site. It is wiser, however, to await the results of many further experiments before accepting that bovine-milk xanthine oxidase is, in fact, a thiol-dependant enzyme.

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Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
London (Great Britain)

D. A. GILBERT

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Uncoupling and inhibition of oxidative phosphorylation by 2-hydroxy-3-alkyl-1,4-naphthoquinones

Various 2-hydroxy-3-alkyl-1,4-naphthoquinones are potent inhibitors of electron transport in the region of the antimycin-inhibitory site of the respiratory chain[1]. The inhibition of respiration in intact mitochondria by 2-hydroxy-3-(3-methylbutyl)-1,4-naphthoquinone may be reversed by the uncoupling agent, 2,4-dinitrophenol[2], suggesting that the quinone might act on energy-conservation reactions. It is of interest to study the influence of naphthoquinones on oxidative phosphorylation under conditions where the inhibition site is bypassed. This may be done by using a system including ascorbate and catalytic amounts of TMPD which appears to supply electrons to the respiratory chain at a locus between the antimycin block and oxygen[3].

Fig. 1 is a polarographic trace of oxygen consumption by rat-liver mitochondria respiring with TMPD and ascorbate. The addition of AMP produced a characteristic stimulation of respiration and this stimulation was completely eliminated by the addition of 2-hydroxy-3-cyclohexyl-1,4-naphthoquinone. Inhibition of State-3 respiration (+ AMP) was approximately to the State-4 (no AMP) level, a property also of the inhibition of coupled respiration by oligomycin[4]. The concentration of CNQ used was sufficient to inhibit succinate oxidation completely.

Abbreviations: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; CNQ, 3-cyclohexyl-1,4-naphthoquinone; DNQ, 2-hydroxy-3-(3,7-dimethyloctyl)-1,4-naphthoquinone.

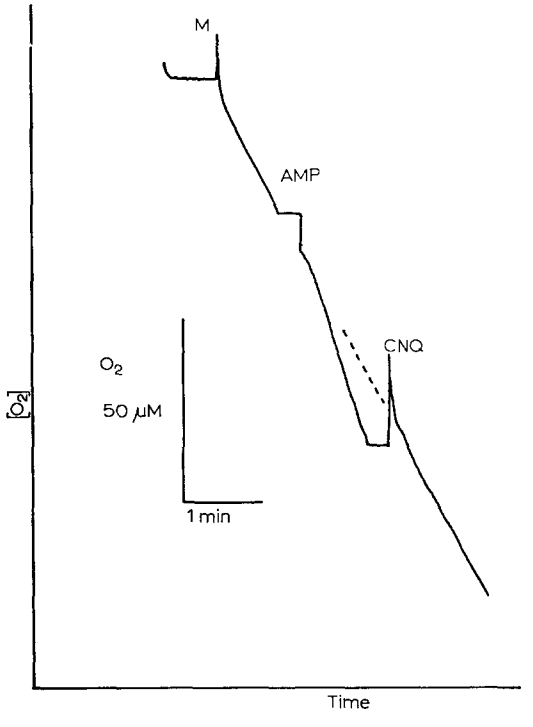

Fig. 1. Inhibition of respiration by CNQ. Reaction mixture contained 15 mM KCl, 2 mM EDTA, 50 mM Tris–HCl, 5 mM $MgCl_2$, 12.5 mM phosphate, 60 μM TMPD, 15 mM ascorbate and 5.4 mg protein in a final volume of 2 ml. The pH was 7.4. Additions were 0.7 μmole AMP and 1.0 μg of CNQ in ethanolic solution.

In Table I it is seen that 2-hydroxy-3-(3-methylbutyl)-1,4-naphthoquinone (hydrolapachol) and 2-hydroxy-3-(3,7-dimethyloctyl)-1,4-naphthoquinone also show oligomycin-like inhibition of State-3 respiration. In contrast to the case of oligomycin[5], inhibition by DNQ is not relieved by 2,4-dinitrophenol. Likewise, inhibition of succinate oxidation by DNQ was previously found not to be released by addition of 2,4-dinitrophenol[2]. Moreover, DNQ does not affect State-4 respiration (nor does hydrolapachol or CNQ). On the other hand, 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (lapachol) and 2-hydroxy-3-(4-hydroxy-3-methyl-2-butenyl)-1,4-naphthoquinone (lomatiol) produce a distinct stimulation, in the manner of an uncoupling agent. Neither compound has a significant effect on State-3 respiration at the concentrations used. In other experiments, it was found that lapachol, like other uncoupling agents[5], releases oligomycin inhibition of respiration.

Table II shows that both DNQ and lapachol prevent the oxidative synthesis of ATP, measured as hexose monophosphate synthesis by the method of SLATER AND HOLTON[6].

Thus, one group of hydroxynaphthoquinones, including DNQ, CNQ and hydrolapachol appears to have the characteristics of inhibitors of oxidative phosphorylation, inhibiting ATP synthesis and preventing the stimulation of respiration by phosphate acceptor. On the other hand, lapachol and lomatiol appear to act as

TABLE I

THE EFFECT OF HYDROXYNAPHTHOQUINONES ON COUPLED RESPIRATION

Conditions were as described in Fig. 1, with 2.8–3.8 mg protein in the different experiments. Oxygen consumption was measured polarographically.

Expt.	*Additions*	O_2 *consumption (μatoms/min)*
1	None	0.073
	0.7 μmole AMP	0.144
	0.7 μmole AMP + 5.3 μg hydrolapachol per mg protein	0.087
2	None	0.107
	0.7 μmole AMP	0.158
	0.7 μmole AMP + 5.3 μg DNQ per mg protein	0.087
	0.7 μmole AMP + 5.3 μg DNQ per mg protein + 50 μM 2,4-dinitrophenol	0.085
3	None	0.078
	0.27 μg DNQ per mg protein	0.078
	0.80 μg DNQ per mg protein	0.078
4	None	0.085
	0.7 μmole AMP	0.133
	0.7 μmole AMP + 0.19 μg CNQ per mg protein	0.077
5	None	0.093
	2.6 μg lapachol per mg protein	0.126
	2.6 μg lapachol per mg protein + 0.7 μmole AMP	0.123
6	None	0.089
	2.6 μg lomatiol per mg protein	0.106
	5.2 μg lomatiol per mg protein	0.112
7	None	0.128
	0.14 μmole AMP	0.188
	0.14 μmole AMP + 2 μg lapachol per mg protein	0.186

uncouplers of oxidative phosphorylation, stimulating respiration in the absence of phosphate acceptor and eliminating ATP synthesis.

Of the hydroxynaphthoquinones studied, those which appear to uncouple possess a 2′,3′-unsaturated side chain at the 3 position (*i.e.* lapachol and lomatiol). Those saturated at this bond (*i.e.* DNQ, CNQ and hydrolapachol) act as phosphorylation inhibitors in much the same manner as oligomycin. The distinction is especially clear in a comparison of lapachol and hydrolapachol which differ only at the 2′,3′ bond of the C-3 side chain.

TABLE II

THE INFLUENCE OF DNQ AND LAPACHOL ON PHOSPHATE ESTERIFICATION

Reaction mixture was as described in Fig. 1 except that, in addition, it contained 0.1 mM ADP, 60 mM glucose, 150 units of hexokinase and 4.9 mg mitochondrial protein in a volume of 1 ml. Oxygen uptake was measured manometrically. The duration of the experiment was 12 min.

Additions	*Hexose monophosphate (μmoles)*	*Δ O (μmoles)*
None	2.54	2.32
DNQ (1 μg per mg protein)	0.06	1.69
Lapachol (1 μg per mg protein)	0.08	2.48

The structural difference between the two classes of hydroxynaphthoquinones discussed above is interesting with regard to various proposed mechanisms, of ATP synthesis[7,8] which involve the formation of rings by condensation of the side chain of a 3-alkyl quinone with O-4. A 2',3' double bond is required for this cyclization. The 2-hydroxyl group in the naphthoquinones used in this work, by contributing an orthoquinoid character, should affect the stability of the ring. The above argument presents an approach to the investigation of the proposed involvement of chromanols or related compounds in mitochondrial oxidative phosphorylation.

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Laboratory of Physiological Chemistry,
University of Amsterdam,
Amsterdam (The Netherlands)

J. L. HOWLAND*

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* Present address: Department of Biology, Bowdoin College, Brunswick, Maine (U.S.A.).

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The effect of glucose analogues on the hepatic glucose-phosphorylating enzymes

Recent work on the nature of the enzymic system catalysing the phosphorylation of glucose by rat liver[1,2] resulted in the demonstration that two enzymes are involved[2,3]. These two enzymes occur in different ammonium sulphate fractions of rat-liver supernatant fractions[4] and have been designated[3,4] as glucokinase (having a low affinity for glucose, $K_{m,1}$, of type EC 2.7.1.2 but not necessarily specific for glucose only[4]) and hexokinase (having a high affinity for glucose, $K_{m,2}$, and probably of non-specific type EC 2.7.1.1). Detailed characterization of the two enzymes will require first their isolation and purification, perhaps following the lead mentioned[4], but considerable information can be obtained by kinetic analysis along the lines used previously[3]. The purpose of this communication is to show how such procedures can be used for a system consisting of two enzymes catalysing the same reaction simul-