RESPIRATORY METABOLISM OF NORMAL AND DIVISIONLESS STRAINS OF CANDIDA ALBICANS*

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ABSTRACT

Respiration of a normal strain of Candida albicans was compared with that of a divisionless mutant which has a biochemical lesion such that metabolically generated hydrogen “spills over,” during growth, for non-specific dye reduction. This waste is not at expense of growth, since both strains grow at essentially similar rates, nor at expense of respiration, since the mutant reduces oxygen more rapidly than the normal strain. Respiration in both strains is qualitatively similar, and seemingly unique among highly aerobic organisms in that it is not mediated by cytochrome oxidase. In resting cells of both strains, respiration is not only resistant to, but markedly stimulated by, high concentrations of cyanide, carbon monoxide, and azide. In contrast, growth of these yeasts is inhibited by low concentrations of cyanide and azide.

Cytochrome oxidase could not be detected in cell-free preparations; reduced cytochrome c was not oxidized by such preparations. Cytochrome bands could not be observed in thick cell suspensions treated with reducing agents. However, incorporation of superoptimal levels of zinc and iron into the culture medium resulted in growth of cells possessing distinct cytochrome bands; respiration of these cells remained insensitive to cyanide, monoxide, and azide, and the bands were maintained in a reduced form on oxygenation.

In the divisionless yeast, tetrazolium dyes compete with oxygen for reduction; this is not the case in the normal strain. The firmness with which hydrogen transfer is channeled in the latter for reduction of disulfide bonds (of importance in the division mechanism) and of oxygen, is contrasted with the lack of such control in the mutant.

INTRODUCTION

Previous work in this laboratory has shown that a divisionless filamentous mutant of a yeast Candida albicans is genetically blocked at a reductive step in such a manner that metabolically generated hydrogen spills over, during growth, for non-specific dye reduction (Nickerson, 1954). This “spill over” is not at the expense of demands for growth, since synthesis of cell mass is

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equally rapid in mutant and normal strains. Resting cells of the mutant rapidly reduce tetrazolium compounds (and dyes with redox potentials down to about $-0.150 \text{v.}$) by their endogenous metabolism. Resting cells of the normal strain carry out such reductions only in the presence of a powerful metal chelating agent. The presence of such an agent in a growing culture of the normal strain induces dye reduction and inhibits cell division. In view of these facts, the biochemical lesion in the genetically blocked strain has been postulated to lie in the alteration of a metallo-flavoprotein (which normally catalyzes the reduction of some "hydrogen acceptor participating in cellular division") to a diaphorase which catalyzes non-specific dye reductions.

The experiments reported in this paper constituted part of an effort to identify the block in hydrogen transport that is responsible for loss of division in this yeast, and deal with the terminal respiratory metabolism of \textit{C. albicans}. The findings demonstrate conclusively that the "waste" of reductive capacity in the divisionless strain is not at the expense of respiratory capacity, since the mutant reduces oxygen even more rapidly than does the normal strain. The findings, furthermore, preclude the operation of cytochrome oxidase as a functional terminal oxidase in either the normal or mutant strain. In all likelihood, the reduction of oxygen is mediated in these yeasts by flavoprotein oxidases. However, despite the absence of a functional cytochrome oxidase system, the synthesis of cytochrome can be induced in \textit{C. albicans} by inclusion of superoptimal levels of zinc in the culture medium.

**EXPERIMENTAL**

\textit{Materials and Methods}.

\textit{Microbiological Methods}.—The procedures employed in characterizing \textit{Candida albicans} (strain 582) and its filamentous mutant (strain 806), have been described (Nickerson and Chung, 1954). Unless otherwise stated, cells employed in manometric experiments were grown in a liquid medium (designated medium 2) of the following composition per liter: glucose, 20 gm.; ammonium sulfate 3.0 gm.; \( \text{KH}_2\text{PO}_4 \) 3.0 gm.; \( \text{CaCl}_2 \) 0.25 gm.; \( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \) 0.25 gm.; biotin 5 \text{ug}. Cells grown in No. 2 medium subjected to continuous agitation for 20 hours at 28°C. were harvested by centrifugation at low speed; the supernatant (growth medium) was discarded, and the cells were washed three times with distilled water by alternate resuspension and centrifugation at low speed. The cells were again suspended in distilled water and used immediately in the experiments outlined below. Dry weights of cell suspensions were determined either gravimetrically on 5 ml. aliquots heated to 90°C. for 24 hours, or turbidimetrically employing a specially constructed weight-turbidity curve.

\textit{Respiration of Growing Cells}.—To study the effect of cyanide, azide, and monoxide on growth and on the respiration of growing cells, the following procedure was employed. Manometer vessels were plugged with cotton and sterilized in an oven by dry heat. Sterile GGY culture medium (2.0 ml.) was added to the main compartment of each vessel. The GGY medium had the following composition per liter: glucose, 10
A washed cell suspension was tipped in from a side arm of a vessel at zero time. The center well of a vessel contained KOH (or KCN, in experiments involving cyanide). Growth was measured by determination of turbidity.

**Nutritional Induction of Cytochrome Synthesis.**—Cells grown in GGY medium or in No. 2 medium were essentially free of absorption bands in the visible region when thick suspensions were examined with a Zeiss hand spectroscope. Following the findings of Grimm and Allen (1954) on *Ustilago sphaerogena*, superoptimal levels of various heavy metals were incorporated into medium 2. One formulation, on which results are reported, was as follows: medium 2 plus per liter; monosodium glutamate, 1.0 gm.; ZnSO₄·7H₂O, 0.010 gm.; and FeCl₃, 0.050 gm. This modification was termed medium 3.

**Preparation of Mitochondria.**—Suspensions of mitochondrial particulates were prepared by the procedure of Nickerson and Falcone (1956 a) from packed washed cells harvested from cultures that had been grown at 28°C. for 48 hours in No. 2 medium with continuous agitation. Cells suspended in 0.25 M sucrose solution were ruptured by mechanical agitation in a Waring blender with glass beads (Minnesota Mining and Manufacturing Co. "glowbrite" beads, size No. 110) according to the technique of Lamanna and Mallette (1954). After 60 to 90 minutes' agitation (with cooling achieved by running tap water), the broken cells were eliminated by centrifugation at 4000 × g for 20 minutes (in refrigerated centrifuge at 5°C.). The turbid supernatant was centrifuged twice more in the same way until practically no sediment was obtained. The supernatant, now free of cells and large particulate matter, was centrifuged for 25 minutes at 23,000 × g at 5°C. The sediment was suspended again in 0.25 M sucrose and spun down twice more in the same manner. The sediment after the final sucrose wash was resuspended in 0.25 M sucrose and examined for cytochrome oxidase activity both manometrically and spectrophotometrically.

Control experiments employing triphenyltetrazolium chloride (TTC) showed a wide range of dehydrogenase activity in mitochondrial particulates prepared in this manner. The presence of certain flavoprotein reductases, including a protein disulfide reductase (PDS reductase), has also been established (Nickerson and Falcone, 1956 b).

**Monomeric Methods.**—Respiratory gas exchange was measured using the Warburg constant volume respirometer at 30°C. Succinate buffer (0.1 M, pH 5.6) was the principal buffer employed; control experiments showed no increase in oxygen uptake over the endogenous in phosphate buffer on the addition of succinate, and no inhibition of endogenous respiration in succinate buffer by malonate (0.01 M). Inhibitors such as cyanide and azide were added as their sodium salts from the side arms of the vessels at zero time. Constant concentrations of cyanide were maintained by placing 2 M KCN (in place of KOH) in the center well (Laties, 1949). Tank nitrogen used in the controls for the carbon monoxide experiments was deoxygenated by passage over hot copper filings. Purified carbon monoxide was obtained in a cylinder from the Mattheson Company. Illumination was provided in experiments involving carbon monoxide by fluorescent lights, constructed in the shape of a semicircle, immersed in a circular water bath. The fluorescent tubing was approximately 1 meter long. The lights were operated from a gaseous tube transformer with 7500 v. output. The tubing provided a brilliant white light with strong emission lines at 436, 542 to 544, and 572 to 574 m."
Oxygen Consumption by Normal and Filamentous Strains of Candida albicans

Previous studies on the respiration of C. albicans by van Niel and Cohen (1942) drew attention to the high endogenous rate exhibited by unstarved cultures of young cells. This rate can be greatly reduced by aeration of washed cells for 24 hours in buffer. The addition of small amounts of glucose to starved cells results in the production of only one-third of the carbon dioxide expected for complete oxidation of the sugar, of which approximately two-thirds can be shown to have been assimilated. This is one of the highest ratios of assimilation to oxidation that has been recorded.

A study by Kluyver and Custers (1940) revealed another aspect of gaseous exchange in which C. albicans is exceptional. Unstarved cells of this organism, as well as certain other species of Candida, exhibit an endogenous fermentation; i.e., anaerobic production of CO₂ in the absence of added substrate. This property is not associated with bakers' yeast, or with other non-filamentous yeasts, and may indicate a spatial relationship between stored reserves and fermentative systems in Candida different from that in most other yeasts (Nickerson, 1947).

With both normal and filamentous strains of C. albicans, the endogenous and exogenous consumption of oxygen are markedly influenced by the age of the culture from which the cells are harvested. Furthermore, the rates of oxygen uptake are affected by the composition and pH of the buffer in which freshly harvested, washed cells are suspended. Data comparing the rates of endogenous consumption by 24 hour cells of both strains are given in Table I for three different suspending media. In all cases, the rate of oxygen consumption per unit weight is higher for the filamentous strain than for the normal strain. This finding demonstrates that the filamentous mutant does not have a block in its respiratory path that would account for its property of rapidly reducing tetrazolium and other foreign hydrogen acceptors. Since the filamentous strain reduces oxygen more rapidly than does the normal strain, this is another indication that the mutant strain has diverted hydrogen from some acceptor (other than oxygen) that is operative in the normal strain.

### Table I

<table>
<thead>
<tr>
<th>Suspending medium</th>
<th>Oxygen consumption (Qₒₒ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
</tr>
<tr>
<td>0.1 M phosphate buffer, pH 7.2</td>
<td>19.4</td>
</tr>
<tr>
<td>0.1 M succinate buffer, pH 5.6</td>
<td>18.5</td>
</tr>
</tbody>
</table>

* All data are averages of four to eight different experiments.
Cyanide.—The data presented in Fig. 1 show that the endogenous respiratory systems of both the normal yeast and its filamentous mutant are insensitive to concentrations of cyanide as high as $10^{-3}$ M. Indeed, cyanide at $10^{-4}$ M or $10^{-5}$ M induces a substantial increase in the rate of oxygen consumption by both strains. Thus it would appear that the function terminal oxidase is not a
Fig. 2. The effect of cyanide on exogenous oxygen uptake by normal strain 582 of *C. albicans* (left) and of filamentous strain 806 (right). Succinate buffer 0.14 M, pH 5.6 with 1.0 mg. glucose per vessel tipped at zero time; 2.6 mg. dry weight cells per vessel for both strains 582 and 806; 0.2 ml. 2 M KCN in center well.
metallo-porphyrin protein, since it is well known that cyanide inhibits the cytochrome oxidase of bakers' yeast, higher plants, and animal tissues, as well as the ascorbic acid oxidase and polyphenol oxidase of higher plants.

![Graph showing the effect of carbon monoxide on endogenous oxygen uptake by normal strain 582 of C. albicans and filamentous mutant strain 806.](image)

Fig. 3. The effect of carbon monoxide on endogenous oxygen uptake by normal strain 582 of C. albicans and filamentous mutant strain 806. Succinate buffer, 0.14 M, pH 5.6. Vessels equilibrated with gas mixtures while shaking in water bath.

The apparent stimulation of oxygen uptake by cyanide can most probably be explained on the basis of its inhibitory effect on catalase activity (Keilin, 1937). Both strains of Candida exhibit appreciable catalase activity which can be effectively inhibited with cyanide. The falling off of the oxygen uptake curve of strain 806 in the presence of 0.01 M cyanide (Fig. 1) may be due to an accumulation of toxic amounts of hydrogen peroxide.
The possibility that the addition of respirable substrate might serve to suppress the endogenous respiration of *C. albicans* and cause the appearance of an exogenous respiration different in kind from the endogenous (as has been indicated for certain microorganisms (Barker, 1936; Taylor, 1950)) is negated since $10^{-8}$ M and $10^{-4}$ M cyanide also stimulate the exogenous respiration due to glucose (Fig. 2). Again, the lesser stimulation obtained with $10^{-2}$ M KCN may be due to the complete inhibition of catalase, with a resultant accumulation of hydrogen peroxide. Other experiments not reported here, using phosphate buffer at pH 7.2, yielded results entirely similar to those shown in Figs. 1 and 2.
Carbon Monoxide.—Carbon monoxide readily forms metal carbonyls and thus in its action also serves to define the operation of metallo-porphyrin protein oxidases. It can be observed (Figs. 3 and 4) that the respiration of both strains of \textit{Candida albicans} is totally insensitive to the action of carbon monoxide whether in the dark or in light. As in the experiments with cyanide, the response of the exogenous respiration to monoxide resembled that of the endogenous respiration.

In addition to demonstrating that the respiration of \textit{C. albicans} is insensitive to monoxide, Figs. 3 and 4 also provide information on the effect of oxygen tension on oxygen uptake. Lowering the oxygen tension from 20 per cent (air) to 5 per cent causes a dramatic inhibition of the exogenous respiration of the filamentous strain, and a noticeable inhibition in strain 582 (Fig. 4). In fact, at the 5 per cent oxygen level the rates of O$_2$ uptake in the presence and absence of substrate are almost exactly the same with either strain. It is well known that the "loading curve" for cytochrome oxidase is independent of the oxygen tension down to about 10$^{-4}$ mm. O$_2$, and the respiration of bakers' yeast (mediated by cytochrome oxidase) has also been shown by Winzler (1941) to be independent of oxygen tension down to about 1 mm. (ca. 0.1 per cent O$_2$). These facts provide additional evidence against the operation of cytochrome oxidase in \textit{C. albicans} and favor the view that a flavin-mediated terminal oxidase(s) is chiefly involved in its oxygen uptake.

Az/de.—The known similarity between the action of cyanide and of azide in their inhibition of metal-containing oxidases would lead one to expect that the respiration of \textit{C. albicans} would be insensitive to azide. However, high concentrations of azide ($10^{-4}$ M and $10^{-3}$ M NaN$_3$) were found to inhibit respiration. Therefore, a wider range of concentration was studied. These results are plotted in Fig. 5. The oxygen uptake of the filamentous mutant is inhibited in this range of azide concentration both with and without added substrate (Fig. 6). The tremendous stimulation observed with strain 582 at a specific concentration of azide ($10^{-4}$ M) initiated further experiments regarding the nature of this stimulation; these studies will be reported in a subsequent paper. Although azide has been demonstrated to inhibit metal oxidases, such as cytochrome oxidase and polyphenol oxidase, this inhibitor has also been shown to act at other loci, particularly those concerned with phosphorylation (Loomis and Lipmann, 1949). Thus, the action of azide on \textit{C. albicans} may not be upon a metal oxidase, but may be inhibiting respiration in some other manner. Indeed, $10^{-4}$ M azide has been shown (Nickerson, 1954) to inhibit diaphorase activity in \textit{C. albicans}, whereas this flavoprotein catalysis is greatly stimulated by $10^{-4}$ M cyanide.

Antimycin A.—This substance has been shown by Potter and Reif (1952) to cause complete inhibition of oxygen uptake by the succinoxidase system. The site of inhibition has been localized at a step involved in the reduction of cyto-
**Candida albicans 582**

**endogenous**

**Candida albicans 582**

**exogenous (glucose)**

- $10^{-4} \text{ M} \text{NaN}_3$
- $10^{-5} \text{ M} \text{NaN}_3$
- $10^{-6} \text{ M} \text{NaN}_3$

**Figure 5.** The effect of sodium azide on endogenous and exogenous respiration of *C. albicans* 582. Succinate buffer, 0.14 M, pH 5.6; 7.1 mg. dry weight cells per vessel for endogenous run; 7.5 mg. dry weight cells and 1.0 mg. glucose per vessel for exogenous measurements.
Fig. 6. The effect of sodium azide on endogenous and exogenous respiration of C. albicans 806. Conditions as stated for Fig. 5, except 2.2 mg dry weight of cells per vessel (endogenous) and 2.0 mg (exogenous).
chrome c (Potter and Reif, 1952; Chance, 1952). At concentrations of 0.1 to 0.5 μg. per ml., antimycin A had no detectable effect on either the endogenous or exogenous (glucose) respiration of \textit{C. albicans}, strain 582. This is additional indication that the cytochrome system does not operate in this yeast.

**Effect of Respiratory Inhibitors on Growing Cells**

In view of the fact that oxygen consumption by resting cells of \textit{C. albicans} is insensitive to cyanide and monoxide, and is apparently not mediated by cytochrome oxidase, it seemed of interest to examine oxygen consumption by growing cells, and to determine whether growth was proportional to oxygen consumption.

As shown in Fig. 7, the oxygen uptake of growing cells of both normal and filamentous strains of \textit{C. albicans} is sensitive to $10^{-4}$ M cyanide. This concentration causes 90 to 95 per cent inhibition of growth (Table II), which is reflected in the curves for oxygen consumption. This sensitivity of growing cells to cyanide is to be compared with the complete insensitivity of oxygen consumption by resting cells to concentrations as high as $10^{-2}$ M cyanide (Figs. 1 and 2). Table II shows that CO has very little effect on the growth of the filamentous strain, but causes a substantial inhibition of strain 582, the inhibition being twice as great in the dark as in light. Oxygen consumption by both strains, curiously, is greater in the dark than in light (Fig. 8).

Lowered oxygen tension is actually about as effective an inhibitor of growth as the presence of carbon monoxide. The following calculations are made from the data in Table II:

<table>
<thead>
<tr>
<th>Oxygen tension</th>
<th>\textit{582} Growth in 4 hrs.</th>
<th>\textit{806} Growth in 4 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 per cent (air)</td>
<td>4.8-fold</td>
<td>5.5-fold</td>
</tr>
<tr>
<td>5 per cent</td>
<td>\begin{align*} \text{Light:} &amp; 3.0-fold \ \text{Dark:} &amp; 2.1-fold \end{align*}</td>
<td>\begin{align*} \text{Light:} &amp; 3.8-fold \ \text{Dark:} &amp; 3.6-fold \end{align*}</td>
</tr>
</tbody>
</table>

Growing cells of the normal and filamentous strains are also distinguishable by their response to sodium azide. Increasing concentrations of azide cause an inhibition of growth of strain 582 that is almost linear over the range $10^{-8}$ M to $10^{-2}$ M. With strain 806 there is an all-or-none effect of azide on growth, with the transition lying between $10^{-4}$ M and $10^{-3}$ M (Fig. 9).

**Attempts to Demonstrate the Presence of Cytochrome Oxidase in Candida albicans**

The oxidation of reduced cytochrome c by homogenates was examined both spectrophotometrically, by measuring the decrease in absorption at 550 μν according to the method of Stern and Timonen (1954), and manometrically...
according to the method of Schneider and Potter (1943), as employed by Grimm and Allen (1954). Completely negative results were obtained with both methods. It must be concluded that neither strain of *C. albicans* contains a functional cytochrome oxidase. Moreover, no cytochrome oxidase has been detected in cells in which the synthesis of cytochrome had been promoted by superoptimal concentrations of zinc and iron. As described in the next section the respiration of "cytochrome-rich" resting cells remained insensitive to cyanide, monoxide, and azide. It is believed that this is the first record of an

![Graph](image-url)
essentially aerobic organism that is lacking in cytochrome oxidase (C. albicans grows scarcely at all in the absence of oxygen).

**Stimulation of Cytochrome Synthesis by Zinc and Iron**

In the early stages of this work it was found that although typical absorption bands could not be detected by examination of packed cells with a Zeiss hand spectroscope, it was sometimes possible to observe weak bands at about 420 mμ and 550 mμ in pyridine extracts of such cells. Extracts of cells grown on medium 2 were found to exhibit bands, whereas extracts from cells grown on GGY medium did not. The effect of nutrition on cytochrome synthesis in C. albicans was, therefore, examined. Grimm and Allen (1954) had found that zinc, supplied at levels superoptimal for growth, dramatically stimulated cytochrome formation in *Ustilago sphaerogena*. A similar situation was found to hold true in *C. albicans*. As shown in Table III, the addition of zinc and iron at levels superoptimal for growth greatly increased cytochrome synthesis in an ammonium-nitrogen medium. The absorption spectra of cell suspensions in which cytochromes had been oxidized or reduced according to the method

<table>
<thead>
<tr>
<th>Addition to vessels containing nutrient medium</th>
<th>Dry weight of cells (mg. per flask)</th>
<th>Strain 582</th>
<th>Strain 866</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initially present After 4 hrs. incubation Inhibition Initially present After 4 hrs. incubation Inhibition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>mg.</td>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>10^-4 M KCN</td>
<td>9.8</td>
<td>45.0</td>
<td>—</td>
</tr>
<tr>
<td>10^-5 M KCN</td>
<td>9.8</td>
<td>12.0</td>
<td>95.8</td>
</tr>
<tr>
<td>None</td>
<td>8.48</td>
<td>42.3</td>
<td>—</td>
</tr>
<tr>
<td>10^-5 M NaNO₃</td>
<td>8.48</td>
<td>36.5</td>
<td>19.2</td>
</tr>
<tr>
<td>10^-5 M NaNO₃</td>
<td>8.48</td>
<td>23.3</td>
<td>56.2</td>
</tr>
<tr>
<td>10^-5 M NaNO₃</td>
<td>8.48</td>
<td>12.1</td>
<td>89.4</td>
</tr>
<tr>
<td>Dark: 95 per cent N₂/5 per cent O₂</td>
<td>4.0</td>
<td>8.6</td>
<td>—</td>
</tr>
<tr>
<td>Dark: 95 per cent CO/5 per cent O₂</td>
<td>4.0</td>
<td>6.5</td>
<td>45.7</td>
</tr>
<tr>
<td>Light: 95 per cent N₂/5 per cent O₂</td>
<td>2.6</td>
<td>6.7</td>
<td>19.6</td>
</tr>
<tr>
<td>Light: 95 per cent CO/5 per cent O₂</td>
<td>2.6</td>
<td>6.7</td>
<td>19.6</td>
</tr>
</tbody>
</table>

* Experiments conducted in Warburg vessels concomitant with gas exchange studies.
of Grimm and Allen are shown in Fig. 10. The typical cytochrome spectrum of cells grown with excess zinc and iron is apparent.

In connection with the data in Table III, it should be noted that the cytochrome bands were present at all times in the cells grown with excess zinc and iron. The bands did not disappear, even on prolonged aeration, but could be oxidized by ferricyanide in heated suspensions. This is additional evidence that a cytochrome oxidase system is not present in *C. albicans*.

The effect of cyanide and monoxide on cells grown with added zinc and iron (so called "cytochrome" cells) was examined. The rates of endogenous and exogenous (glucose) oxygen uptake for such cultures were essentially the same as those found with cultures from ordinary culture medium. No inhibition of oxygen uptake was observed in the presence of monoxide or of cyanide. In one experiment, in which cells from 48 hour cultures were employed, the results shown in Table IV were obtained.


Competition between Oxygen and Dyes for Reduction by Flavoprotein Oxidases

It will be observed (Table V) that the addition of $4 \times 10^{-4} \text{M}$ TTC to resting cells of the filamentous mutant results in a 50 per cent inhibition of oxygen consumption, but causes only half as great inhibition of CO$_2$ production. The R.Q. is thus increased from essentially 1.0 to 1.5 as a result of competition between TTC and oxygen as hydrogen acceptors. However, the formazan
produced accounts for only about 36 per cent of the decrease in oxygen uptake. The competition between oxygen and TTC for reduction by flavoprotein oxidases is expressed in reaction 1 vs. reaction 2.

\[
\text{Candida albicans 582}
\]

![Absorption spectrum of thick cell suspensions of C. albicans 582 reduced with sodium hydrosulfite. Cells grown for 24 hours in medium 2 with addition to medium of zinc and iron (top curve), zinc alone (middle curve—note difference in ordinate), or iron alone (lower line). For top curve, absorption maxima 525, 553, and 605 m\(\mu\).]

(1) \[2H(2e) + O_2 \rightarrow H_2O_2\]

(2) \[2H(2e) + TTC \rightarrow TPF \text{ (triphenylformazan)}\]

In the experiment detailed in Table V, there was present 1.2 \(\mu\)M TTC, the equivalent of 27\(\mu\)l \(O_2\). Assuming that after 75 minutes' reaction time all of the TTC had been reduced (actually the case, for all practical purposes),
oxygen uptake should be decreased only by 27 µl., if the TTC has no other action. Actually, oxygen uptake has decreased 76 µl. from the controls; thus the TTC reduced accounts for only 36 per cent of the decrease. It must be noted, however, that at 75 minutes' reaction time the sum of O₃ consumed (61.5 µl.) and TTC reduced (27 µl.) approximates almost exactly the amount of CO₂ produced (86.8 µl.) to give a modified r.q. (CO₂/O₃ + TTC) = 0.98. These data are taken to mean that TTC competes directly with oxygen for reduction by flavoprotein oxidases, and is without inhibitory effect at the

<table>
<thead>
<tr>
<th>Addition to basal medium</th>
<th>Growth (optical density)</th>
<th>Cytochrome bands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain 582</td>
<td>Strain 806</td>
</tr>
<tr>
<td>None</td>
<td>0.976</td>
<td>0.750</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O (10 mg./liter)</td>
<td>0.980</td>
<td>0.810</td>
</tr>
<tr>
<td>FeCl₃ (50 mg./liter)</td>
<td>0.886</td>
<td>0.722</td>
</tr>
<tr>
<td>Zinc and iron</td>
<td>0.976</td>
<td>0.756</td>
</tr>
</tbody>
</table>

* Medium 2 + 100 mg. per cent Na glutamate.
† 24 hour cultures, blue filter.

<table>
<thead>
<tr>
<th>Concentration of cyanide</th>
<th>Endogenous Qₒ₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>11.3</td>
</tr>
<tr>
<td>10⁻³ M</td>
<td>11.4</td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>10.5</td>
</tr>
</tbody>
</table>

* Cultures grown for 48 hours in medium 3.
TTC + Na$_2$EDTA, although marked reduction of TTC occurred in the presence of Na$_2$EDTA (Table VI).

**TABLE V**

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>O$_2$ uptake</th>
<th>CO$_2$ production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µl.</td>
<td>µl.</td>
</tr>
<tr>
<td>30</td>
<td>54.8</td>
<td>54.4</td>
</tr>
<tr>
<td>60</td>
<td>112.7</td>
<td>102.0</td>
</tr>
<tr>
<td>75</td>
<td>137.8</td>
<td>121.0</td>
</tr>
</tbody>
</table>

Cells = 2.9 mg. dry weight per vessel; TTC = 4.3 × 10$^{-4}$ M.

**TABLE VI**

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Endogenous</th>
<th>+TTC</th>
<th>+EDTA</th>
<th>+TTC + EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O$_2$</td>
<td>CO$_2$</td>
<td>r.q.</td>
<td>O$_2$</td>
</tr>
<tr>
<td>30</td>
<td>43.8</td>
<td>43.3</td>
<td>1.00</td>
<td>48.5</td>
</tr>
<tr>
<td>60</td>
<td>84.7</td>
<td>94.8</td>
<td>0.99</td>
<td>98.6</td>
</tr>
<tr>
<td>90</td>
<td>123.5</td>
<td>120.7</td>
<td>0.99</td>
<td>147.0</td>
</tr>
</tbody>
</table>

Final concentrations: TTC = 4.3 × 10$^{-4}$ M; Na$_2$EDTA = 10$^{-4}$ M; cells = 6.3 mg. dry weight per vessel; total volume 2.8 ml.

**DISCUSSION**

There have been at least two reports of organisms which possess functional cytochrome oxidase systems (as demonstrated with cell-free extracts) but in which oxygen uptake by intact cells is not inhibited by cyanide, azide, or monoxide. Darby and Goddard (1950) uncovered this anomalous behavior in a fungus Myrothecium verrucaria, and Grimm and Allen (1954) have observed a comparable situation in a basidiomycete Ustilago sphaerogena. Oxygen uptake in Candida albicans is not inhibited by cyanide, monoxide, or azide; but we have been unable to demonstrate the presence of any component of the cytochrome system in cells grown aerobically under conditions in which growth is both rapid and abundant. In such material, oxidation of reduced cytochrome c by cell-free preparations is not demonstrable, nor can the characteristic bands of cytochrome components be detected spectroscopically.

Nevertheless, both normal and divisionless strains of C. albicans can be induced to synthesize components of the cytochrome system. Incorporation of
superoptimal concentrations of zinc and iron into the growth medium results in a slight inhibition of growth, but the cells exhibit an intense multi-banded absorption in the visible region that is of a characteristic cytochrome nature. Cells possessing a cytochrome spectrum did not consume oxygen more rapidly than cells without evident cytochrome, nor was oxygen consumption by the "cytochrome cells" inhibited by $10^{-2} \text{M}$ cyanide. The cytochrome bands in these cells were in a continuously reduced state (in the absence of cyanide) and did not disappear on strong aeration of cell suspensions. In 

It has been known for many years that environmental factors can markedly affect the synthesis of heme compounds in yeasts and in animal tissues. Elvehjem (1931) showed that copper and iron play a role in the synthesis of cytochromes in bakers' yeast; and catalase activity in yeasts grown on an iron-deficient medium is lowered (Yoshikawa, 1937). Cytochrome synthesis in yeast is also greatly affected by aeration of the cells. Bakers' yeast develops a 2-banded cytochrome spectrum if grown anaerobically (Fink, 1932), whereas the 2-banded spectrum of brewers' yeast can be converted to the 4-banded spectrum characteristic of bakers' yeast by cultivating brewers' yeast (top or bottom strains) aerobically (Fink and Berwald, 1933), or merely by aerating a suspension of non-multiplying cells (Chin, 1950; Ephrussi and Slonimski, 1950).

The stimulation of cytochrome synthesis in 

It was shown that the addition of triphenyltetrazolium chloride (TTC) to the divisionless strain of 

It has been demonstrated (Nickerson and Falcone, 1956b) that the disulfide bonds in a mannan-protein component of the cell wall comprise a hydrogen acceptor participating in cellular division in 

In the divisionless mutant, protein disulfide reductase activity is negligible and, as a consequence, a substantial fraction of substrate-generated hydrogen normally transported
to protein disulfide reductase is diverted in the growing cell for reduction both of oxygen and other H-acceptors, such as dyes (Fig. 11). This concept is formally analogous to that evoked to explain the increase in respiration consequent on the uncoupling of oxidative phosphorylation by dinitrophenol (Thieman, 1955). In both instances, regulator processes are affected: a hydrogen acceptor in the one, and a phosphate acceptor in the other. In both instances, the affected cell responds by increased utilization of oxygen and decreased glycolysis, i.e. the appearance of a Pasteur effect may result from the removal of a control, and be but a symptom of the loss of control, not a cause thereof. Thus, one might view the differences between dividing and non-dividing cells (whether "normal" vs. divisionless yeasts, or tumorous vs. "normal" tissue cells) not only in terms of "damaged" respiration and "deficient" Pasteur mechanism of the dividing cells, but also of the loss of regulative control in the non-dividing cells that results in increased diversion of substrate-generated hydrogen to oxygen as acceptor. Bearing in mind the nature of hydrogen transport systems demonstrated for C. albicans, ascites tumor cells, and anaerobic bacteria, the impression is gained that specificity of reduction processes is controlled at the flavoprotein level, with the specificity maintained in large measure by the associated metal of the flavoprotein.

Fig. 11. Diagram of terminal oxidations in cytochrome-less cells of Candida albicans. A competition for reduction appears between —O—O— and —S—S—. Dye reduction is prominent only in cells in which metallo-flavoprotein has been converted to diaphorase.
REFERENCES