in the subunits on dissociation as evidenced by the dichroism and electron-spin-resonance measurements.

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Cytochrome *c* Redox Potentials as a Function of the Energy State in Chromatophores

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Spectroscopic assay of *c*-type cytochrome (α -peak 551nm) in the Ga mutant of *Rhodospseudomonas spheroides* and potentiometric analysis (Caswell & Pressman, 1968) of wild-type *Rhodospirillum rubrum* in the potential range of cytochrome c_2 have been employed to monitor electron flow in chromatophores. Both methods showed that *c*-type cytochromes were oxidized on illumination. The rate and extent of oxidation were increased and reduction in the dark was slower in the presence of antimycin A, consistent with the site of inhibition suggested by Nishimure (1963). Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone decreased cytochrome *c* oxidation, indicating at least one coupling site before cytochrome *c*. Subsequent addition of antimycin raised cytochrome *c* oxidation to a value less than that in the presence of antimycin alone, suggesting some 'leak' through the inhibited site.

Addition of ADP during illumination in the presence of Mg^{2+} and phosphate at pH 7.6 showed cycles of reduction in the potentiometric recording that were inhibited by oligomycin.

Valinomycin added before illumination, especially in KCl medium, inhibited the initial rate of cytochrome *c* oxidation and also, though less, the extent. In KCl media valinomycin greatly slowed the reduction in the dark. When added during illumination valinomycin produced transient lowering of cytochrome *c* oxidation. Nigericin had little effect on the initial rate of cytochrome *c* oxidation, but slightly lowered the steady-state value in the presence of K^+ . When both antibiotics were present simultaneously in the presence of K^+ , the steady-state

oxidation rate was lowered to that obtained on the addition of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. We have previously discussed effects of these agents on photophosphorylation and the protonmotive force (Jackson, Crofts & von Stedingk, 1968; Jackson & Crofts, 1969).

Synergistic uncoupling of electron flow and photophosphorylation were observed with actin or, at higher concentrations, with valinomycin and NH_4Cl . This combination collapses the electrochemical potential of an H^+ gradient because NH_3 is able to cross the membrane (Crofts, 1968) and equilibrate the chemical H^+ gradient and NH_4^+ -antibiotic is able to cross the membrane and equilibrate the electrical potential (see also Cockrell & Racker, 1969; McCarty, 1969). A synergism explicable in similar terms was observed with nigericin, together with Triton X-114 at low concentrations that render the chromatophore membrane permeable to small ions (Crofts & Jackson, 1969).

These observations suggest that the membrane-potential component of the protonmotive force (Mitchell, 1966) might control electron flow.

Measurement of initial rates of H^+ uptake with Bromocresol Purple and electron flow through the coupling sites by subtraction of initial rates of cytochrome *c* changes in the presence and absence of antimycin yielded values of $H^+/2e \approx 4$ over a range of light-intensities and in the presence and absence of valinomycin. This is compatible with two phosphorylation sites of adenosine triphosphatase type II (Mitchell, 1966) in the chromatophore cyclic electron-transport pathway.

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