Effect of Exogenous Substrates on the Endogenous Respiration of Bacteria

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The effect of exogenous substrates upon endogenous respiration differs among organisms from inhibition, to no effect, to enhancement (E. A. Dawes and D. W. Ribbons, Ann. Rev. Microbiol. 16:241, 1962). The technique suggested by Burris (W. W. Umbreit, R. H. Burris, and J. F. Stauffer, Manometric Techniques, p. 97, Burgess Publishing Co., Minneapolis, 1949) has been employed extensively to distinguish endogenous from exogenous respiration. In the current work, the time course of oxidation and its response to aerobic and anaerobic conditions, inhibitors, and uncouplers was followed in four bacterial species totally labeled with $^{13}$C.

*Escherichia coli* (Gratia), *Aerobacter aerogenes* (UW), *Bacillus cereus* (USDA), and *Rhizobium leguminosarum* (128 C 53) were cultured in an extract from wheat plants grown from seed for 2 weeks in an atmosphere containing $^{14}$CO$_2$. All organic constituents of the cells were labeled with $^{14}$C, as the labeled wheat extract was their sole source of carbon.

Oxygen uptake was measured manometrically. The exogenous substrate was tipped from the side arm after 10 min of equilibration. CO$_2$ was trapped in the center well in ethanolamine-ethylene glycol (1:2, v/v). At the termination of the experiment, this solution was transferred to a vial for liquid scintillation counting, and toluene-ethylene glycol monomethyl ether (10:7, v/v) plus 5.5 g of 2,5-diphenyloxazole per liter was added (H. Jeffay and J. Alvarez, Anal. Chem. 33:612, 1961). Nitrogen was determined with Nessler's reagent and carbohydrate by the method of W. E. Trevelyan and J. S. Harrison (Biochem. J. 50:298, 1952).

The results of typical experiments of 1-hr duration for each organism studied are given in Table 1. Both *E. coli* and *A. aerogenes* had low endogenous respiration rates compared with their respiration rates in the presence of added glucose, and the glucose enhanced the endogenous respiration of these organisms (Clifton also observed this; J. Bacteriol. 85:1371, 1963).

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TABLE 1. Effect of various exogenous substrates on the endogenous respiration of bacteria

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Escherichia coli</th>
<th>Aerobacter aerogenes</th>
<th>Bacillus cereus</th>
<th>Rhizobium leguminosarum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\Delta Q_{\text{O}}(N))</td>
<td>(\frac{\Delta Q_{\text{O}}(N)}{Q_{\text{O}}(N)})</td>
<td>(\Delta Q_{\text{O}}(N))</td>
<td>(\frac{\Delta Q_{\text{O}}(N)}{Q_{\text{O}}(N)})</td>
</tr>
<tr>
<td>None</td>
<td>105</td>
<td>3.600</td>
<td>0</td>
<td>188</td>
</tr>
<tr>
<td>Glucose</td>
<td>677</td>
<td>9,440</td>
<td>+160</td>
<td>212</td>
</tr>
<tr>
<td>Na pyruvate</td>
<td>555</td>
<td>4,600</td>
<td>+28</td>
<td>187</td>
</tr>
<tr>
<td>Na succinate</td>
<td>140</td>
<td>3,000</td>
<td>-17</td>
<td>135</td>
</tr>
<tr>
<td>Na glutamate</td>
<td>100</td>
<td>2,940</td>
<td>-19</td>
<td>22</td>
</tr>
<tr>
<td>Na acetate</td>
<td>(163)</td>
<td>(3,500)</td>
<td>(-3)</td>
<td>(80)</td>
</tr>
</tbody>
</table>

* \(Q_{\text{O}}(N)\) means the \(^{14}\text{CO}_{2}\) liberation in counts per minute per milligram of total cell nitrogen per hour.

* The per cent change indicates the percentage of increase (+) or decrease (−) in the total counts per minute of \(^{14}\text{CO}_{2}\) in the presence of each substrate as compared with the control without added substrate. In all experiments, each respirometer flask contained 0.8 ml of cell suspension in the main compartment and 0.2 ml of a 0.05 m solution of the tested substrate which was tipped from the side arm after 10 min of equilibration. Suspensions were incubated for 1 hr at 30 C. The E. coli suspension contained 400 \(\mu\)g of total nitrogen per 0.8 ml; A. aerogenes, 450 \(\mu\)g; B. cereus, 150 \(\mu\)g; and R. leguminosarum 150 \(\mu\)g. Data in parentheses for E. coli were taken from another experiment.

**FIG. 1.** Time course of respiration in the absence (control) and in the presence of 0.01 \text{m} glucose. Each point was taken from a different flask. \(^{14}\text{CO}_{2}\) values were corrected for the 10-min period of equilibration.
to reduce glucose oxidation in E. coli by about 50%, the enhancement of endogenous respiration is almost abolished. Both endogenous and exogenous respiration of E. coli and A. aerogenes are enhanced by 2,4-dinitrophenol.

The response of endogenous respiration to glucose varied with the pH and temperature. In E. coli and A. aerogenes at pH 5.0 to 5.5, the liberation of ^14CO_2 in the presence of glucose was less than in the controls without added glucose. With increasing pH, there was a sharp increase

### TABLE 2. Effect of cyanide and 2,4-dinitrophenol (2,4-DNP) on the endogenous respiration of bacteria in the presence or absence of exogenous glucose

<table>
<thead>
<tr>
<th>Organism and condition</th>
<th>^14CO_2 liberation (counts per min per mg of N per hr)</th>
<th>Per cent change</th>
<th>Endogenous (control) with 0.01% glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>1,620</td>
<td>+50</td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td>1,160</td>
<td>-80</td>
<td></td>
</tr>
<tr>
<td><strong>Aerobacter aerogenes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>1,075</td>
<td>+40</td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td>600</td>
<td>-50</td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>11,600</td>
<td>-16</td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td>800</td>
<td>-50</td>
<td></td>
</tr>
</tbody>
</table>

* The per cent change indicates the change which occurred in the ^14CO_2 liberation in the presence of exogenous glucose, as compared with the control (endogenous) without added substrate. The conditions of these experiments were essentially the same as those of Table 1, except that B. cereus was incubated for 2 hr. Aerobic conditions refer to incubation in air.

### TABLE 3. Effect of cyanide and 2,4-dinitrophenol (2,4-DNP) on the endogenous and concurrent exogenous respiration of bacteria

<table>
<thead>
<tr>
<th>Concn added</th>
<th>E*</th>
<th>G*</th>
<th>E</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyanide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>60</td>
<td>1,000</td>
<td>3,580</td>
<td>8,420</td>
</tr>
<tr>
<td>1.0 × 10^-4</td>
<td>71</td>
<td>1,020</td>
<td>4,200</td>
<td>7,820</td>
</tr>
<tr>
<td>2.5 × 10^-4</td>
<td>53</td>
<td>734</td>
<td>3,550</td>
<td>6,510</td>
</tr>
<tr>
<td>5.0 × 10^-4</td>
<td>71</td>
<td>458</td>
<td>4,120</td>
<td>4,300</td>
</tr>
<tr>
<td>1.0 × 10^-3</td>
<td>63</td>
<td>245</td>
<td>3,850</td>
<td>3,550</td>
</tr>
</tbody>
</table>

| **2,4-DNP** |    |    |   |   |
| 0           | 40 | 683  | 4,150 | 7,480 |
| 1.0 × 10^-4 | 50 | 720  | 6,460 | 8,360 |
| 2.5 × 10^-4 | 66 | 741  | 9,460 | 9,000 |
| 5.0 × 10^-4 | 89 | 925  | 12,550| 10,120|
| 7.5 × 10^-4 | 85 | 1,685 | 4,940 | 5,450 |
| 1.0 × 10^-3 | 100| 925  | 12,400| 12,000|

* The same experimental conditions were used as in Tables 1 and 2. Cyanide or 2,4-DNP was mixed with the cell suspensions inside the main compartment of the Warburg flasks prior to the addition of glucose (final concentration always 0.01 M). The incubation of B. cereus was for 2 hr, and the data were calculated for a 1-hr period.

<table>
<thead>
<tr>
<th>Glucose concn</th>
<th>A. aerogenes</th>
<th>E. coli</th>
<th>B. cereus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qo_2(N)</td>
<td>Qo_2(N)</td>
<td>Qo_2(N)</td>
<td>Qo_2(N)</td>
</tr>
<tr>
<td>aerobic</td>
<td>anaerobic</td>
<td>aerobic</td>
<td>anaerobic</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
<td>40</td>
<td>500</td>
</tr>
<tr>
<td>0.001</td>
<td>495</td>
<td>400</td>
<td>500</td>
</tr>
<tr>
<td>0.002</td>
<td>681</td>
<td>600</td>
<td>500</td>
</tr>
<tr>
<td>0.003</td>
<td>531</td>
<td>600</td>
<td>500</td>
</tr>
<tr>
<td>0.005</td>
<td>723</td>
<td>600</td>
<td>500</td>
</tr>
<tr>
<td>0.010</td>
<td>810</td>
<td>700</td>
<td>500</td>
</tr>
<tr>
<td>0.020</td>
<td>892</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>0.036</td>
<td>849</td>
<td>600</td>
<td>500</td>
</tr>
</tbody>
</table>

* The same experimental conditions were used as described in Tables 1 and 2, except for the glucose concentration.
in endogenous respiration, and it was enhanced by glucose between pH 6.5 and 7.5. The enhancement of endogenous respiration is about the same for all glucose concentrations above 0.001 M (Table 4).

Apparently, the respiration of *E. coli* and *A. aerogenes* is under a control mechanism which normally conserves the limited supply of endogenous substrates. When substrate is added, the control is released and both the exogenous and endogenous substrates are oxidized rapidly. The control of endogenous oxidation was re-imposed after about 1 hr under our experimental conditions. *B. cereus* and *R. leguminosarum* exhibit the opposite control response; exogenous substrate partially suppresses their oxidation of endogenous substrates.

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*E. coli*, *A. aerogenes*, and *B. cereus* stock cultures were supplied by W. B. Sarles from the collection of the Department of Bacteriology, University of Wisconsin. The culture of *R. leguminosarum* was supplied by J. C. Burton of the Nitragin Co., Milwaukee, Wis.

Copies of a complete manuscript describing this work are available upon request.