THE INTERPRETATION OF CHANGES IN ELECTRICAL RESISTANCE ACCOMPANYING THE DEATH OF BACTERIAL CELLS

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Of the four or five methods that have been employed for ascertaining the permeability of living cells to salts, the determination of their electrical resistance is usually regarded as the most direct. This view is based on the assumptions that the passage of ions propelled by a given force into a cell is conditioned by the degree of permeability of the cell membrane, that an electric current is conveyed through an aqueous medium by the bodily migration of ions, and that, in accordance with Ohm's law, $E = CR$, the current flowing between two platinum electrodes connected to a source of constant $EMF$ is inversely proportional to the resistance of the medium between those electrodes. Resistance, or rather its reciprocal, conductivity, is thus said to be a direct measure of the migration of ions, and consequently, also, of the permeability of the cell.

On the basis of these theoretical views Osterhout (1918; 1919; 1920) carried out an extensive series of determinations of the electrical conductivity of various tissues—algal tissues, and one animal tissue, frog skin—when placed in various saline solutions. He concluded from his results that when tissues die, their permeability to ions is increased, that death and increased permeability can be brought about by immersion in solutions of monovalent ions, and that this effect can be antagonised by the addition of divalent ions. He showed moreover, that his results agreed with results obtained by determinations of plasmolysis, tissue tension, and diffusion through membranes of living tissues.

The same method has been applied, and on the same theoretical
assumptions, to suspensions of single cells. A number of investigators have worked with red blood cells, Gray (1916) with echinoderm eggs, and Shearer (1919) with suspensions of bacteria. Their results are neither as constant nor as conclusive as are those of Osterhout with tissues. The earlier workers, among them Stewart (1899; 1909) believed that red blood cells have a remarkably high electrical resistance. Brooks (1925) however, has shown that this view is incorrect. Gray reported an increase in the permeability of eggs at fertilisation, and Shearer claimed to have demonstrated the usual salt antagonisms with suspensions of Bacillus coli, and arrived at the conclusion that dead cells offer no resistance to the passage of ions.

The reason why people who worked with suspensions obtained results that were either contradictory or inexplicable, was because they committed the error of adopting unquestioningly and in toto the theoretical assumptions that had been shown by Osterhout to be generally applicable to tissues, and applying them similarly to suspensions. On careful consideration it becomes apparent that a suspension of cells is a different, and in a sense a more complicated, element of electrical resistance than is a block of tissue, and that the interpretation of resistance measurements requires a rather special treatment.

DISCUSSION OF METHOD

The first fact that emerges from a critical consideration of a suspension of cells is that one is measuring, in part at least, the resistance of the suspending medium as well as that of the suspended cells. It therefore becomes necessary to determine the changes that may occur in the resistance of the solution under any given experimental conditions. Further it is necessary to establish the ratio between the volume of cells and the volume of solution in the suspension, and the relation of that ratio to the resistance of the system. And finally it is important to know what significance the size and shape of cells may have in determining the total resistance.

Even when all these factors are determined there still remains the question—to what extent does the resistance of the cell
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actually measure the permeability of the cell membrane? If a direct current were used, the resistance, and consequently the conductivity, of the cell could by hypothesis be considered to be a direct measure of permeability, since conductivity would be conditioned by the direct migration of ions. But in practice the use of a direct current is not feasible because of polarisation effects. To obviate these effects an alternating current is used. The theoretical advantage of an alternating current lies in the hypothesis that under the influence of such a current ions do not migrate bodily, but vibrate symmetrically, and that the current flows by the passage of charges from ion to ion down the line of fall of potential. On this assumption there seems to be no necessity and no justification for supposing that the passage of a current through a cell has any direct relation to the permeability of that cell, but depends rather on its dielectric constant. In practice, however, a true alternating current is rarely attained. The mathematical expressions for the growth and fading of a current in an ordinary induction coil show that the current fades much more rapidly than it develops. This fact is emphasised in many books on physics. Therefore the current that is obtained from a coil and interruptor does not follow a sinusoidal curve, as an alternating current should, but is, in effect, a series of unidirectional shocks. It is possible to compensate for these factors, and apparatus has been devised, and is used in modern physical laboratories which does give a true sinusoidal current, but, as far as I know, it has not as yet been applied to biological investigation. It seems not improbable, therefore, that the results that have been obtained with resistance measurements of tissues and cell suspensions, and which have been interpreted as measuring the permeability of cells to ions, have been due to the fact that these measurements have been made with what was virtually an intermittent direct current, and that the use of a true alternating current might yield results of quite a different nature.

EXPERIMENTAL

At the outset it has to be stated that the results herewith presented are provisional in character because the modern appara-
tus referred to above was not available, and measurements have been made with a simple Kohlrausch bridge.

In these experiments suspensions of *Bacillus cereus* were used. The organism was grown by means of surface inoculation on beef-peptone agar, with an incubation period of twenty-four hours at 20°C. The membranous growth was washed off with a balanced Ringer solution of the following composition:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
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<tbody>
<tr>
<td>1/8 M NaCl</td>
<td>960 cc</td>
</tr>
<tr>
<td>1/8 M CaCl₂</td>
<td>15 cc</td>
</tr>
<tr>
<td>1/8 M KCl</td>
<td>25 cc</td>
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<td></td>
<td>1,000 cc</td>
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The solution was buffered with 4 drops per liter of saturated Na₂HPO₄ solution, the resulting pH being 7.2.

The bacterial cells were washed by repeated centrifuging and resuspension until the resistance of the suspension attained a constant value, and the resistance of the supernatant liquid after centrifuging was the same as the resistance of the pure solution. Four centrifugings were usually sufficient. Growth from 35 agar plates was found to yield 7.5 cc. of centrifuged cells, and this volume was suspended in 7.5 cc. of solution, yielding 15 cc. of suspension. A rigid electrolytic cell (cell constant 0.3072) could be immersed in the suspension and withdrawn from it. It was thus possible to make measurements actually in the centrifuge tube, the bacteria remaining in the same vessel throughout an experiment. Temperature was regulated by means of a simple water bath, heated with an electric bulb, and was controlled by means of a sensitive thermometer inserted into the bacterial suspension. Maximum fluctuations amounted to 0.05°C.

The changes in resistance that occurred when the suspension was killed by heat are shown in figure 1. The suspension was autoclaved at 15 pounds for ten minutes. The continuous lines represent the resistance of the suspension, the dotted lines the resistance of the suspending medium after centrifuging. It is seen that the resistance of the suspension fell considerably after heating, but this was accompanied by a corresponding drop in
the resistance of the suspending medium. On continued resuspension in 1/8 M Ringer the resistance of the suspension again rose but did not attain the value of the resistance of the living suspension. This probably was due to the change in volume produced by heating. Before heating, the volume of the centrifuged cells was 7.5 cc., after heating it was 5 cc. These results can be explained if it is assumed that the death of the cells by heat resulted in a destruction of the impermeability of the cell membrane, and that there was consequently free diffusion of electrolytes in and out of the cell. If the external solution is hypotonic to the cell contents, then there would be a diffusion of salts out of the cell, and the resistance of the suspension would drop owing to an increase in the concentration of salts in the suspending liquid. On repeated resuspension the concentration of salts would again be lowered, and the resistance raised. If this hypothesis is accepted then it becomes evident that in a hypertonic solution the process would be reversed, salts would diffuse into the cell,
and the resistance of the suspension and of the suspending medium would rise. A number of experiments were carried out attempting to demonstrate this phenomenon, but no rise in resistance was observed. In 1/2 M Ringer there was still a slight drop in resistance (fig. 1) in M Ringer no change in resistance was observed, and in higher concentrations up to 2 M there was also no change.

In considering these results it is important also to examine the relation of resistance of a pure solution to the concentration of the salts which it contains. It is well known that resistance plotted against concentration yields an exponential curve, and that at concentrations greater than about 1/4 M resistance is very much less sensitive to changes in concentration than it is at greater dilutions. It follows therefore that in the experiments in which 1/2 M and M Ringer were used, changes in concentration may have occurred of the same order of magnitude as in the case of dilute solutions,—these changes corresponding however, to such small variations in resistance that the apparatus used was not sensitive enough to record them.

**Fig. 2. Effect of Mercuric Chloride and Heat upon Resistance of Suspensions of B. cereus**

Continuous lines indicate resistance of suspension; dotted lines, resistance of supernatant.
Figure 2 shows the resistance curve of a suspension which was killed with mercuric chloride, and then resuspended repeatedly in $1/8$ M Ringer. The death of the cells in mercuric chloride solution was accompanied by a drop in resistance, and, incidentally, the death curve is a typical diffusion curve. When the dead cells were resuspended with washing in Ringer it was found that the resistance of the dead suspension was identical with the resistance of the living suspension, viz., 30.5. The dead suspension was then heated, and a drop in resistance resulted, but this drop was less by 4 ohms than the drop which occurred when a living suspension was killed by heat. The heating caused a reduction in cell volume from 7.5 to 4 cc., and this reduced volume is believed to account for the drop in resistance.

**DISCUSSION**

It is apparent from these results that changes in the resistance of the suspension are always accompanied by changes in the resistance of the suspending medium, and that the bacterial substance offers a seemingly constant resistance to an electric current, a resistance which is quite independent of the permeability or impermeability of any hypothetical enveloping membrane, and which is not affected by the death of the cell. It would seem, however, that the living cell does possess a mechanism for opposing changes in the osmotic pressure of the surrounding medium, and that this mechanism is broken down at death, thus permitting the free diffusion of salts under a concentration gradient. This phenomenon was observed both in suspensions killed by heat, and in those killed with mercuric chloride. Heating, however, causes in addition a reduction in cell volume, due possibly to the coagulation and dehydration of proteins, and this shrinkage serves to decrease still further the resistance of the suspension.

In conclusion it is desired to emphasize the distinction between the migration of ions under the influence of a potential gradient, and their migration under the influence of an osmotic gradient. The one is conductivity, the other diffusion. Diffusion, we know, is conditioned by the permeability of the cell, we do not
At XADER ZOOND know what actually determines conductivity. There are no indications in any available experimental data that the resistance, and therefore the conductivity, of bacterial cells is affected or modified by external conditions or experimental manipulation. But there is ample evidence to support the conclusion that the bacterial cell is comparatively sensitive to osmotic changes in its environment, and the careful and accurate measurement of the electrical resistance of the suspension and the suspending medium offers a valuable method for investigating these phenomena.

ACKNOWLEDGMENT

After the completion of these experiments the work of Green and Larson (Jour. Infect. Dis., 1922, 30, 550) came to the writer’s notice. These investigators, working with suspensions of the colon bacillus, arrived at essentially the same results and conclusions. The data presented in this paper offer an independent confirmation of their findings.

REFERENCES

SHEARER, C. 1919 Jour. Hygiene, 18, 337.