BBA 45956

INHIBITION OF RESPIRATION IN YEAST BY LIGHT

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(Received December 15th, 1969)

SUMMARY

Irradiation of starved cultures of *Saccharomyces cerevisiae* with blue light under aerobic conditions inhibited the capacity of the yeast cells to respire added substrates (*e.g.*, ethanol) and stimulated endogenous respiration. Spectroscopic examination of the cells showed that the irradiation destroyed both cytochrome *a* and a_3 components of cytochrome oxidase and a part of the cytochrome *b*. Irradiation under anaerobic conditions had no effect on the respiratory capacity or the cytochrome content of the cells. Under aerobic conditions cytochrome a_3 was protected against photodestruction when complexed with cyanide and cytochrome *a* was protected when complexed with azide.

INTRODUCTION

The effects of visible light on growth and respiration in yeast and other fungi were studied extensively in the latter part of the 19th century and the first part of this century[1–12,26] but the contradictory reports from many of these studies tended to raise more questions than they settled. The challenge of resolving the early contradictions and the amenability of yeast to studies of growth and respiration led to recent reinvestigations of the effects of light on yeast. MATILE AND FREY-WYSSLING[13], working with both growing and starved cultures of *Saccharomyces cerevisiae*, reported that strong white light inhibited O_2 uptake, but not CO_2 evolution, in glucose-supported respiration. They concluded that light inhibited some process in the Krebs cycle. MATILE[14], in addition, found that light caused an initial stimulation of endogenous respiration followed by subsequent inhibition. EHRENBERG[15,16] reported that strong visible light, with blue being the effective spectral region, inhibited protein synthesis, growth, cell division, and respiration of *S. cerevisiae*. She attributed the inhibition of respiration to the inhibition of protein synthesis but included the possibility of light affecting respiration enzymes or cofactors directly. Light at much lower intensities also inhibits the respiratory adaptation which occurs when anaerobically grown cultures of yeast are exposed to air[17,18] but it is not clear how the photoinhibition of respiratory adaptation is related to the photoinhibition of respiration in fully adapted cells.

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MATILE AND FREY-WYSSLING[13] also observed in a spectroscope that the irradiation treatment caused the cytochromes to go reduced and that the reduced cytochromes were not readily reoxidized by aeration in the dark. They postulated a direct light-induced reduction of the cytochromes but did not report or consider photodestruction of any of the cytochromes.

More recently, EPEL AND BUTLER[19,20] investigated the effects of light on the colorless alga *Prototheca zopfii*. Light inhibited protein synthesis, nucleic acid synthesis, growth, cell division, and respiration in growing cultures and the respiratory capacity of starved cultures. Light also affected some of the cytochromes of this organism which included two *c*-type cytochromes, three *b*-type cytochromes and cytochromes *a* and a_3. The photoinhibition of respiration was correlated with a photodestruction of cytochrome a_3, cytochrome $b(559)$ and cytochrome $c(551)$. Photoinhibition of respiration, growth, and the synthetic processes was considered to be a consequence of the inhibition of the respiratory electron transport chain. It was also reported preliminarily that light destroyed cytochrome oxidase in yeast and cytochrome a_3 in beef-heart mitochondria. The present paper reports in detail the effect of light on respiration and the cytochrome composition of the electron transport chain in yeast.

METHODS

A stock culture of starved yeast was prepared by washing 30 g of baker's yeast twice with 100 ml 0.05 M KH_2PO_4 (cells were collected by centrifugation for 10 min at 12000 $\times$ *g*), suspending the cells in 100 ml of the same buffer and aerating for 18 h with humidified air. The stock suspension ($2 \cdot 10^9$ cells/ml) was diluted 1:20 for irradiation experiments and the same dilution was used for measurements of respiration and absorption spectra.

O_2 uptake was measured at 27° with a Clark-type oxygen electrode (Yellow Springs Instrument Co., Model 4004). Samples were placed in a lucite cuvette of 3.3-ml capacity and stirred with a magnetic stirrer and small teflon-coated stirring bar. The current output of the electrode was taken through a 25-kΩ potentiometer adjusted to give full scale deflection on a 10-mV strip chart recorder with an air-saturated sample (250 μM O_2). Endogenous respiration was measured before adding substrate to the starved cells; exogenous, after adding 25 μl of 10% ethanol to the 3.3-ml sample in the cuvette.

A moderate intensity of white light was obtained from two banks of four 40-W GE-F40 cool white fluorescent lamps 15 inches apart on either side of a water bath. The energy in the middle of the water bath coming from one side was $1.5 \cdot 10^4$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1} of white light or $3 \cdot 10^3$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1} through a broad pass band (10% transmission at 350 and 510 nm, 50% at 370 and 485 nm) blue Corning glass filter, 5562. A high-intensity source of blue light (up to $2.5 \cdot 10^6$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}) was obtained with a 1000-W high-pressure mercury lamp (GE AH-6) focused with two glass lenses through the Corning 5562 glass filter into a water bath (27°). The high-pressure mercury lamp was also used with a 436-nm interference filter, a 404-nm interference filter and a Corning filter (either a 5840 or a 9863) which, in combination with the glass lenses, limited light to the 365-nm region. This lamp was also used with a series of Corning cut-off filters. Measurements of intensities were made with

a Yellow Springs Radiometer through a $CuSO_4$ solution that absorbed 99 % of the light at 650 nm.

Humidified air was bubbled through the yeast suspensions during irradiation to stir the cells. For anaerobic experiments, the concentrated yeast suspension was allowed to go anaerobic before diluting 1:20 with deoxygenated medium. Humidified Ar was bubbled through the suspension 30 min before and during the irradiation period.

Viability of yeast cultures was determined by plating the yeast in petri dishes on a sterilized medium consisting of 1 % yeast extract, 2 % Bacto peptone, 2 % dextrose and 2 % agar. The plates were incubated 24 h at 28° before counting the colonies.

Absorption spectra were measured with a single-beam recording spectrophotometer[21]. A potentiometer geared to the wavelength drive was used for baseline compensation over limited spectral regions. The signal from the potentiometer which was a linear function of wavelength was added to the photometric signal to compensate for the steep background slope of the absorption spectra. The baseline compensation was applied over the region of 510–580 nm for measurement of the *b*- and *c*-type cytochromes and 570–650 nm for cytochrome oxidase.

Absorption spectra were measured at 77°K on 1 ml of yeast suspension with 0.5 g of Al_2O_3 powder (1-μ particle size) or $CaCo_3$ added as a light-scattering agent. The light scatter in the sample intensified the absorption bands by increasing the optical path[22] thus permitting measurements to be made on the dilute suspension of cells ($1 \cdot 10^8$ cells/ml) used in the irradiation treatments. Cells in the Al_2O_3 slurry were reduced with dithionite in the presence of 1 % ethanol. More reproducible reduction results were obtained when dithionite was added to cells which were respiring substrate. KCN and NaN_3, when used, were added 3 min before the dithionite and 30 sec after the ethanol and the cells were allowed to incubate 2 min after addition of dithionite before freezing.

RESULTS AND DISCUSSION

The respiratory capacity of starved cultures of yeast was measured as the rate of O_2 uptake after addition of ethanol (final concentration 0.076 %, v/v). Endogenous respiration was measured as the rate of O_2 uptake in the absence of added substrate. The respiratory capacity of starved cultures of yeast was inhibited by irradiation with visible light. Irradiation with a moderate intensity of white light from fluorescent lamps resulted in an approximate first-order decay with a half-time of 15 h following an initial lag phase (Fig. 1A). The dark control in Fig. 1A showed no loss of respiratory capacity over a period of 100 h. With the higher-intensity source of blue light, the decay was more rapid ($t_{1/2}$ = 8 min) and there was no lag phase (Fig. 1B). At high intensities, the rate of inhibition was, in fact, more rapid during the initial phase. It was also found in the high-intensity irradiation experiment (Fig. 1B) that the photoinhibition process required the presence of O_2. Irradiation under anaerobic conditions did not impair the capacity of the cells to respire substrate under aerobic conditions. Essentially the same results were obtained previously with starved cultures of *P. zopfii*[19,20].

Endogenous respiration was stimulated during the initial part of the irradiation

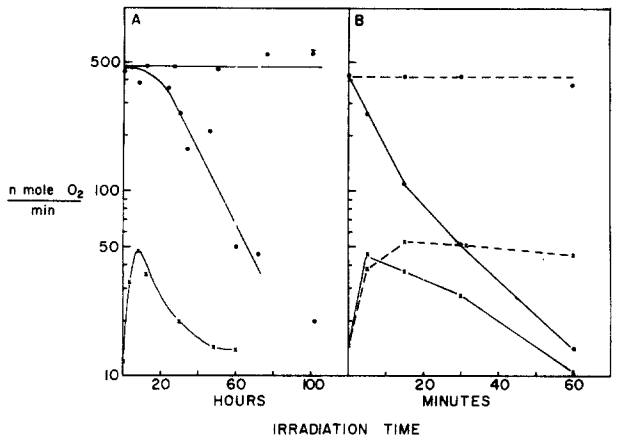
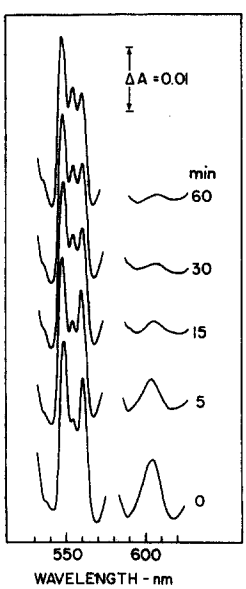


Fig. 1. Effect of light on respiration of yeast. Starved cultures of yeast ($1 \cdot 10^8$ cells/ml) irradiated (A) with moderate-intensity ($3 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}) white light from fluorescent lamps and (B) with high-intensity ($1 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}) blue light from high-pressure mercury lamp and Corning 5562 filter. Rate of exogenous (0.076% ethanol) respiration (○—○) and endogenous respiration (×—×) *vs.* time of irradiation under aerobic conditions. Rate of exogenous respiration (○- - - -○) and endogenous respiration (×----×) *vs.* time of irradiation under anaerobic conditions. Exogenous respiration of nonirradiated cells *vs.* time in dark (●—●).



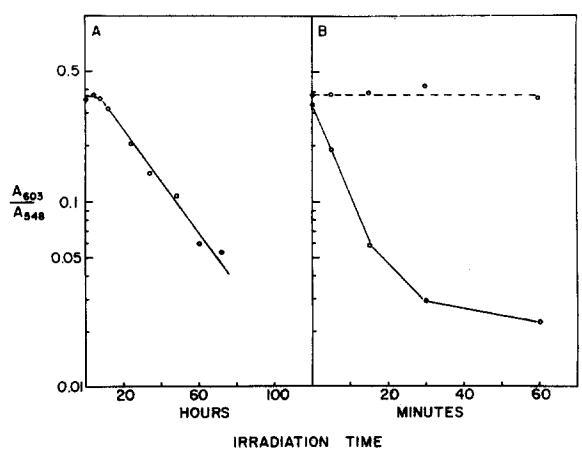


Fig. 2. Absorption spectra of yeast at 77° K after various periods of irradiation with high-intensity ($1 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}) blue light. Cells were reduced with dithionite. $CaCO_3$ was added to 1-ml suspension of cells as a light-scattering agent.

Fig. 3. Ratio of absorbances at 603 and 548 nm *vs.* time of irradiation (A) with the moderate-intensity ($3 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}) source of white light and (B) with the high-intensity ($1 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}) source of blue light. ○—○, aerobic conditions; ○----○, anaerobic irradiation.

but in the presence of O_2 the stimulation was overcome at longer irradiation times. Under anaerobic conditions (Fig. 1B), endogenous respiration was also stimulated but the stimulated respiration was not inhibited by continued irradiation.

The viability of the yeast was not impaired by the irradiation treatment. The colony forming capacity of an aliquot of cells from a nonirradiated culture was 45 colonies per plate while that of cells irradiated 1 h with $1.8 \cdot 10^6$ ergs·cm^{-2}·sec^{-1} of blue light (Corning 5562 filter) was 44 and 42 colonies per plate for two replicates. The respiratory capacity of the irradiated cells was 5 % of dark control.

Absorption spectra of the yeast at various stages of inhibition were measured at 77° K after the cells had been reduced with dithionite. The low temperature spectra showed cytochrome *c* with an absorption maximum at 548 nm, cytochrome c_1 at 554 nm, cytochrome *b* at 559 nm, and cytochrome oxidase at 603 nm. The spectra of the cells after various periods of irradiation with the high-intensity source are shown in Fig. 2. The absorption band of cytochrome oxidase was particularly sensitive to the irradiation treatment: about half of the absorption band at 603 nm was lost after a 5-min irradiation. A part of the cytochrome *b* absorption band was also lost. The loss of the absorption band will be equated with destruction of the pigment without implying the nature of the destruction. Cytochrome *c* was unaffected and cytochrome c_1 appears to have increased somewhat although the latter observation may result from an unmasking of the absorption band. The same spectral

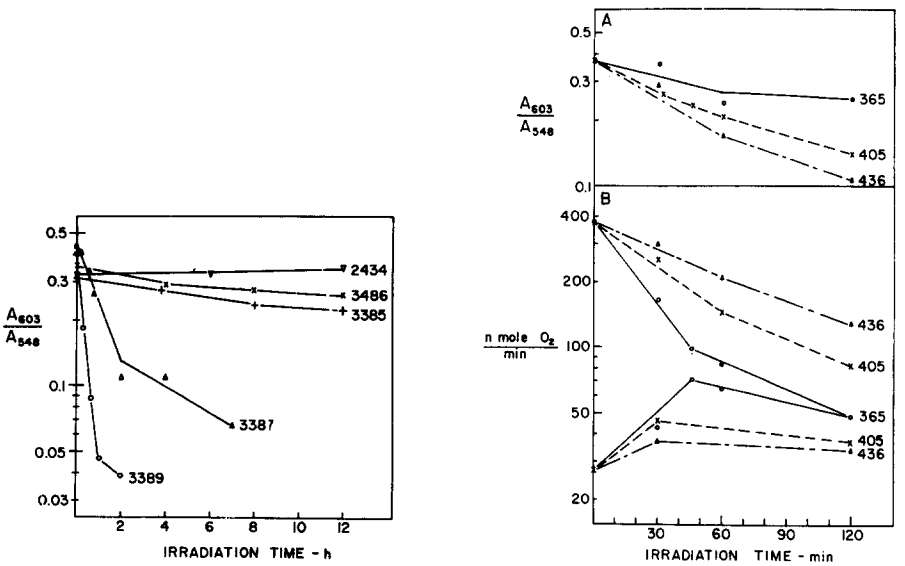


Fig. 4. Ratio of absorbances at 603 and 548 nm *vs.* time of irradiation with the high-pressure mercury lamp and the various Corning cut-off filters indicated. 2434, $T = 50$ % at 598 nm, $2 \cdot 10^5$ ergs·cm^{-2}·sec^{-1}. 3486, $T = 50$ % at 530 nm, $2 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}. 3385, $T = 50$ % at 485 nm, $2 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}. 3387, $T = 50$ % at 450 nm, $3 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}. 3389, $T = 50$ % at 425 nm, $4 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}. All intensity measurements were made with a $CuSO_4$ filter (99 % absorption at 650 nm). The same $CuSO_4$ filter was used for irradiation with all Corning filters except 2434 and 3486.

Fig. 5. A. Ratio of absorbances at 603 and 548 nm *vs.* time of irradiation with monochromatic light at 436, 405, and 365 nm each at $2 \cdot 10^5$ ergs·cm^{-2}·sec^{-1}. B. Rate of O_2 uptake for exogenous (0.076 % ethanol) respiration (three upper curves) and endogenous respiration (three lower curves) *vs.* time of irradiation with the same sources.

changes were noted after longer irradiation periods with the lower intensity source of fluorescent lamps. The time-courses for the loss of absorbance at 603 nm (plotted as the ratio of absorbances at 603 and 548 nm) with the moderate-intensity source and the high-intensity source are shown in Figs. 3A and 3B, respectively. The absorbances were estimated as the height of the absorption maximum above the horizontal line drawn through the adjacent minima. This estimate is subject to increasing error as the absorption band of cytochrome oxidase decreases. Nevertheless, a reasonably close correspondence between the time-course of cytochrome oxidase disappearance and the time-course of photoinhibition of exogenous respiration was found (compare Figs. 3A and 3B with Figs. 1A and 1B). Irradiation with the high-intensity source under anaerobic conditions (Fig. 3B) had no effect on the absorption spectrum.

Yeast suspensions were irradiated with the high-pressure mercury lamp through a series of cut-off filters to determine the effective wavelength regions for the photodestruction of cytochrome oxidase (Fig. 4). Essentially no effect was observed on the cytochromes with wavelengths longer than 485 nm even though the irradiation times were long (up to 12 h) and the intensities were high ($2 \cdot 10^6$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}). An appreciable effect was found at the next lower cut off wavelength, 450 nm, obtained with a Corning 3387 filter, and still greater effects with a Corning 3389 filter which transmitted wavelengths longer than 425 nm. The effect of light on cytochrome oxidase was clearly limited to the blue and near ultraviolet.

The relative effectiveness of 436, 405, and 365 nm at $2 \cdot 10^5$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1} was tested for the photodestruction of cytochrome oxidase (Fig. 5A) and the photoeffects on respiration (Fig. 5B). Both 436 and 405 nm were more effective than 365 nm in destroying cytochrome oxidase but 365 nm was more effective than the other two wavelengths for inhibiting exogenous respiration and stimulating endogenous respiration. Preliminary measurements at 330 nm indicated an effective inhibition of respiration with little destruction of cytochrome oxidase. The action spectra for photo-

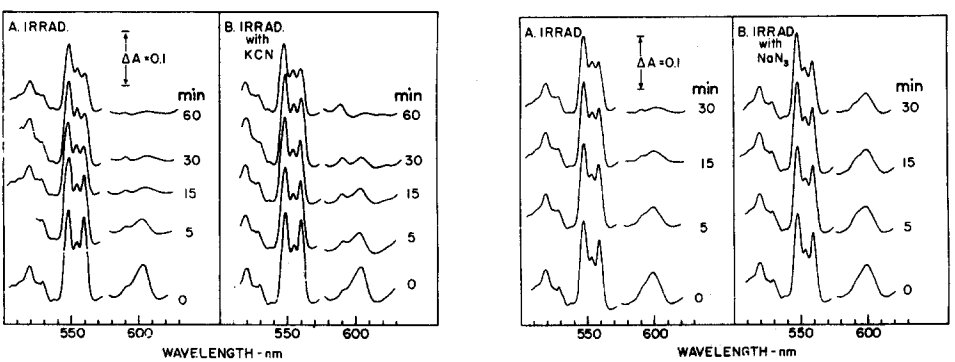


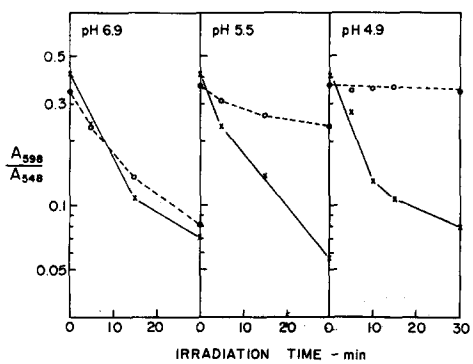
Fig. 6. Absorption spectra of yeast at 77°K after various periods of irradiation (pH 6.9) with high-intensity ($2.5 \cdot 10^6$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}) blue light (A) in the absence of cyanide and (B) in the presence of 1 mM KCN. All spectra measured in the presence of 1 mM KCN and dithionite with Al_2O_3 as a light-scattering agent.

Fig. 7. Absorption spectra of yeast at 77°K after various periods of irradiation (pH 5.5) with blue light ($1.5 \cdot 10^6$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}) A. In the absence of azide. B. In the presence of 1 mM NaN_3. All spectra measured in the presence of 1 mM NaN_3, 1 mM KCN and dithionite with Al_2O_3 added as a light-scattering agent.

inhibition of respiration and photodestruction of cytochrome oxidase do not appear to coincide in the near ultraviolet regions apparently because near-ultraviolet radiation can also inhibit respiration by inactivating some component, possibly a quinone[23], in addition to cytochrome oxidase. However, the primary inhibitory action of blue light (passed by a 5562 Corning filter) on respiration can be attributed to photodestruction of cytochrome oxidase.

The *a* and a_3 components of cytochrome oxidase were examined separately by measuring the spectra of cells in the presence of cyanide and dithionite. The absorption band of the reduced cyanide–cytochrome a_3 complex appeared as a shoulder at 590 nm on the reduced cytochrome *a* absorption band with a maximum at 603 nm. Methanol, which was required in *P. zopfii*[19] in order for the cyanide–cytochrome a_3 complex to be reduced by dithionite, was not required in yeast. Irradiation of the yeast (Fig. 6A) destroyed both cytochrome *a* and cytochrome a_3. Fig. 6B shows the absorption spectra of the yeast after various periods of irradiation with high intensity in the presence of cyanide. The cytochrome a_3 was completely protected against photodestruction as the cyanide–cytochrome a_3 complex. It was also found previously with *P. zopfii* that cyanide protected cytochrome a_3 against photodestruction[20]. A close comparison of the spectra in Figs. 6A and 6B (*e.g.* the 30-min spectra) indicates that the photodestruction of cytochromes *a* and *b* was somewhat slower in the presence of cyanide.

Spectra were also measured after various times of irradiation in the absence (Fig. 7A) and presence (Fig. 7B) of azide at pH 5.5. The site of azide inhibition of the respiratory electron transport chain is a question open to some controversy. GILMOUR *et al.*[24] presented evidence that azide complexes with the reduced form of cytochrome *a* while MUIJSERS *et al.*[25] reported a complex between azide and the oxidized form of cytochrome a_3. The results of Fig. 8 show clearly that azide protects cytochrome *a* against photodestruction and thus may be taken as presumptive evidence that azide complexes with cytochrome *a*. Any protective effect of azide on cytochrome a_3 is less certain because azide shifted the absorption band of cytochrome *a* to



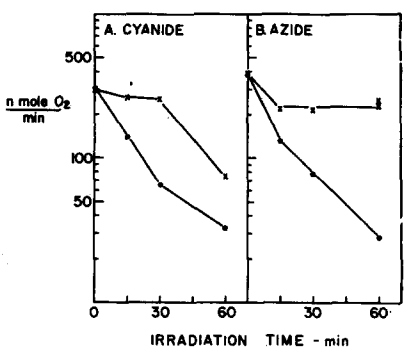


Fig. 8. Ratio of absorbances at 598 and 548 nm *vs.* time of irradiation with blue light ($1.5 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}). ○---○, irradiated in the presence of 1 mM NaN_3; ×—×, irradiated in the absence of azide.

Fig. 9. Rate of O_2 uptake for exogenous (0.076% ethanol) respiration of yeast ($1 \cdot 10^8$ cells/ml) *vs.* time of irradiation with blue light ($1 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}). A. ○—○, irradiated in absence of cyanide; ×—×, irradiated in the presence of 1 mM KCN with subsequent washing (washed 3 times with buffer, cells centrifuged 10 min at 5000 rev./min) to remove the cyanide. B. Same as A but with 1 mM azide.

598 nm which decreased the spectral resolution of the reduced cyanide–cytochrome a_3 complex. The cytochrome *b* appeared to be partially protected by azide similar to the partial protection by cyanide. Fig. 8 is a plot of the ratio of absorbances at 598 and 548 nm as a function of irradiation time in the presence and absence of azide at pH 6.9, 5.5, and 4.9. The protection of cytochrome *a* by azide (as well as the inhibition of respiration by azide) requires acid conditions so that the unionized form of the azide can penetrate the cell.

Cyanide and azide can be washed out of yeast cells with restoration of most of the respiratory capacity of the cells. Fig. 9 is a plot of the respiratory capacity of the yeast cells after various periods of irradiation in the absence of cyanide and azide (circles) or in the presence of cyanide or azide with a subsequent wash treatment to remove the inhibitors just prior to the respiratory measurement (crosses). Cyanide protected the respiratory capacity of the cells against photoinhibition for irradiation periods up to 30 min. Fig. 6B indicates that about one third of the cytochrome *a* was present after 30 min of irradiation in the presence of cyanide. During the next 30 min of irradiation in the presence of cyanide cytochrome *a* continued to be destroyed and the respiratory capacity decreased markedly. With azide 65 % of the respiratory activity was regained after washing the azide out of the cells following an irradiation of 1 h. These results on the protection of respiratory activity by azide suggest at least a partial protection of cytochrome a_3 as well as cytochrome *a*.

ACKNOWLEDGMENTS

This work was supported by a Dernham Fellowship of the American Cancer Society (H.N.), a National Science Foundation Grant GB-6557X, and a U.S. Public Health Service Fellowship, National Institutes of Health Grant GM-33,055 (B.L.E.).

REFERENCES

1 G. BONNIER AND L. MANGIN, *Ann. Sci. Nat. Botan. VI*, Ser. 17 (1884) 210.
2 F. ELFVING, Dissertation, Helsingfors, 1890.
3 W. SHORAWSKY, *Trav. Soc. Natl. Varsovie. Compt. Rend. Sect.* 1–2, 6 (3) (1894) 49.
4 R. KOLKWITZ, *Jahrb. Wiss. Botan.* 33 (1899) 128.
5 N. A. MAXIMOV, *Zentr. Bakteriol. Parasitenk. Abt. II*, 9 (1902) 193.
6 W. LOHMANN, Inaugurale Dissertation, Rostock, 1896.
7 L. BUCHTA, *Zentr. Bakteriol. Parasitenk. Abt. II*, 41 (1914) 340.
8 A. H. HUTCHINSON AND D. NEWTON, *Can. J. Res.*, 2 (1930) 249.
9 P. SCHUTZENBERGER AND E. QUINQUAUD, *Compt. Rend.*, 77 (1873) 272.
10 L. KNY, *Ber. Deut. Botan. Ges.*, 2, Heft 3 (1884).
11 O. WARBURG, *Biochem. Z.*, 177 (1926) 471.
12 G. GUERRINI, *Mem. Acad. Sci. Inst. Bologna*, IX, 7 (1940) 179; cited from E. BÜNNING, *Fortschr. Botan.*, 11 (1944) 143.
13 P. MATILE AND A. FREY-WYSSLING, *Planta*, 58 (1962) 154.
14 P. MATILE, *Ber. Schweiz. Botan. Ges.*, 72 (1962) 236.
15 M. EHRENBERG, *Arch. Microbiol.*, 54 (1966) 358.
16 M. EHRENBERG, *Arch. Mikrobiol.*, 61 (1968) 20.
17 E. SULKOWSKI, B. GUERIN, J. DEFAYE AND P. SLONIMSKI, *Nature*, 202 (1964) 36.
18 B. GUERIN AND R. JACQUES, *Biochim. Biophys. Acta*, 153 (1968) 138.
19 B. L. EPEL AND W. L. BUTLER, *Science*, 166 (1969) 621.
20 B. L. EPEL AND W. L. BUTLER, *Plant Physiol.*, in the press.
21 K. H. NORRIS AND W. L. BUTLER, *IRE Trans. Bio-Med. Electronics*, BME-8 (3) (1961) 153.
22 W. L. BUTLER, *J. Opt. Soc. Am.*, 52 (3) (1962) 292.
23 E. R. KASHKET AND A. F. BRODIE, *J. Bacteriol.*, 83 (1962) 1094.
24 M. V. GILMOUR, D. F. WILSON AND R. LEMBERG, *Biochim. Biophys. Acta*, 143 (1967) 487.
25 A. O. MUIJSERS, E. C. SLATER AND B. F. VAN GELDER, in B. CHANCE, R. W. ESTABROOK AND T. YONETANI, *Hemes and Hemoproteins*, Academic Press, New York, 1966, p. 467.
26 N. A. MAXIMOV, *Zentr. Bakteriol. Parasitenk. Abt. II*, 9 (1902) 261.