STUDIES OF THE EFFECT OF SODIUM AZIDE ON MICROBIC GROWTH AND RESPIRATION

IV. THE EFFECT OF SODIUM AZIDE ON GLUCOSE FERMENTATION AND LACTIC ACID PRODUCTION BY STREPTOCOCCI AND LACTOBACILLI

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A study of the effects of sodium azide on the gas metabolism of Bacillus subtilis and Pseudomonas aeruginosa under aerobic conditions has been described (Lichstein and Soule, 1944c). It was shown that the chemical exerted an inhibitory effect on the oxygen consumption of these bacteria, and the respiratory quotients obtained suggested that the organisms were respiring anaerobically. The fact that P. aeruginosa is able to grow readily in the absence of free oxygen, while B. subtilis is more strongly aerobic, may explain the differences in susceptibility of these two species of germs to the chemical.

Since certain strains of streptococci, and lactobacilli are markedly resistant to the action of sodium azide using growth in the presence of the chemical as an index (Lichstein and Soule, 1944a), it appeared desirable to investigate the action of sodium azide on the metabolism of representative members of this genus.

EXPERIMENTAL

Two strains of streptococci, Streptococcus hemolyticus (Dochez) and S. hemolyticus (G7), and two strains of lactobacilli, Lactobacillus casei and L. fulleri were investigated.

Preliminary tests of the gas exchange of these germs on glucose infusion agar were made by the methods of Novy, Roehm, and Soule (1925). It was quickly ascertained that the gas exchange was very small; indeed there was no appreciable change at the end of 96 hours. It appeared evident that these germs were growing anaerobically even in the presence of an ample amount of free oxygen. Because of the failure to demonstrate any gas exchange, further gas metabolism studies were discontinued. However, explanations for the above performance are readily found in the literature.

Anderson (1938) in his book states that the streptococci cannot utilize oxygen, the best of all hydrogen acceptors from the energy point of view. This is not due to any actual sensitivity to oxygen, since streptococci grow readily in the presence of free oxygen. Other investigators, namely Farrell (1935), Chu and Hastings (1938), and Sevag and Shelburne (1942) reported the oxygen uptake of washed suspensions of streptococci on a glucose substrate to be negligible.

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The latter authors, however, showed that when certain coenzyme factors were added to the washed suspensions, the aerobic and anaerobic respiration rates were several fold accelerated. Barron and Jacobs (1938) found that of 7 strains of streptococci, 3 consumed oxygen in the presence of glucose.

More pertinent to the present study is the report of Friedemann (1939) who investigated actively growing strains of streptococci. He showed that 80–95 per cent of the glucose was converted into lactic acid, acetic acid, and ethyl alcohol. The most important single product was lactic acid and this substance accounted for 74–91 per cent of the glucose utilized by the pathogenic strains of streptococci studied. Since the conversion of glucose to lactic acid is an anaerobic one, the germs were apparently utilizing an anaerobic respiratory mechanism even when grown under aerobic conditions.

The carbohydrate metabolism of the lactobacilli is very similar to that of the streptococci.

Because of these findings an experiment was outlined to study the effect of sodium azide on the utilization of glucose and the production of lactic acid by these germs.

A solution of chemically pure glucose was prepared and sterilized apart from the extract broth. An accurately measured and equal volume of the sterile glucose was added aseptically to 30 ml. of media contained in large culture tubes. The concentrations of sodium azide employed were 0.005, 0.01, and 0.02 per cent, corresponding to M/1300, M/650, and M/325. Each tube of medium contained approximately 0.5 gram of calcium carbonate to prevent the accumulation of acid which would otherwise result in either a bacteriostatic or bactericidal effect. Each culture tube was inoculated with 0.1 ml. of a 24-hour infusion-broth culture of the organism, and incubated at 30°C for 20 days. The tubes were agitated several times a day to insure thorough mixing.

At the end of 20 days the glucose in the cultures and in the uninoculated controls was determined by employing a modification of the method of Shaffer-Hartman-Somogyi. Glucose was still present in some of the cultures. Since glucose interferes with the determination of lactic acid, it was removed from the cultures by the method of Friedemann and Graeser (1933). The technics outlined by these investigators were also used for the lactic acid determinations.

The results of the glucose and lactic acid determinations are given in table 1.

The data show that sodium azide, in the concentrations employed, apparently had no influence on the utilization of the glucose by the streptococci. At the time of making the analyses, all of the cultures of the streptococci, whether sodium azide was present or not, were found to be glucose-free. Likewise, in the control cultures of lactobacilli there was no glucose, but in the presence of sodium azide L. fuller had utilized only about 43 per cent, and L. casei, 30 per cent of the quantities originally present. It seemed desirable to employ an excess of glucose with the streptococci in order better to ascertain the effect of the chemical on the utilization of this carbohydrate. In order to gain this information the experiment was repeated. The procedure employed was exactly the same as already described, except the period of incubation was shortened to 8 days. At
this time the results of the glucose determinations showed the amount of sugar fermented to be the same whether sodium azide was present or not (table 2). These results indicate more clearly that sodium azide in the concentrations employed had no effect on the utilization of glucose by the streptococci.

The yields of lactic acid under these conditions with the two strains of streptococci growing in the absence of sodium azide accounted for 72 per cent of the glucose. Such a value is in keeping with the results of other investigators (Friedemann, 1939). The lactic acid production in the presence of sodium azide gave values distinctly higher than those of the controls. It was interesting to note the marked similarity of the results for both strains of streptococci.

Glucose was still present in the cultures of the lactobacilli containing sodium azide, whereas the controls were glucose-free. The yields of lactic acid in the latter case accounted for 49 and 50 per cent of the glucose, values distinctly below those of the streptococci, while in the presence of sodium azide the figures were somewhat higher, but did not even approach those of the streptococci.

### TABLE 1

*Effect of sodium azide on glucose utilization and lactic acid production by streptococci and lactobacilli, 20 days, 30°C.*

<table>
<thead>
<tr>
<th>ORGANISMS</th>
<th>CONCENTRATION OF SODIUM AZIDE</th>
<th>ANALYSES AT END OF INCUBATION PERIOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>Millimoles glucose present</td>
</tr>
<tr>
<td>S. hemolyticus (strain G7)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>S. hemolyticus (strain Dochez)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>L. Fulleri</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>1.56</td>
</tr>
<tr>
<td>L. Casei</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>1.94</td>
</tr>
<tr>
<td>Uninoculated control (1)</td>
<td>0.00</td>
<td>2.70</td>
</tr>
<tr>
<td>Uninoculated control (2)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Since the quantity of growth of the lactobacilli in the presence of sodium azide compared favorably with the growth in the controls, the differences in the residual glucose were not anticipated. It is to be noted that the millimoles of lactic acid produced in the presence of the chemical were lower than in its absence since the utilization of the glucose was also markedly reduced.

TABLE 2
Glucose utilization by streptococci in the presence of sodium azide, 8 days, 30°C.

<table>
<thead>
<tr>
<th>ORGANISMS</th>
<th>CONCENTRATION OF SODIUM AZIDE</th>
<th>ANALYSES AT END OF INCUBATION PERIOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>Millimoles of glucose present</td>
</tr>
<tr>
<td>S. hemolyticus (Deches)</td>
<td>0.00</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>1.41</td>
</tr>
<tr>
<td>S. hemolyticus (G7)</td>
<td>0.00</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>1.07</td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>0.00</td>
<td>8.55</td>
</tr>
</tbody>
</table>

DISCUSSION

The conclusions which may be drawn from this experiment are limited. It would be desirable to make complete analyses of the end products of the fermentation of glucose so that the significance of the increased lactic acid yields in the presence of sodium azide could be established. However, it seems quite evident from the data presented that the enzyme system involved in the conversion of glucose to lactic acid by streptococci and lactobacilli is resistant to sodium azide. This is particularly well indicated by the results obtained with the lactobacilli, since, even though the amount of glucose fermented was reduced in the presence of the chemical, the percentage yield of lactic acid remained the same or was slightly increased. Since the conversion of glucose to lactic acid constitutes the major source of energy of these germs in the presence of this carbohydrate, it is perhaps an important reason for their marked resistance to the growth-inhibiting action of sodium azide. Although these bacteria are markedly saccharolytic in their food requirements, it is generally stated that they are also mildly proteolytic. A study of their metabolism in media free of glucose would therefore be desirable before any generalizations can be drawn.

CONCLUSIONS

1. Sodium azide in concentrations of 0.005, 0.01, and 0.02 per cent in glucose extract broth had no effect on the utilization of this sugar by two strains of
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streptococci. The yields of lactic acid from glucose were slightly higher in the presence of the chemical.

2. In the presence of these same concentrations of sodium azide and under similar conditions, Lactobacillus casei consumed about 30 per cent of the glucose, and L. fuller i utilized about 43 per cent of the sugar. On a percentage basis, the yields of lactic acid were not decreased in the presence of the chemical.

REFERENCES


