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# Respiratory Development in *Saccharomyces cerevisiae* Grown at Controlled Oxygen Tension

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*Saccharomyces cerevisiae* was grown in batch culture over a wide range of oxygen concentrations, varying from the anaerobic condition to a maximal dissolved oxygen concentration of 3.5 μM. The development of cells was assayed by measuring amounts of the aerobic cytochromes $aa_3$, *b*, *c*, and $c_1$, the cellular content of unsaturated fatty acids and ergosterol, and the activity of respiratory enzyme complexes. The half-maximal levels of membrane-bound cytochromes $aa_3$, *b*, and $c_1$, were reached in cells grown in $O_2$ concentrations around 0.1 μM; this was similar to the oxygen concentration required for half-maximal levels of unsaturated fatty acid and sterol. However, the synthesis of ubiquinone and cytochrome *c* and the increase in fumarase activity were essentially linear functions of the dissolved oxygen concentration up to 3.5 μM oxygen. The synthesis of the succinate dehydrogenase, succinate cytochrome *c* reductase, and cytochrome *c* oxidase complexes showed different responses to changes in $O_2$ concentration in the growth medium. Cyanide-insensitive respiration and $P_{450}$ cytochrome content were maximal at 0.25 μM oxygen and declined in both more anaerobic and aerobic conditions. Cytochrome *c* peroxidase and catalase activities in cell-free homogenates were high in all but the most strictly anaerobic cells.

The genus *Saccharomyces* has been used extensively to study the effects of anaerobiosis and catabolite repression on mitochondrial development. Organisms from this genus provide some of the few systems available among eukaryotes in which gene expression is clearly regulated by these environmental factors. There is, however, a great deal of uncertainty as to the precise role of oxygen or the energy source in determining the morphological and biochemical changes consequent on change of these environmental variables. It is well known, for instance, that the synthesis of unsaturated fatty acids, sterol, ubiquinone, and a number of hemoproteins is inhibited when oxygen is excluded from the culture medium. This is a consequence of the direct involvement of molecular oxygen in the biosynthetic pathways for these substances in the cell (6). However, other changes occur in these yeasts as a consequence of the absence of oxygen, for example, those involving enzymes of the tricarboxylate and glyoxylate cycles (5, 20).

Whether oxygen is involved as a regulator of development by acting at the gene level, or whether it acts indirectly as a reactant in the synthesis of particular components of the types mentioned above, which in turn act as regulators, is a question about which little is known at present. One approach to this question is to consider which developmental changes induced by oxygen in yeast are most sensitive to oxygen. From this might come some indication of the sequence of developmental events initiated by oxygen. In this investigation we report experiments in which *Saccharomyces cerevisiae* has been grown at different dissolved oxygen concentrations. Respiratory development in cells equilibrated with a range of oxygen concentrations up to 4 μm has been assessed by measurement of enzymes, cytochromes, and lipids whose synthesis and function in this yeast are dependent on molecular oxygen.

## MATERIALS AND METHODS

**Organism and culture conditions.** The organism used and composition of the growth medium have been described previously (7).

**Measurement and control of dissolved oxygen concentration.** The dissolved oxygen in the culture medium was measured with a Clarke-type oxygen electrode (YSI, Yellow Springs, Ohio) when the oxy-

gen concentration was above 1.5 μM oxygen. Below this value, a MacKereath oxygen electrode, constructed basically according to Johnston (1967), was used. The MacKereath electrode's silver cathode was made from 22-gauge silver wire in the shape of a spiral, 1.5 cm in diameter, and the thickness of the Teflon membrane was 0.012 mm. A block diagram of the overall control system is shown in Fig. 1. The signal from the electrode was amplified with a Copenhagen radiometer (PM26) pH meter connected to a Servogor Recorder (1-mV scale) using the external meter connection (0–10 mV). After each specified time interval, the oxygen controller compared the signal from the oxygen-sensing electrode with a reference voltage which could be set to any value. For our apparatus, a time delay of 4 s was chosen since the oxygen electrode required 3 s to register approximately 80% of the increase in oxygen concentration after a pulse of air was admitted to the carrier gas stream. If the oxygen concentration was lower than the reference value, either (i) a solenoid valve was opened to admit air to a constant nitrogen gas stream (300 ml per min), or (ii) a peristaltic pump was switched on to pump air into the carrier gas when low oxygen concentrations were maintained. The duration of the air pulse was proportional to the difference between the reference voltage and the signal from the oxygen electrode. As the sensing electrode's signal approached the reference value, the duration of the pulse similarly decreased. The system of control was highly reliable and avoided excessive fluctuation about the set reference even when the oxygen demand of the culture was high. Two modifications enhanced the stability of the controller. Firstly, the length of tubing between the culture vessel and the point of air admission was as short as practicable, and secondly the gas space above the culture was very small. The volume of the culture vessel was 2.8 liters and this was filled to 2.6 liters with medium. Agitation was provided by a magnetic bar (6-by 1-cm diameter) rotating at 200 to 300 rpm. Nitrogen (from Commonwealth Industrial Gases, Sydney, Australia) was "oxygen free" grade but contained 1 to 7 μliters of oxygen per liter. This nitrogen was successively passed through a packed glass-fiber filter, through 0.5 M potassium hydroxide and finally passed through a vanadyl sulfate-zinc amalgam trap (15). Air-saturated growth medium was assumed to contain 230 μM oxygen (11, 16). The electrodes were calibrated with gas mixtures supplied from Commonwealth Industrial Gases, 1% air in nitrogen and 5% air in nitrogen, and also with lower partial pressures of oxygen, by mixture of these standard gas compositions with nitrogen. Gas flow rates were measured with calibrated flow meters (Roger Gilmont Instruments, New York).

After calibration of the oxygen electrode, the medium was inoculated with cells to an initial cell density of 0.02 to 0.04 mg (dry wt) per ml, and the medium was flushed with nitrogen to reduce oxygen tension near to that required for a particular experiment.

**Preparation of sub-cellular fractions.** Cells were collected, then disrupted by using the mechanical method described by Gordon and Stewart (7).

A large particle fraction was prepared from the cell-free homogenate by centrifuging for 20 min at 25,000 × *g*. A small particle fraction was obtained from the supernatant fluid by centrifuging for 90 min at 100,000 × *g*. These two fractions correspond approximately to mitochondria and microsomes.

**Enzyme assays.** Enzymatic assay of cytochrome *c* peroxidase (ferrocytochrome *c*:$H_2O_2$ oxidoreductase; EC 1.11.1.5) was carried out essentially by the method of Yonetani and Ray (24), except that sodium acetate buffer (pH 6.0) was used instead of potassium phosphate.

Succinate dehydrogenase (succinate: cytochrome *c* oxidoreductase; EC 1.3.99.1) was measured as follows. The reaction mixture consisted of 1.0 ml of 200 mM potassium phosphate (pH 7.2); 0.5 ml of water; 0.1 ml of 0.6 M sodium succinate; 0.2 ml of 3 mM sodium ethylenediaminetetraacetic acid (EDTA) (pH 7.2); 0.1 ml of 20 mM potassium cyanide; and 0.1 ml of oxidized cytochrome *c* (10 mg/ml). The reaction was started by adding enzyme.

Cytochrome *c* oxidase (ferrocytochrome *c*:$O_2$ oxidoreductase; EC 1.9.3.1) was assayed by the method of Smith (19). Activities of these three enzymes are given as initial velocity of cytochrome *c* oxidation or reduction, as appropriate.

Other enzymes were assayed by standard procedures: succinate dehydrogenase by the method of Arrigoni and Singer (1); fumarate hydratase (L-malate hydro-lyase; EC 4.2.1.2) according to Vary et al. (20); catalase ($H_2O_2$:$H_2O_2$ oxidoreductase; EC 1.11.1.6) as described by Bonnichsen et al. (3). Protein was determined by the method of Lowry et al. (14). The measurement of cell respiration has been described previously (7).

**Measurement of cytochromes.** Cytochromes were determined using a Cary 14R spectrophotometer fitted with a scattered-light transmission accessory. Spectra were measured at room temperature and at −196 C.

The concentration of cytochrome $aa_3$ was estimated from the difference in absorbance at room temperature between reduced and oxidized pigment at 605 minus 630 nm using an millimolar absorbancy coefficient of 16 $cm^{-1}$ Cytochromes *b*, *c*, and $c_1$ were then determined by using the resolved spectra obtained at low temperature and referring to the $aa_3$ peak (4). Millimolar specific absorbancies and wave-length

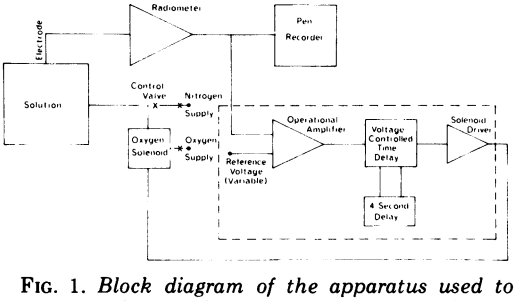


FIG. 1. *Block diagram of the apparatus used to measure and control oxygen concentration in the culture medium.*

pairs used were: *b*, 17.9 $cm^{-1}$, 559–575 nm; *c*, 19.0 $cm^{-1}$, 547–540 nm; $c_1$, 19.0 $cm^{-1}$, 553–540 nm.

**Extraction of ubiquinone, lipids, and cytochrome c.** The cellular ubiquinone, fatty acid, and sterol contents were determined by methods previously described (7). Cytochrome *c* was extracted from whole cells according to the method of Sels et al. (17).

**Cytochrome $P_{450}$ content of cells.** Cytochrome $P_{450}$ was estimated from the carbon monoxide plus dithionite-reduced versus dithionite-reduced spectra of whole cell suspensions at room temperature. The concentration of $P_{450}$ was calculated from the difference in absorbancy at 450 minus 490 nm using the specific absorbancy coefficient quoted by Ishidate et al. (10).

**Measurement of oxidation-reduction potential.** The redox potential of the culture medium was monitored during growth with a platinum electrode and a calomel electrode as reference connected to a radiometer (Copenhagen) pH meter.

## RESULTS

**Growth characteristics.** In Fig. 2, growth curves for cultures grown under anaerobic conditions (i.e., without air added to the nitrogen stream) and at an oxygen concentration of 2.8 $\mu$M are shown. Growth under anaerobic conditions proceeds until a cell density of approximately 1 mg (dry wt) per ml is reached (Fig. 2 a). Thereafter, the cells cease growing and, subsequently, viability decreased as reported previously (7). Small amounts of respiration are detected even in these cells, and this is maximal at about mid-exponential phase and decreases slightly by the beginning of stationary phase. The oxidation-reduction potential of the medium during cell growth follows the respiration curve closely, reaching a maximum at about the same time.

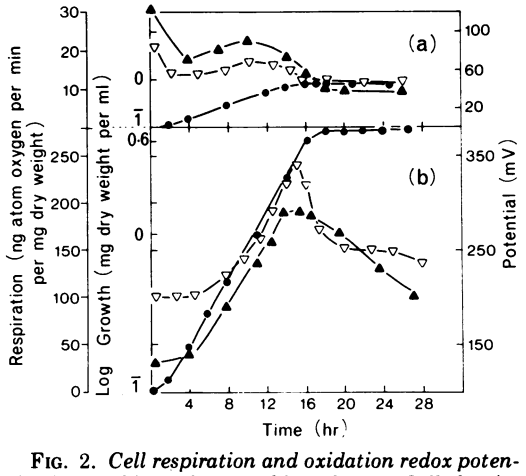


FIG. 2. *Cell respiration and oxidation redox potential in aerobic and anaerobic cultures. Cell density* (●); *cell respiration* (▲); $E_h$ (▽).

Cells grown in 2.8 $\mu$M oxygen also show a maximum in cell respiration in late exponential phase, with a maximum potential developed in the medium at the same time (Fig. 2b). Increasing the oxygen concentration to approximately 9 $\mu$M did not affect the respiratory activities developed. Respiration in these cells is similar to that found in shaking cultures of this organism. Changes in cell respiration appears to develop over a narrow range of oxygen tension. The two examples described above represent the region in which the effect of oxygen tension on cellular growth and development was examined. When cells were grown at intermediate oxygen concentrations, similar shaped curves relating respiration, potential, and growth were obtained. For a particular dissolved oxygen concentration, the maximum value of respiration developed is quoted throughout the text and in diagrams. This was also the point at which redox potentials were measured, and when cultures were harvested for assay of the enzymes, cytochromes, and lipids.

In Fig. 3 the effect of dissolved oxygen concentration on the maximal respiration developed, and on growth rate, are shown. Under anaerobic conditions, generation times are of the order of 5 h, and the respiration developed is about 5% of the maximum rate achieved in the most aerobic condition. At 3.5 $\mu$M oxygen, respiration is 180 to 200 ng atoms of oxygen per mg per min and the doubling time is a little over 1 h. There is almost a linear relationship between redox potential and oxygen tension in the cultures, and, considering the stable nature of the potential measurement, it is a useful alternative to the measurement of oxygen tension as conditions of anaerobiosis are approached. As a consequence of this near linear relationship, results expressed below as a function of oxygen concentration would show essentially the same pattern if plotted in terms of the oxidation-reduction potential of the medium.

**Respiratory components in cells grown at low oxygen concentrations. (i) Cyanide-insensitive respiration, $P_{450}$ and cytochrome c.** A maximum activity of cyanide-insensitive respiration occurs in cells grown in 0.2 to 0.5 $\mu$M oxygen (Fig. 4). The synthesis of $P_{450}$, a pigment that may be associated with non-mitochondrial respiration (9) is also maximal in this region of oxygen concentration. Cytochrome *c* synthesis shows approximately an opposite relationship and, above 0.4 $\mu$M oxygen, the amount of cytochrome *c* increases almost linearly with oxygen concentration.

**(ii) Unsaturated fatty acids and ergosterol.** One of the more sensitive responses to low

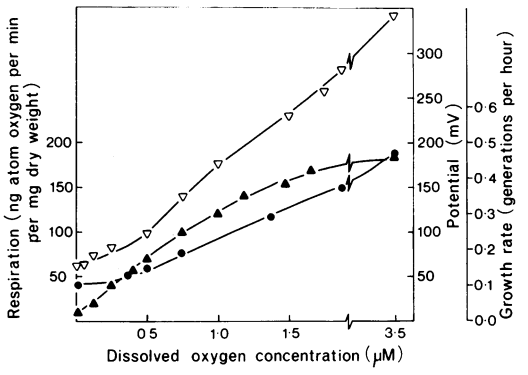


FIG. 3. *Effect of dissolved oxygen concentration on the maximum respiration developed (▲) and on the growth rate (●) of cells. The potential ($E_h$) of the culture at the time maximum respiration is developed is also shown (▽).*

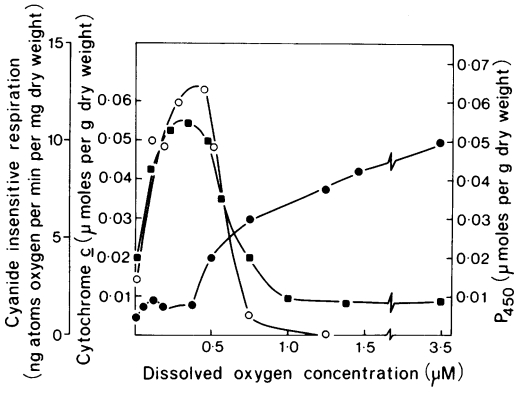


FIG. 4. *Cellular content of cytochrome c (●), $P_{450}$ (○), and cyanide-insensitive respiration (■) of cells as a function of oxygen concentration in the growth medium.*

oxygen tension in the medium is seen in the unsaturated fatty acid and sterol content of the cells (Fig. 5). Aerobic cells contain approximately 90 μg of total fatty acid per mg (dry wt); about 80% of this is accounted for as monounsaturated fatty acids (palmitoleic and oleic). In anaerobically cultured cells the total fatty acid content is much lower (20 μg per mg dry wt), and in addition the proportion of unsaturated fatty acids to total fatty acids is less than 20%. The sterol content of the cells is also low: 0.3 to 0.5 μg per mg of cells, compared with about 3 μg per mg in cells grown aerobically. As the dissolved oxygen concentration is increased, the total fatty acid and the unsaturated fatty acid increase in a similar fashion, whereas sterol synthesis lags initially, but then increases rapidly as oxygen tension rises. Above an oxygen concentration of 0.75 μM, the cellular content of these lipids increases more gradually.

**(iii) Enzyme content of cells grown at low oxygen tension.** The activity of a number of enzymes associated with mitochondrial and extramitochondrial respiratory processes was measured in cells grown at various oxygen concentrations to determine differences in the extent of synthesis of different parts of the respiratory chain in response to oxygen (Fig. 6). Succinate dehydrogenase was measured as a representative flavoprotein of the mitochondrial respiratory chain; succinate-cytochrome *c* reductase and cytochrome *c* oxidase as cytochrome-containing complexes of the respiratory chain; and fumarase as a representative soluble enzyme of the tricarboxylate cycle. Cytochrome *c* peroxidase and catalase were also measured in these experiments; the former may also be a mitochondrial enzyme (2, 23), and the latter is probably peroxisomal in origin (9). In our preparations, however, these two hemoproteins were recovered predominantly in the cytosol fraction described below.

Of the mitochondrial respiratory chain components measured, the synthesis of succinate-cytochrome *c* reductase was most sensitive to environmental oxygen. Development of activity was maximal at 0.35 μM oxygen. Cytochrome oxidase and succinate dehydrogenase were somewhat less sensitive, there being little further increase above 1 μM oxygen.

The synthesis of both cytochrome *c* peroxidase and catalase are particularly sensitive to oxygen. In the case of the former, activity is fully developed in cells grown at 0.23 μM oxygen

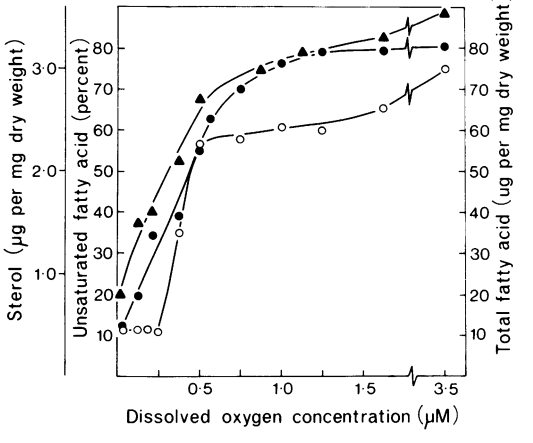


FIG. 5. *Sterol content (○), total fatty acid content (▲), and proportion of unsaturated fatty acids to total fatty acid (●) in cells grown at different dissolved oxygen concentrations.*

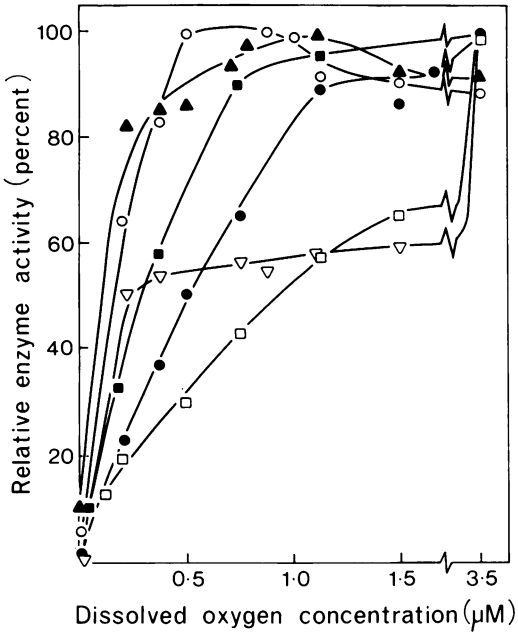


FIG. 6. *Enzyme activities in cell-free homogenates of cells grown at different oxygen concentrations. Succinate dehydrogenase (■); succinate-cytochrome c reductase (○); cytochrome oxidase (●); fumarase (□); cytochrome c peroxidase (▲); and catalase (▽). Enzyme activities have been expressed as percentage of the activity measured in cells grown in 3.5 μM oxygen. The activities (micromoles per minute per milligram of protein) in these cells were: succinate dehydrogenase, 0.12; succinate-cytochrome c reductase, 0.016; cytochrome oxidase, 0.065; fumarase, 0.275; cytochrome c peroxidase, 0.24; catalase, 4.0.*

and above. The synthesis of catalase showed a biphasic response, being very sensitive at low concentrations of oxygen then showing a further increase at the higher concentrations of oxygen used. The synthesis of fumarase, on the other hand, was almost linear in its response to dissolved oxygen, and in this respect is similar to cytochrome *c* (Fig. 4) and ubiquinone as described below.

**Subcellular distribution of respiratory enzymes.** In all cell types examined, more than 60% of the total cytochrome *c* oxidase was recovered in the 25,000 × *g* fraction, the remainder being associated with the 100,000 × *g* particles. In the more aerobic cells, approximately 80% of the total succinate-cytochrome *c* reductase activity occurred in the 25,000 × *g* fraction. A smaller proportion of this enzyme was recovered in this fraction from the more anaerobic cells.

Cytochrome *c* peroxidase and catalase occurred almost exclusively in the 100,000 × *g* supernatant fraction, with one exception. In anaerobic cells (respiration rates of 10–15 ng atoms of oxygen per min per mg) the small amount of catalase in the cell was predominantly associated with the microsomal fraction. Of the fumarase activity in cell homogenates, 10 to 20% was recovered in particles, mostly the 25,000 × *g* fraction.

The changes in distribution of enzymes between the two particulate fractions and the supernatant fraction in response to altered oxygen tension may reflect real differences in distribution existing in the cell, or changes in the tightness of binding to sub-cellular structures, or both. The mechanical method of cell disruption used in these experiments is not the best means of measuring changes in compartmentation of enzymes. However, less disruptive techniques of cell fractionation, using preliminary formation of spheroplasts, were not satisfactory because enzyme levels change during the long incubation periods involved.

**Spectra of cell homogenates and of cell-free particle fractions.** The results described above suggest that the synthesis of the succinate-cytochrome *c* reductase complex is signficantly more sensitive to oxygen than is cytochrome oxidase. However, since catalytic activity rather than total enzyme protein was measured, this could be a consequence of a more effective integration of cytochromes *b* and $c_1$ into the catalytic complex, rather than being due to a greater synthesis of these cytochromes at low oxygen tension. The total amount and distribution of individual cytochromes was therefore measured from difference spectra of cell-free homogenates and particle fractions.

Examples of the dithionite-reduced minus ferricyanide-oxidized difference spectra of cell-free homogenates from cells grown at several different oxygen tensions are shown in Fig. 7. Cells grown at 3.5 μM oxygen have a spectrum typical of aerobically grown cells and contain cytochromes $aa_3$, *b*, $c_1$, and *c* (low-temperature α-absorption maxima at 601, 559, 553, and 547 nm, respectively, and β-bands at 528 [b] and 520 nm [$c + c_1$], respectively). When the oxygen tension is decreased to 0.7 μM, the cytochrome *c* content falls relative to *b* and $c_1$. As the oxygen tension is lowered still further, the 547-nm band decreases until it is no longer visible in the anaerobic cell. In the range of oxygen concentration below about 0.5 μM, the 559-nm band is progressively shifted to a lower wavelength and is located at 557.5 nm in anaerobically grown cells; simultaneously, the 553-nm peak decreases to 551.5 nm. These trends are also apparent in the β-region; the absorption bands of cytochromes *b* and $c + c_1$ also decrease, whereas bands at 526 and 531 nm become more

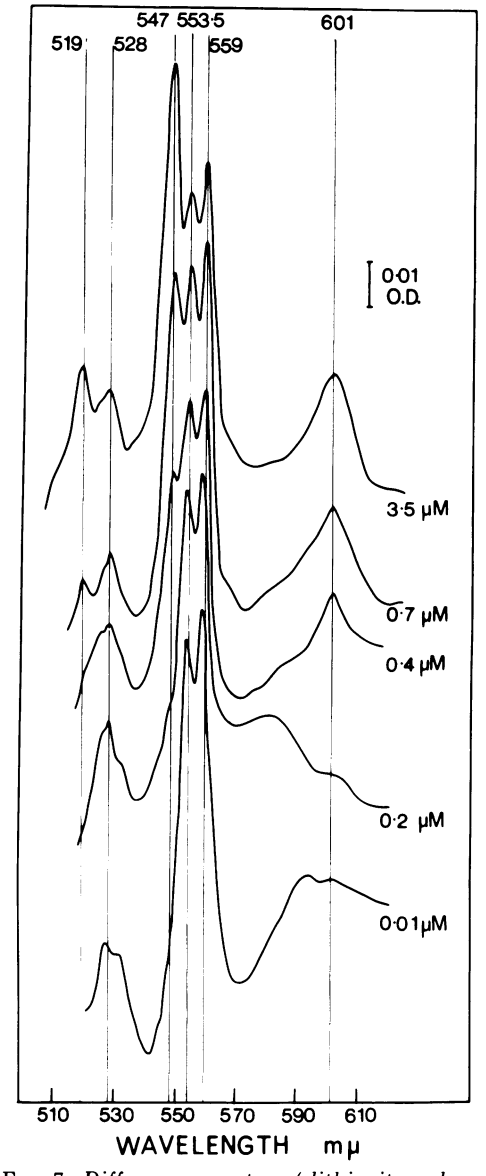


FIG. 7. *Difference spectra, (dithionite-reduced minus ferricyanide-oxidized, −196 C) of cell-free homogenates, from cells grown at different oxygen tensions.*

prominent. Thus, the anaerobic cell is characterised by two $\alpha$-bands of almost equal intensity (557.5 and 551.5 nm), and these may represent the split absorption band of a *b* type cytochrome, possibly cytochrome $b_1$ (12). When endogenous reductants were allowed to reduce the cytochromes, the results were essentially the same as those seen with dithionite.

Figure 8 shows spectra of low-speed fractions from cells grown in 0.4 $\mu$M oxygen (respiration rate: 65 ng atoms of oxygen per min per mg of cells). When reduced with succinate in the presence of cyanide, cytochromes *a* (601 nm), *b* (559 nm), $c_1$ (553 nm), and *c* (547 nm) can be seen. By adding antimycin A (final concentration 5 $\mu$g/ml) to oxidized particles prior to reduction with succinate, absorption bands due to cytochrome *b* (559 nm with $\beta$-band at 528 nm) are seen. Addition of ascorbate under these conditions results in cytochrome $c_1$ (553 and 520 nm) becoming reduced. Cytochromes *c* and *a* are also reduced. When the particles were reduced with dithionite, there was an increase in the intensity but no change in the position of the absorption bands.

When the same treatments were applied to the 100,000 × *g* fraction the same results were obtained if reduction was carried out with succinate and ascorbate, though the intensity of the absorption bands was lower. With dithionite as reductant, a slight shift in the position of the $\alpha$-bands occurred, and the 559-nm band was depressed to 558 nm and the 553-nm peak was depressed to 552.5. This would suggest that the anaerobic *b*-type cytochrome ($\alpha$ bands at 557.5 and 551.5 nm) is present in this fraction and although not reduced by succinate, it becomes apparent when dithionite is used.

The same approach was used to examine particles from cells grown at 0.17 $\mu$M dissolved oxygen (respiration rate: 30 ng atoms of oxygen per min per mg of protein). The amount of cytochrome *c* in comparison to cytochromes *b* and $c_1$ is particularly low in both the 25,000 × *g* and 100,000 × *g* particle fractions, as is the case in the cell homogenates; the anaerobic *b*-type cytochrome appears to be concentrated in the 100,000 × *g* fraction as judged by a shift to lower wavelengths of the maxima when dithionite is used (Fig. 9).

Spectra of homogenates from anaerobic cells (respiration rate, 10–15 ng atoms of oxygen per min per mg) suggest that these cells contain only the anaerobic cytochrome $b_1$ (Fig. 7). Yet a 25,000 × *g* fraction from these cells, when reduced with succinate plus cyanide, does contain small amounts of cytochromes $aa_3$, *b*, and *c*. The 100,000 × *g* microsomal fraction contains virtually only anaerobic *b*-type cytochrome as shown by the split $\alpha$-band (557.5 and 551.5 nm) and a Soret band at 424 nm.

A quantitative assessment of the relative amounts of the aerobic cytochromes formed in response to altered oxygen tension is shown in Fig. 10. Values given are for cell-free homogenates except for cells grown at 0.17 and 0.35 $\mu$M oxygen, in which case the cytochrome content of the two particulate fractions has been summed. There appears to be little difference in synthesis of cytochromes $aa_3$ compared with *b* and $c_1$ in

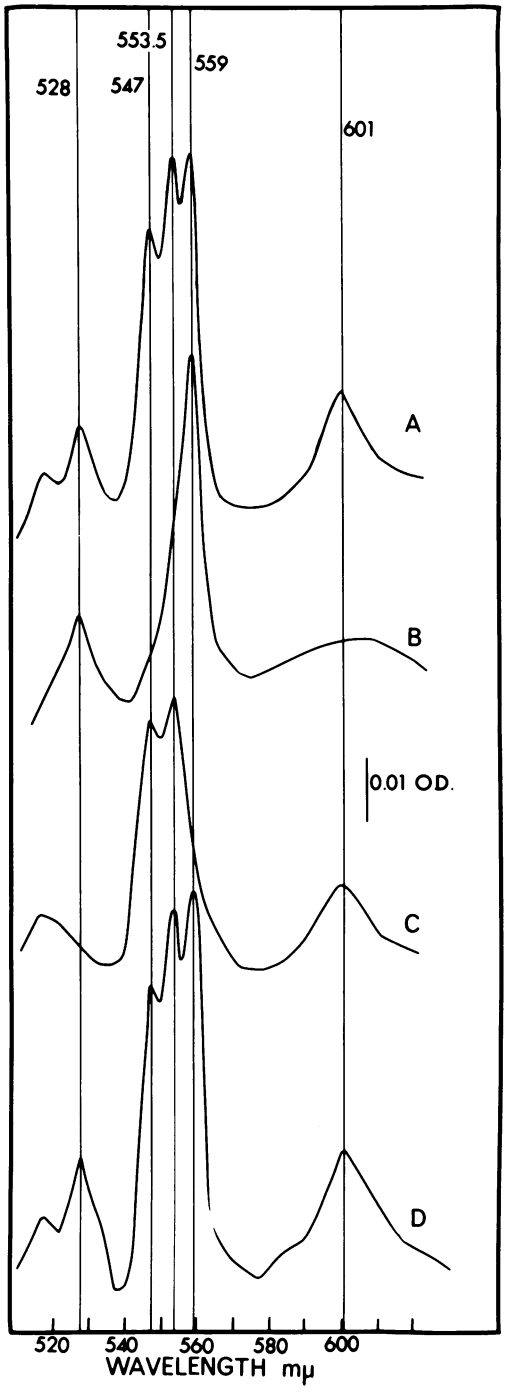


FIG. 8. *Difference spectra (−196 C) of the 25,000 × g fraction from cells grown in 0.4 μM oxygen (respiration rate: 65 ng atoms of oxygen per min per mg of cells). A, Particles reduced with succinate in the presence of cyanide. B, Particles reduced with succinate in the presence of antimycin A. C, Particles reduced with ascorbate in the presence of antimycin A. D, Particles reduced with dithionite.*

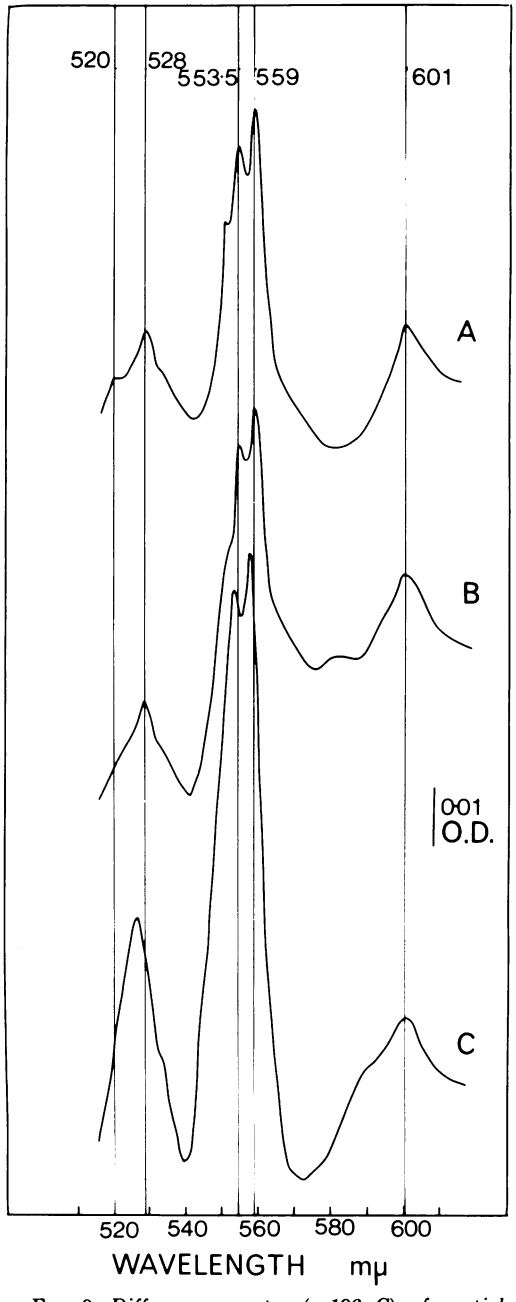


FIG. 9. *Difference spectra (−196 C) of particles from cells grown in 0.17 μM oxygen. A, Particles (25,000 × g) reduced with succinate with cyanide present. B, Microsomal fraction (100,000 × g) reduced with succinate with cyanide present. C, Microsomal fraction reduced with dithionite.*

response to increasing oxygen tension.

The synthesis of cytochrome *c*, as measured in these spectroscopic experiments, was less sensitive to oxygen and approximately proportional to the external oxygen tension. In this respect the results are similar to those shown in Fig. 4. The results obtained by the spectroscopic method, however, are somewhat lower than those found by extraction. Ubiquinone, extracted from the cells, showed a linear response to oxygen concentration similar to that of cytochrome *c*.

## DISCUSSION

In this study of the relationship between environmental oxygen tension and the development of respiratory pathways (mitochondrial and extra-mitochondrial) in *S. cerevisiae*, two questions have been asked: firstly, how sensitive quantitatively is the development of respiratory pathways in this organism to external oxygen; secondly, among the cellular developmental events considered, is there a particular sequence that might provide evidence as to the action of molecular oxygen as an initiator and regulator of development?

The synthesis of enzymes such as cytochrome *c* peroxidase and succinate-cytochrome *c* reductase is particularly sensitive to oxygen in that the activities of these enzymes are near those found in aerobic cells at oxygen concentrations of about 0.02 μM (equivalent to one-thousandth part of air saturation of the medium). This degree of sensitivity explains the observation of Sels and Cocriamont (18) that cytochrome *c* peroxidase synthesis is not repressed in *S. cerevisiae* at oxygen tensions down to one-hundredth that of air saturation. It may also account for the high levels of this enzyme found in "anaerobic" cells by others (2, 13). The recovery of the peroxidase almost entirely in the cytosol fraction in the present experiments may be a consequence of the mechanical method of cell breakage used, since there is good evidence that the enzyme is localized in mitochondria in this yeast (2, 24).

The synthesis of membrane lipids in the cells and membrane-bound mitochondrial cytochromes shows a half-maximal response to oxygen concentrations of 0.1 to 1 μM oxygen. This is significantly lower than the corresponding value for growth rate (>2 μM oxygen); these values will be referred to as "apparent $K_m$'s" for oxygen. The latter value is similar to the $K_m$ for respiratory oxygen consumption by *S. cerevisiae* of (2.8 μM) reported by Winzler (22) which suggests that the growth rate of this organism at

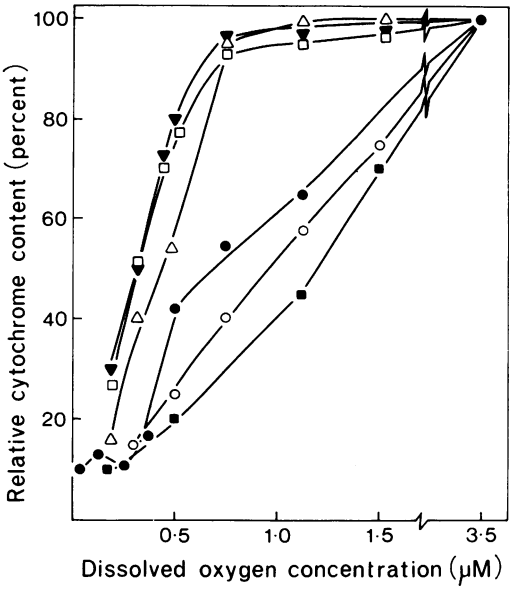


FIG. 10. *Cytochrome and ubiquinone content of cell-free homogenates prepared from cells grown at different oxygen concentrations. Symbols:* ○, *Cytochrome c extracted from whole cells;* ○, *cytochrome c determined spectrophotometrically;* ■, *ubiquinone;* △, *cytochrome* $aa_3$; □, *cytochrome b;* ▽, *cytochrome* $c_1$. *Cytochrome levels are expressed as percentage of cytochrome content found in cells grown at 3.5 μM oxygen concentration. The cytochrome content (nanomoles per milligram of protein) of these cells was:* $aa_3$, *0.065; b, 0.13; c, 0.12;* $c_1$, *0.08. The ubiquinone content of these cells was 0.18 mg per g (dry wt) of cells.*

low oxygen tension is determined by the rate of respiratory energy production in the cell, rather than by the synthesis of specific cell components. The obligately aerobic yeast *Candida utilis*, on the other hand, has an apparent $K_m$ for oxygen related to growth of 0.5 μM (11). This may indicate that in this organism, oxygen for biosynthetic reactions may be the rate-limiting factor for growth at low oxygen tension.

The developmental pattern of mitochondrial components seen in this organism suggests that there is a differential rather than a co-ordinate response to environmental oxygen with respect to the particulate lipids and cytochromes on the one hand, and the mobile electron carriers and accessory enzymes on the other. The half-maximal levels of membrane-bound cytochromes $aa_3$, *b*, and $c_1$ were observed in cells grown in oxygen concentrations around 0.4 μM, and the apparent $K_m$ of 0.5 μM oxygen for the synthesis of ergosterol and unsaturated fatty acids is not significantly different, suggesting a co-ordinate

synthesis of the particulate elements of the inner mitochondrial membrane. Some small, but reproducible differences in oxygen sensitivity were evident between some of these components. The synthesis of cytochrome *b* and $c_1$ was more sensitive to oxygen in the culture medium compared to cytochromes $aa_3$, and this may reflect the assembly sequence during membrane development. Similar considerations may apply to the observation that unsaturated fatty acid synthesis is more sensitive to oxygen than is ergosterol synthesis.

The results obtained from assays of the respiratory enzyme complexes which contain the particulate cytochromes showed a less than co-ordinated synthesis of cytochrome oxidase, succinate cytochrome *c* reductase, and succinate dehydrogenase, and needs to be explained in terms other than a differential synthesis of the component cytochromes. The results in Fig. 6 showed that the synthesis of succinate cytochrome *c* reductase very nearly paralleled the development of the particulate cytochromes *b* and $c_1$, whereas the greatest discrepancy occurred between cytochrome $aa_3$ synthesis and the development of cytochrome *c* oxidase activity. It is probably not surprising that respiratory enzyme activity does not exactly parallel variation in the component cytochromes since other components are also part of the complete enzyme systems. The synthesis of these additional components may be uncoupled from cytochrome synthesis. In the case of cytochrome *c* oxidase, the synthesis of the catalytically active system may be imagined in three separate steps: firstly, the synthesis of the apoprotein components; secondly, the association of the specific prosthetic group with these apoproteins; and lastly, the production of structural building blocks to enable the integration of the holoenzyme into the mitochondria. It has been reported that some constituent proteins of cytochrome *c* oxidase in yeast are made on the mitochondrial ribosomes (8). When cells are grown at low oxygen concentrations, the synthesis of essential structural proteins or protein constituents of cytochrome *c* oxidase may subsequently limit the development of the catalytically active system.

The plot of cellular respiration rate versus oxygen concentration in the medium (Fig. 3) shows an almost linear relationship, and is quite similar to the curve relating the cellular levels of ubiquinone and cytochrome *c* to the oxygen concentration used for growth. The synthesis of these two constituents of the electron chain, therefore, reflects the functional condition of the electron transport chain, and does not follow the same response pattern to environmental oxygen as the particulate cytochromes *b*, $c_1$, and $aa_3$. The dissociation of ubiquinone synthesis from that of other components of the respiratory chain has also been shown by using inhibitors of protein synthesis in nongrowing systems (7). Ubiquinone synthesis and incorporation into mitochondria could occur even in the absence of particulate cytochrome synthesis and the synthesis of both cytochrome *c* oxidase and succinate dehydrogenase. It appears from the results presented that there is an apparent priority for oxygen for the synthesis of the particulate elements of the inner membrane of mitochondria, compared to the synthesis of ubiquinone, cytochrome *c*, and fumarase.

It is not possible from the experiments described to determine the relative importance of oxygen tension and oxidation-reduction potential as regulators of development in this yeast, since change in oxygen tension resulted approximately in a proportional change in redox potential. This appears to be in contrast to the situation that exists in chemostat cultures of *Escherichia coli* and *Klebsiella aerogenes*, where at low oxygen tension redox potential may increase very considerably without equivalent change in oxygen tension of the culture (21).

These experiments have defined the range of oxygen concentration over which the synthesis of mitochondrial components occurs. Further experiments to relate mitochondrial biosynthesis with the development of peroxisomes in continuous culture are in progress.

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