PHOSPHORYLATION COUPLED TO ELECTRON TRANSPORT MEDIATED BY SHORT CHAIN DERIVATIVES OF COENZYME Q

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It has been shown by Hatefi (1959) and Hatefi, Lester, Crane, and Widmer (1959) that mitochondria can directly oxidize the reduced form of coenzyme Q_{10}. We have carried out a study on the efficiency of phosphorylation coupled to electron transport initiated by short chain derivatives of coenzyme Q in rat liver mitochondria. In the experiments of Hatefi, $Q_{10}H_2$ was added to mitochondria and the extent of its oxidation determined spectrophotometrically. In the experiments described in this communication catalytic quantities of the Q derivatives were used to couple the oxidation of external DPNH to initiation of mitochondrial electron transport. In previous experiments of this kind (Jacobs and Sanadi (1960)) it was shown that while external DPNH alone could not initiate electron transport in undamaged rat liver mitochondria, this process could be mediated with catalytic amounts of such redox systems as cytochrome c, ferricyanide, silicomolybdate and Vitamin K_3, which were maintained reduced in the presence of external DPNH by the "external DPNH-cytochrome c reductase". The data of Table I show that Q_0, Q_2 and duroquinone, but not Q_{10}, were also capable of acting as electron shuttles

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TABLE I

OXYGEN UPTAKE AND P/O RATIOS

Quinone	Oxygen Uptake μatoms 25 min.	P/O
-	0.0	-
Q_0 0.1 mg.	5.6	0.80
0.25 mg.	10.5	0.74
0.25 mg.*	0.0	-
0.5 mg.	10.8	0.41
Q_2 .066 mg.	4.5	0.13
.132 mg.	9.7	0.09
.330 mg.	18.1	0.00
.330 mg.*	0.0	-
Duroquinone 0.25 mg.	8.4	0.68
0.5 mg.	12.5	0.53
0.5 mg.*	3.3	0.00
Q_{10} 0.5 mg.	0.0	-

Complete reaction mixture contained DPNH (7.5×10^{-3} M), potassium phosphate (7.5×10^{-3} M, pH 7.0), $MgCl_2$ (7.5×10^{-3} M), ATP (1.5×10^{-3} M), glucose (6×10^{-2} M), sucrose (0.25 M), hexokinase (0.2 mg. protein), rat liver mitochondria (7-9 mg. protein) and additions indicated in Table. Final Reaction volume, 3.3 ml. Quinones added in minimal volume of absolute ethanol.

*+ 1 γ antimycin A

between the external reductase and the internal cytochromes. Although ascorbate could replace DPNH as a passive source of substrate quantities of reducing electrons, its use required higher levels of Q derivatives to obtain a given oxidation rate. Antimycin A completely inhibited electron transport initiated by the Q derivatives and almost completely inhibited that initiated by duroquinone. In view of this observation it was expected that

a P/O ratio of 2.0 could be approached at quinone concentrations which did not markedly uncouple phosphorylation from succinate oxidation. However, a comparison of the data in Tables I and II clearly shows that under such conditions the upper limit is only 1.0. The reason for this discrepancy is not obvious. It should

TABLE II

UNCOUPLING ACTION OF QUINONES

Quinone	Oxygen Uptake μatoms 15'	P/O
-	9.8	1.70
Q_0 .25 mg.	6.8	1.43
-	11.2	1.80
Q_2 .066 mg.	12.3	1.57
.330 mg.	8.1	0.0
-	13.5	1.65
Duroquinone 0.25 mg.	14.2	1.52
0.50 mg.	10.9	1.60
-	12.7	1.76
Q_{10} 0.5 mg.	11.9	1.65

Complete reaction mixture contained potassium succinate (1.5×10^{-2} M), potassium phosphate (1.0×10^{-2} M, pH 7.0), $MgCl_2$ (7.5×10^{-3} M), ATP (1.5×10^{-3} M), glucose (6×10^{-2} M), sucrose (0.25 M), hexokinase (0.2 mg. protein), rat liver mitochondria (7-9 mg. protein) and additions indicated in Table.

be noted that Q_2 is a more powerful uncoupler than Q_0 (Table II). Cross, Taggart, Covo, and Green (1949) observed that when the 4-nitro group of 2,4-dinitrophenol was replaced with a fatty chain (n-octyl), the resulting compound acted as an efficient uncoupler. The structural resemblance between Q_2 hydroquinone and this substituted nitrophenol might be sufficient to impart strong un-

coupling action to Q derivatives with short side chains.

Of all the compounds which were examined for their capacity to act as electron shuttles in the above-described system (complex inorganic anions, substituted benzo and naphthoquinones, quinoneamides) only short chain derivatives of coenzyme Q and plastoquinone initiated antimycin-sensitive electron transport. Such specificity may signify that these external quinones can interact with the electron transport chain only at the level of endogenous coenzyme Q, which apparently lies before the antimycin sensitive site (Hatefi, 1959). Further work bearing on this question is in progress.

References

Cross, R. J., Taggart, J. V., Covo, G. A., and Green, D. E., J. Biol. Chem., 177, 655 (1949).

Hatefi, Y., Biochim. Biophys. Acta, 34, 183 (1959).

Hatefi, Y., Lester, R. L., Crane, F. L., and Widmer, C., Biochim. Biophys. Acta, 31, 490 (1959).

Jacobs, E., and Sanadi, D. R., Biochim. Biophys. Acta, 38, 12 (1960).