physiological pH succinate oxidation is limited by its entry.

Short exposure to pH 6.3 with succinate and uncoupler present did not significantly increase membrane permeability to a sulphonated azo-dye. Lowering the pH to 6.3 changes the quantities of ionic species $\text{H}_2\text{succinate}:\text{Hsuccinate}^{1-}:\text{succinate}^{2-}$: from 0.1mM:2.5mM:37.4mM to 0.4mM:6.2mM:33.4mM and the faster succinate entry is probably due to the increased proportion of uncharged molecules, which can permeate the relatively hydrophobic osmotic barrier by a diffusion pathway. It appears that the membranes of these cells, grown in suspension in modified Waymouth medium (Sinclair, Reid & Mitchell, 1963), are virtually impermeable to succinate²⁻, which predominates at physiological pH. This is in contrast with the apparent ability of succinate²⁻ to permeate the mitochondrial membrane by a carrier mechanism (Chappell & Haahrhoff, 1967).

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Indirect Evidence that Light-Induction of Adenosine Triphosphate Hydrolysis by Chloroplasts Depends on Electron-Transfer Reactions

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The dark hydrolysis of ATP by spinach or lettuce chloroplasts, after activation by light in the presence of cysteine (80mM), is stimulated by ammonium chloride (5mM) added after illumination; the ATP hydrolysis then becomes progressively slower, thus confirming earlier observations with dithiothreitol (Carmeli & Avron, 1969; Carmeli, 1969; cf. also Marchant, 1969). A distinct difference is found between the use of *N*-methylphenazonium methyl sulphate on the one hand and FMN on the other as added redox substance. If the reaction mixture is not stirred during the illumination period (5min) when *N*-methylphenazonium methyl sulphate was used the initial stimulated rate is high and falls off relatively slowly, whereas with FMN the initial rate is lower and falls off rapidly. If on the other hand the mixture is continuously stirred during the light phase, the initial high rate with *N*-methylphenazonium methyl sulphate now falls off more rapidly, whereas with FMN the initial rate is now considerably higher and no longer falls off so rapidly. This higher rate with FMN is even

observed if a few seconds' agitation is given during the latter part of the illumination period. Neither stirring nor agitation are necessary for these higher rates with FMN if the illumination period is shortened to 2min.

The effects with FMN can be attributed to the need for either oxygen or oxidized FMN for electron transfer, the FMN having become reduced during early illumination and not then available in the oxidized form unless the mixture is stirred. With *N*-methylphenazonium methyl sulphate a possible explanation is that stirring in from the surface layers of the oxidized cofactor itself, or a derivative of it, is responsible for the decreased response to ammonium chloride subsequently, assuming that maintenance of efficient cyclic electron transfer throughout illumination depends on the nature and redox state of this cofactor (cf. Zweig & Avron, 1965). Both of these explanations would imply that a maintained stimulation by ammonium chloride depends on prior electron transport.

Further support for the involvement of electron transfer in the light-induction is given by the absence of inhibition by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (0.1mM), with or without ammonium chloride, when *N*-methylphenazonium methyl sulphate is used (cf. Carmeli & Avron, 1966), but very clear inhibition with FMN, as is found for photophosphorylation.

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Preparation of Human Pathological γ -Macroglobulins by Rate Zonal Centrifugation in a BXV Ti Rotor

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Zonal rotors (Anderson, 1966) have mostly been used for the preparation of cell particles, but both Fisher & Canning (1966) and Anderson *et al.* (1967) used the separation of a macroglobulin peak from rat plasma as a test of resolution for zonal rotors. Their results suggested that the technique could be