The potentiating effect of digitoxin and quinidine on dinitrophenol uncoupling of oxidative phosphorylation*

Concentrations of digitoxin which *per se* have little action upon respiration or P:O ratios measured in mitochondrial preparations of guinea pig liver or rabbit heart markedly potentiate the uncoupling effect of DNP** upon oxidative phosphorylation. This potentiating effect is shown in Table I, which shows also a similar effect by quinidine. Of special interest is the marked inhibitory effect of quinidine upon respiration as shown in Table I, since at a concentration of $1 \cdot 10^{-3}$ *M* it completely abolishes respiration of heart mitochondrial preparations under similar conditions. The respiration of liver mitochondria, although affected by quinidine appears to be less sensitive ($1 \cdot 10^{-3}$ *M* will inhibit 40%) (unpublished experiments of the author).

TABLE I

THE EFFECT OF DIGITOXIN AND QUINIDINE UPON DNP UNCOUPLING OF OXIDATIVE PHOSPHORYLATION

Each vessel contained the following expressed in micromoles per 2 ml: potassium phosphate buffer pH 7.45, 30; tris-HCl buffer pH 7.5, 100; potassium α-ketoglutarate 30; sodium ATP 4; $MgSO_4$ 15; glucose 100, rabbit heart mitochondria 0.7 mg nitrogen per vessel added in 0.5 ml of 0.25 *M* sucrose. Enough hexokinase to transfer 150 micromoles of high energy phosphate per ten minutes at 38°. Hexokinase and DNP tipped after equilibration (ten minutes) 16 minutes incubation at 38°. Air, NaOH in center well.

Exp.	*Conditions*	*O uptake*	*P:O*
1	Control	6.2	2.60
1	+ DNP $1.25 \cdot 10^{-5}$ *M*	5.6	2.36
1	+ DNP $5 \cdot 10^{-5}$ *M*	6.2	1.89
1	+ Digitoxin $3.3 \cdot 10^{-5}$ *M*	6.6	2.40
1	+ Digitoxin $3.3 \cdot 10^{-5}$ *M* + DNP $1.25 \cdot 10^{-5}$ *M*	6.3	1.91
1	+ Digitoxin $3.3 \cdot 10^{-5}$ *M* + DNP $5 \cdot 10^{-5}$ *M*	6.8	0.76
1	+ Digitoxin $6.6 \cdot 10^{-5}$ *M*	6.2	2.40
1	+ Digitoxin $6.6 \cdot 10^{-5}$ *M* + DNP $1.25 \cdot 10^{-5}$ *M*	5.5	1.64
1	+ Digitoxin $6.6 \cdot 10^{-5}$ *M* + DNP $5 \cdot 10^{-5}$ *M*	7.4	0.72
2	Control	6.9	2.60
2	+ DNP $1.25 \cdot 10^{-5}$ *M*	6.8	2.40
2	+ DNP $5 \cdot 10^{-5}$ *M*	7.3	1.46
2	+ Quinidine $5 \cdot 10^{-4}$ *M*	3.9	2.80
2	+ Quinidine $5 \cdot 10^{-4}$ *M* + DNP $1.25 \cdot 10^{-5}$ *M*	3.6	2.15
2	+ Quinidine $5 \cdot 10^{-4}$ *M* + DNP $5 \cdot 10^{-5}$ *M*	4.1	0.81

Other experiments some of which are shown in Table II, indicate that the potentiating effect is dependent on the composition of the medium particularly of sodium and potassium, as is the oxidative phosphorylation mitochondrial system of guinea pig liver. Complete replacement of potassium by sodium showed a drop in respiration of approximately 50% with a drop in P:O ratios of 60% as shown in Table II.

The observation of the digitoxin potentiating effect appears to be the first demonstration of a biochemical effect of digitoxin at a subcellular level; further it is strongly reminiscent of the action of digitalis and related compounds in the whole animal or in the isolated organ. Similar effects can be demonstrated with triiodothyronine replacing DNP***. Although the mechanism of action of DNP is yet unknown it is attractive to interpret the potentiating effect of digitoxin and quinidine upon uncoupling of oxidative phosphorylation by DNP as an indication of the effect of these compounds upon mitochondrial permeability.

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** The following abbreviations are used throughout this paper: DNP, 2,4-dinitrophenol; ATP, adenosine triphosphate; P:O, micromoles of inorganic phosphate uptake per microatom of oxygen uptake; tris, (hydroxymethyl) aminomethane.

TABLE II

THE EFFECT OF SODIUM AND POTASSIUM CONCENTRATION UPON OXIDATIVE PHOSPHORYLATION AND DIGITOXIN POTENTIATION OF DNP UNCOUPLING

All components were as described for the experiments of Table I, except that α-ketoglutarate was replaced by L-glutamate and that 0.027 micromoles of cytochrome *c* were added to each vessel. In experiment 2A, 90 micromoles of KCl were added. In experiment 2B potassium was entirely replaced by sodium. Guinea pig liver mitochondria, 1.7 mg nitrogen for experiment 1, 1.8 for experiments 2A and 2B. Fifteen minutes incubation at 38°.

Exp.	*Conditions*	*O uptake*	*P:O*
1	Control	5.6	2.2
1	+ DNP $1.25 \cdot 10^{-5}$ *M*	5.6	2.1
1	+ DNP $5 \cdot 10^{-5}$ *M*	6.1	1.9
1	+ Digitoxin $3.3 \cdot 10^{-5}$ *M*	6.2	2.0
1	+ Digitoxin $3.3 \cdot 10^{-5}$ *M* + DNP $1.25 \cdot 10^{-5}$ *M*	7.3	1.5
1	+ Digitoxin $3.3 \cdot 10^{-5}$ *M* + DNP $5 \cdot 10^{-5}$ *M*	7.3	1.3
2A	Control	7.9	2.2
2A	+ DNP $1.25 \cdot 10^{-5}$ *M*	7.5	2.2
2A	+ DNP $5 \cdot 10^{-5}$ *M*	8.9	1.5
2A	+ Digitoxin $3.3 \cdot 10^{-5}$ *M*	8.9	2.2
2A	+ Digitoxin $3.3 \cdot 10^{-5}$ *M* + DNP $1.25 \cdot 10^{-5}$ *M*	9.2	1.8
2A	+ Digitoxin $3.3 \cdot 10^{-5}$ *M* + DNP $5 \cdot 10^{-5}$ *M*	9.6	1.5
2B	Control	3.2	0.8
2B	+ DNP $1.25 \cdot 10^{-5}$ *M*	3.1	0.8
2B	+ DNP $5 \cdot 10^{-5}$ *M*	4.3	0.4
2B	+ Digitoxin $3.3 \cdot 10^{-5}$ *M*	4.2	0.6
2B	+ Digitoxin $3.3 \cdot 10^{-5}$ *M* + DNP $1.25 \cdot 10^{-5}$ *M*	3.6	0.6
2B	+ Digitoxin $3.3 \cdot 10^{-5}$ *M* + DNP $5 \cdot 10^{-5}$ *M*	3.5	0.1

sources: DNP, from Matheson, Coleman and Bell; ATP, from Pabst Laboratories; quinidine, from Merck Laboratories. Hexokinase was purified to stage 3A essentially by the method of BERGER[1] *et al.*; cytochrome *c* was prepared by the method of KEILIN AND HARTREE[2], inorganic phosphate was measured by the method of GOMORI[3], nitrogen by a standard micro-Kjeldahl.

Heart and liver mitochondria were prepared by an abridged modification of the method of SCHNEIDER[4]; liver cells were homogenized with a Potter-Elvehjem homogenizer to make a 10% homogenate; heart cells were homogenized by blenderizing a fine scissors mince of the ventricular portion of two rabbit hearts for 75 seconds in a small Waring blendor (model with constricted base of about 50 ml capacity, total capacity 250 ml) with 40 ml of ice-cold 0.25 *M* sucrose. An additional 20 ml of sucrose were added and the homogenate was filtered through four layers of gauze. The homogenates were centrifuged at 600 × *g* for ten minutes, the sediment rehomogenized for 25 seconds with 50 ml of 0.25 *M* sucrose and recentrifuged at 600 × *g* for ten minutes. The combined supernatant fluids were centrifuged at 6000 × *g* for 20 minutes and taken up in 0.25 ml sucrose. No rehomogenization of the liver was carried out and the higher speed centrifugation was 8500 × *g*.

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