HYDROQUINONE MONOPHOSPHATES AND OXIDATIVE PHOSPHORYLATION

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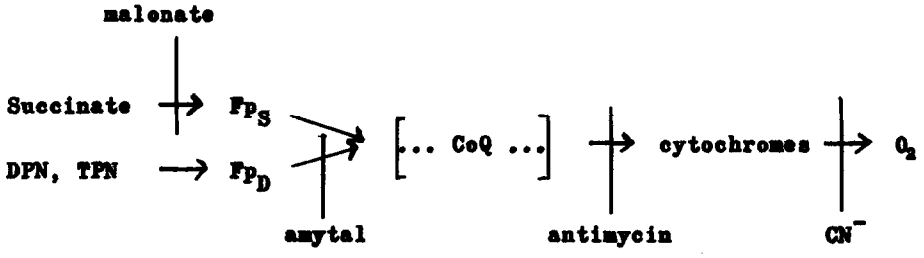
The ubiquinones (CoQ) occuring in mammalian mitochondria in high concentration (Lester and Crane, 1959; Pumphrey and Redfearn, 1960) are most probably concerned with electron transfer in the respiratory chain (Green, 1961). From experiments with inhibitory substances the position of CoQ is supposed to be between the amytal-sensitive and the antimycin-sensitive reactions.



As a result of the investigations of Martius and Nitz-Litzow (1954) of the role of vitamin K, monophospho-dihydro K$_1$ (K$_1$H$_2$-P, II) and monophospho-menadiol (I), as well as monophosphates of CoQ-H$_2$ (III) were considered as intermediates in oxidative phosphorylation. During chemical oxidation of certain hydroquinone monophosphates "energy-rich" phosphate is formed (Wieland and Pattermann, 1958, 1959; Clark, Kirby and Todd, 1958). In order to test the enzymatic synthesis of ATP from

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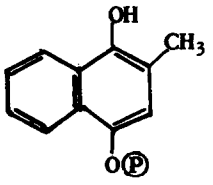
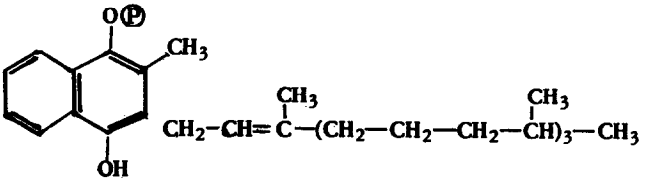
[1] CoQ$_{4,0}$H-P has been synthesized by another route by C.H.Shunk, J.F. McPherson and K.Folkers, Biochim.Biophys. Research Comm.6, 124 (1962).

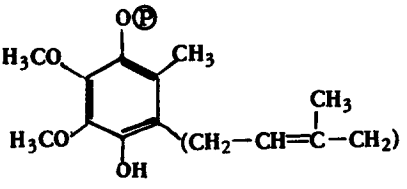
ADP in the presence of mitochondrial preparations, the phosphoric esters I, II and III (n = 6; CoQ_6H-P[1]) were synthesized (Buck, 1961; Höhl, 1962) by Andrew's (1961) method.



I



II



III

Ⓟ=PO_3H_2

Materials and methods. - Vitamin K_1 was a preparation of Hoffmann-La Roche, Grenzach, CoQ_6 of Cutolo Calesi, Naples. The esters I, II and III are oily compounds, only soluble in water if alkali or an organic solvent is present. They were used as ethanolic solutions. ^{32}P-containing CoQ_6H-P was synthesized from $^{32}PCl_3$, obtained from Radiochemical Centre, Amersham, England. Hexokinase, glucose-6-phosphate dehydrogenase (G-6-PD), and TPN were preparations of C.F. Boehringer, Mannheim-Waldhof. Mitochondria from rat heart were prepared according to Chance and Hagihara (1961), from liver according to Schneider (1948), frozen mitochondria after Weinbach (1959) and digitonin particles after Devlin and Lehninger (1959). The O_2-consumption was measured manometrically and the reduction of cytochrome c by scanning the absorption at 530 mμ.

In our experiments the suspension of mitochondria (4-5 mg protein)

together with other additions, auxiliary enzymes and substrates (1-2 mM) in 1-2 ml buffer was incubated for 2,5 hours at 30° C. ATP formed was determined optically at 366 mμ by way of G-6-P with TPN and G-6-PD (Lamprecht and Trautschold, 1958).

Results. - Menadiol phosphate (I) and KH-P (II) were found to inhibit the formation of ATP from ADP during the oxidation of endogenous or of added substrate. In the presence of 3 μmoles/ml the amount of ATP was reduced to 50 percent. Menadiol and KH_2 did not show any inhibitory effect.

CoQ_8 H-P (III) is oxidized slowly (ca. 20 μl/hour) with simultaneous phosphorylation of ADP. Table 1 shows P:O ratios obtained with III as sole electron donor in the presence of several mitochondrial preparations.

Table 1

Oxidative phosphorylation in different rat mitochondria preparations with CoQ_8 H-P as sole substrate.

No	Preparation (rat)	Oxidant	P:O ratio
1	Heart sarcosomes, intact	O_2	1,5
2	Liver mitochondria, intact	O_2	1,25
3	Liver mitochondria, frozen	O_2	0,4 - 0,7
4	Liver mitochondria, frozen	Cytochrome c	0,9
5	Digitonin particles (liver)	O_2	1,8

The effects of inhibitory and uncoupling substances shown in Table 2 suggest that CoQ_8 H-P enters the respiratory system at the ubiquinone level.

Radioactive G-6-^{32}P was formed in an experiment in which 3 μmoles of CoQ_8 H-^{32}P were incubated in citrate-NaOH buffer (25 mM), ADP (2,5 mM), glucose (10 mM), EDTA (1 mM), histidine (10 mM), serum albumin (5 mg/ml),

Table 2

Effect of inhibitory substances on ATP-formation from ADP during oxidation of CoQ_9H-P by rat liver mitochondria.

Inhibitor	Conc.(μmoles/ml)	% ATP
—	—	100
KCN	1,6	0,0
Antimycin A	(10 μg/mg protein)	30
Amytal	6	100
Malonate	10	100
Dinitrophenol	1	0,0

suspension of hexokinase (0,02 ml) with digitonin particles (ca. 4 mg protein) under oxygen at 30° C for 2 hours. The P:O ratio was about 1. The G-6-P separated from other P-containing substances by paper electrophoresis at pH 4,2 exhibited a specific radioactivity equal to the CoQ_9H-^{32}P oxidised. Dilution of ^{32}P-content did not occur if ^{31}P inorganic phosphate was added before incubation. Therefore the phosphate moiety of III is not incorporated into ATP after hydrolysis but CoQ_9H-P seems to be an immediate phosphorylating agent on oxidation in the respiratory chain.

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