Metabolism of the Mutant *Saccharomyces cerevisiae* R 12 A

II. Endogenous Metabolism

A. KOTYK

Laboratory for Cellular Metabolism, Institute of Biology, Czechoslovak Academy of Sciences, Prague

Received September 1, 1960

The previous paper (Kotyk, 1961) contained data on the lack of effect of oxygen on the metabolism of glucose in the mutant *Saccharomyces cerevisiae* R 12 A which is not able to oxidize glucose even under aerobic conditions.

It is known that in normal yeast incubated without substrate oxygen will cause a marked drop of inorganic phosphate level, accompanied by oxygen consumption and carbon dioxide production. The respiratory quotient of this process is generally less than one, e.g. Stickland (1956) gives values of about 0.85. In this laboratory, values ranging between 0.89 and 1.06 were found both for baker's yeast and for the pure strain R 12. There has been some disagreement about the nature of processes taking place during endogenous respiration but the latest work of Eaton (1960) indicates that yeast cells contain two separate glycogen pools, only one of which can be utilized anaerobically but both aerobically. In addition to this, ethanol is accumulated during anaerobic endogenous metabolism, which can then be broken down aerobically. This explains the relatively low respiratory quotient of endogenous respiration (*RQ* of 0.66 would correspond to full oxidation of ethanol) and puts an end to speculations about the character of the substrate broken down (lipids, proteins etc.).

As we are dealing here with processes different from glucose degradation, the effect of oxygen on the endogenous metabolism of the respiration-deficient mutant was taken up.

MATERIALS AND METHODS

Manometric methods and fractionation of phosphate compounds used here were the same as described in the previous paper (Kotyk, 1961).

RESULTS AND DISCUSSION

As follows from Table 1, if a greater amount of yeast cells is used a definite production of carbon dioxide and con-

<table>
<thead>
<tr>
<th>Gas phase</th>
<th>Inhibitor</th>
<th>( Q_{O_2} )</th>
<th>( Q_{CO_2} )</th>
<th>Apparent RQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0</td>
<td>0.22–0.35</td>
<td>1.09–1.55</td>
<td>4.4–5.4</td>
</tr>
<tr>
<td>Air</td>
<td>5 \times 10^{-4}_M\ DNP</td>
<td>0.30–0.58</td>
<td>5.6–7.8</td>
<td>13.5–18.5</td>
</tr>
<tr>
<td>Air</td>
<td>10^{-4}_M\ KCN</td>
<td>0.77–0.83</td>
<td>0.80–1.26</td>
<td>1.2–1.7</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0</td>
<td>0.60–1.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>5 \times 10^{-4}_M\ DNP</td>
<td></td>
<td>5.4–7.6</td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>10^{-4}_M\ KCN</td>
<td></td>
<td>1.09–1.36</td>
<td></td>
</tr>
</tbody>
</table>
Consumption of oxygen may be demonstrated manometrically under conditions when no added substrate is utilized. The value of $Q_{CO_2}$ is positively influenced by $5 \times 10^{-4}$M 2,4-dinitrophenol which is analogous to the situation observed in normal yeast under anaerobic conditions when dinitrophenol makes it possible to tap the "aerobic" polysaccharide reserve substrate. Oxygen consumption is only little affected by dinitrophenol, being occasionally lower, at other times higher than in the control. The fact that potassium cyanide has no inhibitory effect on the metabolic quotients even aerobically indicates that an auto-oxidizable enzyme is involved which is not blocked by cyanide (possibly cytochrome $b_6$).

![Diagram of glucose metabolism](image)

**Fig. 1.** Intracellular inorganic phosphate (lower curves) and total labile phosphate (upper curves), aerobically (full line) and anaerobically (broken line), without substrate (open circles) and with 5% glucose (full circles). Arrows indicate the points of changing incubation conditions.

The $RQ$ value of endogenous respiration has but an illustrative character; perhaps it would be more correct to consider simply the difference in $Q_{CO_2}$ between aerobic and anaerobic conditions and to relate it to the corresponding $Q_{O_2}$ — in such a case the $RQ$ value would average at about 1.0.

These findings alone indicate that the endogenous metabolism of the mutant $R_{12}A$ differs from that of the parent strain, very likely on account of the fact that only a part, or possibly different components of the cytochrome system are involved here.

In another set of experiments the changes in the levels of different phosphate compounds following the introduction of air into an anaerobic suspension were investigated. As may be seen in Fig. 1, which comprises inorganic phosphate and the sum of labile phosphate, oxygen has a very appreciable effect on the individual levels of phosphate compounds (unlike in the presence of glucose). Inorganic phosphate decreases and high-energy phosphate bonds are formed, roughly as in normal yeast cells, but on account of the very low value of $Q_{CO_2}$ the P/O ratios calculated from the initial rate of phosphorylation are very high and exceed those commonly found even in purified enzyme systems. As was shown by experiments using radioactive phosphate, however, the introduction of oxygen does not bring about a gradual increase in the specific activity of labile phosphate as might be expected but only a sudden increase in the content, as well as in total activity of high-energy phosphates takes place. This indicates a very rapid change in the content (or possibly only distribution) of high-energy bonds which, however, cannot be set equal to steadily proceeding phosphorylation.

That we are not dealing here with oxidative phosphorylation in the usual sense of the word is manifested by the lack of effect of dinitrophenol which occasionally increases the utilization of inorganic phosphate, unlike in normal yeast cells, where it blocks it at the concentration used ($5 \times 10^{-4}$M). On the other hand, potassium cyanide ($10^{-4}$M) suppresses this process completely, which again demonstrates that it is not coupled with the consumption of oxygen proceeding endogenously, which is even enhanced by cyanide.