CONDUCTIVITY STUDIES OF BACTERIAL SUSPENSIONS

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In recent physical-chemical studies of the bacterial cell little attention has been
devoted to the effect of concentrated cell suspensions on the ability of the sus-
pending medium to conduct an electric current. Oker-Blom (1912) reported
that bacterial cells increased resistance. He concluded that the mass of the cells
was sufficient to hinder the free movement of the ions carrying the current and
hence caused a decrease in conductivity. Shearer (1919) reported that heavy
suspensions of living bacterial cells increased resistance but suspensions of dead
cells showed little effect. Green and Larson (1922) found that killing bacteria
by heat decreased the resistance. They concluded that conductivity is not a
measure of the permeability of the bacterial membranes but that it merely indi-
cates the extent of diffusion of salts from the killed cells. Brooks (1923) found
the conductance of living tissue to be closely related to the surrounding fluid with
which it is in equilibrium. All cells dead or alive were observed to offer greater
resistance to the passage of current than the surrounding medium. Fricke and
Curtis (1934) studied a paste of yeast cells suspended in dilute sodium chloride
and observed an increase in resistance with frequencies up to 128,000 cycles per
second. Above that frequency they report that the cellular membranes allow an
appreciable part of the current to pass through the cells, resulting in lower resis-
tance values. For tissue cells and cells of larger microorganisms, it has been
established that the surface of the protoplasmic membrane offers high resistance
to moderate frequencies of electrical current (Cole, 1933).

EXPERIMENTAL RESULTS

Cell suspensions were prepared using the usual methods for growing massive
cultures in aerated liquid media. Ordinary nutrient broth with 1 per cent sucrose
and potassium phosphate added as additional buffer was used as the growth
medium. All cultures were incubated at 30 C. The usual time of incubation
was 48 hours, though shorter growth periods and growth of the organisms on agar
media did not influence the type of results obtained. The cells were harvested
by centrifugation and washed three or more times to remove the components of
the medium and metabolic products from the cells. Cells were stored at 3 C and
used within 48 hours. Quantitative estimates of the number of cells present
were made by dry weights at 110 C, by direct microscopic counts, and by poured
plates. Poured plate counts indicated that a large proportion of the cells used
were viable.

Electrical measurements of resistance were made by the usual Wheatstone

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periment Station.
bridge circuit with a vacuum tube oscillator giving an alternating current of approximately 1,000 cycles (Dole, 1935). This frequency was high enough to avoid serious polarization errors and yet did not cause changes in membrane potentials (Fricke and Curtis, 1934). Most measurements were made in open beakers with a dip type conductivity cell, though some were made in closed vessels to avoid the solution of atmospheric gases. Resistance measurements were made at 25 °C. The results of these measurements are expressed as the specific resistance of the solution studied.

Preliminary experiments indicated that the effect of adding washed cells to an electrolyte solution gave variable results. In some instances there was a slight increase in conductivity; other times the resistance of the suspension increased. In certain cases a marked increase in the resistance resulted. Among the factors that seemed to influence the resistance were the concentration of cells, the concentration of the electrolyte, and the type of electrolyte. Table 1 shows the resistance of several electrolytes when a heavy suspension of Bacillus megatherium was added. The cells had been washed four times in a dilute sodium chloride solution of the same concentration as used in the experiment. The control consisted of an equal amount of the washing solution containing no cells. All the electrolytes were adjusted to approximately the same resistance. In the case of the hydrogen, iron, and mercuric chlorides the presence of cells increased resistance. With the calcium and ammonium salts no change in resistance was noted, whereas the other salts gave a slight decrease. In this experiment the organisms were viable in the presence of certain salts, e.g., calcium, and not viable in the presence of others, e.g., mercury. However, no relation between the killing effect of the electrolyte and a change in resistance was evidenced. In other experiments no significant differences were observed in the resistance offered by heavy suspensions of heat-killed and living organisms.

The observed increases in resistance following the addition of bacterial cells may have been due to the nonconducting cell membranes preventing free passage
of the ions through the suspension (Oker-Blom, 1912). In this case, however, the bacterial cells occupied only a small part of the total volume of the suspensions despite the fact that the number of cells was about 30 billion per milliliter. It seems more logical that the increased resistance resulted from the removal of ions from the solution by the bacterial cells. Evidence indicating this to be the case was obtained by centrifuging the suspension and comparing the conductivity of the supernatant solution with that containing cells. In all instances, the supernatant liquid gave conductivity values similar to those of the cell suspensions regardless of whether the addition of cells to the original electrolyte solution increased or decreased resistance. These data indicate that the increases in resistance resulting from the presence of cells are due to the removal of electrolyte from the solution by the cells, and not to the impedance offered by the cellular entities.

If the bacterial cells were combining with the electrolyte, then repeated washing of the cells in solutions of the ions to be studied should result in saturating the combining capacities of the cells. Cells repeatedly washed and suspended in various chloride solutions were found, after a few such treatments, to give constant resistance readings of the same value as the washing solution. Further evidence that the increase in resistance was due to the removal of ions was obtained by quantitative measurement of the electrolyte before and after the addition of the cells. In table 2 are shown the decrease in hydrogen ion concentration and increased resistance when increasing volumes of water-washed cells were added to a hydrochloric acid solution. The pH values were determined with a glass electrode potentiometer. Parallel with an increase in the resistance of the suspension an increase in the pH was recorded, indicating the removal of the hydrogen ions from solution.

McCalla (1940) has shown that the process of adsorption is one of the mechanisms by which the bacterial cell may combine with ions from the surrounding environment. This process is an exchange phenomenon in which more readily adsorbed ions in the solution replace those less tightly held by the cellular surfaces. McCalla observed that the hydrogen ion readily replaces most of the cations which might normally be located on the cell. Since cations vary in equivalent conductance, such an exchange process could cause changes in resistance of the cell suspensions. The hydrogen ion possesses an equivalent

<table>
<thead>
<tr>
<th>VOLUME OF CELL SUSPENSION ADDED</th>
<th>RESISTANCE OF HCl SOLUTION</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5,918</td>
<td>3.32</td>
</tr>
<tr>
<td>2</td>
<td>7,084</td>
<td>3.49</td>
</tr>
<tr>
<td>5</td>
<td>8,878</td>
<td>3.80</td>
</tr>
<tr>
<td>10</td>
<td>12,333</td>
<td>4.23</td>
</tr>
</tbody>
</table>
conductance of 350 at 25°C, which is much higher than the values of other cations normally present. Ammonium ions, for instance, have an equivalent conductance of 74.5. Thus, if an ion with a high conductance value, such as hydrogen, displaces an ion on the surface of the cell possessing a lower conductance value, such as ammonium, the result should be to decrease the conductivity of the solution.

To test this hypothesis the resistance and hydrogen ion and ammonium ion concentrations of a 0.001 N HCl solution were measured. To the HCl solution was then added Aerobacter aerogenes cells that had been washed in a 0.001 N NH₄Cl solution until equilibrium in resistance was reached. The resistance and hydrogen ion and ammonium ion concentrations of the HCl were again measured and the following alterations recorded:

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in resistance</td>
<td>3,030 ohms</td>
</tr>
<tr>
<td>Decrease in H⁺ concentration</td>
<td>4.8 × 10⁻⁴ N</td>
</tr>
<tr>
<td>Increase in NH₄⁺ concentration</td>
<td>3.8 × 10⁻⁴ N</td>
</tr>
</tbody>
</table>

When similar determinations were made on a 0.001 N NH₄Cl solution before and after the addition of Aerobacter aerogenes cells saturated with hydrogen ions in a 0.001 N HCl solution, the results were as follows:

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in resistance</td>
<td>65 ohms</td>
</tr>
<tr>
<td>Increase in H⁺ concentration</td>
<td>3.0 × 10⁻⁴ N</td>
</tr>
<tr>
<td>Decrease in NH₄⁺ concentration</td>
<td>6.0 × 10⁻⁴ N</td>
</tr>
</tbody>
</table>

In the first of these experiments the hydrogen ion, being more readily adsorbed, replaced the ammonium ion on the cell in nearly quantitative proportions. Since the ammonium ion is a less efficient carrier of the electrical current than the hydrogen ion, the resistance of the suspension increased correspondingly. Conversely, an increased hydrogen ion concentration in the second experiment was accompanied by a decrease in the ammonium ion concentration and in resistance. Since the ammonium ion is adsorbed much less readily than the hydrogen ion, the cationic exchange was considerably less in the second experiment though the cell concentrations were the same (8 billion per ml) in both cases.

Although these studies have indicated that the conductivity of a bacterial cell suspension is influenced by exchange phenomena of electrolytes with the cell, it is apparent that the value of this procedure in measuring the concentration of specific ions is definitely limited since it indicates only changes in the total electrolyte concentration. These experiments involved only studies with cations of the chloride salts. The use of different anions would probably give comparable results. Further studies of conductivity, coupled with adequate quantitative measurements of the ionic composition of the cells and the suspending medium, might yield useful information about the nature of the uptake of inorganic food substances by microorganisms.

**SUMMARY**

Conductivity studies with cell suspensions of a number of bacterial species have shown that changes in resistance of the suspension are related to the type
of cation in the solution. Greatest increases in resistance were observed with readily adsorbed cations. The process of base exchange is suggested as responsible for the observed resistance changes.

REFERENCES


