Relationship of the P:O Ratio to Thyroid Hormone Content of Isolated Rat Liver Mitochondria[1] (34419)

RICHARD W. HENINGER,[2] FAITH N. MONG, AND EDWIN C. ALBRIGHT
(Introduced by D. M. Donaldson)

Departments of Physiology and Medicine, University of Wisconsin Medical School, Madison, Wisconsin 53706

The enhanced oxidative metabolism characteristic of hyperthyroidism is well documented. Furthermore, the literature contains an abundance of data relating to the effect of exogenous thyroid hormone administration both *in vivo* and *in vitro* on respiration and phosphorylation of rat liver mitochondria and submitochondrial particles under a variety of experimental conditions (1–4). Although a few investigations have involved the effect of thyroidectomy and either thyroxine or triodothyronine replacement *in vivo* on oxidative phosphorylation (5–8), no studies have been conducted which relate physiologic variations in mitochondrial thyroid hormone content to the P:O ratio.

With the advent of the isotopic equilibrium technique (9) it became possible to quantitatively measure endogenous thyroxine and triiodothyronine in mitochondrial preparations (10), thus making possible a correlation between endogenous thyroid hormone levels and P:O ratios in mitochondria. The experiments reported here were designed to study this relationship.

Materials and Methods. The isotope equilibrium method (9) for the determination of iodine-containing compounds in tissues was used for mitochondria as previously described (10).

Diets. All rats were initially fed an unlabeled iodine deficient ration[3] containing 0.06 μg of iodine[4]/g for 4 weeks. This was followed by the labeled ration to which sodium iodine (^{127}I) was added to raise the iodine content to 0.22 μg/g.

The ^{125}I (half-life 60 days) was obtained carrier-free from a commercial source[5]. Its purity was confirmed by chromatographic analysis. Approximately 30 mCi (1 mCi/animal) of the radioisotope were mixed as previously described (11), with the quantity of the ration required to feed the rats throughout the experiment. On the first day of feeding the labeled diet, it contained approximately 1.5 μCi of ^{125}I/g. The amount of iodine added to the diet as ^{125}I was negligible. Weighed aliquots were counted to establish the ^{125}I:^{127}I ratio and to check uniformity of mixing. The coefficient of variation of radioactivity in samples of labeled diet was 6%.

Preparation of animals. Male albino rats (Holtzman) with an initial weight of 150 g were used in all experiments. On arrival in the laboratory all animals were fed the unlabeled diet for 4 weeks. At the end of this time 22 rats were subjected to surgical thyroidectomy of varying completeness, while 8 nonoperated animals served as euthyroid controls. Following thyroidectomy all rats were fed the labeled diet for 30–45 days before sacrifice, a period sufficient to establish equilibrium between the specific activity of the diet and that of the iodine pools in the body (11). All animals received 0.5% calcium gluconate in the drinking water. Rats were killed by exsanguination, the livers were excised, blotted free of residual blood, rinsed with water, and chilled.

Preparation of mitochondria. Mitochondria from a weighed sample were prepared in

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[2] Present address: Dept. of Zoology, Brigham Young University, Provo, Utah.

[3] General Biochemicals Corp., Chagrin Falls, Ohio.

[4] Assayed by the Wisconsin Alumni Research Foundation, Madison, Wisconsin.

[5] Union Carbide Corp., Tuxedo, New York.

0.25 *M* sucrose by the method of Schneider (12) with the following modifications: (i) the nuclear fraction was rehomogenized once instead of twice, and (ii) the final mitochondrial sediment was made up to a volume equal to the initial weight of the tissue sample. Throughout the fractionation procedure, care was taken to obtain a pure preparation rather than a quantitative yield. All manipulations were performed in tubes cooled in ice.

The mitochondria (0.5 ml in 0.25 *M* sucrose) were added to chilled Warburg vessels containing 6 μM ATP, 50 μM phosphate buffer, pH 7.4, 15 μM $MgSO_4$ and 30 μM glutamate. The side arm contained 50 μM glucose and 0.5 mg of yeast hexokinase preparation. A final volume of 3.0 ml was made with 0.25 *M* sucrose. KOH (0.5 *N*) was placed in the center well. Incubation was carried out at 30°, with shaking, after an equilibration period of 8 min. The hexokinase was added to the reaction mixture after equilibration and zero-time flasks were removed for inorganic phosphate analysis according to the method of Lowry and Lopez (13). Oxygen consumption was measured during the next 14 min and net phosphate was calculated from the difference between zero-time flasks and the 14-min incubation flasks. From these data P:O ratios (μmoles of phosphate/μatoms of oxygen) were calculated.

Determination of mitochondrial thyroid hormone content: A duplicate of the original mitochondrial suspension was counted in a well-type scintillation counter to determine total radioactivity. Duplicate 0.1-ml aliquots were analyzed for total nitrogen content by the micro-Kjeldahl method. The remainder of the suspension was extracted for chromatography. Extraction and separation of labeled compounds was carried out as described previously (10). Data are expressed as mμg of thyroxine plus triiodothyronine per mg of nitrogen.

Results. Representative radiochromatograms of mitochondria from normal and completely thyroidectomized rats are shown in Fig. 1. The radioactivity in the mitochondria from the normal rats was present as iodide, thyroxine, and triiodothyronine whereas mitochondria from completely thyroidectomized rats showed only iodide. On the chromatograms from the partially thyroidectomized rats a substantial amount of the radioactivity was present as iodide but the mitochondria also contained varying amounts of thyroxine and triiodothyronine. In many of the latter samples triiodothyronine was not detectable since the total thyronine content was considerably reduced.

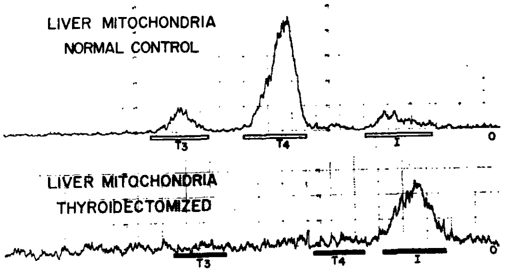

FIG. 1. Radiochromatograms of liver mitochondria from normal and completely thyroidectomized rats. O, origin; I, iodide; T_4, thyroxine; T_3, triiodothyronine; solvent front now shown; solvent system: tertiary amyl alcohol saturated with 2 *N* ammonium hydroxide.

The relationship between mitochondrial thyroxine plus triiodothyronine annd P:O ratio is illustrated in Figs. 2 and 3. In Fig. 2, individual values for each animal are shown. As shown, there is a progressive decrease in the P:O ratio as the hormone content of

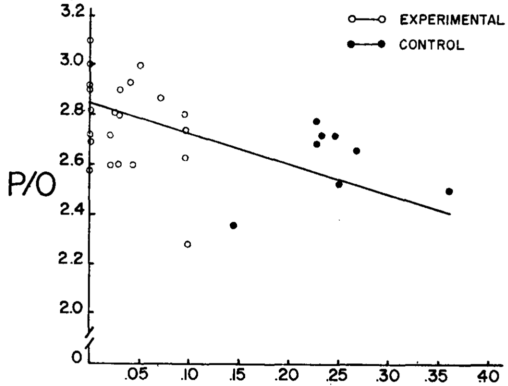

FIG. 2. Relationship between P:O ratio and mitochondrial thyroxine + triiodothyronine content. This represents a plot of data from individual rats. Each point is the mean of four P:O determinations.

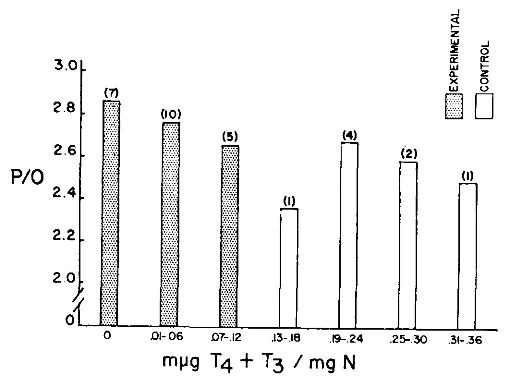

FIG. 3. Relationship between P:O ratio and mitochondrial thyroxine+ triiodothyronine content. This represents an interval plot of data from Fig. 2. The numbers in parentheses shows the number of animals within the designated interval.

the mitochondria increases. This is described by the regression equation $y = 2.85 - 2.02\ x$. When the data are grouped according to a range of mitochondrial hormone content (Fig. 3) the decrease in P:O ratio is more clearly evident.

The decrease in the P:O ratio seen in mitochondria from normal rats as compared to mitochondria from completely thyroidectomized rats was due to an increase in oxidation occurring in the normal rats; phosphorylating efficiency remained unchanged. This is shown in Table I.

In a previous experiment (14) a hyperthyroid condition was induced in rats by subjecting them to a cold environment (0–4°) for 2 weeks. In some of these animals mitochondria were prepared as described in this report and P:O ratios were determined; we have no data on the mitochondrial thyroid hormone content. The results of this experiment are shown in Table II. The mean P:O ratio of the control rats was 2.64 while the ratio for the hyperthyroid animals was significantly less at 2.24. The change in the ratio was brought about by an increase in oxidation and a decrease in phosphorylating efficiency.

Discussion. Reports in the literature up to this point have indicated that liver mitochondria from thyroidectomized rats have P:O ratios not significantly different from mitochondria of intact rats. Bronk and Bronk (7) found mitochondria from normal and thyroidectomized rats with succinate as substrate to have P:O ratios of 1.43 and 1.44, respectively, although oxygen consumption was less in the thyroidectomized animals. When thyroidectomized rats were given "physiological" doses of thyroxine or when the mitochondria were incubated *in vitro* with thyroxine there was an increase seen in both oxidation and phosphorylation with no change in P:O ratio (6, 7). Maley and Lardy (8) also observed in four experiments with thyroidectomized rats that there was no difference in P:O ratios from those of the control group. They did, however, observe that mitochondria from thyroidectomized rats exhibited a greater degree of acceleration of respiration when dinitrophenol was added than did the con-

TABLE I. The Influence of the Thyroid on the Oxidation of Glutamate and Phosphorylation.[a]

Nonthyroidectomized				Completely thyroidectomized			
Rat no.	O_2 uptake (μatoms)	Phosphates fixed (μmoles)	P:O	Rat no.	O_2 uptake (μatoms)	Phosphates fixed (μmoles)	P:O
N-1	10.46	24.76	2.36	T-1	8.92	25.97	2.91
N-2	10.25	25.92	2.53	T-4	8.82	27.32	3.10
N-3	9.90	26.31	2.69	T-16	9.35	27.26	2.92
N-4	9.87	24.64	2.49	T-18	8.52	23.86	2.82
N-5	9.46	24.94	2.66	T-20	9.91	25.44	2.58
N-6	9.98	27.13	2.72	T-22	9.06	27.23	3.01
N-7	9.00	24.94	2.78	T-24	7.70	20.88	2.72
N-8	9.65	24.26	2.52				
Mean	9.82	25.35	2.59		8.90	25.42	2.86

[a] A 14-min incubation period.

TABLE II. P:O Ratios in Mitochondria from Normal and Cold-Induced Hyperthyroid Rats.[a][b]

	Normal				Hyperthyroid		
Rat no.	O_2 uptake (μatoms)	Phosphates fixed (μmoles)	P:O	Rat no.	O_2 uptake (μatoms)	Phosphates fixed (μmoles)	P:O
1	9.65	26.34	2.75	5	9.47	23.06	2.44
2	9.13	23.85	2.63	6	10.76	24.10	2.24
3	10.57	27.54	2.61	7	10.78	22.62	2.10
4	9.52	24.57	2.58	8	10.27	22.50	2.19
Mean	9.72	25.57	2.64		10.32	23.07	2.24

[a] Each value represents the mean of 4 separate determinations on a single rat.

[b] A 14-min incubation period.

trols. It was concluded that normally occurring concentrations of thyroid hormone uncouple an oxidative phosphorylation reaction.

The present data indicate that the P:O ratio of thyroidectomized rats is significantly higher than that found in normal rats. Furthermore, there appears to be an inverse linear relationship between mitochondrial thyroid hormone content and P:O ratio. This would suggest that under a physiologic condition where the animal secretes less than normal amounts of thyroid hormone the tightness of the coupling of oxidation to phosphorylation is increased.

Maley and Lardy (8) also prepared mitochondria from thyrotoxic rats and reported P:O ratios 50% lower than controls. Smith and Fairhurst (15) observed that cold-adapted rats had an increased oxygen consumption and a decreased P:O ratio when compared with controls. Our data from the cold-induced hyperthyroid rats are in general agreement with these findings. Although we do not have the data on the mitochondrial thyroid hormone content in hyperthyroidism induced by cold exposure, we have observed in other experiments (14) that whole liver hormone content is elevated under these conditions. Thus it is reasonable to assume that the decrease in P:O ratio seen under these conditions is associated with an increase in liver mitochondrial hormone content.

Summary. An isotope equilibrium technique was used to measure mitochondrial thyroid hormone concentration in thyroidectomized, partially thyroidectomized, and intact rats in order to correlate this with the P:O ratios in the mitochondria from these animals. The data suggest that thyroidectomized rats have P:O ratios higher than normal rats. Furthermore, there appears to be an inverse linear relationship between mitochondrial thyroid hormone content and the P:O ratio.

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