The action of thyroxine on mitochondria and oxidative phosphorylation

It has been demonstrated in several laboratories that *in vitro* addition of thyroxine or related thyroid-active compounds to isolated mitochondria causes uncoupling of phosphorylation from electron transport under certain experimental conditions, an action also shown by a variety of structurally similar nitro- and halophenols[1-3]. Such experiments have suggested that uncoupling of oxidative phosphorylation, with consequent compensatory changes in respiratory rate, represents the physiological mode of action of the thyroid hormone.

Work of MARTIUS AND HESS has shown that thyroxine uncouples oxidative phosphorylation in *rat* liver mitochondria only if the hormone is preincubated with the mitochondria[1]. Some difficulty in reproducing these experiments with rat liver mitochondria led HOCH AND LIPMANN to study such uncoupling effects with mitochondria isolated from hamster liver[2], which are presumably more permeable to thyroxine. We have found that uncoupling of oxidative phosphorylation in *rat* liver mitochondria by thyroxine and triiodothyronine may be regularly and reproducibly observed if the mitochondria are first suspended briefly in hypotonic sucrose, a treatment already known to cause alterations in the permeability and in the enzymic organization of mitochondria[4]. In Table I it is seen that the *in vitro* addition of thyroxine does not uncouple phosphorylation in rat liver mitochondria held in isotonic media. However, following a short preincubation under hypotonic conditions the addition of thyroxine causes immediate and considerable uncoupling, which in many experiments is complete. In confirmation of BAIN[5], magnesium was found to reverse the thyroxine-induced uncoupling, but does not affect that produced by 2,4-dinitrophenol (DNP).

TABLE I

EFFECT OF HYPOTONIC PRETREATMENT OF MITOCHONDRIA

L-Thyroxine	P:O		
	0.25 M Sucrose	*0.15 M Sucrose*	*0.075 M Sucrose*
None	1.4	1.3	1.1
$7.5 \cdot 10^{-5}$ *M*	1.4	1.1	0.4

Rat liver mitochondria were suspended for 10 minutes prior to phosphorylation test in varying sucrose concentrations at 0° C. The phosphorylation test medium consisted of 0.01 *M* BOH, $1.5 \cdot 10^{-5}$ *M* cytochrome *c*, 0.001 *M* DPN, 0.01 *M* KF, 0.005 *M* ADP, 0.0125 *M* phosphate buffer pH 7.4, and thyroxine as shown. Incubated 8 min at 30° C.

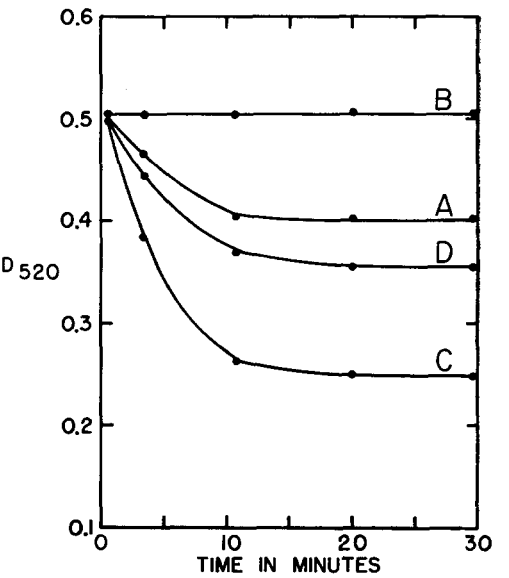

Fig. 1. Effect of thyroxine and DNP on swelling. Mitochondria were suspended in 0.3 *M* sucrose and 0.02 *M* tris pH 7.4 (total volume 4.5 ml) at 23–25° C; additions were made as shown below. The first reading was taken 10 seconds after addition of mitochondria to medium. Curve A, control; curve B, 10^{-4} *M* DNP; curve C, $3 \cdot 10^{-5}$ *M* L-thyroxine; curve D, 10^{-4} *M* DNP plus $3 \cdot 10^{-5}$ *M* L-thyroxine.

The association of the uncoupling effects of thyroxine with alterations in mitochondrial structure as described above and the report by AEBI AND ABELIN that the mitochondria of hyperthyroid tissues are "swollen"[6] have suggested the possibility that thyroxine might primarily affect some aspect of mitochondrial structure. By following the changes in light-scattering of mitochondrial suspensions[7], we have found that thyroxine and triiodothyronine in very low concentrations caused rapid swelling of mitochondria in an isotonic medium[8]. In striking contrast, DNP, to which thyroxine has often been compared in its action *in vitro*, caused no swelling whatsoever, but was in fact a potent stabilizing agent (Fig. 1). The swelling effects of thyroxine were exerted by concentrations one-tenth those required to cause uncoupling of phosphorylation (*i.e.* $1 \cdot 10^{-6}$ *M*). The swelling action of thyroxine could be prevented by addition of Mg^{++}, DNP, or ATP. These findings thus suggest that DNP and thyroxine differ radically in their mode of action on mitochondria, although both uncouple oxidative phosphorylation.

Such a fundamental difference between DNP and thyroxine was more strikingly and conclusively demonstrated by study of the uncoupling of oxidative phosphorylation as it occurs in a multi-enzyme complex isolated from digitonin extracts of rat liver mitochondria[9]. This enzyme preparation does not possess the highly developed morphology and highly complex organization of the intact mitochondrion. Phosphorylation coupled to the oxidation of β-hydroxybutyrate (BOH) by molecular oxygen in such isolated enzyme preparations was found to be completely uncoupled by low concentrations of DNP, pentachlorophenol, and gramicidin, for instance, but was not uncoupled in the presence of D- or L-thyroxine in concentrations up to $5 \cdot 10^{-3}$ *M* under any circumstances tested (Table II), which included preincubation with the enzyme, variation of pH, and variation of Mg^{++} concentration. Thyroxine thus resembles Ca^{++} in that it causes swelling and uncouples phosphorylation in mitochondria but has no uncoupling effect on the isolated phosphorylating enzyme complex[9,10].

TABLE II

EFFECT OF UNCOUPLING AGENTS ON THE ISOLATED ENZYME COMPLEX

Addition	*None*	$5 \cdot 10^{-5}$ *M DNP*	$5 \cdot 10^{-3}$ *M L-Thyroxine*	$5 \cdot 10^{-3}$ *M L-Triiodothyronine*
P:O	2.1	0.0	2.0	2.5

The medium consisted of 0.01 *M* DL-BOH, $2.4 \cdot 10^{-3}$ *M* ADP, 0.01 *M* phosphate pH 6.5. Enzyme preincubated with phosphate and addition for 5 min. Final volume, 3.0 ml. Incubated 20 min at 25° C.

These findings, which will be described more fully elsewhere, therefore indicate that uncoupling of phosphorylation induced by thyroxine in mitochondria does not occur by direct interaction of the hormone with the enzymes of oxidative phosphorylation. Rather they suggest that the *in vitro* action of thyroxine is concerned with control of some structural property of the mitochondrion, or some other enzymic function. Although it is not yet proved that any of the *in vitro* effects of thyroxine on mitochondria yet described is related to the physiological mode of action of the thyroid hormone, other experiments to be reported have revealed that mitochondria isolated from hyperthyroid rats swell more readily and those from hypothyroid rats swell less readily than those from normal animals.

This investigation was supported by grants from the U.S. Public Health Service, The Nutrition Foundation, Inc., and the National Science Foundation.

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Received October 7th, 1955

* Life Insurance Medical Research Fund Fellow.
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