THE RESPIRATION OF SALMONELLA IN THE PRESENCE OF AGGLUTINATING SERUM

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The literature concerning the effect of immune serum upon the activities of microorganisms has been fully reviewed and discussed by Sevag (1945). Sevag and Miller (1948) recently reported that the agglutination of typhoid organisms does not inhibit oxygen uptake by the cells, whereas lysed cells show an immediate increased rate of oxygen consumption followed by a marked decrease. The work herein reported consisted of a series of experiments to determine whether combination of agglutinins with the cells of a number of Salmonella species had any effect on their respiratory activity.

Cells for studies were obtained by culturing the organism on glycerol thiosulfate agar. Sufficient growth occurred in 24 hours to yield a heavy suspension of cells suitable for use either as antigens or for enzyme studies. Cells were suspended in a balanced salts-buffer solution (Harris and Gainey, 1944) and washed twice by centrifuging, after which they were suspended in a concentration of approximately 100 times the turbidity of MacFarland nephelometer tube no. 1, and cooled to 3 C.

Normal adult rabbits were immunized by a series of intravenous injections of heat-killed cultures. The immune serum was obtained by centrifugation following clotting of blood removed by cardio-puncture. All tests were conducted the same day the rabbits were bled. Agglutination tests to determine the antibody titer followed the usual procedure for tube tests.

Oxygen utilization was measured in the Warburg microrespirometer at 37 C, following the usual technique of Dixon (1934). Twentieth molar glucose, buffered at pH 7.0 with phosphate salts, was used as the respiration substrate in all experiments. Cells grown and harvested in the manner described were quite active physiologically and exhibited little or no endogenous respiration. The methylene blue reduction tests followed the Thunberg technique as described by Quastel and Whetham (1925). Visual judgment of the complete reduction of the dye was taken as the end point. Although this method is subject to error, significant differences in the rate of reduction of the methylene blue can be observed.

In measuring oxygen uptake by microorganisms with the manometric method, the formation of hydrogen gas by the cells may lead to significant errors. However, in no instance in this investigation was a positive gaseous pressure recorded during an experimental period of 2 hours when nitrogen replaced the air in the respiration flasks and alkali was present to absorb carbon dioxide. Hence it may be assumed that no appreciable volume of hydrogen was liberated by the

1 Contribution 231, Bacteriology Department, Kansas Agricultural Experiment Station.
cells under the experimental conditions. This failure to liberate hydrogen was probably due to the organism's inability to form the necessary adaptive enzyme, hydrogenlyase, following growth on the glucose-free glycerol thiosulfate medium (Stephenson and Stickland, 1933). The experiments were of such short duration that no appreciable subsequent growth and enzyme formation could be expected to take place in the respirometer.

When either normal or immune serum was added to the bacterial cells, buffer, glucose system in the respirometer, an appreciable increase in the rate of oxygen uptake was observed as compared to that found when glucose alone was used. Warren (1945) found that addition of immune serum to luminescent bacteria gave increased light production. He attributed this increased activity to the utilization of metabolites present in the serum. In order to eliminate variations due to such differences, comparisons were made of oxygen uptake of Salmonella in normal and immune sera and in mixtures of these. When the quantity of immune serum was less than 0.1 ml, normal serum was added to bring the total volume of serum to 0.1 ml. In this way the quantities of metabolites and the quantity of the natural complement added to the respirometer flasks were held fairly constant.

**DISCUSSION OF RESULTS**

The cubic millimeters of oxygen utilized in 1 hour by eight species of Salmonella in the presence of varying quantities of immune and normal rabbit serum are shown in table 1. The italicized values indicate experiments in which sufficient agglutinin was present for complete agglutination of the cells. The agglutination titers of the sera varied from relatively low titer in the case of Salmonella gallinarum to relatively high titer for Salmonella schottmuelleri. The quantities

<table>
<thead>
<tr>
<th>SPECIES STUDIED</th>
<th>CELLS PER FLASK</th>
<th>O2 UPTAKE IN MM³ PER HOUR IN THE PRESENCE OF RABBIT SERUM*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>billions</td>
<td>Normal</td>
</tr>
<tr>
<td>S. schottmuelleri</td>
<td>1.45</td>
<td>78</td>
</tr>
<tr>
<td>S. paratyphi</td>
<td>1.67</td>
<td>134</td>
</tr>
<tr>
<td>S. california</td>
<td>1.17</td>
<td>105</td>
</tr>
<tr>
<td>S. stanley</td>
<td>1.43</td>
<td>139</td>
</tr>
<tr>
<td>S. derby</td>
<td>1.53</td>
<td>115</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>2.03</td>
<td>107</td>
</tr>
<tr>
<td>S. gallinarum</td>
<td>1.27</td>
<td>45</td>
</tr>
<tr>
<td>S. pullorum</td>
<td>1.60</td>
<td>70</td>
</tr>
</tbody>
</table>

* Normal rabbit serum added to give total volume of serum equal to 0.1 ml; values italicized represent flasks with sufficient agglutinin to give complete agglutination.
of agglutinin added to the respiration flasks ranged from a concentration which
gave no visible clumping of the cells to 500 times the quantity necessary for com-
plete agglutination.

The quantities of oxygen utilized in normal and in immune serum show slight
variations, but there was no consistent decrease in respiratory activity that
could be attributed to antibodies. The recorded differences might be due to
slight variations in the quantity of available metabolites in the sera. Varying
the ratio of antibody to antigen did not give a zone of optimal inhibition of respi-
ration.

In the experiments just described, the rabbits were immunized with heat-
killed bacterial cells, which would give rise only to antibodies against the somatic
antigens of the dead organisms. In order to determine whether living bacteria,
with and without flagellar antigens, might produce different results, rabbits
were immunized first with killed cultures and then were subjected to inoculations
with live broth cultures. In other experiments, adult hens were given intra-
peritoneal inoculations of live pullorum organisms. Respiration studies using
the antisera from these animals gave results similar to those shown in table 1.

The results obtained in these experiments agree with those reported by Sevag
and Miller (1948) for the intact sensitized typhoid organisms. None of the
strains used exhibited marked bacteriolysis in the presence of the fresh immune
serum. In several instances the complement concentration was increased by
the addition of fresh guinea pig serum without affecting the rate of oxygen utiliza-
tion or causing lysis of the sensitized cells.

Preliminary studies of dehydrogenation of various organic substrates by
Salmonella species in the presence of blood serum indicated that normally the
quantity of metabolizable compounds in the serum alone was sufficient to allow
rapid reduction of the methylene blue. To avoid this complication, cell suspen-
sions were mixed with an equal quantity of fresh normal or immune serum and
incubated at 37 C for 3 hours. The cells were then removed by centrifuging and
were washed twice in the balanced salt solution. Cells from the immune serum
were still combined with enough agglutinin to agglutinate on standing a few
minutes, but neither those from normal nor immune serum reduced methylene
blue more rapidly in the absence of added substrate than did untreated control
cells.

Methylene blue reduction time data in the presence of 0.1 molar glucose,
sodium lactate, and sodium formate are recorded in table 2. These three com-
ounds were rapidly oxidized by all the species studied. Reduction was almost
as rapid in the presence of glycerol and mannitol as in the presence of glucose,
but sodium succinate, sodium gluconate, ethyl alcohol, and sodium acetate were
utilized only slightly or not at all. Treatment of cells with fresh immune serum
gave no evidence of inhibition of enzymic activity by antibody in the presence of
any substrate studied.

Measurement of both oxygen uptake and the dehydrogenase activity of intact
cells has demonstrated no significant differences between the effect of normal and
immune serum on the respiratory activity of the salmonellae. Apparently,
combination of the specific agglutinins with the bacterial cell and their subsequent agglutination have no direct effect upon enzymes responsible for the activation of the organic substrate. Also, agglutination apparently does not interfere with the mechanism for transporting electrons between the substrate-activating portion of the respiratory system and that part combining with molecular oxygen.

**TABLE 2**

*Time in minutes required for the reduction of methylene blue by Salmonella species treated with normal and immune serum*

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CELLS TREATED WITH NORMAL SERUM</th>
<th>CELLS TREATED WITH IMMUNE SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium lactate</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. pullorum</em></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><em>S. paratyphi</em></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><em>S. stanley</em></td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. pullorum</em></td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><em>S. paratyphi</em></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><em>S. stanley</em></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>Sodium formate</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. pullorum</em></td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td><em>S. paratyphi</em></td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td><em>S. stanley</em></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

**SUMMARY**

Oxygen uptake, with glucose as the substrate, by eight species of *Salmonella* occurred at essentially the same rate when the cells were in the presence of fresh immune rabbit serum as when in fresh normal serum. Varying the proportions of agglutinin to cells over a wide range, with sera of both high and low titer, gave no indication of a zone exhibiting antienzyme activity affecting respiration. Dehydrogenase activity of four species was studied upon nine organic substrates. Reduction of methylene blue in the presence of a specific substrate occurred at the same rate when the cells were treated with fresh immune serum as with normal agglutinin-free serum.

**REFERENCES**