The electron-bypass and displacement concepts were investigated by measuring the sensitivity of an acetone-extracted heart-muscle preparation at different concentrations of inhibitors and in the presence of various concentrations of quinone.

Succinate oxidase activity of acetone-extracted heart-muscle preparation reconstituted with coenzyme Q-0 or methyl-coenzyme Q-0 was not completely abolished even by high concentrations of antimycin. High antimycin concentrations would, however, completely abolish coenzyme Q-2- or Q-3-stimulated succinate oxidase of acetone-extracted heart-muscle preparation. The pattern of inhibition appears consistent with the operation of an electron-bypass mechanism in coenzyme Q-0- and methyl-coenzyme Q-0-mediated electron transport and of an inhibitor-displacement mechanism of succinate oxidase stimulation in preparations treated with coenzyme Q-2 or Q-3.

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Inhibitory Effects of Uncoupling Agents on Systems I and II of Photosynthetic Electron Transport in Chloroplasts

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The materials carbonyl cyanide *m*-chlorophenylhydrazine (CCCP), dicoumarol, pentachlorophenol and 2,4-dinitrophenol have been reported to uncouple photophosphorylation and to inhibit the Hill reaction in chloroplasts, at similar concentrations (de Kiewiet, Hall & Jenner, 1965; Jagendorf & Neumann, 1965; Krogman, Jagendorf & Avron, 1959; Neumann & Jagendorf, 1964). Their effects on system I are more difficult to observe, since the absorption by the reagents themselves masks the reduction of NADP in the spectrophotometer. The following method makes use of the reduction of methyl viologen, observed by Arnon (1963).

Air-tight stoppered cuvettes contained broken chloroplasts equivalent to 0.05 mg. of chlorophyll, 1.5 μ moles of methyl viologen, 30 μ moles of cysteine and 0.3 μ mole of 2,6-dichlorophenol-indophenol in 3 ml. of 0.04 M-phosphate buffer, pH 6.8. They were illuminated in saturating red light for 20 sec. periods, and read in the spectrophotometer at 607 m μ against unilluminated controls. For system II, a Clark oxygen electrode was used to measure the Hill reaction with 2 mM-K₃Fe(CN)₆-1 mM-KCl, containing 0.05 mg. of chlorophyll in 3 ml. of the same buffer.

*K'*_i values for CCCP, dicoumarol, pentachlorophenol and dinitrophenol in the Hill reaction were

8.9, 20, 1.7 and 40 μ M respectively. In system I, the value for CCCP was 260 μ M, and no inhibition was observed with dicoumarol up to the limit of solubility, about 100 μ M at pH 7.1. Pentachlorophenol and dinitrophenol both caused a decrease in rate of some 40% at about 10 μ M, but this lower rate was not inhibited further at concentrations up to 0.5 mM. Since dinitrophenol can be reduced by system I (Wessels, 1960) the inhibition may be overestimated.

It is unlikely that these agents are acting on pigment system II, since system I would be thought similar, and it is hardly affected. There may be a specific inhibition of a reaction near system II that has no counterpart in system I. It is also possible that the action on system II is the same phenomenon as the uncoupling of phosphorylation on the hypothesis of Mitchell (1966), namely the destruction of some pH gradient or electrochemical gradient on which the Hill reaction might depend.

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The Mechanism of the Phosphoglycerate Mutase Reaction

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Muscle phosphoglycerate mutase was thought to catalyse the intermolecular transfer of phosphate from 2,3-diphosphoglycerate (2,3-diPGA) to 2- and 3-phosphoglycerate (2-PGA and 3-PGA), but Grisolia & Cleland (1968), who studied the effect of 2,3-diPGA concentration on the reaction rate proposed that the mechanism involved a phosphoenzyme as in the phosphoglucomutase reaction. However, such kinetic observations are compatible with both mechanisms (Britton & Clarke, 1968) and a phosphoenzyme has not been isolated. To distinguish between the mechanisms, induced-transport tests have been carried out (Britton & Clarke, 1968). These tests depend on the fact that when one substrate is converted into the other by the intermolecular transfer of phosphate from