The uncoupling of oxidative phosphorylation by thyroxine and triiodothyronine in ultrasonic extracts of liver mitochondria*

Recent reports[1,2,3] have described methods for preparing fragmented mitochondria in which phosphorylation is coupled to electron transport. The preparations obtained by treatment with digitonin[1] or sonic oscillation[3] differed from intact mitochondria in that oxidative phosphorylation was not uncoupled by thyroxine. In this communication we wish to report that mitochondrial fragments prepared in the sonic oscillator by a slight modification of the procedure of KIELLEY AND BRONK[3] are uncoupled by thyroxine and triiodothyronine. The effects of the hormones on the fragments are compared to the results obtained with intact mitochondria.

The mitochondria from about 35 g of rat liver were isolated in sucrose according to the method of SCHNEIDER[4] and were then washed once with a 0.012 *M* KF – 0.050 *M* *K* phosphate (pH 7.0) solution and resuspended in 10 to 15 ml of the same media. The mitochondria were treated in a 10-kc Raytheon oscillator for 45 sec and the suspension was centrifuged at 25,000 × *g* for 20 min. The supernatant was spun again at 25,000 × *g* for 20 min and the supernatant from the second centrifugation was used for the determination of oxidative phosphorylation. The O_2 uptake was measured by the conventional Warburg technic, and the phosphate esterification was estimated by the disappearance of inorganic phosphate using glucose and hexokinase as the acceptor system. The sonic extract had approximately the same Q_{O_2} as the intact mitochondria. In agreement with KIELLEY AND BRONK[3], P:O ratios greater than 1.0 have not been obtained with either succinate or β-hydroxybutyrate (Table I). The phosphorylation associated with the oxidation of succinate was almost completely uncoupled by $3.3 \cdot 10^{-5}$ *M* dinitrophenol.

TABLE I

THE UNCOUPLING OF OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIAL FRAGMENTS

The test system contained 50 μmoles K phosphate (pH 7.0), 12 μmoles KF, 15 μmoles $MgCl_2$, 60 μmoles succinate, 3 μmoles adenosine diphosphate, 150 μmoles glucose, and purified yeast hexokinase. When β-hydroxybutyrate (60 μmoles) was substrate, 2 μmoles diphosphopyridine nucleotide were added. Final volume, 3.0 ml. Temperature 28°. The incubations were continued until the O_2 consumption reached about 8.5 μatoms, which required from 12 to 18 min.

Substrate	*Uncoupling reagent*	*Concentration (M)*	*O_2 uptake (μatoms/15 min)*	*Phosphate esterification (μmoles/15 min)*	*P:O*
Succinate	None		8.84	8.81	1.0
	Dinitrophenol	$3.3 \cdot 10^{-5}$	10.02	0.86	0.09
	None		11.30	9.15	0.81
	Thyroxine	$1 \cdot 10^{-4}$	10.40	6.53	0.63
		$2 \cdot 10^{-4}$	9.85	2.29	0.23
	Triiodothyronine	$1 \cdot 10^{-4}$	10.78	6.21	0.58
		$2 \cdot 10^{-4}$	9.28	1.67	0.18
	Triiodothyro-acetic acid	$1 \cdot 10^{-4}$	5.76	1.75	0.30
		$2 \cdot 10^{-4}$	4.32	0.43	0.11
β-Hydroxy-butyrate	None		7.22	6.55	0.91
	Thyroxine	$2 \cdot 10^{-4}$	8.70	5.57	0.64
		$3 \cdot 10^{-4}$	7.43	3.51	0.47
		$4 \cdot 10^{-4}$	8.28	2.36	0.28

In contrast to the earlier preparations[1,3], phosphorylation in these fragments was sensitive to thyroxine** and triiodothyronine. Using succinate as substrate, both hormones lowered the P:O ratio from 0.8 to values of approximately 0.2, the extent of lowering depending on the concentration of the hormone employed. The 4–15% decrease in O_2 uptake was small compared to the reduction in the phosphate esterification. Triiodothyroacetic acid not only blocked the phosphorylation but also decreased O_2 consumption by 50% or more. The oxidation of β-hydroxybutyrate required the addition of diphosphopyridine nucleotide and twice as much extract in order to obtain sufficiently rapid rates for manometric measurement. Since the degree of uncoupling is related to the concentration of extract, twice as much thyroxine, $4 \cdot 10^{-4}$ *M*, was needed to produce

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** The samples of thyroxine, triiodothyronine, and triiodothyroacetic acid were gifts from Smith, Kline & French Laboratories.

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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