THE PREADAPTIVE OXIDATION OF GALACTOSE BY YEAST

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INTRODUCTION

It has been demonstrated (1) that the adaptation of yeast to ferment galactose involves a modification of the apoenzymatic or protein portion of the enzyme system. A transformation of this kind would presumably require an energy supply.

This conclusion is supported by a number of facts. Adaptation of yeast to ferment a substrate such as galactose is relatively rapid under aerobic conditions (2, 3). Under anaerobic conditions, adaptation by cells from a 48 hour culture is either enormously slower or does not occur at all. This can be correlated with the finding (4-6) that the intact yeast cell is unable to use its endogenous carbohydrate reserves anaerobically, whereas the latter are quite readily oxidized aerobically. The correlation was established more directly by experiments in which the carbohydrate stores of the cells were exhausted to various degrees by varying times of aeration in the absence of exogenous substrate. During this process, the rate of endogenous respiration diminishes. The time of adaptation increases steadily with increasing exhaustion of the endogenous reserves, and rises quite sharply as the endogenous approaches zero.

Finally, it has been shown (7) that, if anaerobically available energy be supplied in the form of a small amount of fermentable substrate, anaerobic adaptation is effected under conditions which would otherwise not support the adaptive process.

As has been pointed out previously, one of the paradoxes of the aerobic adaptation lies in the fact that it can occur after virtually complete exhaustion of the carbohydrate reserves. While it is possible that stored fat and protein could under such condition be utilized by the cell as a source of energy for synthetic activity, observation of oxygen consumption and CO₂ production gives no evidence that such is the case. A resolution of this paradox was offered by the finding (8) that oxidation of the adaptive substrate could occur before the appearance of any of the enzymes necessary to its fermentation. This

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capacity of unadapted cells to oxidize the substrate suggested the possibility that this preadaptive oxidation was the source of energy for the adaptive process.

It is the purpose of the present paper to provide further evidence for the existence of this preadaptive oxidation as well as to explore some of the details of the reaction.

**Methods and Materials**

The strains employed and the methods of handling the cultures are similar to those detailed in the previous paper. The same can be said concerning the manometric measurements and methods of preparing standard suspensions. The galactose employed in the present investigations was purified by the method previously described. The purity of the resulting preparation was tested by an analysis for the presence of contaminating fermentable substances by the method of Winzler (9). Less than 0.05 per cent of such material was present in the galactose used in the experiments reported here. Reducing sugar was determined by the method of Folin and Malmros (10). When galactose was being determined, galactose standards were employed.

In the experiments in which carbon balances were sought, the following procedure was used. Subsequent to deproteinization the several groups of components were separated by a number of steps according to the following plan.

1. A distillation of a measured quantity was made at an acid pH (acid to Congo red). The condenser (water-cooled) was equipped with an adapter which dipped below the surface of a few milliliters of ice cold distilled water in the receiver, the latter being surrounded by an ice bath. The solution was distilled down to one-third of its original volume. This distillation separates part of the volatile organic acids and all the ethyl alcohol and acetaldehyde from non-volatile substances such as galactose.

2. The distillate was made alkaline (a small amount of solid powdered phenolphthalein was used as an indicator) with NaOH, and redistilled in the same fashion as above. This separates the alcohol and acetaldehyde from the volatile acids, whose Na salts are non-volatile. The distillate was made up to a definite volume, to be further analyzed for alcohol.

3. The residues of the first and second distillations were again made acid to Congo red, and once more distilled. The combined residue, whose volume might be 25 to 50 ml., was first distilled down to a volume of 15 ml., and then subjected to a steam distillation until ten volumes (150 ml.) had come over. The combined distillate contains the volatile acid fraction.

4. The residue from the third distillation was subjected to a continuous ether extraction overnight (12 to 18 hours). The ether extract was then evaporated on the steam bath, and the residue taken up in distilled water and brought to a definite volume. This fraction contains non-volatile but ether-soluble organic compounds, particularly the dicarboxylic and lactic acids. The ether-insoluble residue of the extraction contains chiefly carbohydrates and inorganic salts.

The point of the procedure just outlined is that, at the cost of some extra steps, it permits the separation of the volatile compounds into the two classes of alcohol plus acetaldehyde and volatile acid without incurring the risk of caramelization of residual carbohydrate which would occur during heating at an alkaline pH.

The steam distillation (step 3) will probably carry over about a third of any pyruvic
acid which may be present. If the amount of pyruvic acid is appreciable, a second steam distillation is required to effect a reasonably good separation of pyruvic from acetic and the other volatile acids. Since qualitative tests for pyruvic acid on aliquots of the original solution and the various fractions by the Na nitroprusside method (11) were completely negative, this additional step was not required.

The alcohol fraction required one further step before analysis. This consisted in oxidizing the contents of a 50 ml. aliquot with acid dichromate by heating for 10 minutes in a boiling water bath. This was followed by a ten volume steam distillation conducted as in step 3 above. The oxidation converts the alcohol and aldehydes into the corresponding acids, which are found in the steam distillate.

Each of the fractions is now determined by titration of the acids present with standard alkali (about 0.02 N NaOH). In the case of the alcohol fraction, the equivalents of acid obtained by titration are divided by 0.984 (if only ethyl alcohol and acetaldehyde are present) to obtain the equivalents of alcohol (12).

The individual acids in the non-volatile fraction were not all determined. Lactic acid was investigated by the colorimetric methods of Barker and Summerson (13) and found to be absent, while pyruvic acid was eliminated as mentioned earlier. This leaves no common monocarboxylic acids to be considered.

Preadaptative Galactose Oxidation

A typical adaptation curve obtained with strain 812 is presented in Fig. 1. The results found with other strains do not differ in any but the minor quantitative aspects to be expected from differences in the induction periods and the levels of the endogenous respiration. As indicated by the arrow, the galactose was added at the very beginning of the experiment. The addition produced no significant change from the endogenous respiration. The $Q_{0.1}$ in the initial portion of the curve of Fig. 1 is close to the value of 28.0 for the endogenous $Q_{0.1}$ obtained in a parallel run with a control set to which galactose was not added. The absence of immediate effects on the addition of galactose is also observed in Fig. 2 where the addition occurred 30 minutes later. Nevertheless, it is evident, particularly from Fig. 2, that the galactose is affecting the physiological characteristics of the cell even before the enzymatic apparatus necessary for its fermentative utilization makes its appearance. Normally the endogenous respiration would have begun to show a sharp decrease at 120 minutes. At the end of 150 minutes the $Q_{0.1}$ of the endogenous control run at the same time had decreased to 40 per cent of its initial value. No such change is observed in Fig. 2 during the same period.

The nature of the phenomenon involved here is more clearly seen in Fig. 3, where the galactose was added after the cells had entered the zero-rate portion of the endogenous curve. Immediately following the addition, there is a considerable burst of $O_2$ consumption and $CO_2$ evolution. Subsequently the respiration rate assumes the value characteristic of the linear portion of the endogenous curve. This is maintained until either adaptation occurs or the substrate is exhausted.
Table I records a more extensive survey of this phenomenon covering the entire endogenous curves of two strains in intervals of 30 minutes. It is clear from the results obtained that the presence or absence of an effect of the addition of galactose on the respiratory rate depends on the level of the endogenous respiration at the time of the addition. If the latter is maximal, no further increase is observed. If it is low, however, the addition of galactose will raise it to the levels attained during the linear portion. It will further be observed that these effects of galactose on the aerobic metabolism are not reflected in any changed capacity to handle the sugar anaerobically. During these periods when active aerobic oxidation was observed with galactose as the sole substrate, the \( Q_{CO_2}^{on} \) values in the presence of galactose remained uniformly negligible. It will further be noted that, in all the cases examined, the R.Q. did not differ

![Graph showing the effect of galactose addition on respiratory rate](https://example.com/graph.png)
significantly from unity, either in the high rate period immediately following
the addition of the galactose or in the subsequent one where $Q_o$ and $Q_{co}$
values characteristic of the constant-rate portion of the endogenous curves
were assumed.

Certain of the details in the response to galactose in the preadaptive period
were different when other strains were used. Fig. 4 illustrates an experiment
of this kind with strain C1, which is a representative of *S. carlsbergensis*. This
culture normally has a rather high endogenous respiration, attaining $Q_o$ values
of 55. In the experiment depicted in Fig. 4, a culture of this kind was dis-
similated until it reached a $Q_o$ value of about 5.0. This is the rate observed
in the first 60 minutes in Fig. 4. On the addition of galactose at 60 minutes
the respiratory rate increases to 28 and subsequently rises higher. Unlike the
cerevisiae strains described in the previous curves, no sudden burst of oxidation

**Fig. 2. Course of aerobic adaptation.** The arrow indicates point at which the
addition of galactose was made. Illustrating the absence of effect of galactose on
$Q_o$ during the linear phase of endogenous respiration.
is observed and the respiratory rate characteristic of the linear portion is not attained until some time after contact with galactose is made. This lowered response of strain C is attributable to the circumstance that this yeast is physiologically less vigorous than the strains of *S. cerevisiae* with which it is compared, and is rather easily injured by the process of dissimilation.

These experiments strongly suggest that in all the strains examined there exists a mechanism for preadaptive utilization of galactose. The unity of the R.Q. and the complete absence of the ability to produce CO₂ anaerobically further points to a purely aerobic metabolism in this period. The fact that the addition of galactose fails to lead to respiratory stimulation during the linear endogenous period would seem to indicate that the preadaptive metabolism of the galactose follows the same pathway as that used in the oxidation of the carbohydrate reserves, and that these kinds of substrates compete for enzyme
TABLE I
Effect of Adding Galactose during Different Periods of the Endogenous Respiration on the 
\(O_2\), \(Q_{O_2}\), and \(Q_{CO_2}\) Values of Unadapted Cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Period at end of which addition was made</th>
<th>(Q_{O_2})</th>
<th>(Q_{CO_2})</th>
<th>(Q_{O_2})</th>
<th>(Q_{CO_2})</th>
<th>(Q_{O_2})</th>
<th>(Q_{CO_2})</th>
<th>(Q_{O_2})</th>
<th>(Q_{CO_2})</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>Before addition</td>
<td>20 min. following addition</td>
<td>40 min. following addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>812</td>
<td>0–30</td>
<td>27.3</td>
<td>27.3</td>
<td>0.01</td>
<td>27.3</td>
<td>27.3</td>
<td>0.1</td>
<td>27.3</td>
<td>27.3</td>
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<tr>
<td></td>
<td>30–60</td>
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<td>27.7</td>
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<td>27.7</td>
<td>27.7</td>
<td>0.1</td>
<td>27.7</td>
<td>27.6</td>
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<td></td>
<td>60–90</td>
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<td>0.3</td>
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<td>26.5</td>
<td>0.0</td>
<td>26.5</td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td>90–120</td>
<td>24.3</td>
<td>24.0</td>
<td>0.08</td>
<td>26.8</td>
<td>27.1</td>
<td>0.2</td>
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<tr>
<td></td>
<td>120–150</td>
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<td>12.1</td>
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<td>0.2</td>
<td>27.5</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td>150–180</td>
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<td>9.0</td>
<td>0.1</td>
<td>38.0</td>
<td>38.2</td>
<td>0.3</td>
<td>26.8</td>
<td>27.2</td>
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<tr>
<td></td>
<td>180–210</td>
<td>6.2</td>
<td>6.2</td>
<td>0.2</td>
<td>56.3</td>
<td>53.3</td>
<td>0.1</td>
<td>29.1</td>
<td>28.3</td>
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<td>210–240</td>
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<td>70.2</td>
<td>65.3</td>
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<td>29.8</td>
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<table>
<thead>
<tr>
<th>Strain</th>
<th>Period at end of which addition was made</th>
<th>(Q_{O_2})</th>
<th>(Q_{CO_2})</th>
<th>(Q_{O_2})</th>
<th>(Q_{CO_2})</th>
<th>(Q_{O_2})</th>
<th>(Q_{CO_2})</th>
<th>(Q_{O_2})</th>
<th>(Q_{CO_2})</th>
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<tr>
<td>LK2G12</td>
<td>0–30</td>
<td>31.2</td>
<td>31.3</td>
<td>0.1</td>
<td>31.3</td>
<td>31.3</td>
<td>0.1</td>
<td>31.2</td>
<td>31.3</td>
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<td>31.1</td>
<td>0.3</td>
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<td>31.1</td>
</tr>
<tr>
<td></td>
<td>60–90</td>
<td>32.4</td>
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<td>0.4</td>
<td>32.4</td>
<td>32.0</td>
<td>0.4</td>
<td>33.0</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>90–120</td>
<td>31.0</td>
<td>30.8</td>
<td>0.8</td>
<td>33.1</td>
<td>32.0</td>
<td>0.6</td>
<td>33.1</td>
<td>32.0</td>
</tr>
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<td></td>
<td>120–150</td>
<td>27.0</td>
<td>27.0</td>
<td>0.1</td>
<td>40.1</td>
<td>39.5</td>
<td>0.2</td>
<td>31.6</td>
<td>32.3</td>
</tr>
<tr>
<td></td>
<td>150–180</td>
<td>15.4</td>
<td>15.5</td>
<td>0.2</td>
<td>53.2</td>
<td>50.7</td>
<td>0.5</td>
<td>32.4</td>
<td>33.1</td>
</tr>
<tr>
<td></td>
<td>180–210</td>
<td>9.0</td>
<td>9.1</td>
<td>0.03</td>
<td>78.9</td>
<td>76.3</td>
<td>0.03</td>
<td>30.5</td>
<td>30.8</td>
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<tr>
<td></td>
<td>210–240</td>
<td>5.2</td>
<td>5.4</td>
<td>0.01</td>
<td>85.6</td>
<td>84.0</td>
<td>0.05</td>
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<td>32.1</td>
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<tr>
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<td>240–270</td>
<td>0.8</td>
<td>1.0</td>
<td>0.03</td>
<td>90.1</td>
<td>87.3</td>
<td>0.07</td>
<td>32.6</td>
<td>31.0</td>
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</table>

Fig. 4. The effect of adding galactose on the aerobic metabolism of a dissimilated culture of S. carlsbergensis.
when both are present together. The fact that the \( Q_{ol} \) can rise above this level lends further support to this interpretation. If such a competitive interaction did exist, a preferential utilization of the carbohydrate reserves would leave the rate unchanged on the addition of galactose during a period when the concentration level of the preferred substrate is non-limiting.

Fig. 5. Preadaptive aerobic metabolism of galactose by dissimilated and undisimilated cells. The ordinate represents the amount of galactose consumed by 1 ml. of the suspension, expressed in milligrams. Illustrating the rapid preadapptive consumption of galactose by dissimilated cells.

Fig. 6. The anaerobic assimilation of galactose by adapted and unadapted cultures. The ordinate represents the residual galactose in milligrams, in 1 ml. of the suspension. Gas phase N₂. Illustrating the inability of unadapted cells to consume any galactose.

Relation between Galactose Oxidation and the Endogenous Respiration

The hypothesis of interaction between the oxidation of galactose and of the endogenous reserves was tested by experiments which examined and compared the metabolism of galactose in cultures prepared under different conditions.

In these experiments 2 day cultures were washed as usual and resuspended in \( \text{M/15 KH}_2\text{PO}_4 \). The resulting suspension was divided into two portions. One received an amount of galactose sufficient to make a final solution containing 25 mg./cc. and was then placed in a water bath at 30.2°C, and shaken. Samples were withdrawn at intervals for sugar analysis. The other portion of the suspension was allowed to dissimilate for 4 hours while shaking in a water bath. At the end of this period the same amount of galactose which was used previously was now added to this portion, and the carbohydrate content was subsequently followed. The results of such an experiment with one of the strains employed are given in Fig. 5. Two other strains were tested in the same manner and gave similar results.
It is evident from Fig. 5 that previous dissimilation, resulting in the exhaustion of the endogenous reserve, facilitates the preadaptive utilization of the galactose. The departure from linearity observed in the lower curve represents the onset of adaptation and the fermentative utilization of the galactose. These results support the conclusion concerning the relation between this preadaptive utilization of the galactose and the endogenous respiration.

This phenomenon of the preferential utilization of the endogenous reserves can cause the appearance of an "induction" period in the utilization of an externally supplied substrate. Such an "induction" may have all the characteristics of adaptation to the utilization of the exogenous substrate. However, such "adaptations" can easily be distinguished from true adaptations, since they can be made to occur by dissimilation in the absence of the substrate to which the cell is presumably becoming adapted.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Strain</th>
<th>Galactose per cc. at 0 time</th>
<th>Time in contact</th>
<th>Galactose per cc.</th>
<th>Galactose assimilated</th>
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</thead>
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<tr>
<td>1a</td>
<td>Db23B</td>
<td>14.7</td>
<td>6.3</td>
<td>14.8</td>
<td>0.</td>
</tr>
<tr>
<td>1b</td>
<td>Db23B</td>
<td>14.7</td>
<td>20.7</td>
<td>14.6</td>
<td>0.1</td>
</tr>
<tr>
<td>1c</td>
<td>Db23B</td>
<td>20.0</td>
<td>4.3</td>
<td>20.0</td>
<td>0.</td>
</tr>
<tr>
<td>2a</td>
<td>LK2G12</td>
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<td>4.7</td>
<td>17.4</td>
<td>0.1</td>
</tr>
<tr>
<td>2b</td>
<td>LK2G12</td>
<td>17.5</td>
<td>4.7</td>
<td>17.3</td>
<td>0.2</td>
</tr>
<tr>
<td>3a</td>
<td>812</td>
<td>20.0</td>
<td>6.0</td>
<td>20.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3c</td>
<td>812</td>
<td>14.8</td>
<td>23.5</td>
<td>14.7</td>
<td>0.1</td>
</tr>
</tbody>
</table>

It was pointed out above that the unity of the R.Q., as well as the absence of any anaerobic CO₂ production during the preadaptive period, strongly suggested that during this period galactose is metabolized by a purely aerobic mechanism. Further experiments were performed to test this supposition more directly.

Suspensions of 2 day cultures were prepared in the usual manner and dissimilated for 3.5 hours at 30.2°C. They were then distributed to Warburg vessels with known amounts of galactose in the side-arms. After flushing with nitrogen, the galactose was tipped and the vessels were allowed to shake. After various intervals, pairs were removed, the yeast quickly centrifuged down, and the supernatant analyzed. The results are shown in Table II.

A variation of this experiment was also carried out which involved longer incubation periods.

Two day glucose-grown cultures were treated and suspended as above. Samples were then distributed to Thunberg tubes containing known amounts of galactose in their side-arms. The tubes were evacuated and filled with nitrogen twice. After
having been brought to the temperature of the water bath the galactose in the side-
arms was tipped into the main compartments of all tubes simultaneously. At in-
tervals, one after the other was removed from the water bath, the yeast quickly
centrifuged, and an aliquot of the supernatant taken for analysis.

Fig. 6 gives the results obtained with one of the strains employed. For purposes
of comparison, data obtained with an adapted culture of equal density under
similar conditions are included. The sole difference in the treatment of the
adapted culture is that it did not undergo any prior dissimilation. Such dis-
similation could not be employed since the galactozymase system is extremely
unstable and tends to disappear rapidly in oxygen in the absence of its substrate.

It is clear, both from Table II and Fig. 6, that no significant metabolism
of galactose occurs under anaerobic conditions with unadapted cells. Fig. 6
further illustrates the inability of certain strains to adapt anaerobically even
with prolonged incubation with substrate.

The Effect of Inhibitors on the Respiration of Galactose by
Unadapted Cells

To check further on the nature of the respiration of the unadapted cell in
the presence of galactose, the effects of the addition of cyanide on the \( Q_{10} \) and
\( Q_{\text{CO}_2} \) were compared.

In these experiments the cells were first dissimilated for 3.5 hours to reduce the
contribution of the endogenous respiration to less than 10 per cent. Sufficient galac-
tose was added to yield a final concentration of 4 per cent. By allowing 30 minutes
to elapse after the addition of the galactose before the experiment was started, the
"burst portion" (see Fig. 3) of the galactose oxidation was avoided. All the data
were obtained during the stable linear portion of the curve. The suspending fluid
in these experiments for both the experimentals and controls was \( \mu/15 \) phosphate
buffer at pH 7.2. The oxygen cup contained appropriate mixtures of KOH and HCN
in the center well to avoid net distillation from the main compartment.

The results of these experiments are given in Table III. They indicate that
the CO\(_2\) produced is purely respiratory in origin, since the effects on the oxygen
consumption and CO\(_2\) production parallel one another.

Lundsgaard (14) demonstrated the specific effect of low concentrations of
iodoacetic acid (IAA) on the fermentative process in yeast. He found that
\( 10^{-4} \) M IAA completely inhibits the fermentation, leaving the respiration un-
hampered, the R.Q. approaching unity. This offered another method of testing
for the existence of fermentative components in the preadaptive metabolism
of galactose. Here again, as in the case of the cyanide experiment, the cells
were previously dissimilated to insure that the major portion of the respiration
being studied was due to the galactose added.
These experiments were performed with cells suspended in M/15 KH$_2$PO$_4$ containing 4 per cent galactose. Precautions, similar to those taken in the cyanide experiment, were observed here to insure that the observations were being made in the linear portion of the curve. Readings were recorded 50 minutes after the introduction of the IAA from the center well into the main compartment. This delay was made to compensate the lag effect often observed in inhibitions of oxygen consumption due to the addition of IAA.

### TABLE III

*Effect of KCN on Respiration of Unadapted Cells in 4 Per Cent Galactose*

<table>
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<tr>
<th>Strain</th>
<th>Control</th>
<th>KCN Concentration</th>
<th>Experimental</th>
<th>Inhibition</th>
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<tr>
<td></td>
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<td>$\text{O}_2$</td>
<td>$\text{CO}_2$</td>
<td>$\text{O}_2$</td>
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<td>1 $\times 10^{-s}$ M</td>
<td>0.3</td>
</tr>
<tr>
<td>812</td>
<td>26.5</td>
<td>27.2</td>
<td>1 $\times 10^{-s}$ M</td>
<td>2.4</td>
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<td>812</td>
<td>26.8</td>
<td>26.5</td>
<td>1 $\times 10^{-s}$ M</td>
<td>0.01</td>
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</table>

### TABLE IV

*Effect of IAA on Respiration of Unadapted Cells Metabolising Galactose*

<table>
<thead>
<tr>
<th>Strain</th>
<th>IAA Concentration</th>
<th>$\text{O}_2$</th>
<th>$\text{CO}_2$</th>
<th>Inhibition</th>
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</tr>
<tr>
<td></td>
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<td>2 $\times 10^{-s}$ M</td>
<td>10.3</td>
<td>10.1</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>2 $\times 10^{-s}$ M</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>31.5</td>
<td>32.1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2 $\times 10^{-s}$ M</td>
<td>31.5</td>
<td>32.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2 $\times 10^{-s}$ M</td>
<td>19.7</td>
<td>9.9</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>2 $\times 10^{-s}$ M</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
</tr>
</tbody>
</table>

From the results summarized in Table IV, it is evident that no selective inhibition on the carbon dioxide production occurs, as would be the case if the origin of the CO$_2$ were fermentative. Figs. 7 and 8 give the detailed results obtained with strain LK2G12. For comparative purposes, the curves obtained with adapted cultures of the same strain are also included. These curves demonstrate even more clearly than is possible by a table the striking difference in the behavior towards IAA of cells fermenting galactose as compared with...
Fig. 7. The effect of IAA on the $Q_{O_2}$ and $Q_{CO_2}$ of adapted and unadapted yeast-metabolizing galactose. The galactose was introduced 40 minutes prior to the first reading. This illustrates the effect of an IAA concentration ($2 \times 10^{-4}$ M) too low to inhibit the purely oxidative component of the metabolism.

Fig. 8. The effect of IAA on the $Q_{O_2}$ and $Q_{CO_2}$ of adapted and unadapted yeast-metabolizing galactose. The galactose was introduced 40 minutes prior to the first reading. This illustrates the effect of an IAA concentration ($2 \times 10^{-3}$ M) high enough to inhibit the oxidative as well as the fermentative component of the metabolism.

those metabolizing it by means of a purely aerobic system. Where the IAA is sufficiently high in concentration to affect the aerobic process, the oxygen
and CO₂ curves parallel each other, as in the case of the unadapted culture oxidizing galactose in the presence of a 2 × 10⁻³ M IAA (lower curve, Fig. 8). Such a parallel response is not seen with the adapted culture (upper curve, Fig. 8). In this latter case CO₂ production responds more quickly and more markedly than the oxygen consumption. It will be noted from Fig. 7 that 2 × 10⁻⁴ M IAA, which is unable to affect the aerobic utilization of galactose by the unadapted cells, causes a decrease in the aerobic fermentation resulting in a final R.Q. of 1. That this concentration of IAA is able completely to inhibit the fermentative utilization of galactose by adapted cultures was further tested by examining its effect on anaerobic production of CO₂. In all cases a Q₁₀ of zero was obtained within 10 minutes following the addition of the IAA.

In general, the response of the galactose respiration is strikingly similar to that found for the endogenous respiration (4, 5).

The Assimilation of Galactose and the Products of Its Utilization by Unadapted Cells

It has been shown in the preceding section that galactose can be oxidized by unadapted cells, and demonstrated directly that galactose disappears from the medium during the preadaptive phase. The question naturally arises whether the oxygen consumed corresponds to the galactose which has disappeared. The usual method for testing the completeness of the oxidation of a substrate involves introducing a known amount and comparing the oxygen consumed with that which would be predicted theoretically from the amount added. In most cases it is possible to determine the point at which all of the added substrate has been consumed by the occurrence of a sharp drop in the respiratory rate to the endogenous level at the point of exhaustion of the exogenous substrate. In the present instance, however, this method is not available, since the respiratory rate of unadapted cells metabolizing galactose does not differ significantly from the endogenous rate. Under these circumstances a very gradual decrease is observed on the exhaustion of the external substrate, and not only is its initiation hard to detect, but it is probable that the fall occurs some time after all of the exogenous substrate is exhausted. In view of this fact it was necessary to make parallel determinations of oxygen consumption and galactose disappearance.

The 2 day cultures used in these experiments were dissimilated for 7 hours to insure negligible contributions from the endogenous respiration. No experiment was run over 3 hours after the introduction of the galactose, in order to avoid overlapping into the adaptive period when fermentative utilization occurs.

The procedure adopted after some preliminary experiments was as follows: Portions of the dissimilated suspensions were set up in Warburg vessels for oxygen consumption and CO₂ production measurements. After the galactose was transferred from the side-arms to the main compartment, the vessels were allowed to shake for various periods of time. During this incubation period the course of the oxygen
consumption and CO₂ production was followed manometrically. When an adequate amount of oxygen had been consumed, the vessels were removed and the contents centrifuged quickly in preparation for an analysis of the supernatant for the remaining galactose. The values thus obtained for two strains are recorded in Table V.

It is evident that a considerable discrepancy exists between the oxygen consumed and that which would be expected on the basis of the amount of galactose which had disappeared. There are several possible ways of interpreting results of this nature. It is conceivable that synthesis of cellular components accompanies the oxidation of the galactose. Such phenomena have been observed by Winzler and Bamberg (15), Winzler (16), and van Niel and Cohen (17) in yeast consuming other substrates. A similar discrepancy between oxygen consumed and substrate metabolized has been noted in other microorganisms by Barker (18), Clifton (19), Clifton and Logan (20), and Doudoroff (21). These authors have also interpreted their observations in terms of the assimilation of the unaccounted for substrate as cellular material. However, it must be noted that, despite the unity of the r.q., all or part of the discrepancy might be ascribed to incomplete oxidation of the galactose.

In order to examine this question further an attempt at a complete carbon balance was made, in which gas production, galactose utilization, polysaccharide storage, and the formation of alcohol and organic acids were studied. At the same time a comparison was made between the preadaptive and postadaptive products of galactose utilization. The following experimental procedure was adopted.

Several Erlenmeyer flasks of 125 cc. capacity, each containing 8 cc. of dissimilated yeast suspension (strain C1) prepared in the usual way, with galactose and water to make a total volume of 16 cc., were shaken in a water bath at 30.2°C. With this preparation, adaptation sets in soon after 90 minutes. Consequently, flasks were

---

**TABLE V**

**Oxygen Consumption Corresponding to the Metabolism of Various Amounts of Galactose**

The theoretical amount of oxygen required for the complete oxidation of 1 mg. of galactose to CO₂ and H₂O at 30°C., the temperature of the experiment, is 748 c.mm.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Galactose consumed</th>
<th>O₂ consumed</th>
<th>r.q.</th>
<th>Theoretical</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>812</td>
<td>2.1</td>
<td>455</td>
<td>1.00</td>
<td>1570</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>406</td>
<td>1.10</td>
<td>1120</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>838</td>
<td>1.08</td>
<td>2620</td>
<td>32</td>
</tr>
<tr>
<td>LK2G12</td>
<td>3.0</td>
<td>695</td>
<td>1.00</td>
<td>2280</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>592</td>
<td>1.08</td>
<td>1660</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>443</td>
<td>1.03</td>
<td>1340</td>
<td>33</td>
</tr>
</tbody>
</table>
removed at 90 minutes for the preadaptive period and at 180 minutes for an examination of the postadaptive period. The contents were quantitatively washed into large centrifuge tubes containing 2 cc. of 10 N H₂SO₄ to stop the reaction and precipitate the proteins. After centrifugation and removal of the supernatant fluid, the residues were washed once on the centrifuge with dilute acid and the washings added to the supernatant. The latter was made up to a definite volume in a volumetric flask for further analysis according to the procedures described in the section on methods. The residue of yeast cells was analyzed for polysaccharides according to a modified Pfliiger method (22).

The results of such balance experiments are presented in Table VI in terms of milliequivalents of products formed and compounds disappeared. Aside from the components specifically mentioned in the table, it might be noted that qualitative tests for pyruvic acid on aliquots of the original solution and the various fractions by the sodium nitroprusside test (11) were completely negative. Further, the fractionation procedure employed determines alcohol and

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Products formed (m. eq.)</th>
<th>Carbon atoms in products (m. eq.)</th>
<th>Compounds disappeared (m. eq.)</th>
<th>Carbon atoms converted (m. eq.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preadaptive (0 to 90 min.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>0.028</td>
<td>0.168</td>
<td>0.050</td>
<td>0.300</td>
</tr>
<tr>
<td>Polysaccharide (as hexose)</td>
<td>0.019</td>
<td>0.048</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>O₂</td>
<td>0.061</td>
<td>0.061</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>0.019</td>
<td>0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.019</td>
<td>0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volatile acids</td>
<td>0.003</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-volatile ether-soluble acids</td>
<td></td>
<td></td>
<td>0.030</td>
<td>0.030</td>
</tr>
<tr>
<td>Total</td>
<td>0.283</td>
<td>0.350</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post-adaptive (90 to 180 min.)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Products formed (m. eq.)</th>
<th>Carbon atoms in products (m. eq.)</th>
<th>Compounds disappeared (m. eq.)</th>
<th>Carbon atoms converted (m. eq.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>0.136</td>
<td>0.816</td>
<td>0.222</td>
<td>1.332</td>
</tr>
<tr>
<td>Glycogen</td>
<td>0.244</td>
<td>0.244</td>
<td>0.172</td>
<td></td>
</tr>
<tr>
<td>O₂</td>
<td>0.076</td>
<td>0.152</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>0.244</td>
<td>0.244</td>
<td>0.172</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.158</td>
<td>0.006</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Volatile acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-volatile acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.212</td>
<td>1.342</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
aldehyde together, hence qualitative tests for acetaldehyde by Rimini's modification of the method of Simon (23, 24) were applied to the original solution and to the various fractions with uniformly negative results. Since an ether extraction was resorted to, the possibility exists that the ether might have carried over acetoain or diacetyl. These were therefore tested for by the modified Voges-Proskauer procedure (25), again with negative results.

The figures in Table VI represent net changes in the corresponding products. The first two columns list those which show a net increase; the last two, a net decrease. An examination of the preadaptive portion reveals that 0.350 milliequivalent of carbon atoms disappeared during the preadaptive period. Of this, 0.283 milliequivalent or 81 per cent was recovered in the carbon fractions examined. The recovery of 81 per cent of the carbon disappearing is fairly satisfactory, considering that no determinations were performed to detect changes in the lipid storage of the cells or non-volatile ether-insoluble materials such as phosphate esters. It is evident that a little more than half of the galactose utilized in the preadaptive period can be accounted for by the increase in the polysaccharide fraction. However, the oxygen consumption still amounts to only about half of the theoretical for complete oxidation of the galactose not stored as polysaccharide (0.022 milliequivalent of galactose should result in the consumption of 0.132 milliequivalent of oxygen, as against 0.058 milliequivalent actually consumed during this period). In view of the fact, however, that we are accounting in our over-all balance for only about 80 per cent of the disappearing carbon, this discrepancy may well be explained by the galactose entering some of the unanalyzed fractions.

The only anomaly in the preadaptive phase is the appearance of some alcohol. This could be accounted for on the supposition that adaptation actually comes somewhat earlier than 90 minutes. More probable, however, is that it arose from the reduction of some volatile acid component by hydrogen donors remaining in the cell at the beginning of the experiment or formed during the galactose metabolism. In this connection it will be noted that 0.05 milliequivalent of volatile acids disappeared during the incubation period which corresponds very well to the 0.048 milliequivalent of alcohol which was formed. Furthermore, the r.q., both on an over-all basis, as is seen from the table, as well as by the continuous manometric determination during the 90 minute period of the experiment, remained close to unity. This would be difficult to understand if the alcohol arose from the metabolism of the galactose, but would be consistent if the former is formed from the volatile acids.

For purposes of comparison similar data for the postadaptive period from 90 to 180 minutes have been included in the table. The carbon recovery here is better than that obtained in the preadaptive period, 91 per cent of the galactose disappearing being accounted for. This would seem to indicate that postadaptively the galactose does not accumulate as much in the unanalyzed.
fractions as is the case in the preadaptive period. Again, as before, the polysaccharide stored represents in terms of carbon about 60 per cent of the galactose which has disappeared. The difference between the oxygen consumed and CO₂ produced is 0.072 milliequivalent, in good agreement with 0.076 milliequivalent of alcohol produced.

The results obtained indicate that galactose cannot only be oxidized in the period before it can be adaptively fermented, but may also be stored in very appreciable quantities as polysaccharide as well as other intermediates.

**The Kinetics of Galactose Oxidation**

The ability of unadapted cells to oxidize galactose in the absence of a previous contact would argue the existence of a preformed enzyme or enzyme system, the specificity of which permitted combination with galactose. Presumably, however, the affinity of such an enzyme for a substrate like galactose would not be as great as that for the endogenous substrate it normally catalyzes. The fact that, in the competitive interaction between galactose and the endogenous polysaccharide during the preadaptive period, it is the latter which is the preferred substrate would support such a contention.

It seemed possible that an examination of the response of the respiratory rate to increasing concentrations of galactose might reveal further information on this question. It would at any rate provide an estimate of the order of magnitude of the dissociation constant of the enzyme-substrate complex. Experiments designed to obtain such information were performed. As in all previous instances where it was desired to study the oxidation of galactose, well dissimilated cultures were used, both to lower the endogenous respiration and to extend the preadaptive period.

Fig. 9 gives a typical rate-concentration curve of an unadapted culture oxidizing galactose. For purposes of comparison there are included similar curves for an adapted culture oxidizing in one case galactose and in the other glucose. It is immediately evident that the nature of the response of the respiratory rate in an unadapted culture to increasing concentrations of galactose is entirely different from that of an adapted one oxidizing either this hexose or glucose. A much higher concentration is required in the unadapted culture in order to arrive at the maximal rate of respiration. Unlike the adapted culture, the unadapted one responds over a very wide range of concentrations to increasing amounts of galactose. It is seen that, comparatively, there is relatively little difference in the behavior of the adapted culture with galactose and glucose insofar as concerns the concentration necessary to arrive at the maximal activity.

The behavior of the unadapted culture is consistent with the interpretation that the enzyme involved has a relatively low combining capacity for galactose. Measurements of the dissociation constant, according to the procedure of
Lineweaver and Burk (26), would tend to bear this out. It was found that galactose being oxidized by an adapted culture gave a value of 0.0045. The same culture oxidizing glucose yielded a value of 0.0025. However, an unadapted culture oxidizing galactose resulted in a dissociation constant of 0.0385 which indicates a more than tenfold difference between the combining capacities of adapted and unadapted cultures for galactose.

![Graph](image-url)

**Fig. 9.** $Q_O$-concentration curves; (A) glucose; (B) galactose with an adapted culture; (C) galactose with an unadapted culture. Each point is the average of closely agreeing duplicate determinations, and represents the steady rate for the corresponding concentration. This rate is always reached within 10 minutes after addition of the sugar to the yeast suspension.

**The Oxidation of Sugars by Non-Adaptable Strains**

A question which arises in connection with the ability of unadapted yeast cells to oxidize galactose is whether this ability is necessarily connected with the ability to adapt to their fermentation. It is therefore of interest to report some observations which answer this in the negative.

Strains are known which will not adapt to galactose fermentation even when incubated with this sugar in a complete medium for long periods of time. Others can give rise to mutants which on incubation with galactose will adapt to its fermentation. Table VII records the results on the measured respiratory rates when galactose is added to a series of non-adaptable strains. All these
experiments were carried out with non-dividing suspensions which rules out any possibility of mutation and subsequent selection. It is important to note further that the stimulation observed in these cases on the addition of galactose was immediate.

It is evident that these non-adaptable strains possess the capacity of oxidizing the galactose although they cannot acquire the ability to ferment. It may be worth noting that, in all the adaptable and non-adaptable strains tested thus far, no exception has been found in this capacity to oxidize the galactose. Clearly, while this physiological characteristic may be necessary for adaptation to occur in the absence of other energy sources, by itself it does not guarantee that adaptation will occur. It merely furnishes a condition under which adaptation is possible if other genetically controlled factors are present.

<table>
<thead>
<tr>
<th>Yeast</th>
<th>$Q_O_2$ before addition</th>
<th>$Q_O_2$ after addition</th>
<th>$Z.Q.$ after addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>2.3</td>
<td>20.3</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>22.9</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>21.0</td>
<td>1.03</td>
</tr>
<tr>
<td>Schizosaccharomyces octosporus</td>
<td>2.0</td>
<td>22.0</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>19.5</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>24.6</td>
<td>1.08</td>
</tr>
<tr>
<td>Saccharomyces ludwigii</td>
<td>1.0</td>
<td>10.4</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>15.6</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>12.1</td>
<td>1.08</td>
</tr>
</tbody>
</table>

It is interesting to note that normally adaptable strains can be treated in such a way that they are incapable of adapting. It has been shown (7) that length of dissimilation increases the adaptation time. If dissimilation is carried on for over 20 hours, adaptation does not occur within 48 hours. Despite this, such cells are perfectly well able to oxidize the galactose during the 48 hour period in which they show themselves unable to adapt to its fermentation.

**DISCUSSION**

(a) *General Biochemical Nature of the Oxidation of Galactose*

Previous workers have offered evidence for the existence of a purely aerobic pathway for carbohydrate breakdown. Among these may be cited Loebel's (27) study of the oxidation of fructose by brain tissue, and Barker, Shorr, and Malam's (28) investigation of the continued respiration in the presence of IAA, which confirmed similar findings by Lundsgaard (14). In bacteria
Barron and Friedemann (29) present striking evidence in the form of some bacterial species which cannot ferment glucose but have no difficulty oxidizing it. The demonstration of a purely aerobic metabolism of galactose by unadapted and unadaptable cells adds another piece of evidence to the collection of evidence against the so-called "unitary hypothesis" (cf. Burk, 30) of the relation between fermentation and aerobic oxidation. The galactose differs from glucose at the fourth carbon. The trioses derived from galactose would hence be identical with those which are formed from glucose. Consequently, a cell possessing, as all unadapted cells examined here do, the full complement of enzymes necessary for fermentation of glucose could not possibly distinguish between the triose phosphate formed by the splitting of glucose and that yielded by the cleavage of galactose. The pathway of galactose oxidation by unadapted cells must therefore diverge before the 3-carbon stage is reached; for, if it did not, the ability to oxidize would have to be accompanied by the capacity to ferment this hexose. This divergence at higher levels is further supported by the investigation of Reiner (31) into the phosphate changes in the preadaptive period. It was found that phosphopyruvic acid phosphate falls markedly during the preadaptive oxidation, indicating an absence of regeneration of this compound during this period.

Since there is no evidence that galactose induces this other pathway, it is evident that it preexists functionally in normal unadapted cells. The data presented here would indicate that it represents the metabolic mechanism employed by the cell for the oxidation of its reserves. What the details of the pathway are cannot as yet be stated. Dickens (32, 33) and Lipmann (34), who have both studied the oxidation of hexose monophosphate, have proposed a scheme for the normal purely aerobic oxidative degradation of glucose which may be summarized as follows:

\[
\begin{align*}
&\text{CHO} \quad \text{COOH} \quad \text{COOH} \\
&\text{HCOH} \quad \text{HCOH} \quad \text{C} = \text{O} \quad \text{CHO} \\
&\text{HOCH} \quad \text{HOCH} \quad \text{HOCH} \quad \text{HOCH} \\
&\text{HGH} \quad \text{HCOH} \quad \text{HCOH} \quad \text{HOCH} \\
&\text{HCOH} \quad \text{HCOH} \quad \text{HCOH} \quad \text{HCOH} \\
&\text{CH}_3\text{PO}_4\text{H}_2 \quad \text{CH}_3\text{PO}_4\text{H}_2 \quad \text{CH}_3\text{PO}_4\text{H}_2 \quad \text{CH}_3\text{PO}_4\text{H}_2
\end{align*}
\]

The first stage is a formation of the hexose-6-phosphate (1) and the oxidation of this to the 6-phosphohexonate (2), then to 2-keto-phosphohexonate (3), and the decarboxylation of the latter to a pentose phosphate. This scheme is based on the observations by Warburg, Christian, and Griese (35) that the hexose-
6-phosphate can be oxidized to the corresponding hexonate by enzyme preparations from yeast. Lipmann (34) obtained further evidence from manometric studies that the hexonate could oxidize to the pentose via the 2-keto-phosphohexonate. Dickens (33), using Lebedew preparations plus purified coenzyme II, confirmed these findings by isolating the phosphoketohexonic acid and a phosphopentonic acid as their barium salts.

The direct application of this scheme without modification to the preadaptive oxidation of the galactose is not possible for several reasons. One would have to assume that an enzyme existed in the cell which could phosphorylate galactose in the 6 position. The recent work of Kunitz and MacDonald (36) rules out the possibility that hexokinase, the enzyme normally involved in this kind of reaction, can effect it with galactose. Furthermore, Grant (37) has shown that a preparation from galactose-adapted yeast, which could ferment galactose, failed to ferment synthetically prepared galactose-6-phosphate. A fully adapted cell would presumably contain all the components of an unadapted one plus those it gained as a result of the adaptation. Since galactose-6-phosphate is not a metabolizable intermediate for an adapted cell, it seems unlikely that it would be one for an unadapted cell.

In looking for the mechanism of galactose oxidation, the relation between the galactose oxidation and the endogenous fermentation should be borne in mind. The data on this problem can at present be best explained by supposing that the galactose is oxidized through the same metabolic mechanism which the cell employs in using its polysaccharide reserves. We may summarize the evidence leading to this conclusion as follows:

1. Both have an R.Q. of unity and a vanishingly small anaerobic CO₂ production.
2. The behavior towards KCN of the O₂ consumption and of the aerobic CO₂ production in both types of respiration is qualitatively the same.
3. IAA in concentration sufficient to inhibit fermentation does not affect O₂ production of either the endogenous respiration or of the preadaptive oxidation of galactose.
4. The response of the O₂ consumption to addition of galactose at various levels of the endogenous respiration of a dissimilating suspension indicates the existence of a competitive interaction between them.
5. Rate of galactose assimilation increases on exhaustion of the reserves by previous dissimilation.

There are two possible mechanisms which would account for the striking similarity between preadaptive galactose oxidation and the endogenous respiration.

1. Galactose may be converted to polysaccharide before being oxidized. This would be a variation of the mechanism proposed by Willstätter and Rohdewald (38) for the utilization of glucose and maltose.
2. That part of the galactose which is oxidized may not pass through polysaccharide but may have at least one rate-limiting step in common with the endogenous polysaccharide in their oxidative pathway.

It is not possible to decide on the precise mechanism involved here on the basis of the evidence as yet available, and it is further conceivable that both of the above may be acting as factors in the preadaptive oxidation. In this connection it should be noted that Kosterlitz (39) found that adapted yeast could utilize galactose-1-phosphate. If unadapted yeast were able to phosphorylate galactose but not able to isomerize it to the corresponding glucose ester, it would have available a close analogue of glucose-1-phosphate, which is the normal substrate of polysaccharide formation. This may explain the capacity of unadapted cells to store polysaccharide during the preadaptive period. The polysaccharide stored by adapted yeast fermenting galactose has, it is true, been found to be a polymer of glucose, and not of galactose. However, the polysaccharide formed preadaptively has never been examined in this way. In favor of the possibility that the polysaccharide formed preadaptively differs from that formed postadaptively is the sharp increase in polysaccharide storage observed after adaptation (see Table VI). This would be reasonable if glucose-1-phosphate could be formed after adaptation but not before, so that the substrate of normal glycogen formation would be available postadaptively. Otherwise, it is not easy to see why acquisition of the ability to ferment galactose should also produce a four- or fivefold increase in the ability to form polysaccharide from it. In any case, it is evident that the identification of the polysaccharide formed during the preadaptive oxidation of galactose could provide an important clue for the elucidation of the biochemical details involved.

(b) Implications of the Preadaptive Oxidation for the Adaptive Process

As was pointed out in the introduction, the existence of this preadaptive oxidation of galactose resolves the problem of how cells can adapt to galactose aerobically even though they have exhausted their endogenous reserves. Evidently the oxidation of the galactose can supply the necessary energy. We have here a situation where one and the same compound supplies both the energy and the stimulus required for a change in the enzymatic constitution of a cell.

Apparently the fermentative utilization of a carbohydrate imposes greater restrictions of specificity than its aerobic metabolism. No enzymatic modification is required for the latter pathway. Whether this is a general property of all adaptations involving carbohydrate substrates it is impossible to say. Preliminary data indicate that the same phenomenon exists in lactose adaptation by \textit{B. coli} and in maltose adaptation in yeast.

The fact that the combining capacity for galactose as measured by the rate-concentration curves is greater with adapted cells than with unadapted cells raises certain questions concerning the nature of the adaptation. This fact might be interpreted to mean that the adaptive process is a consequence of
increasing the specificity of some preexisting enzymatic component which already possesses some capacity for combining with the adapting substrate. Such an interpretation would be consistent with Monod's (40) hypothesis of adaptation, which assumes that adaptation consists in increasing the specificity of some relatively non-specific protein precursor under the influence of the combining substrate. While such phenomena may take place and play a role in enzymatic adaptations, it seems difficult to explain the observations presented on this basis alone.

If this specificity modification were the only process involved, it would be expected that as a result of the adaptation a rise in $Q_{O_2}$ would be the only thing observed. While such an increase in respiration in the presence of galactose is attained, by far the most unique characteristic of the adaptation is the appearance of the capacity to ferment the galactose. It would be difficult to argue that an increase in specificity of the enzyme involved in the oxidation of the galactose should result in the appearance of the capacity to ferment this hexose. This conclusion is made even more unlikely by the fact that the rise in $Q_{O_2}$ invariably follows the appearance of the adaptive enzyme activity and never precedes it. This is seen in Figs. 1, 2, and 3, in which it may be observed that the rise in the rate of oxygen consumption always occurs after the CO$_2$ curve has diverged to give an R.Q. above 1, indicating aerobic glycolysis and hence adaptive enzyme activity. While such a change in specificity cannot be excluded, it seems likely that the rise in $Q_{O_2}$ following adaptation is in part at least a result of the accumulation of oxidizable intermediates, as a result of the onset of the adaptive metabolism. This would explain the time relations of the two phenomena.

In any event it seems clear that the adaptation results in the appearance of an enzymatic activity which is essential for the anaerobic utilization of the galactose but not for its aerobic metabolism.

SUMMARY

A preadaptive purely aerobic utilization of galactose by yeast cells has been demonstrated. Hence, the adaptation by yeast to galactose is not to its utilization per se, but specifically to its metabolism by a glycolytic mechanism. An examination of this preadaptive oxidation of galactose reveals that it has many characteristics in common with the endogenous metabolism of yeast. Included among these are the similarities of the R.Q. values and the response of the $O_2$ and $Q_{CO_2}$ to KCN and iodoacetic acid. Further, a competitive interaction appears to exist between the endogenous respiration and the preadaptive oxidation of the galactose. The latter can replace the endogenous respiration as a source of energy for the adaptation to the fermentation of the galactose. Carbon balance studies of the galactose oxidation revealed that polysaccharide could be formed as a result of this metabolism during the preadaptive period.
PREADAPTIVE OXIDATION OF GALACTOSE BY YEAST

Non-adaptable cells were also found to possess the capacity to oxidize galactose in the complete absence of any ability to metabolize it anaerobically.

The significance of these findings for the biochemistry and physiology of the adaptation is discussed.

BIBLIOGRAPHY