OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA FROM ANIMALS TREATED WITH 2-CHLORO-3-PHYTYL-1,4-NAPHTHOQUINONE, AN ANTAGONIST OF VITAMIN K_1

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Possible function of certain quinones as coenzymes in the multienzyme system of the respiratory chain in electron transport and in oxidative phosphorylation has been considered repeatedly. Such a role for vitamin K was suggested first by Martius and Nitz-Litzow (1954a, 1954b), who reported a reduced efficiency of phosphorylation associated with the oxidation of β-hydroxybutyrate in mitochondria from vitamin K-deficient chicks. This defect could be corrected by the addition of vitamin K_1. Also, they observed that dicoumarol and other substances related to vitamin K could uncouple the phosphorylation associated with the oxidation of β-hydroxybutyrate (Martius and Nitz-Litzow, 1953).

It has been shown recently (Lowenthal *et al.* 1960) that 2-chloro-3-phytyl-1,4-naphthoquinone, a structural analogue of vitamin K_1, is an antagonist of the effects of vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) on blood coagulation. In the normal animal the antagonist causes an anticoagulant effect, reversible by vitamin K_1, and in the coumarin anticoagulant treated animal it inhibits the antidotal effect of vitamin K_1. Because of the above considerations it was of interest to investigate oxidative phosphorylation of liver mitochondria isolated from animals pretreated with the vitamin K_1 antagonist (Parmar and Lowenthal 1961).

EXPERIMENTAL

Rats weighing 175-250 g. received 0.5 mg/100 g. of 2-chloro-3-phytyl-1,4-naphthoquinone intravenously, the highest dose which did not cause death from

hemorrhage within 24 hours. The animals were killed 24 hours later, when the prothrombin time had increased to over 600 seconds, compared to a normal value of 38–40 seconds. Solubilization of the antagonist for intravenous administration with the aid of Tween 80 (polyoxyethylene sorbital mono-oleate) and determination of prothrombin time in the rat have been described previously (Lowenthal and Taylor, 1959).

Livers from experimental and control animals were homogenized in ice-cold 0.25 M sucrose (10% w/v) and the mitochondria isolated by differential centrifugation by the method of Hogeboom et al. (1948) as modified by Hawkins (1952). The mitochondria were washed twice and then suspended in 0.25 M sucrose so that 10 ml of the suspension was equivalent to 10 g. of fresh tissue. Standard procedures for measurement of oxidative phosphorylation were employed using the hexokinase–glucose system to trap phosphate. P/O ratios were determined for the oxidation of β-hydroxybutyrate, succinate and L-glutamate.

RESULTS AND DISCUSSION

The results show that the vitamin K_1 antagonist in a dose which causes a very marked decrease of the plasma prothrombin level as indicated by prolongation of the prothrombin time had no effect on the efficiency of oxidative phosphorylation associated with the oxidation of β-hydroxybutyrate, succinate and L-glutamate by liver mitochondria isolated from such animals (Table I). While these findings fail to indicate that vitamin K_1 is involved in either electron transport or in oxidative phosphorylation, they do not necessarily disprove the suggestion of Martius. For example, it is possible that the analogue interferes selectively with the action of vitamin K_1 on blood coagulation but not with that on oxidative phosphorylation, or that the anticoagulant effect is produced before any interference with oxidative phosphorylation can be detected.

TABLE I

Oxidative Phosphorylation in Rat Liver Mitochondria

Substrate	$-\Delta P_i^*$ (μ moles)		$-\Delta O_2^*$ (μ atoms)		P/O Ratio	
	C	E	C	E	C	E
β-hydroxy butyrate (12)	16.78 ±4.48	15.68 ±4.8	6.29 ±1.83	5.83 ±1.73	2.68 ±0.10	2.69 ±0.13
Succinate (6)	16.73 ±1.1	16.72 ±0.93	9.97 ±1.03	10.21 ±0.9	1.68 ±0.07	1.64 ±0.08
L-glutamate (6)	24.5 ±1.0	22.63 ±1.1	9.76 ±1.4	8.99 ±1.36	2.51 ±0.08	2.52 ±0.12

* Corrected for endogenous $-\Delta P_i$ and oxygen uptake. C and E denote control and experimental animals respectively.
The incubation mixture contained in a final volume of 3 ml, 30 μ moles of orthophosphate, pH 7.4; 30 μ moles of substrate; 20 μ moles of $MgCl_2$; 2 μ moles of ATP; 0.04 μ moles of cytochrome C; 150 μ moles of glucose; 0.5 mg hexokinase (Sigma Chemical Company Type II); 30 μ moles of KF (added last); 0.5 ml (500 mg wet weight of liver) of mitochondrial suspension. Glucose and hexokinase were present in the side arm and were added at the zero time. Incubated in air at 30° for 20 minutes. Mean and standard deviations are given. Number of experiments are shown in parentheses in the Table.

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