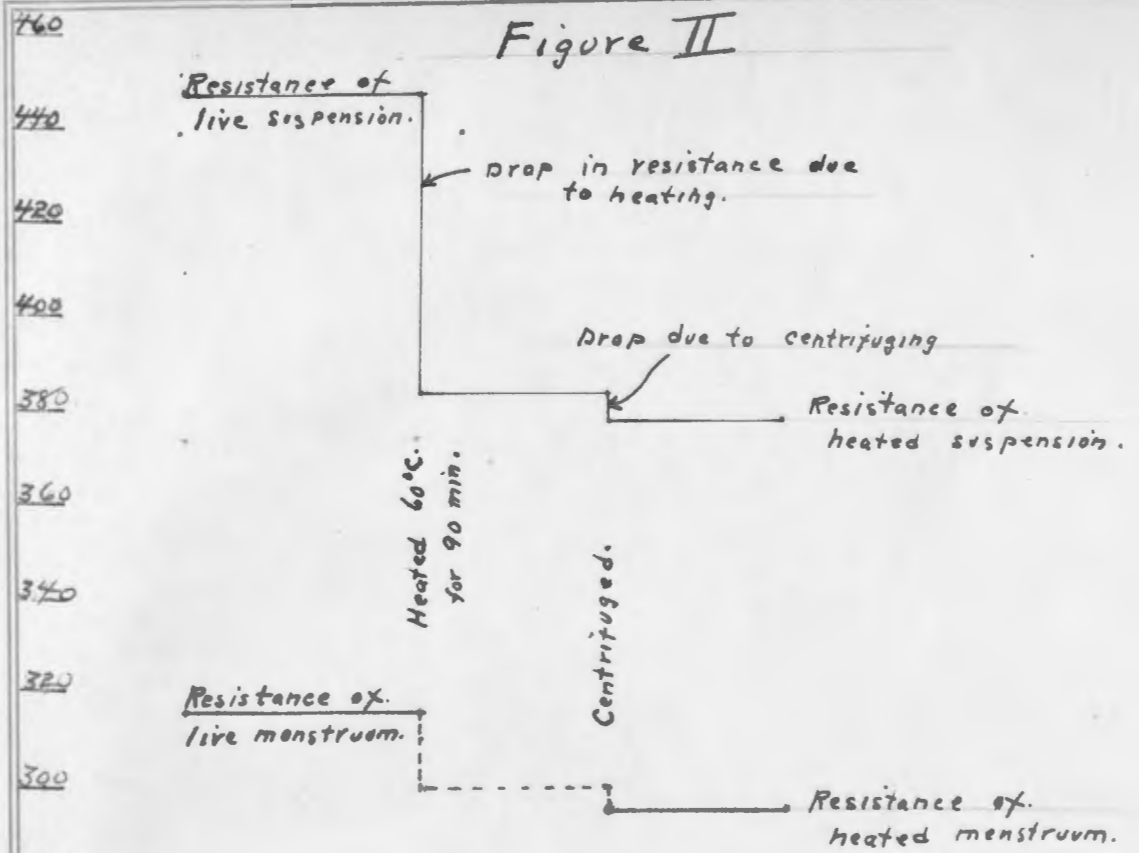
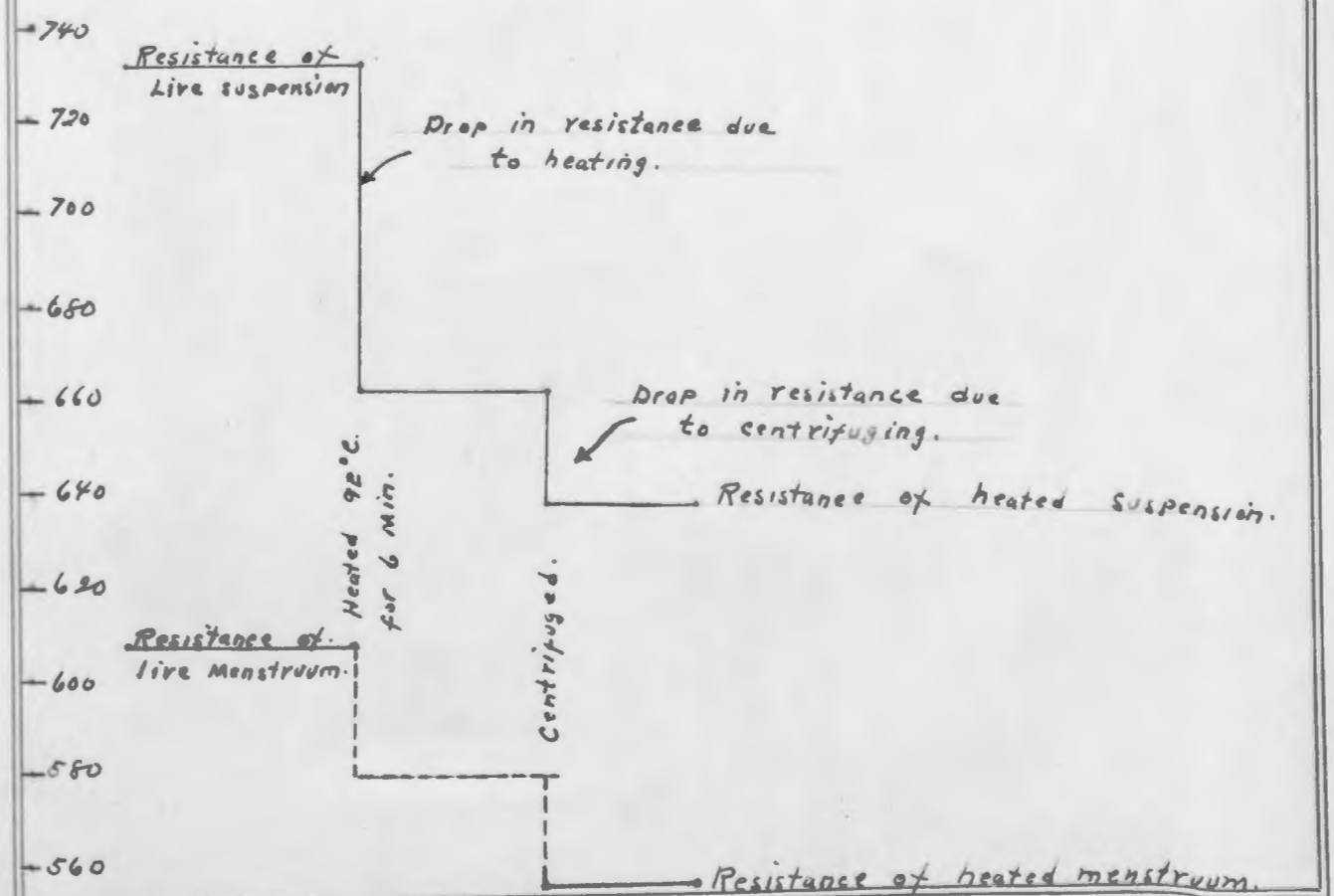


Figure III



condenser, that red blood cells contain ionized salts in solution equivalent in effect to a KCl solution between .1 and .01 normal. Stewart showed that in hemolysis of red blood cells the release of electrolytes from the cell preceded that of the hemoglobin. McClendon observed, as far back as 1910, that eggs of fundulus lose magnesium in pure NaCl solution; he has recently observed the exit of chlorides from cells upon their death. These observations appear to establish without doubt that cells contain electrolytes in solution which are held inside the cell by the semipermeable membrane which, if destroyed, releases the contained salts and allows them to come to equilibrium with the salts in the surrounding medium. This appears at once to have an important bearing upon the interpretation of results obtained in conductivity experiments with bacteria. The decrease in resistance observed after heating a suspension of bacteria may not only be due to an increased permeability of the cell membrane for ions traveling under the influence of electrical potential, but may also be a direct increase in the carrying capacity of the liquid due to additional ions obtained from the cell contents. Both of these factors will depend upon the concentration of salts in the conducting liquid for the magnitude of their effect. A decrease in the number of ions in proportion to the cells present will result in an increase of the resistance offered by the cells in that the possibility of an ion to travel a given distance unimpeded is proportionally decreased. Also the nearer the degree of ionization of the external liquid to the ionization of the cell sap, the less will be the effect of the latter when it is given freedom of diffusion. It is clear that if cells are killed in a solution isotonic with the cell sap, the effect of the salts contained within will be nil. This quantitative relation will be discussed more fully with a suc-

ceeding experiment.

It is also noticeable in Figs. II and III that there is considerable difference in the resistance offered by the menstruum of the heated suspension and the heated suspension itself, the difference between 377 ohms and 295 ohms, or 82ohms, in II and between 638 and 557 or 81 ohms in III. From this it is apparent that dead bacterial cells must offer resistance to ions traveling in an electric current. That the membrane is permeable is shown by the issue of the salts from the cell interior. The degree of heat with the tendency to agglutination of the cells is proof the cells were dead. Stewart showed that the resistance of red blood cells was due to the membrane alone and this is understandable if the delicate structure of that cell is considered, it being hardly more than a gel surrounded by a semipermeable membrane. The volume of solid material in the case of hemolysis of red blood cells shrinks to almost nothing. The solid volume of a quantity of bacteria, on the other hand, is, if any, very slightly decreased by destroying the permeability of the membrane, and each bacterium retains its morphology, due to a large amount of strong structural constituents. In Osterhout's work of 1916 on the permeability of Laminaria, he points out the decrease in resistance of a cylinder of the tissue, saturated with sea water, to the resistance of sea water alone upon death of the cells, and he concludes from this that the dead cells offer no resistance. It is to be remembered that similar results were found in the first experiments offered in this discussion and it is now apparent that an interpretation according to Osterhout's findings leads to an erroneous conclusion. Every experiment performed upon the resistance of bacterial suspensions in which the bacteria were removed so that the conductivity of the liquid could be measured and then added again to form the suspen-

sion which was measured, the presence of the dead bacteria caused an increase in resistance which was 50 to 80% that offered by the same bacteria when in the same amount of solution and alive.

It was found that if the suspension of bacteria was measured directly after heating, centrifuged, and remixed, a drop in resistance occurs. That this is a further change in the amount of obstruction by the bacterial cells is not considered, but is believed to be a still further decrease in the resistance of the solution. Heating surely destroys the semipermeability of the membrane and allows diffusion to take place. Evidently equilibrium of salt concentration on both sides of the membrane is not reached at once. In centrifuging, each bacterium is driven on the average thru the water a distance equal to half the depth of the fluid height in the tube. This motion of flowing liquid can be considered as washing out the salts and bringing the concentration to an equilibrium. In Fig. II, where the bacteria were heated at 60°C for 90 minutes the change of resistance in centrifuging was small, being 8% of the total decrease. In Fig. III, where the bacteria were heated at 92°C for 6 minutes, the decrease of resistance due to centrifuging is 30% of the total. It can be seen from the figures that the decrease in resistance due to heating is comparable in the two cases. Time for diffusion is seen to be the main factor in the establishment of equilibrium on the two sides of the membranes. It is to be supposed that the diffusion would take place much faster at a high temperature but as in the second case the temperature was 92°C, the effect of this factor appears limited. When one considers the large surface area of a bacterium in proportion to the volume of the cell and the small distance that the diffusing ions have to travel to reach the surrounding solution, the rate of diffusion seems very slow. This seems to

offer an opportunity to speculate upon the gross structure of the bacterial membrane.

The bacterial cell is specialized in having a structure strong enough to retain its morphology in spite of severe physical treatment. It may be that this inert structural material in fact makes up in large part the limiting membrane. The osmotically active membrane which carries on the exchange of ions in the process of metabolism would then have to be considered as only being a part of the limiting membrane. Such an idea of the bacterial membrane, while without proof, at least gives a point of view that allows explanation of the slow diffusion of salts from the interior of the cell and only assumes the presence in the limiting membrane of the non-conducting material that gives to dead bacteria their resistance to an electric current.

In the experiments already discussed, considerable variation in the degree of resistance change is noted. The lowering of the resistance of a suspension of bacteria is seen to be due to two main factors, the actual increase in permeability of the bacterial cells and an increase in conductivity of the solution carrying the bacteria. The first factor in the case of actual killing of the bacteria would seem to depend only upon the number of the cells present. The increase in conductivity would seem to depend upon the relation of the saline concentration within the cell to that of the solution surrounding it, as well as upon the proportion of cells to the menstruum in which they are suspended.

Experiments which are conducted on a series of bacterial suspensions made up from different growths of cells on various batches of media are hardly comparable because of the variation of these factors. The number of cells in a thick suspension is difficult to determine

as a routine. The concentration of the menstruum can be controlled but the concentration of the salts within the cell is an unknown quantity. It is known that bacteria can accommodate for growth in a great range of salt concentration. B. anthracis flourishes on agar containing 8 to 10% sodium chloride. Analyses of bacterial ash have shown the salt content to vary with the medium upon which they were grown. The increase in salt content is probably due to increase of salts in the cell sap which acts to equalize the osmotic pressure on both sides of the membrane. To determine the relation of the salts diffusing from the cells to the effect produced in different concentrations of menstruums, a series of experiments were performed on equal numbers of bacteria from a single growth, suspended in equal amounts of different salt concentrations.

The colon bacilli from a 24-hour growth on 25 Kolle flasks were washed in 120 cc of distilled water and, after centrifuging, resuspended in 15 cc of distilled water. This suspension was divided into 5 equal parts and to each part was added a solution of NaCl of a different concentration. Each suspension was washed twice more in its respective concentration of NaCl, so that a series of bacterial suspensions in 1/10, 3/10, 5/10, 7/10 and 9/10% NaCl solutions was obtained. From the work of Osterhout, Loeb, Gray and others, it was considered necessary to add a small amount of $CaCl_2$ to each solution to antagonize the toxic action of sodium for membranes. The bacteria were again centrifuged down and the resistance of the supernatant menstruum measured. An even suspension of the bacteria in the same liquid was again made and its resistance taken. This procedure was followed with each of the suspensions after which they were placed in a water bath at 65°C for 20 minutes. After centrifuging, the resistances of the various supernatant liquids were

measured. Again even suspensions were made and their resistances measured. Fig. IV shows the following results graphically expressed.

Table A,

Concentration of NaCl	Resistance of suspension : Alive	Dead	Resistance of menstruum : Alive	Dead
0.1%	1625 ohms	1203 ohms	1463 ohms	1083 ohms
0.3%	645	555	560	495
0.5%	399	362	350	325
0.7%	303	283	263	250
0.9%	314	295	263	253

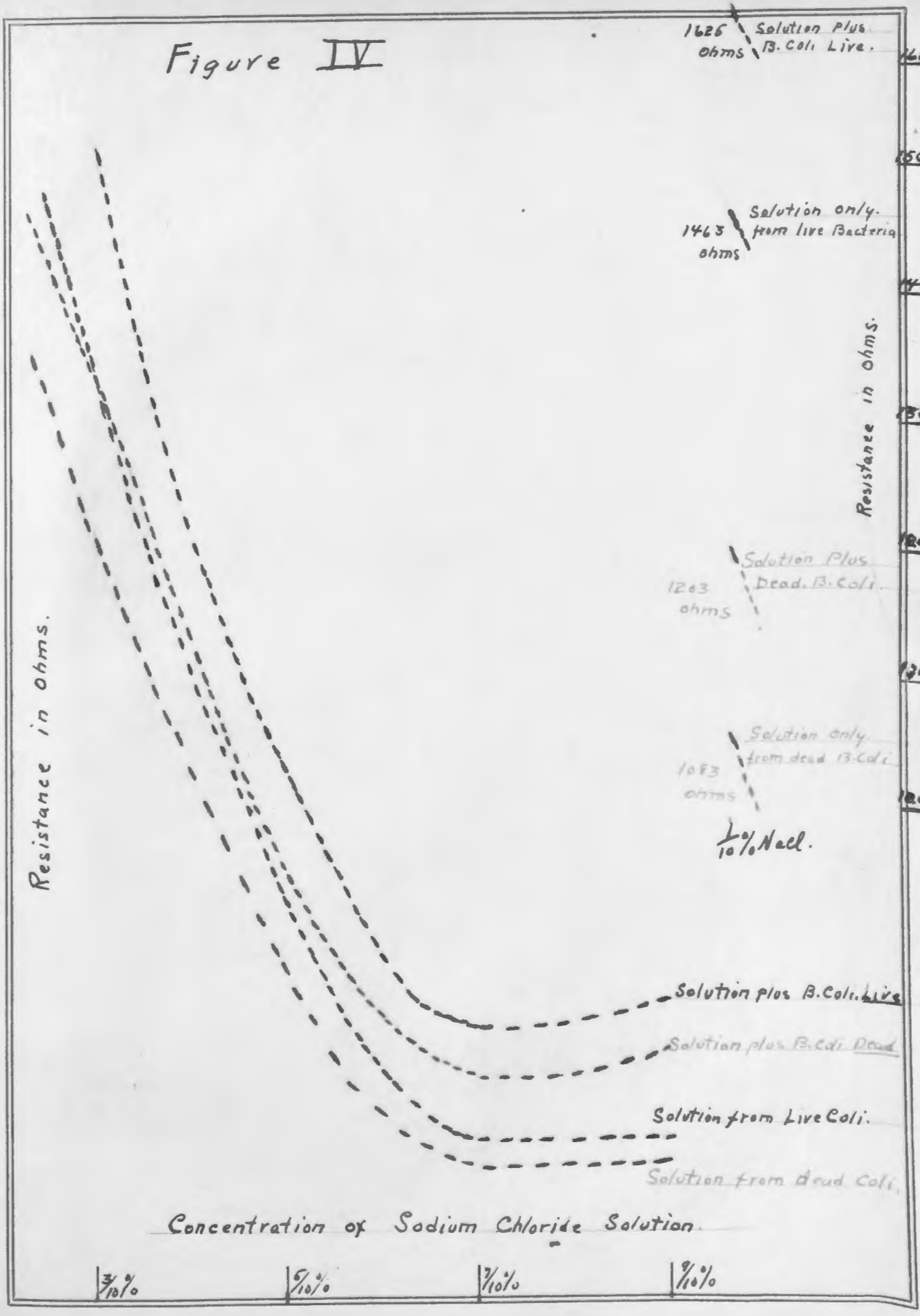
The most evident result obtained from this series is the reduplication of the results of former experiments to show that dead bacteria do offer resistance which is less than that offered by the same organisms when alive. The following table shows the relative proportion in the different concentrations.

Table B.

Concentration of NaCl solution	Resistance of living bacteria in ohms	Resistance of dead bacteria in ohms	Rd/Rl
	Rl	Rd	
0.1%	162	120	70%
0.3%	85	60	72%
0.5%	49	37	75%
0.7%	40	33	82%
0.8%	51	42	82%

It will be noticed that a definite decreasing series is found except for the last case. The basis for this variation from the series is seen on the graph where both the dead and living bacteria offer a greater resistance in the 0.9% solution than in the 0.7%,

Figure IV


1626 ohms
Solution Plus B. Coli Live.
1463 ohms
Solution only from live Bacteria
1203 ohms
Solution Plus Dead. B. Coli.
1063 ohms
Solution only from dead B. Coli
1/10% NaCl.
Resistance in ohms.
650
600
550
500
450
400
350
300
250
1600
1500
1400
1300
1200
1100
1000
Solution plus B. Coli. Live
Solution plus B. Coli. Dead
Solution from Live Coli.
Solution from dead Coli.
Concentration of Sodium Chloride Solution.
3/10%
5/10%
7/10%
9/10%

while the resistance of the solutions does not change in either case. The changes in resistance in the 0.9% NaCl suspension, however, are exactly analogous to those taking place in the other members of the series and they are included to show other relations which they depict. The last column of Table 5 shows the percentage resistance of the dead bacteria to that of the same cells when alive, and this percentage is an increasing value corordinately with the increasing salt concentration of the solution. An attempt to find some explanation of this increasing value is given in the following experiment.

A pair of electrodes wired into a Wheatstone bridge was immersed in a solution of 1/10% NaCl. A copper ferrocyanide membrane made by depositing the copper ferrocyanide into a parchment thimble as a base was slipped over each electrode and the resistance measured. The membranes were removed and the resistance again measured. This procedure was repeated for 5/10 and 1% NaCl solutions with results as follows.

Table C.

Concentration of NaCl solution	Resistance with membrane Rv	Resistance with no membrane Rc	Rc/Rv
0.1%	542 ohms	474 ohms	87.6
0.5%	130	110	84.6
1.0%	68	56	82.3

One can consider the resistance of a solution as the resistance of an identical solution with the electrodes surrounded by a completely permeable membrane. No matter how the concentration of the solution is varied, the relation of the resistance of the solution to the resistance of the solution with a permeable membrane around the electrodes will be a constant as the resistances will be identic-

al. Then if a semipermeable membrane be substituted for the permeable, its effect upon the resistance of the solutions may be compared with the resistance of the solution as a constant. This comparison may be made from the data of the preceding experiment and is given in the column Rc/Rv, where Rc represents the constant quantity, the resistance of the same solution containing a hypothetical permeable membrane and Rv represents the resistance of the same solution containing a semipermeable membrane depending upon the size of its pores for its function. When Rc is divided by Rv, a variable quotient is produced and Rv must therefore be a variable quantity. In the equation Rc/Rv = Q, as Rc is a constant and Rv a variable, the quotient Q will decrease as Rv increases. An actual division of the resistances as indicated above shows the quotient Q to decrease as the percentage of the NaCl solution increases. Therefore, Rv increases with the percentage of NaCl, which is to say that a copper ferrocyanide membrane offers relatively more resistance the higher the concentration in which it is functioning.

The analogy of this experiment to the results given in Table B lies in the fact that the ratio, Rd/Rl may similarly be considered the ratio of a variable and a constant. As long as the bacterial cell holds within it free ionized salts, it may be considered completely impermeable and a constant, as was assumed for a solution containing a completely permeable membrane in the experiment with the copper ferrocyanide membrane. We then have the equation Rd/Rl = Q, where Rd is the resistance of a solution containing dead bacterial membrance and Rl containing living bacterial membranes, and Q is the resulting quotient. Rl as a living cell suspension is considered a constant. Q is found from the experimental data to be an increasing variable. This leaves Rd as a variable and as the con-

stant is in the denominator, Q and Rd vary directly with each other. It appears then that a copper ferrocyanide membrane between two electrodes, or a quantity of dead bacterial cells between two electrodes, gives a relative increase in resistance when the concentration of the conducting solution is increased. The essential structure of a copper ferrocyanide membrane is a nonconducting framework with numerous small holes that allow ions to pass thru. A similar structure for dead bacterial membranes was speculated upon previously from other considerations.

In the experiment graphed as Fig. IV the salts contained within the cells is considered a fairly constant quantity as the bacteria in all suspensions are from a single planting on the same cultural medium containing 1/2% NaCl. The effect of freeing this constant quantity of contained salts in solutions of varying concentration is shown by the difference in the resistance of the menstruums before and after heating. This relation is shown by the following table of such differences.

Table D.

Concentration of NaCl	Decrease in resistance of menstruum
0.1%	380 ohms
0.3%	65
0.5%	25
0.7%	13
0.9%	10

Previous experiments indicated that the decrease in resistance of bacterial suspensions in salt concentrations favorable for bacterial life was due in the largest part to an issue of salts from the cell interior. These findings are substantiated by the above results

which show that the effect of the issue of such salts conforms with the general laws of ionization. Further this experiment shows that the concentration of free salts within the bacterial cells used in this experiment is above a concentration equivalent to a 0.9% salt solution in that the conductivity of a 0.9% salt solution was increased when the bacteria were killed in it, altho less than 25% of the suspension was bacterial cells. A method is herewith given, then, by which the diffusible salts contained within a given type of cell may be determined directly and a new field of investigation opened up in the study of salts contained in the cell fluids.

It is noticeable on Fig. IV that the resistance of the dead coli suspension is far below that of the menstruum of the live suspension in the 1/10% NaCl, but in the 5/10% NaCl the resistance of the dead coli suspension is the greater. This is again the effect of the diffusion of salts from the cell interior. This is shown clearly by the graphic description of changes taking place in the different suspensions upon heating, as given in Figs. V, VI and VII which represent the 1/10, 3/10 and 9/10% solutions, respectively. In Fig. V the resistance of the heated suspension drops far below the unheated menstruum, due to the great degree of ionization which takes place when the salts diffuse out into such a dilute solution. In Fig. VI the resistance of the heated suspension is equal to and in VII greater than that of the unheated menstruum, as the more concentrated solution allows less ionization of the salts diffusing from the bacteria. It is evident that at some higher concentration of the menstruum, the destruction of the membrane will bring about no change in its conductivity and the value of the solution concentration will represent very closely the equivalent value of the concentration of total diffusible salts in the cell liquid. At such

Figure V.

B. Coli suspended in 1/10% NaCl.

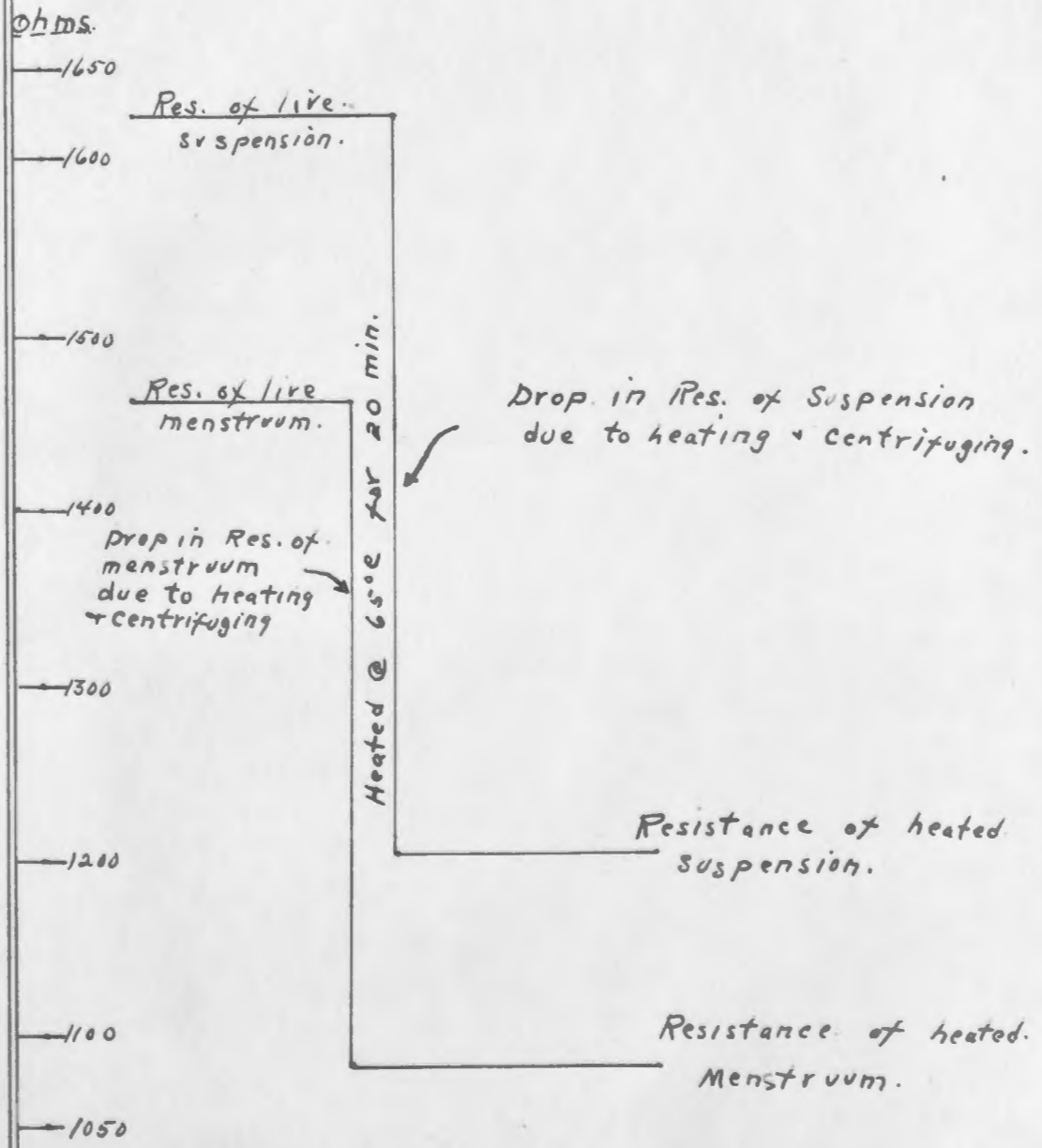


Figure VI.

B. coli suspended in 3/10 NaCl.

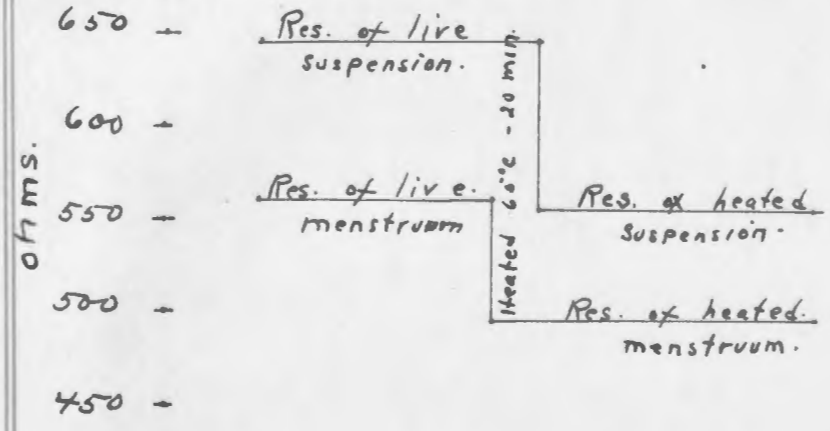
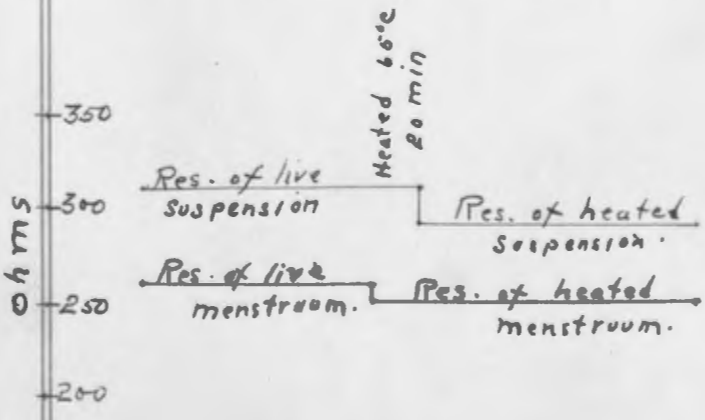


Figure VII

B. coli suspended in 7/10 % NaCl.



concentration the decrease in resistance will represent wholly an increase in the permeability of the bacterial membrane.

Attention is called to the specific experimental conditions in the work described above. The bacteria were killed by temperatures ranging from 58°C to 96°C. The degree of heat used could not be demonstrated to be responsible for any variation in results. The properties ascribed to dead bacteria concerns bacteria killed by heat alone and at the temperatures given.

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