On the Relationship of Liver Mitochondrial Coenzyme Q to Hyperthyroidism in Rats[1] (34848)

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Although the overt physiological effects of thyroid hormones have been known for over 70 years, the mechanisms by which these effects are produced still are not understood in fundamental terms. It has been shown that in some systems at least, thyroxine has an anabolic effect in enhanced biosynthesis of mitochondrial protein (1). This could be explained in part by enhanced production of ATP, which is required for activation of amino acids for protein synthesis. The authors suggested that the acceleration of metabolic rate by thyroxine may be secondary to the stimulation of energy-requiring reactions such as protein synthesis.

Yet it is inescapable that at excessive levels thyroid hormones have a net catabolic action. There is excessive oxidative activity and heat production representing inefficient energy transduction from calorigenic components of the animal's diet and of his own tissues. This leads to loss in weight or, in young animals, to lessened gain in weight. In biochemical terms this probably means that the thyroid hormones "uncouple" oxidative phosphorylation by releasing electron transport from its usual regulation by sufficient ATP levels and sufficiently lowered levels of ADP.

That coenzyme Q has a role in electron transport appears to be established (2). In derived form it is considered by some to be a phosphorylated intermediate for generation of ATP (3–7). If both of these postulates are correct, one can logically assume that in efficient, tightly coupled phosphorylation, mitochondrial content of coenzyme Q should not be below a certain functional level, and that if thyroidal uncoupling action is exerted at this site, mitochondrial content of coenzyme Q should be lowered by excess of thyroid hormones.

One must consider the possibility that excess thyroid hormones increase measured coenzyme Q levels while at the same time uncoupling phosphorylation. This would be the result if the analysis for coenzyme Q were responsive to coenzyme Q but not to the phosphorylated coenzyme Q derivative which has been postulated.

It also has been proposed (8, 9), however, that the role of coenzyme Q is to provide an alternate (to cytochrome *b*) route between flavoproteins and cytochrome *c* and that this is a nonphosphorylating route. If correct, thyrotoxic levels of thyroid hormones might in this case also be expected to produce higher levels of coenzyme Q rather than lower levels. It has, in fact, been reported to do so in rat tissues (10, 11).

The main purpose of the present study was to ascertain the effect of excess thyroid hormones on specifically mitochondrial coenzyme Q and to relate this, if possible, to functional efficiency of the mitochondria as judged by P/O ratios. In view of the derivation of O-bound methyl groups of coenzyme Q from methionine (12) and reports that methionine has an antithyrotoxic action in chicks (13), methionine supplementation has been included in the study to determine if it has any ability to reverse the effects of excessive thyroid hormones in rats at the mitochondrial coenzyme Q level.

Materials and Methods. Weanling male white rats (National Animal Laboratories)

[1] This study was supported in part by Public Health Service Research Grant AM-09246 from the National Institutes of Health. Published with the approval of the Director of the Colorado State University Experiment Station as Scientific Series Paper No. 1515.

were individually marked, caged in groups of five (two such groups, or ten rats, per treatment) in an air-conditioned rodent room according to dietary treatments being employed, and given their assigned diets and water *ad libitum* until sacrificed. To assess body weight gains on the different regimens, they were weighed weekly or oftener and at time of sacrifice.

The four dietary treatments were (a) basal (deficient in methionine–cystine but otherwise nutritionally balanced); (b) basal plus 0.25% DL-methionine (Met); (c) basal plus 0.40% desiccated thyroid (DT); and (d) basal plus both DL-methionine and desiccated thyroid at the same respective levels.

At 3, 5, and 7 weeks after placement on diets, three rats were sacrificed each time from each treatment. The entire experiment was performed three times so that three replicate samplings were available from each of the treatments at each age on experiment.

After terminal weighing, the rats were sacrificed by exposure to chloroform. Livers were removed immediately and weighed. A 5-g portion of each was homogenized in a glass Tri-R homogenizing tube containing 30 ml of chilled 0.25 *M* sucrose. Mitochondria were separated immediately by differential ultracentrifugation. The nuclei, unbroken cells, erythrocytes, and any other large particles were centrifuged down at 600*g* for 10 min in a refrigerated centrifuge (2500 rpm, head 856, International HR-1). The decanted supernatant fluid was recentrifuged at 8500*g* (8250 rpm, head 858) for 10 min, sedimenting the mitochondria. The sediment was washed twice by resuspension in 0.25 *M* sucrose (2 ml/g liver represented) and finally resuspended in 0.25 *M* sucrose. Oxygen uptakes of portions of the suspension were measured in a Warburg constant-volume respirometer at 5 hr and again at 7 hr after sacrifice. The mitochondrial suspension was kept as near 0° as possible during centrifugation and subsequent storage until coenzyme Q extraction was begun.

Phosphorus uptake was determined by measurement of inorganic phosphate before and after the periods of incubation for oxygen uptake by the method of Fiske and SubbaRow (14). Extraction of coenzyme Q was begun 24 hr after sacrifice of the rats following the procedure of Beyer *et al.* (9). The final purified extract was subjected to spectrophotometric assay using Craven's test (15). Nitrogen content of the mitochondrial suspension was determined by the method of Koch and McMeekin (16) to provide a comparative and accurate basis for expression of results.

The data were tabulated and subjected to

TABLE I. Effects of Dietary Desiccated Thyroid and Methionine on Body Weight Gain and Efficiency of Feed Utilization by Rats.

	Av body wt gain at 2 weeks (g)[a]				Group apparent feed conversion (%)[b]			
	Basal	DT	Met	Combination	Basal	DT	Met	Combination
Exp. 1	74.1	57.8	82.9	65.7	31.6	22.7	47.3	29.3
Exp. 2	80.0	69.5	84.5	80.4	36.3	29.2	39.4	31.1
Exp. 3	81.4	72.6	84.9	79.1	33.7	34.4	35.4	34.2
All	78.5	66.6	84.1	75.1	33.9	28.8	40.7	31.5

[a] There were 10 rats per treatment in each experiment.

[b] Determined at 2 weeks in Exp. 1 and at 3 weeks, just prior to first sampling, in Exp. 2 and 3.

Mean body weight gain differences were subjected to the F test for significance, with results as follows:

for Basal vs Dt, $F = 21.7$
for Basal vs Met, $F = 8.7$
for Combination vs DT, $F = 7.7$
for Combination vs Met, $F = 11.2$
} All highly significant. With 59 degrees of freedom, $F_{0.01} = 7.1$

for Combination vs Basal, $F = 1.65$ (not significant)

TABLE II. Effects of Dietary Desiccated Thyroid and Methionine on Rat Liver Mitochondrial P/O Ratio and Content of Coenzyme Q.

Treatment	Av liver mitochondrial P/O ratio			Av liver mitochondrial coenzyme Q (μg/mg mitochondrial N)		
	3 weeks	5 weeks	7 weeks	3 weeks	5 weeks	7 weeks
Basal	2.475 (9)[a]	2.71 (9)	2.94 (10)	7.64 (9)	15.1 (9)	5.39 (11)
+ 0.40% desiccated thyroid	1.14 (9)	1.05 (9)	.687 (7)	9.42 (9)	22.2 (9)	14.4 (11)
+ 0.25% DL-methionine	2.23 (9)	2.42 (9)	2.54 (11)	6.19 (9)	9.30 (9)	5.08 (11)
Combination	1.32 (9)	1.08 (9)	1.05 (7)	10.3 (9)	18.1 (9)	13.4 (11)

[a] Numbers in parentheses are numbers of suspensions observed. For P/O ratios, measurements were made at 5 and 7 hr, and the data for both times are here combined.

analysis of variance using a computer.

Results and Discussion. Average body weight gains and group apparent feed conversions at 2 weeks on experiment are shown in Table I. The data demonstrate that DT and methionine influenced body weight gains in the living animals and that their effects were in opposite directions, as reported previously for chicks (*13*). This is borne out further by the data on apparent feed conversion, though these were not subject to statistical evaluation.

Liver mitochondrial P/O ratios and contents of coenzyme Q are shown as averages in Table II. The data were subjected to analysis of variance with results as discussed in the following.

P/O ratios. The methionine-sufficient and-deficient diets yielded P/O ratios approaching the theoretical maximum of 3, whereas these diets with DT added yielded considerably lower P/O ratios, particularly at 7 weeks of age. Comparing all P/O ratios from non-DT rats to all from DT-treated rats, keeping separate the 5-hr and 7-hr determinations, gave rise to Table III. Examination of this table shows that DT had a significant effect on P/O ratios and that methionine did not.

It appears that no effects other than that of DT were significant, save possibly that of increasing age of the rats as indicated by P/O ratios measured at 7 (but not 5) hr. This second (7-hr) measurement was designed to be a duplication of P/O ratios measured at 5 hr after sacrifice. The same mitochondrial suspension was used for both measurements and was kept from 0–4° during the 2-hr period between the two Warburg runs. But the results of the two runs did not correlate at all satisfactorily. It is assumed that despite the precautions taken, a functional change occurred in the mitochondria during the 2-hr period or as a result of that much additional handling. Notwithstanding this, the principal conclusion to be drawn is the same and survives statistically from either the 5-hr or the 7-hr P/O data (see Table III). Only DT significantly affected P/O ratios. To provide the broadest available base, the P/O data in Table II are a composite of both 5-hr and 7-hr data.

Coenzyme Q contents. Coenzyme Q values were determined on the same mitochondrial suspensions used for P/O determinations. The average values are shown, along with the P/O average ratios in Table II. It appears that DT caused increased levels of coenzyme Q in the liver mitochondria with methionine either sufficient or deficient and that meth-

TABLE III. Analysis of Variance. P/O Ratios of Liver Mitochondria.

Source	*df*	*F*	
		5-hour	7-hour
DT	1	53.720[a]	15.944[a]
Met	1	.00005	3.646
DT × Met	1	3.063	.991
Age	2	.907	4.152[a]
Age × DT	2	2.349	.712
Age × Met	2	.300	1.054
Age × DT × Met	2	.985	.413

[a] Significant at 5% level.

TABLE IV. Analysis of Variance. Coenzyme Q Contents of Liver Mitochondria.

Source	*df*	*F*
DT	1	33.729[a]
Met	1	3.030
DT × Met	1	.097
Age	2	20.385[a]
Age × DT	2	.567
Age × Met	2	1.724
Age × DT × Met	2	.279

[a] Significant at 5% level.

ionine was without effect. This appears to be warranted as a conclusion by results of analysis of variance, shown in Table IV.

The age of the rats was a determining factor for coenzyme Q content of mitochondria. There was also a suggestion of an age effect on P/O ratios. This was equivocal, but the effect on coenzyme Q content seems definite. Evidently, from Table II, liver mitochondrial content of coenzyme Q reached a peak at about 5 weeks of age. There was no corresponding inflection in P/O values.

Correlation of P/O ratios and coenzyme Q contents. Because DT decreased P/O ratios and increased coenzyme Q contents, the possibility of a causal relationship between these effects was of crucial interest. A negative correlation between P/O and coenzyme Q content of the mitochondria appears obvious in Table II, but the individual data were quite variable within treatments. Therefore, product-moment correlation coefficients were computed from those data. Negative correlations between P/O ratios and coenzyme Q contents were confirmed at the 5% confidence level for the effects of DT on these two variates, with and without supplemental methionine (Table V).

TABLE V. Correlation of Effects of Desiccated Thyroid on P/O Ratios and Coenzyme Q Contents of Liver Mitochondria.

	r			
	5-hr P/O	7-hr P/O	*df*	$r_{.05}$
Met-deficient	—.699	—.631	7	—.666
Met-supplemented	—.711	—.766	7	—.666

Thus, the possibility is presented that excess thyroid hormones administered to young rats decrease the efficiency of their liver mitochondria by a mechanism related to higher levels of coenzyme Q therein. This could be by increased utilization of the postulated nonphosphorylating coenzyme Q bypass for electron transport.

Methionine supplementation of the methionine-deficient diet was without effect on either P/O ratios or coenzyme Q content of rat liver mitochondria. Thus, there is no explanation in these terms of the ability of methionine to exert effects on overt animal performance in a direction opposite to those of excess thyroid. The notion of a specific antithyrotoxic effect of methionine is not supported by this study.

Summary. A study in young rats (weanling to 7 weeks of age) has shown that: (1) Liver mitochondria from rats with induced hyperthyroidism were less efficient as energy transducers than controls, as judged by lower P/O ratios. (2) Liver mitochondria from hyperthyroid rats contained more coenzyme Q than did controls. (3) Accordingly, coenzyme Q probably is not involved as an intermediate for phosphorylation; but rather, in the hyperthyroid state is in an alternate pathway of electron transport by-passing phosphorylation. (4) Methionine, under the conditions of the study, exerted no antithyrotoxic effect in terms of coenzyme Q content or P/O ratios of liver mitochondria.

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Received Feb. 9, 1970. P.S.E.B.M., 1970, Vol. 134.