EFFECT OF VISIBLE LIGHT ON THE MITOCHONDRIAL INNER MEMBRANE

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SUMMARY: Exposure of mitochondria to visible light in the presence of riboflavin resulted in the initial release of respiratory control, followed by inhibition of electron transport and dissolution of structural integrity. Under these conditions, however, cytochrome c oxidase activity remained unchanged. ATPase activity was stimulated initially and remained in this activitated state even under continued illumination. In submitochondrial preparations, both electron transport and ATPase declined as a function of illumination time; cytochrome c oxidase was not sensitive to light. Enzyme inactivation also occurred to a lesser extent in the absence of riboflavin.

INTRODUCTION: Simultaneous exposure to visible light and air, both in the presence and in the absence of exogenous photosensitizers results in the killing of microorganisms (1-4). Furthermore, mammalian cells, both in suspension (5) and in tissue culture (6-9), are sensitive to visible light. Chance and Hess (10) have demonstrated that the absorption of light by ascites tumor cells in the green to red portion of the spectrum is qualitatively identical to that of the isolated mitochondria. Although this study was not concerned with photokilling, the results allude to the possible involvement of mitochondria in this phenomenon. In addition, the light sensitivity of the respiratory chain in both microorganisms and eukaryotes has been reported (11-13) and the role of endogenous photosensitizers assigned to flavins and hemes.

The present report describes the effect of visible light on isolated mitochondria. These studies should yield further information

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Abbreviations: *SMP (submitochondrial preparations), TMPD (N,N,N'N' tetramethyl-p-phenylenediamine).

into the possible involvement of mitochondria in the photokilling of cells.

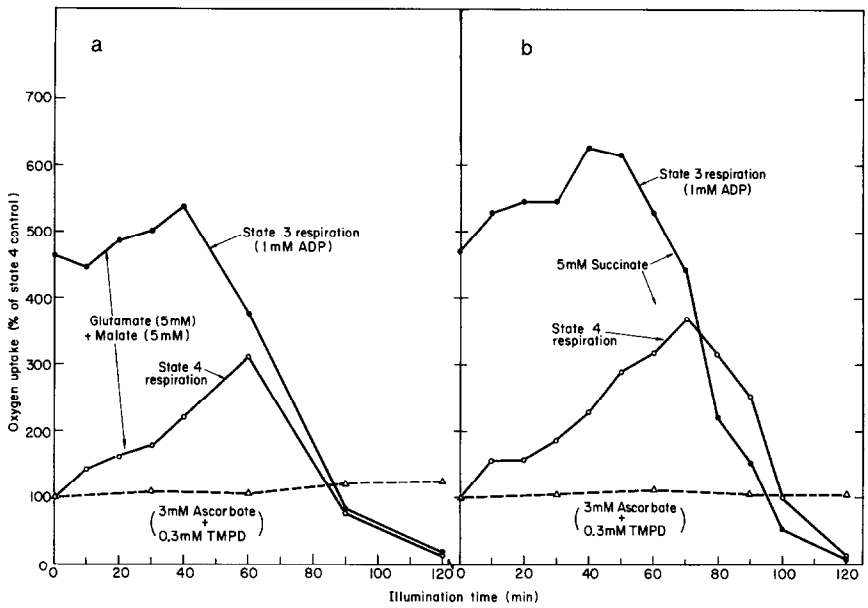
METHODS: Isolation Procedures. Rat liver mitochondria were isolated in 0.25 M sucrose (14) and submitochondrial preparations prepared as previously described (15).

Illumination. Samples (25 mg protein/ml) were irradiated at 3-5^{0}C in 0.25 M sucrose containing riboflavin (25 µg/ml). The light source used was a 300 watt projection lamp (tungsten filament) which emitted a net 8000 foot candles of light. The sample and light source were separated by a 1% solution of copper sulphate to reduce infra-red light and a glass filter to reduce ultraviolet light.

Enzyme Assays. All assays were carried out at room temperature in a medium containing 240 mM sucrose, 5 mM Tris-HCl, 2 mM potassium phosphate and 2 mM potassium chloride. In the ATPase assay, potassium phosphate was replaced by 0.2 mM magnesium chloride in the above medium.

Oxygen consumption was measured polarographically. Succinate dehydrogenase was assayed as described (16). NADH dehydrogenase was measured as described (17), but using an NADH regenerating system (50 µM NAD^+, 0.4 units alcohol dehydrogenase/ml, and 15 mM ethanol). ATPase activity was determined by measuring inorganic phosphate release (18). Protein determination was by a standard method (19).

RESULTS: Electron Transport. Mitochondrial suspensions were illuminated for various lengths of time and the effect on electron transport determined (Fig. 1a, 1b). The oxidation of NAD^+-linked substrates (malate+glutamate) and of succinate was stimulated during the first hour of illumination. This stimulation was much more striking in the absence of phosphate acceptor (state 4) than in its presence (state 3). Under these conditions, however, continued illumination resulted in the gradual inhibition of oxygen uptake in both respiratory states. Cytochrome c oxidase activity (substrate: ascorbate + TMPD) was not affected by light. Light treatment was accompanied by optical changes in the mitochondrial suspension. No change was noted during the first 120 min. of illumination, followed by a gradual decrease over the next 180 min. In addition, the decrease in absorbance (measured at 650 nm) was coincident with a release of protein into a form non-sedimentable by centrifugation (87500 g-min). Examination of treated suspensions with the electron microscope (J.R. Walton collaboration) indicated a transition of mitochondria from a "condensed" configuration before illumination, to a partly condensed and partly "expanded" structure at



Figs. 1a,1b. Effect of illumination on respiration of rat liver mitochondria. Respiratory substrates were added at the concentrations indicated. Details are given in Methods.

the onset of absorbance changes, to a grossly swollen condition at the end of illumination (for the nomenclature see reference 20).

Illumination of inner membrane suspensions resulted in the parallel inactivation of NADH oxidase, succinate oxidase, and succinate dehydrogenase (Fig. 2). NADH dehydrogenase activity, however, was relatively less sensitive to inactivation than the corresponding oxidase activity; furthermore, inactivation of this activity involved a short lag period. Cytochrome c oxidase was not inhibited by illumination and in some experiments was even slightly stimulated.

ATPase. In intact mitochondria, the latent ATPase was stimulated during the initial phase of illumination with maximum activity reached at a point corresponding to the complete release of respiratory control. Little change in activity occurred with further illumination (Fig. 3). In SMP, however, ATPase activity declined as a function of illumination time, with nearly complete inactivation occurring at 240 min.

All the experiments described above on respiration and on ATPase were carried out employing riboflavin as an exogenous photosensitizer. However, since mitochondrial membranes contain many compounds representing potential photosensitizers, experiments were also carried out in SMP in the absence of riboflavin (Table I). Photoinactivation of electron transport and ATPase also occurred under these conditions. However, at comparable illumination times, inactivation was greater in the presence

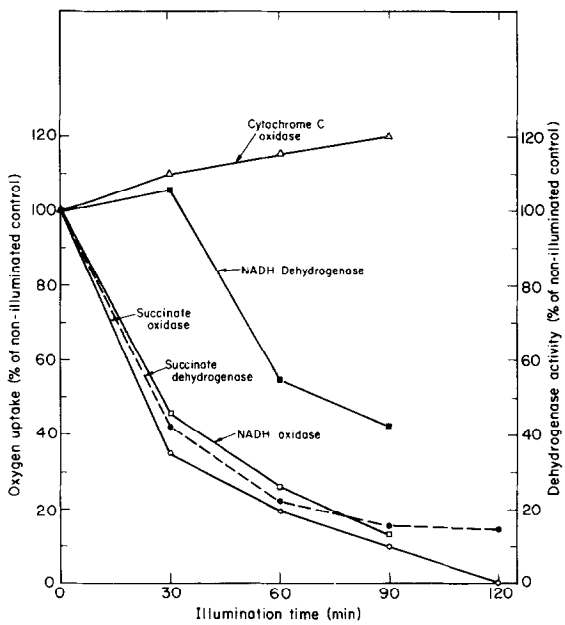
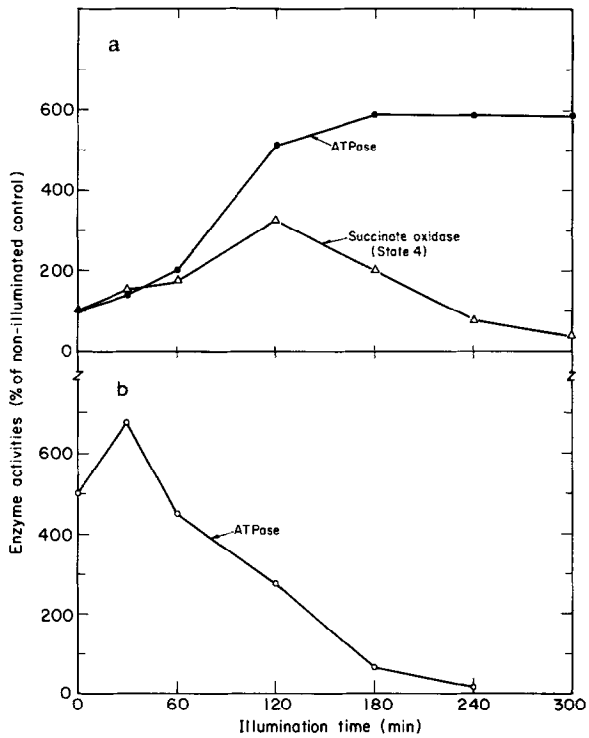


Fig. 2. Effect of illumination on electron transport in submitochondrial preparations. Concentration of succinate and ascorbate + TMPD as in Fig. 1. Details are in Methods.

of riboflavin.

DISCUSSION: The present study has demonstrated that exposure of rat liver mitochondria or SMP to visible light results in the inactivation of several inner membrane enzyme activities. Although the inclusion of riboflavin into the system gave rise to more pronounced levels of inhibition, these effects were also obtained with illumination alone,



Figs. 3a, 3b. Effect of illumination on ATPase activity of mitochondria (Fig. 3a) and of submitochondrial preparations (Fig. 3b). The concentration of succinate was as in Fig. 1.

indicating the presence of endogenous photosensitizers. The inactivation process in mitochondria was complex , involving at least two separate phases. The first, occuring during the initial 60-90 min of illumination, consisted of a loss of energy coupling reflected by stimulation of both electron transport and ATPase. The second phase was distinguished by an inhibition of respiration and an eventual dissolution of mitochondrial integrity.

ATPase activity was effected by light differently in mitochondria and SMP. In the former system, the stimulated activity was resistant to extended illumination while a steady decline leading to complete inactivation occurred in SMP. It is possible that sonication used to prepare SMP causes a reorganization of membrane components resulting

in an increased sensitivity to light. Alternatively, some matrix components absent in SMP may protect the ATPase in mitochondria.

The mechanism of inactivation of mitochondrial function by light is at present being studied. However, the data obtained suggest that the site of inhibition of electron transport apparently exists prior to cytochrome c since cytochrome c oxidase activity was unaffected by illumination in both intact mitochondria and SMP. Inactivation of cytochrome c oxidase activity observed by Epel (2) could be due

TABLE 1

Effect of Illumination on Electron Transport and ATPase of Submitochondrial Preparations in Absence and in Presence of Riboflavin

	Residual Activity[a] (% of non-illuminated control)	
	- Riboflavin	+ Riboflavin
Succinate oxidase	48	10
Succinate dehydrogenase	18	8
NADH oxidase	70	13
NADH dehydrogenase	19	15
Cytochrome c oxidase	136	120
ATPase	61	36

[a]For conditions of illumination (90 min) and of assays see Methods.

to differences in intensity and quality of light used. Moreover, the inactivation was not restricted to flavoenzymes since ATPase was also affected. One explanation for our findings involves the flavin photosensitized production of reactive compounds such as singlet oxygen and superoxide ion which in turn may inactivate enzymes directly. Furthermore, these compounds may induce lipid peroxidation thereby generating additional reactive molecules capable of affecting enzyme activity. Enzyme inactivation may also be due, in part, from light

induced polymerization of polypeptides as preliminary studies using sodium dodecyl sulphate-polyacrylamide gel electrophorsis have indicated.

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