an effect comparable to that with succinate as substrate. The rate of oxidation of β-hydroxybutyrate was unaffected or slightly increased by these levels of the hormone.

It is apparent that the effects of these uncoupling reagents on oxidative phosphorylation in the fragments are very similar to their actions in intact mitochondria. The effective concentrations and the order of activity among the compounds is about the same, with dinitrophenol being the most active agent. The decrease in the rate of succinate oxidation in the presence of thyroxine and an acceptor system has been observed in intact mitochondrial preparations[5] as well as the fragments. The rate of β-hydroxybutyrate oxidation was not diminished in either system. Preliminary experiments, using the free radical tetramethyl-*p*-phenylenediamine, have shown that this reagent uncouples oxidative phosphorylation in the sonic extracts as had been noted previously with intact mitochondria[7]. A possible difference in the systems is the greater inhibition of O_2 consumption in the particles by triiodothyroacetic acid.

Since TAPLEY, COOPER, AND LEHNINGER[8] could not find any effect of thyroxine on the enzyme complex prepared with digitonin, but did observe effects of the hormone at very low concentrations on the osmotic swelling of whole mitochondria, they have suggested that uncoupling may be secondary to structural changes in the mitochondria and not due to a direct action on the enzyme system responsible for phosphorylation. In view of the present results and the possibility that digitonin itself may interfere with the thyroxine effect, a direct action of the hormone must still be considered as a definite possibility.

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Vitamin K_1, a component of the mitochondrial oxidative phosphorylation system**

Since the original implication of a role for vitamin K_1 in the mitochondrial oxidative phosphorylation system by MARTIUS AND NITZ-LITZOW[2] and MARTIUS[2], several studies have appeared which have attempted, by direct and indirect means, to extend the primary observations. MARTIUS[2] proposed a site for vitamin K_1 activity between DPN and cytochrome *b* and hypothesized that dicoumarol uncouples oxidative phosphorylation in the electron-transport chain by competitive inhibition of vitamin K_1. CHANCE AND WILLIAMS[3] have criticised this interpretation on the grounds that a vitamin K_1 effect on phosphorylative efficiency does not necessitate an electron-transport function. COOPER AND LEHNINGER[4] have proposed that dicoumarol acts at the phosphorylation level and not directly on an electron-transport component between DPN and cytochrome *c*. BRODIE *et al.*[5] have recently presented evidence for functional roles of vitamin K_1 in both electron transport and coupled oxidative phosphorylation in bacterial systems. DALLAM AND ANDERSON[6] have also recently reported restoration of uncoupled oxidative phosphorylation by vitamin K_1 after ultraviolet (2537 Å) treatment of mitochondria and thus support the original finding of MARTIUS AND NITZ-LITZOW[1]. COLPA-BOONSTRA AND SLATER[7,8] have reported phosphorylation of ADP accompanying the oxidation of reduced vitamin K_3 (menadione) by heart-muscle mitochondria and present evidence that electrons from reduced menadione enter the electron-transport chain in the region of flavoprotein. These authors do not exclude the possibility that the reactions studied may be artificial due to non-specificity. At present, both the role and the location of vitamin K_1 in oxidative phosphorylation are open questions.

Twice-washed rat-liver mitochondria were prepared by differential centrifugation in 0.25 *M* sucrose and irradiated with ultraviolet light (2600 Å or 3600 Å) in a rotary irradiator[9]. Quartz tubes were employed for experimental samples and control mitochondria were irradiated in either pyrex tubes (2600 Å) or in pyrex tubes covered with aluminum foil. Oxygen consumption was

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measured by the conventional Warburg technique and phosphorylation according to MARTIN AND DOTY[10] as modified for tracer determination by LINDBERG AND ERNSTER[11]. Vitamin K_1 was emulsified in 0.25 *M* sucrose in the presence of crystalline serum albumin (ultraviolet irradiated). Following irradiation the mitochondria were reisolated and half of each group incubated with the vitamin K_1-albumin emulsion for 5 min. All operations prior to the final Warburg incubation were carried out in the cold.

Irradiation with 3600 Å light severely affects the oxidation of both succinate and glutamate and the accompanying phosphorylations (Table I). Values for identical experiments after exposure

TABLE I

THE EFFECT OF VITAMIN K_1 ON 3600 Å EXPOSED MITOCHONDRIA

Each Warburg vessel contained: Mitochondria, 0.05 rat-liver equivalent; KCl, 150 μmoles; orthophosphate, 50 μmoles; adenylic acid, 4.3 μmoles; glucose, 60 μmoles; sucrose, 125 μmoles; succinate or glutamate, 30 μmoles; Mg^{++}, 7.5 μmoles; hexokinase, in excess. Reaction vol. 2 ml. When present, vitamin K_1, 10^{-8} *M*; crystalline serum albumin, 4 mg; cytochrome *c*, $4 \cdot 10^{-5}$ *M*.

System	*Substrate*	*Additions*	*Oxygen consumption (μatoms)*	*P esterification (μmoles)*	*P/O*
Control	succinate		16.9	32.2	1.90
Control	succinate	K_1 + cyt. *c*	16.1	29.6	1.84
Irrad.	succinate		7.9	5.3	0.67
Irrad.	succinate	albumin	7.9	5.8	0.74
Irrad.	succinate	K_1	6.4	3.9	0.59
Irrad.	succinate	cyt. *c*	14.7	23.4	1.59
Irrad.	succinate	K_1 + cyt. *c*	15.1	22.7	1.50
Control	glutamate		17.5	45.6	2.61
Control	glutamate	K_1 + cyt. *c*	16.4	41.3	2.52
Irrad.	glutamate		4.7	3.7	0.79
Irrad.	glutamate	albumin	3.9	2.8	0.71
Irrad.	glutamate	K_1	9.4	14.6	1.55
Irrad.	glutamate	cyt. *c*	4.2	2.8	0.67
Irrad.	glutamate	K_1 + cyt. *c*	14.9	34.4	2.31

to 2600 Å light are not presented since all efforts to restore oxidation and phosphorylation have been without success. Following 3600 Å exposure, cytochrome *c* is capable of restoring oxidation and phosphorylation in the succinate, but not in the diphosphopyridine nucleotide (DPN)-dependent glutamate system. Vitamin K_1 alone partially restores the glutamate system and vitamin K_1 plus cytochrome *c* restores this system to near control values. These preliminary data tend to support the thesis of BRODIE *et al.*[5] that "vitamin K_1 is involved as a coenzyme in both electron transport and coupled oxidative phosphorylation", and place the site of action of vitamin K_1 between DPN-dependent dehydrogenase and the common link between the DPNH and succinic oxidase systems. Under the conditions of these experiments neither menadione nor α-tocopherol will replace vitamin K_1.

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