compound was generously supplied by Dr S. G. Waley).

Two characteristics of cataractous lenses favour mixed-disulphide formation. First, the protein thiol groups become more accessible and, secondly, there is relatively little glutathione present to reduce mixed disulphides. Huisman & Dozy (1962), working on haemoglobin, predicted that mixed-disulphide formation would occur when the concentration of glutathione was low.

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Diquat Cataract in the Rat

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Rats fed on a diet containing 0.05–0.075% of the herbicide diquat (1,1'-ethylene-2,2'-dipyridylum dibromide) slowly develop lens opacities while remaining healthy in other respects (Howe & Wright, 1965; A. Pirie & J. R. Rees, unpublished work). The lens opacity starts in the posterior cortex, spreads to the central core and finally becomes complete. Mitosis in the lens epithelium is initially depressed, but recovers and no cellular damage remains. Intraperitoneal injection of diquat labelled with ^{14}C in the ethylene bridge leads to appearance of radioactivity in the lens and other parts of the eye.

The herbicidal action of diquat is considered to be due to its reduction to a bright-green free radical in photosynthesizing cells (Homer, Mees & Tomlinson, 1960; Davenport, 1963). The reaction is reversed by O_2 with formation of H_2O_2 . Gage (1968) has described several enzyme systems in liver that can form the free radical, and Orr (1966) found that ascorbic acid can reduce diquat to the free radical in air while being oxidized itself. Present experiments have shown that the ascorbic acid of the lens and of intraocular fluids was less in diquat-

fed rats than in litter-mate controls. We confirmed the finding by D. G. Clark (personal communication) that the GSH concentration of the lens remained at the normal value during cataract development. This marks diquat cataract off from many other types in which the GSH concentration of the lens decreases.

We have investigated enzymic and non-enzymic reactions of diquat with constituents of lens, aqueous humour and vitreous humour. A freshly prepared lens extract will reduce diquat to the free radical anaerobically in the dark, probably largely through the action of glutathione reductase and NADPH. The free radical is also formed photochemically in sunlight and can be demonstrated anaerobically in the presence of electron donors such as EDTA or amino acids, or with aqueous humour. Sunlight will also catalyse aerobic oxidation of ascorbic acid by diquat in aqueous humour. H_2O_2 is formed. The maintenance of GSH in the cataractous lens may be connected with the ability of the diquat free radical to reduce GSSG in the presence of glutathione reductase and NADP.

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Spontaneous Reversal of Arsenate-Induced Swelling of Mitochondria

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Hunter & Ford (1955) and Packer (1961) have described mitochondrial swelling induced by arsenate and prevented by EDTA. Ter Welle & Slater (1967) found that arsenate produces a time-dependent appearance of oligomycin-insensitive respiration and that this also is prevented by EDTA.

Addition of arsenate (1.25 mM) to rat liver mitochondria oxidizing β -hydroxybutyrate in an Mg^{2+} -free medium results in the following events: a lag phase in which the rate of oxygen uptake is approximately doubled and during which little or no swelling occurs; a phase of marked swelling and acceleration of oxygen uptake; a phase of temporary reversal of swelling (by about 20% of the total) during which the high rate of oxygen uptake is maintained; a phase of slow steady swelling during which the rate of oxygen uptake declines; a small amplitude burst of swelling when the medium goes anaerobic (or if antimycin A or an uncoupling agent

is added), followed by a return to the slow steady rate of swelling.

Antimycin, added at the point of maximum swelling, prevented the reversal phase, and slow steady swelling resulted. Addition of oligomycin, 2,4-dinitrophenol or carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone at the same point did not prevent reversal of swelling, which thus appears to be dependent on respiration but not high-energy intermediates.

Addition of ethylenedioxybis(ethyleneamine)-tetra-acetic acid before arsenate prevented significant swelling, and addition after arsenate apparently caused a return to the original volume. In the latter case this was accompanied by a decrease in the rate of oxygen uptake and restoration of some respiratory control.

These results are interpreted as follows. During the phase of accelerating swelling and respiration Ca^{2+} is being released and β -hydroxybutyrate equilibrates across the membrane. When respiration reaches a very high rate, product can leave faster than β -hydroxybutyrate can enter, possibly because the membrane is damaged, allowing outward leakage of K^+ . This results in reversal of swelling, superseded by a slow degradation of the membrane. Respiration can, however, still maintain some electrochemical gradient between the inside of the mitochondrion and the medium. When respiration ceases this gradient disappears and there is a small burst of swelling due to re-equilibration of ions across the membrane.

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Proton Transport in Mitochondrial Oxidative Phosphorylation

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At physiological pH values phosphorylation of ADP produces approximately equimolecular amounts of OH^- ion. Since the inner mitochondrial membrane is not highly permeable to H^+ or OH^- ions, this internal production of OH^- ions must be compensated by some associated process. P_i is effectively exchanged for two OH^- ions (Chappell & Crofts, 1965), thus overcompensating for the internal production of OH^- ions. If the ATP-ADP exchange-diffusion carrier exchanged ATP^{4-} for ADP^{3-} the overall process would be balanced, but this mechanism would be thermodynamically un-

satisfactory since a pH differential that favoured P_i uptake would also favour retention of ATP.

If the ATP-ADP exchange-diffusion carrier exchanged ATP^{4-} for ADP^{3-} and overcompensation by the $\text{P}_i\text{-OH}^-$ ion exchange were compensated by a respiration-driven proton pump, then conditions of raised internal pH and negative internal electrical potential would favour uptake of P_i and exchange of internal ATP^{4-} for external ADP^{3-} .

This suggests a mechanism of oxidative phosphorylation in which production of non-phosphorylated high-energy intermediates and proton pumping proceed in parallel. Such a scheme has features that contrast with those of the chemical and chemiosmotic mechanisms proposed for oxidative phosphorylation.

Production of the chemical intermediate and the electrochemical potential difference at a particular phosphorylation site could result from the same electron-transfer step, but could also result from neighbouring electron transfers.

The magnitude of the electrochemical potential difference across the mitochondrial membrane would be small, except in the presence of substances, such as Ca^{2+} ions, that discharge the non-phosphorylated high-energy intermediate, when all the energy from electron transfer becomes available for generating electrochemical potential differences.

Uncouplers have to produce both proton permeability in the membrane and discharge of the non-phosphorylated high-energy intermediate, unless some other process can compensate for the proton pumping. 2,4-Dinitrophenol affects the activity of the mitochondrial adenosine triphosphatase coupling factor (Pullman, Penefsky, Datta & Racker, 1960; Selwyn, 1968) and also renders lipid membranes permeable to protons (Hopfer, Lehninger & Thompson, 1968). Ca^{2+} ions uncouple only in the presence of ions such as acetate, which can discharge the proton gradient (Lehninger, Carafoli & Rossi, 1967). Acetate ions alone do not uncouple and are not accumulated, since they do not discharge the chemical intermediate.

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