# RECOVERY OF RESPIRATION CAPACITY OF YEAST AFTER PHOTOINHIBITION

Helga NINNEMANN
*Institut für Chemische Pflanzen-physiologie,*
*74 Tübingen, W. Germany*

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## 1. Introduction

Respiration of non-photosynthetic organisms and of isolated mitochondria can be inhibited by irradiation with blue light as was shown in the colorless green alga *Prototheca* [1, 2], in *Saccharomyces cerevisiae* [3] and isolated beef heart mitochondria [4]. This decrease in respiration rate is paralleled by a decrease of cytochrome $a_3$ in *Prototheca* and beef heart mitochondria and of cytochromes $a$ and $a_3$ in yeast.

It was proposed that light destroyed cytochrome oxidase and thus inhibited oxygen consumption of the organisms. With a partially purified preparation of cytochrome oxidase the Soret band of the oxidized form of cytochrome $a_3$ was shown to be the light absorbing pigment for the photoinhibition of respiration [4].

In the current paper it shall be shown that irradiated yeast cells can regain their respiratory capacity in an energy-dependent process involving protein synthesis.

A short report of these results was given before at the First European Congress of Biophysics, Vienna 1971 (Proceedings Vol. IV).

## 2. Material and methods

Baker's yeast, *Saccharomyces cerevisiae*, was grown at room temperature as described previously [3] in 50 mM $KH_2PO_4$ (pH 4.5) or in 50 mM $KH_2PO_4$–$K_2HPO_4$ (pH 7.0) under aerobic conditions, starving the cells for at least 18 hr in darkness. The stock suspension contained $2 \times 10^9$ cells/ml. For irradiation experiments a 20-fold dilution of the stock was used. The cells were irradiated for 30 min in the aerated starvation buffer. Ethanol (EtOH) as substrate was added only to the measuring cuvette. During the respiration recovery period ethanol was repeatedly added as indicated in the graphs to prevent exhaustion of the substrate. Uncouplers or inhibitors of protein synthesis were added to the culture only after irradiation.

The light source used was a XBO 501 Osram Xenon lamp of 450 W with a blue Corning filter No. 5562. A water filter of 6 cm was placed between lamp and sample. The intensities of blue light were $1.0–2.5 \times 10^6$ ergs $cm^{-2}$ $sec^{-1}$. Oxygen consumption was measured with a Yellow Springs Clark-type oxygen electrode YSI 4004 fitted into a 4.0 ml plexiglass cuvette.

The growth rate of yeast suspensions was determined in a Metrohm colorimeter E 1009 (Herisau, Switzerland) at 620 nm. Budding cells were counted microscopically with a "Thoma Neu" counting cell. Distribution of cell size could be observed with a Coulter Counter of Coulter Electronics, Inc., England.

The inhibitors of protein synthesis, chloramphenicol (CAP) and cycloheximide (CH) were purchased from Serva, Heidelberg. CAP was dissolved in the medium with gentle heating.

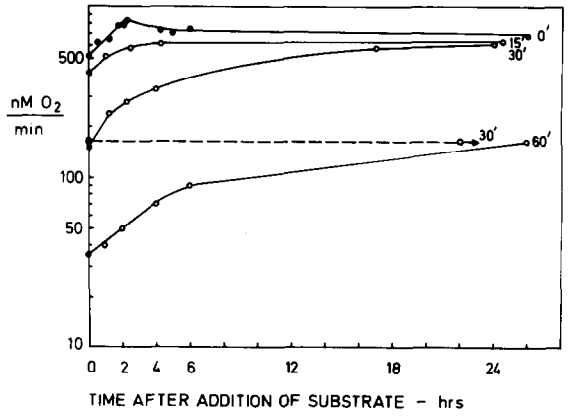


Fig. 1. Rate of recovery of respiratory activity in yeast ($10^8$ cells/ml) *vs.* time after addition of substrate (0.5% ethanol). Substrate added immediately after irradiation. Irradiation for 0 min, 15 min, 30 min or 60 min, respectively (solid lines) with 2.5 X $10^6$ ergs $cm^{-2}$ $sec^{-1}$ of blue light from a 450 W Xenon lamp and Corning filter Nr. 5562. Dashed curve: respiration capacity of irradiated yeast (30 min blue, 2.5 X $10^6$ ergs $cm^{-2}$ $sec^{-1}$) cultured without substrate for 90 hr.

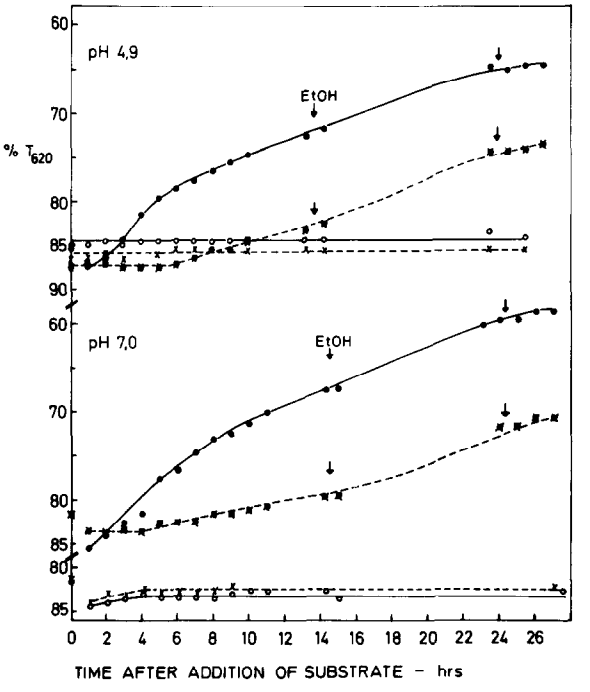


Fig. 2. Growth rate of yeast determined colorimetrically at 620 nm *vs.* time after addition of 0.5% EtOH in media of pH 4.9 and pH 7.0. (●—●—●): Non-irradiated yeast with EtOH. (○—○—○): Non-irradiated yeast without EtOH. (⊗—⊗—⊗): Irradiated yeast (30 min, 2.5 X $10^6$ ergs $cm^{-2}$ $sec^{-1}$) with EtOH. (X—X—X): Irradiated yeast without EtOH. Arrows indicate repeated addition of EtOH (0.5%).

## 3. Results

It has been shown previously that 1 hr of the relatively high intensities of blue light used in our experiments did not affect the viability of irradiated yeast [3]. In cells which were illuminated for 30 or 60 min and subsequently kept dark and aerated in starvation medium the respiratory capacity did not increase for at least 90 hr after illumination (fig. 1, dashed curve). If, however, yeast irradiated for 15–60 min was cultured in the presence of 0.5% ethanol in phosphate buffer, the cells regained their respiratory capacity within 18–24 hr partly or totally, depending on the length of the previous irradiation (fig. 1).

Restoration of the respiratory rate commences immediately after addition of substrate. By following the growth rate of irradiated yeast colorimetrically after addition of ethanol it can be seen that irradiated cells do not grow within the first 6 hr after addition of the substrate and only later resume growth (fig. 2). The percentage of budding cells was counted in the microscope with irradiated and non-irradiated yeast. In both cases only 0.5–1.5% of budding cells could be observed.

Following the size distribution of irradiated and non-irradiated yeast in presence of ethanol with a Coulter Counter showed no distinct new class of smaller cells made up from newly released buds over 24 hr. It can be concluded from these observations that restoration of respiratory capacity occurs in the actually irradiated yeast cells.

That the restitution of respiratory capacity is energy-dependent was shown by adding the uncoupler of oxidative phosphorylation, 2,4-dinitrophenol (2,4-DNP) to the irradiated culture in presence of substrate. Already 5 X $10^{-6}$ M 2,4-DNP which stimulates respiration in non-irradiated cells, inhibits recovery of irradiated cells about 40%, higher concentrations almost completely prevent the cells from regaining their normal respiration rate. The previous observation on the decrease of absorption of cytochrome oxidase after irradiation with blue light led us to assume a

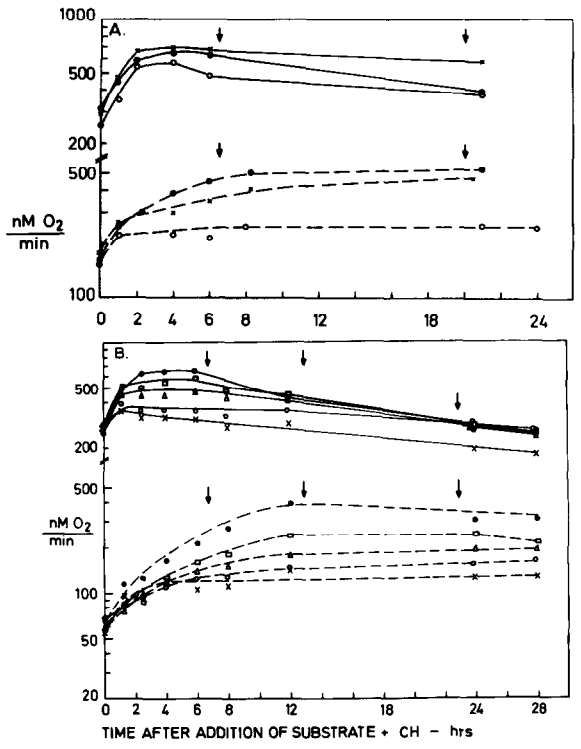


Fig. 3. Rate of recovery of respiratory activity in irradiated yeast (30 min blue, 2.5 X $10^6$ ergs $cm^{-2}$ $sec^{-1}$) and rate of respiration in non-irradiated cells in presence of chloramphenicol (CAP) or cycloheximide (CH) *vs.* time after addition of substrate. Solid lines: non-irradiated yeast. Dashed lines: irradiated yeast. A) 20 Hr preincubation with CAP. (●—●—●, ● - - ● - - ●): Control without CAP, non-irradiated and irradiated, respectively. (X—X—X, X - - X - - X): CAP 1.5 X $10^{-3}$ M. (○—○—○, ○ - - ○ - - ○): CAP 1.2 X $10^{-2}$ M. B) Cycloheximide. (●—●—●, ● - - ● - - ●): Control without CH, non-irradiated and irradiated, respectively. (□—□—□, □ - - □ - - □): CH 6 X $10^{-7}$ M, (△—△—△, △ - - △ - - △): CH $10^{-6}$ M, (○—○—○, ○ - - ○ - - ○): CH 3 X $10^{-6}$ M, (X—X—X, X - - X - - X): CH 6 X $10^{-6}$ M. ↓ Indicates repeated addition of EtOH (0.5%).

destruction of the enzyme though we were unable to define the term biochemically. Experiments with inhibitors of protein synthesis indicate that in fact one or – more probably – several proteins are destroyed by light and can be re-synthesized, provided a substrate like ethanol or low concentration (0.7–1.0%) of glucose is present.

Chloramphenicol (CAP) is known to inhibit protein synthesis of bacteria, chloroplasts, and mitochondria [5–7]. As low concentrations of the inhibitor (6–12 X $10^{-5}$ M) are ineffective, we used concentrations as high as 1.5 X $10^{-3}$ M and 1.2 X $10^{-2}$ M (cf. [8]) and had to preincubate the cells for 20 hr with the antibiotic. These high concentrations of the drug have essentially no effect on the respiratory rate of non-irradiated yeast. But they strongly suppress the respiration recovery in irradiated cells (fig. 3A). The resistance of the irradiated cells towards the inhibitor might be due to penetration difficulties of the drug into starved yeast.

Cycloheximide (CH) which inhibits specifically cytoplasmic protein synthesis in yeast and higher organisms prevents respiration recovery as well. Already low concentrations (0.6–1.0 X $10^{-6}$ M) of this inhibitor, applied together with ethanol to irradiated yeast, prevent the cells markedly to regain their respiratory activity (fig. 3B).

## 4. Discussion

Upon addition of substrate the respiration of photoinhibited starved yeast can recover to about its normal rate within 24 hr (fig. 1). The respiratory restoration in irradiated cells begins before growth is resumed (fig. 2), and thus occurs in the irradiated cells themselves. This observation is of interest because it explains why colorless organisms can grow in continuous daylight in spite of the fact that the intensities of normal daylight suffice to destroy their cytochrome oxidase provided the cells are exposed to it long enough [3]: under growing conditions irradiated cells are able to resynthesize the destroyed enzyme(s) in a short time. Photoinhibition of respiration and respiratory recovery are wide spread phenomena in colorless cells [9].

The recovery process is energy-requiring since it is prevented by 2,4-DNP and involves protein syntheses sensitive both to chloramphenicol and cycloheximide (fig. 3). The inhibitory action of chloramphenicol (CAP) on 70 S ribosomes is well documented [10, 11]. Wintersberger [12] showed that incorporation of amino acids into isolated yeast mitochondria is inhibited by CAP, whereas the antibiotic is without effect on amino acid incorporation by the cytoplasmic 80 S ribosomal system [13, 14].

It may be assumed that the necessity for preincubation with CAP in the experiments described here is

due to changes in cell membrane permeability induced by the physiologically stressing starvation conditions. This assumption finds support in work of Linnane and his group [15] who described CAP-resistant yeast mutants and found their resistance to be caused by a decreased plasma membrane permeability to the drug.

In contrast to chloramphenicol, cycloheximide (CH) inhibits specifically protein synthesis of cytoplasmic 80 S ribosomes in mammalian tissue, many higher plants and yeast [16]. In irradiated yeast cells low concentrations of CH ($0.6–3 \times 10^{-6}$ M) which have little or no effect on respiration of non-irradiated cells, suppress the substrate-induced recovery of respiration.

The membrane-bound cytochrome oxidase is, at least in its final functional form, believed to be synthesized in the mitochondria [8]. The ability of irradiated yeast to regain its respiratory capacity and its prevention by CAP indicate that after addition of substrate cytochrome oxidase is resynthesized in photoinhibited cells. The sensitivity of the recovery process to CH suggests either that cytoplasmic (structural?) protein synthesis is required for resynthesis of mitochondrial proteins destroyed by light or that the blue irradiation, besides its destructive effect on protein(s) synthesized by the mitochondrial system, also destroys protein(s) synthesized by the cytoplasmic ribosomes which may or may not be part of cytochrome oxidase.

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