THE ENDOGENOUS RESPIRATION OF MICROORGANISMS

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In respiration studies and work on oxidative assimilation by microorganisms it is often of interest to know whether or not the endogenous respiration continues in the presence of an external substrate. Indirect methods, such as those first described by Barker (1936), have frequently been used in attempts to answer this question, but they are not entirely satisfactory. A more direct approach has been made recently by the use of cells which have incorporated C\(^{14}\), so that the carbon dioxide produced by endogenous respiration is radioactive. An unlabeled substrate is then added, and the total radioactivity of the carbon dioxide produced is determined. This carbon dioxide should be inactive if the endogenous respiration is completely suppressed by the added substrate. Experiments of this type with the green alga Chlorella vulgaris, the fungus Zygorhyncus moelleri, and baker's yeast are reported here.

EXPERIMENTAL METHODS

Two experiments were carried out with C. vulgaris. In the first the cells were grown in light in a medium containing 1 per cent glucose (the medium of Pearsall and Loose (1937) with the addition of trace elements). After growth the cells were washed and resuspended in 0.067 M KH\(_2\)PO\(_4\) and placed in a closed flask with 20 \(\mu\)C of BaC\(^{14}\)O\(_2\) in a center well. C\(^{14}\)O\(_2\) was liberated from this with acid. The flask was shaken in the light for 2 hours so that the cells could incorporate the C\(^{14}\)O\(_2\). The cells were then washed, resuspended in 0.067 M KH\(_2\)PO\(_4\), and pipetted into Warburg flasks. In the second experiment the cells were grown in closed flasks in a medium containing 1 per cent KHCO\(_3\) as well as other essential elements. The gas phase originally consisted of 95 per cent (v/v) air and 5 per cent (v/v) CO\(_2\); to this was added 140 \(\mu\)C of C\(^{14}\)O\(_2\). The cells were grown for 6 days with C\(^{14}\) present. They were then centrifuged, washed, and resuspended in 0.067 M KH\(_2\)PO\(_4\), pH 4.5, for the experiment.

Z. moelleri was grown as described by Moses (1954). After growth the crop from 50 ml of growth medium was washed and suspended in 0.067 M phosphate buffer, pH 6.8, and 140 \(\mu\)M of glucose, containing 50 \(\mu\)C of uniformly labeled C\(^{14}\)-glucose were added. After 3 hours the cells had all been metabolized as determined manometrically, and the cells were washed, resuspended in 45 ml of buffer, and dispensed into Warburg flasks.

D. C. L. baker's yeast (0.28 g fresh weight) was suspended in 50 ml of 0.067 M KH\(_2\)PO\(_4\), and 140 \(\mu\)M of glucose containing 50 \(\mu\)C of uniformly labeled C\(^{14}\)-glucose added. After 3 hours the cells were washed, resuspended in 26 ml of KH\(_2\)PO\(_4\), pH 4.5, and dispensed into Warburg flasks.

Evolved CO\(_2\) was trapped in 0.15 ml or 0.2 ml of 10 per cent (w/v) KOH in the center wells of the Warburg flasks provided with filter paper concertinas. At the end of the experiments the concertinas were removed and placed in graduated tubes. The center wells were washed several times with distilled water, and the washings added to the tubes. The pooled washings together with the concertinas were diluted to 2.0 ml with water; 0.05 ml of this solution was pipetted onto recessed aluminum plates (2.2 cm diam), the bottoms of which had been covered with a layer of collodion. A circle of lens tissue paper (1.9 cm diam) was placed over the drop of liquid on the aluminum plate, and the whole dried for 4 minutes under an infrared lamp (Calvin et al., 1949). The samples were counted in a proportional gas (methylene)-flow counter connected to a scaler. Replicate samples showed good agreement in counting rates by this method. In 6 replicate determinations on the same sample the standard deviation was 3.16 per cent of the mean.

RESULTS AND DISCUSSION

The results are recorded in table 1. Apart from a 10 per cent suppression when
yeast, respectively, to the found C14 led or produced; C. vulgaris was produced; microorganisms.

Gibbs and Wood (1952) obtained a similar difference using Pseudomonas fluorescens which was either grown on uniformly labeled glucose or made radioactive after growth by the addition of labeled substrates. Reiner et al. (1949) also found that supplying unlabeled acetate or glucose to yeast which had recently assimilated C14 led to increased C14O2 production. Sodium azide and 2:4-dinitrophenol stimulated the endogenous respiration of Z. moelleri and yeast, respectively, and this stimulation was reflected in the increased total activity of the CO2 produced; these substances did not greatly alter the final rates of oxygen uptake when glucose was added. The addition of glucose, however, did reduce the C14O2 production.

Cochrane and Gibbs (1951) concluded from work with C14-labeled Streptomyces coelicolor that the endogenous respiration was not suppressed when glucose or pyruvate was added. They propose, therefore, that the endogenous respiration should be deducted when interpreting oxidative assimilation experiments with this organism. Santer and Ajl (1954), using Pasteurella pestis, came to a similar conclusion.

This conclusion does not necessarily follow, however, from the fact that the C14O2 production is unchanged when glucose is added as Reiner et al. (1949) have pointed out. It is possible that the relationship between endogenous and glucose respiration can be expressed in a simplified form by the schema:

\[ Z \xrightarrow{X} Y \rightarrow CO_2 \]

**TABLE 1**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>pH</th>
<th>μL O₂ Absorbed</th>
<th>μL CO₂ Evolved</th>
<th>Total Activity of Evolved CO₂</th>
<th>cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>Endogenous</td>
<td>4.5</td>
<td>57</td>
<td>57</td>
<td>30,700</td>
<td></td>
</tr>
<tr>
<td>(i) treated with C14O₂ after growth</td>
<td>Glucose</td>
<td>4.5</td>
<td>472</td>
<td>548</td>
<td>43,000</td>
<td></td>
</tr>
<tr>
<td>(ii) grown on C14O₂</td>
<td>Endogenous</td>
<td>4.5</td>
<td>83</td>
<td>77</td>
<td>48,040</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>4.5</td>
<td>357</td>
<td>445</td>
<td>44,880</td>
<td></td>
</tr>
<tr>
<td><em>Zygorhyncus moelleri</em></td>
<td>Endogenous</td>
<td>6.8</td>
<td>283</td>
<td>230</td>
<td>24,192</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>6.8</td>
<td>1,312</td>
<td>1,453</td>
<td>24,615</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>6.8</td>
<td>1,357</td>
<td>1,019</td>
<td>21,346</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Succinate</td>
<td>3.4</td>
<td>225</td>
<td>218</td>
<td>21,423</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endogenous + NaN₂</td>
<td>6.8</td>
<td>720</td>
<td>540</td>
<td>53,577</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose + NaN₂</td>
<td>6.8</td>
<td>1,574</td>
<td>1,638</td>
<td>28,538</td>
<td></td>
</tr>
<tr>
<td><em>Baker's yeast</em></td>
<td>Endogenous</td>
<td>4.5</td>
<td>69</td>
<td>77</td>
<td>185,880</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>4.5</td>
<td>401</td>
<td>453</td>
<td>220,960</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endogenous + DNP</td>
<td>4.5</td>
<td>184</td>
<td>167</td>
<td>415,120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose + DNP</td>
<td>4.5</td>
<td>661</td>
<td>1,038</td>
<td>328,760</td>
<td></td>
</tr>
</tbody>
</table>

Buffer concentration 0.067 M in each experiment, and pH as indicated. In the C. vulgaris experiments, the glucose concentration was 0.056 M and the total volume of liquid 2.3 ml. In the yeast experiment, the glucose concentration was 0.01 M, the dinitrophenol (DNP) concentration 6 × 10⁻⁴ M, and the total volume of liquid 3.0 ml. In the Z. moelleri experiment, the substrate concentrations were 0.02 M, the NaN₂ concentration 1.33 × 10⁻⁴ M, and the total volume of liquid 3.0 ml. Gas phase, air; temp, 25 C. The experiments were duplicated or triplicated, and the mean values are given here.

acetate and succinate were supplied to Z. moelleri, the addition of substrates had little effect on C14O₂ production unless azide or 2:4-dinitrophenol was present. This result contrasts with that of Wiame and Doudoroff (1951), using Pseudomonas saccharophila, but agrees with those of Cochrane and Gibbs (1951), Gibbs and Wood (1952), Blumenthal et al. (1951), Reiner et al. (1949), and Santer and Ajl (1954) with other microorganisms.

When glucose was added in the yeast and first C. vulgaris experiments, more C14O₂ was produced; this effect was not found in the second C. vulgaris experiment using cells grown in C14O₂. Gibbs and Wood (1952) obtained a similar difference using Pseudomonas fluorescens which was either grown on uniformly labeled glucose or made radioactive after growth by the addition of labeled substrates. Reiner et al. (1949) also found that supplying unlabeled acetate or glucose to yeast which had recently assimilated C14 led to increased C14O₂ production.
where $X$ is the labeled substrate of endogenous respiration, $Z$ a product of oxidative assimilation which may or may not be identical with $X$, and $Y$ an intermediate common to both endogenous and glucose respiration. The addition of glucose, by increasing the concentration of $Y$, may increase the rate of the back reaction $Y \rightarrow X$; if the rate of the forward reaction $C^{14}X \rightarrow Y$ is unchanged, the carbon dioxide produced will still contain $C^{14}O_2$ although there is no net breakdown of $X$. If this is so, the endogenous respiration is suppressed for the purposes of oxidative assimilation calculations, yet there is little change in the production of $C^{14}O_2$. Moreover, since the concentration of $Y$ is raised by the formation of unlabeled $Y$ from glucose, the $Y$ now converted to $X$ by the back reaction, although greater in amount, may contain less $C^{14}$ than it did before glucose was added. If the labeled $X$ continues to break down at the same rate as before, this will mean that the rate of $C^{14}O_2$ production will increase when glucose is added: this was found, in fact, in the yeast and first $C. vulgaris$ experiments.

Azide and dinitrophenol probably stimulate endogenous respiration by uncoupling phosphorylation (Simon, 1953) which checks the respiration rate between $Y$ and $C^{14}O_2$. If this is so, and the rate of the forward reaction $X \rightarrow Y$ remains unaltered, little $Y$ may be reconverted to $X$ by the back reaction in the presence of these substances. The addition of glucose by increasing the concentration of $Y$ will increase the rate of this back reaction, and this could mean that more $C^{14}$ was reconverted to $X$ and less appeared as $C^{14}O_2$. Whether or not this was so would depend on the relative rates of the reactions $Y \rightarrow X$ and $Y \rightarrow CO_2$.

Thus this admittedly highly simplified schema will explain the main features of the present results. It is concluded that studies with $C^{14}$-labeled cells will show that the endogenous respiration is suppressed if the addition of substrate is found to depress greatly the rate of $C^{14}O_2$ production, but if the $C^{14}O_2$ production remains unchanged, no definite conclusion can be drawn. In fact, previous indirect studies with the organisms used in the present work have suggested that the endogenous respiration of yeast (Winzler, 1940) and $Chlorella$ spp. (Myers, 1947; Syrett, 1951) is suppressed by the addition of glucose, while that of $Z. moelleri$ continues (Moses, 1955). This contradiction between the results of the indirect method and those with $C^{14}$-labeled cells may possibly be due to the situation discussed here.

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SUMMARY

Cells of $Chlorella vulgaris$, $Zygoryhynchus moelleri$, and baker's yeast were made radioactive with $C^{14}$. The effect of the presence of various substrates and inhibitors on the rate of evolution of $C^{14}O_2$ was investigated. Generally, the $C^{14}O_2$ production in the presence of an external oxidizable substrate was either unaffected or was somewhat stimulated. Sodium azide and 2:4-dinitrophenol stimulated both the endogenous respiration and the $C^{14}O_2$ evolution; the presence of glucose together with inhibitor suppressed the increased rate of $C^{14}O_2$ evolution.

Although these results appear to indicate that the endogenous respiration is not suppressed by the presence of an external substrate it is pointed out that other interpretations are possible.

REFERENCES


Moses, V. 1955 Glucose respiration in _Zygorhyncus moelleri_; the entry of glucose into the cells. J. Exptl. Botany, 6, 222–234.


