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Effect of experimentally induced thyrotoxicosis on oxidative energy metabolism in rat heart mitochondria

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Abstract. Treatment of rats with T_3 resulted in a significant decrease in body weight, while the heart weight increased. T_4 treatment had less marked effect on body weights but resulted in decreased heart weights. Serum T_4 levels decreased significantly with simultaneous increase of T_3 level following T_3 treatment, whereas with T_4 treatment, levels of both T_4 and T_3 increased in the serum. Low doses of T_3 (0·5 μg) caused decrease in mitochondrial protein content while high dose of T_4 (1 μg), caused significant increase in mitochondrial mass. The state 3 respiration rates were significantly depressed following T_3 and T_4 treatments, in a substrate specific manner with the effects being more pronounced with T_3; these responses with T_4 were dose-dependent for succinate and ascorbate + N,N,N',N'-tetramethyl-*p*-phenylenediamme. State 4 respiration rates also exhibited similar corresponding changes. ADP/O ratios were not changed but ADP-phosphorylation rates were decreased significantly particularly so with the T_3-treated animals. Treatment with T_3 also resulted in lowering of intramitochondrial cytochrome contents. Similar effects were seen also with higher doses of T_4. The results thus indicate that T_3- and T_4-thyrotoxicosis results in impaired energy metabolism in heart mitochondria.

Keywords. Thyrotoxicosis; heart mitochondria; energy metabolism.

Introduction

Role of thyroid hormones in regulation of mitochondrial energy metabolism and their energy-linked functions and on the basal metabolic rate (BMR) is now well established (Tata *et al.,* 1963; Tata, 1964; Gustafsson *et al.,* 1965; Oppenheimer *et al.,* 1976; Oppenheimer, 1979; Bernal and De Groot, 1980). Deficiency of thyroid hormones causes decrease in metabolism with concomitant lowering of the basal metabolic rate (Tata, 1964). Treatment of hypothyroid animals with thyroid hormones restores these activities to normal or near normal levels, which is also accompanied by increase in the concentrations of specific respiratory components such as cytochromes and protein synthesis (Tata *et al.,* 1963; Tata, 1964; Gustafsson *et al.,* 1965; Katyare *et al.,* 1970).

Thyrotoxicosis—a condition of excessive levels of thyroid hormones—on the other hand, increases BMR and causes fast pulse (tachycardia), anxiety, palpitation, increased sweating, loss of weight, general muscular weakness, elevation of

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Abbreviations used: BMR, Basal metabolic rate; MOPS, 3-(N-morpholino) propanesulfonic acid; TMPD, N, N, N′, N′-tetramethyl-*p*-phenylenediamine; RIA, radioimmunoassay; RCR, respiratory control ratios; T_3, 3,3',5-triiodo-L-thyronine; T_4, L-thyroxine.

lysosomal cathepsin D activity and uncoupling of oxidative phosphorylation, which results in hyperthermia (Williams and Bakke, 1962; Johansen *et al.,* 1978; Irvine *et al.,* 1979; De Visscher and Burger, 1980; Jabbari and Huot, 1980; Satav and Katyare, 1981, 1982). Many reports are available on the effects of thyrotoxicosis on energy metabolism in mitochondria from tissues such as liver, kidney, brain and skeletal muscle (Maley and Lardy, 1953; Hoch and Lipmann, 1954; Dow, 1967; Stocker *et al.,* 1968; Winder *et al.,* 1975; Winder and Holloszy, 1977; Satav and Katyare, 1981, 1982). It has been shown that excessive levels of thyroid hormones affect the rates of coupled phosphorylation, ADP/O ratios, cytochrome contents and ATPase activity in mitochondria (Winder *et al.,* 1975; Winder and Holloszy, 1977; Satav and Katyare, 1982).

The effects of thyroid hormones on energy metabolism in the heart are not well understood. Tachycardia associated with thyrotoxicosis is accompanied by persistent atrial fibrilation resulting in transient episodes of arrhythmia (Hoffman and Lowery, 1960). Recent studies indicate that congestive heart failure can be produced in experimental animals by administering thyroxine (Shapiro *et al.,* 1975) and that infants with neonatal thyrotoxicosis without underlying cardiac disease may develop congestive heart failure (Shapiro *et al.,* 1975). The frequency of mitral valve prolapse is twice as much in hyperthyroid than in euthyroid individuals (Channide *et al.,* 1981).

The energy metabolism of the heart relates essentially to the contractile mechanisms of the myofibrils and oxidative phosphorylation in the mitochondria (Mela-Riker and Bukoski, 1985). In the present study an attempt has therefore been made to assess the effect of thyrotoxicosis on energy metabolism in heart by studying mitochondrial oxidative phosphorylation using experimental animal model. Results of the present studies together with those on altered membrane structure-function relationships (Dave *et al.,* 1989) have helped in illustrating and explaining the underlying biochemical lesions in heart pathology.

Materials and methods

Chemicals

Sodium salts of succinic acid, L-glutamic acid and ascorbic acid as well as 3,3′, 5-triiodo-L-thyronine (T_3), L-thyroxine (T_4), ADP, rotenone, Triton X-100, 3-(N-morpholino) propanesulfonic acid (MOPS), EDTA, EGTA and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. N,N,N′N′-tetramethyl-*p*-phenylenediamine (TMPD) was from British Drug Houses, Poole, Dorset, UK. All other chemicals used were of analytical-reagent grade. Radioimmunoassay (RIA) kits for thyroxine (code RIA-K5) and tri-iodothyronine (code RIA-K_4) were obtained from the Radiopharmaceuticals Division of Bhabha Atomic Research Centre, Bombay.

Animals

Male albino rats of Wistar strain of average weight 205·0 ± 4·4 g were used. The rats were made thyrotoxic by injecting subcutaneously 0·5 or 1 μg of L-T_3 or L-T_4/g

body weight (dissolved in 0·9% NaCl solution containing 5 mM NaOH) per day for 4 days (Carter *et al.,* 1981, 1982; Satav and Katyare, 1981, 1982). The animals were killed on the 5th day. Control animals received the corresponding amounts of saline vehicle. Concentrations of the hormones were adjusted such that the animals received 100 μl of hormone solution or saline vehicle per 100 g body weight. A record of initial and final body weights together with heart weight of both normal and thyrotoxic animals was maintained.

Serum T_3 and T_4

Serum samples were collected from control and thyrotoxic animals on the fifth day of the treatment and the contents of T_4 and T_3 in the sera were determined by RIA.

Isolation of mitochondria

Isolation of rat heart mitochondria was carried out essentially by the procedure described earlier (Doussiere *et al.,* 1984; Katyare, S. S., Rajan, R. R. and Gopalswamy, U. V., unpublished results). The rats were killed by decapitation and their hearts were quickly removed and placed in beakers containing chilled isolation medium (0–4°C), consisting of 0·225 M mannitol, 0·075 M sucrose and 1 mM EDTA, pH 7·4. The tissue was washed repeatedly with the isolation medium to free it from blood and was minced into small pieces (1·5 × 1·5 mm) and homogenized in a glass teflon Potter-Elvehjem homogenizer (wall clearance 0·18 mm) giving two up and down strokes. The homogenate (10% w/v) was centrifuged at 700 *g* for 10 min in a Sorvall RC5-C refrigerated centrifuge (0–4°C) to sediment nuclei and cell debris. The supernatant was subjected to a further centrifugation at 10,000 *g* for 10 min to sediment mitochondria. The mitochondrial pellet was washed once. Finally, the mitochondrial pellet obtained from 1 g of tissue was suspended in 1 ml of isolation medium and was used for the study of oxidative phosphorylation and measurement of cytochrome contents. All operations were carried out at 0–4°C.

Oxidative phosphorylation

Measurements of coupled phosphorylation were carried out at 25°C with a Clark type oxygen electrode essentially as described by Katyare *et al.* (1977). The medium (in a total volume of 1·3 ml) contained 225 mM sucrose, 20 mM KCl, 1 mM EGTA, 10 mM MOPS buffer (pH 7·4), 5 mM potassium phosphate buffer pH 7·4 and approximately 1 mg of mitochondrial protein. Concentrations of the substrates used were: glutamate (10 mM), pyruvate + malate (10+lmM), succinate (10 mM) and ascorbate (10 mM) plus TMPD (0·1 mM); with succinate and ascorbate + TMPD, 1 μM rotenone was also included in the reaction medium. About 150–250 nmol of AD P in 10–20 μl were added, and the rates of respiration in the presence of ADP (state 3) and after its depletion (state 4) were recorded. ADP/O ratios and the ADP-phosphorylation rates were calculated as described (Ferreira and Gil, 1984; Katyare *et al.,* 1977).

Mitochondrial cytochrome contents

Approximately 6–10 mg of mitochondrial proteins were solubilized using freshly prepared Triton X-100 solution at a final concentration of 0·1% and the volume was made up to 6 ml with isolation medium in which 10 mM potassium phosphate buffer pH 7·4 was also included. The difference spectra of dithionite-reduced minus ferricyanide-oxidized cytochromes were recorded as described by Satav and Katyare (1982). Contents of intra-mitochondrial cytochromes were calculated using the wavelength pairs and extinction coefficients for cytochromes aa_3, b and $c + c_1$, respectively, as described (Satav and Katyare, 1982).

Protein was estimated by the method of Lowry *et al.* (1951).

Results

The effects of T_3- and T_4-induced thyrotoxicosis on the body weights of the animals and heart weight are summarised in table 1. It is evident that treatment with T_3 brought about significant decrease in the body weights (17·8 and 28·0% decrease respectively for two doses employed). Treatment with T_4 was less effective and resulted in 8·5 and 16·7% decrease respectively with the two doses. The results thus emphasize the catabolic role played by T_3 in the experimental thyrotoxicosis. Similar findings have been reported by earlier research workers (Winder *et al.*, 1975; Satav and Katyare, 1982; Cote' and Boulet, 1985).

Table 1. Effect of thyrotoxicosis on body weight and heart weight.

		Heart weight	
Treatment	Body weight (g)	(g)	Body weight (%)
Control (12)	250·4 ± 3·3	0·863 ± 0·011	0·344 ± 0·006
0·5 μg T_3 (10)	205·9 ± 5·3***	1·149 ± 0·039***	0·556 ± 0·016***
1·0 μg T_3 (10)	180·0 ± 2·1***	0·990 ± 0·010***	0·551 ± 0·008***
0·5 μg T_4 (10)	229·1 ± 7·0**	0·878 ± 0·020^{NS}	0·385 ± 0·007***
1·0 μg T_4 (19)	208·7 ± 4·3***	0·679 ± 0·016***	0·326 ± 0·005*

Animals were given T_3 or T_4 (dissolved in 0·9 % NaCl containing 5 mM NaOH) in doses indicated/g body weight subcutaneously for 4 consecutive days.
Results are given as means ± SEM of the number of observations indicated in the parentheses.
*P<0·05, ** P< 0·02 and ***P<0·001 compared with control.
NS, Not significant.

Thyrotoxicosis also brought about marked changes in the heart weight in these animals (table 1). Thus treatment with T_3 resulted in cardiac hypertrophy and the absolute weight of the heart tissue increased by 15–33% with the lower dose of T_3 being more effective than the higher dose. The effects of T_4 treatment were less marked. Cardiac hypertrophy in rabbits receiving repeated injections of T_4 has been reported by Sinha *et al.* (1982).

The data on the content of T_4 and T_3 in the serum are given in table 2. It may be noticed that treatment with T_3 brought about a 50–57% decrease in serum T_4 levels. Lower dose of T_3 (0·5 μg) resulted in a small but reproducible increase in

Table 2. Effect of experimental thyrotoxicosis on serum T_3 and T_4 levels.

Treatment	T_4 (μg/dl)	T_3 (ng/dl)
Control	6·80 ± 0·81	120·4 ± 4·3
0·5 μg T_3	3·40 ± 0·32* [a]	134·0 ± 1·87**
1·0 μg T_3	2·94 ± 0·14***	1560·0 ± 67·82****
0·5 μg T_4	9·40 ± 1·10[NS]	752·0 ± 14·97****
1·0 μg T_4	15·70 ± 1·03****	1100·0 ± 57·01****

Experimental details are as given in table 1.
Results are given as mean ± SEM of 5 independent observations. *P< 0·01, **P< 0·02, ***P< 0·002 and ****P< 0·001 compared with control.
NS, Not significant.

serum T_3 levels (11% increase) while at the higher dose of 1 μg, the increase in serum T_3 levels was 13-fold. Levels of serum T_4 increased steadily with increasing doses of injected T_4 and amounted to 38 and 130% increase at the two doses employed. Serum T_3 levels also increased by 6-9-fold under these conditions.

The effect of thyrotoxicosis on protein content in the heart and the yield of mitochondrial proteins was next examined. These results are given in table 3. It can be seen that both T_3 as well as T_4 treatment caused significant increase in the protein content in the heart (23–86% increase) with only marginal changes in the yield of mitochondrial proteins except in the case of higher doses of T_4 where this value increased by almost 75%.

Table 3. Effect of thyrotoxicosis on protein content and mitochondrial yield in rat heart.

Treatment	Tissue protein content (mg/g tissue)	Yield of mitochondrial protein (mg/g tissue)
Control (4)	135 ± 9	10·0 ± 0·6
0·5 μg T_3 (4)	182 ± 6*	7·2 ± 0·6**
1·0 μg T_3 (4)	182 ± 3***	9·0 ± 0·8[NS]
0·5 μg T_4 (5)	167 ± 2**	8·6 ± 0·4[NS]
1·0 μg T_4 (5)	251 ± 15****	18·1 ± 0·8****

Experimental details are as given in table 1.
Results are given as mean ± SEM of number of observations indicated in the parentheses.
*P< 0·01, **P<0·02, ***P< 0·002 and ****P< 0·001 compared with control.
NS, Not significant.

The results on effects of thyrotoxicosis on oxidative phosphorylation are given in tables 4–7. The data in table 4 summarize the effects of thyrotoxicosis on oxidative phosphorylation in rat heart mitochondria with glutamate, as the substrate. It is evident that T_3 treatment resulted in approximately 70–76% decrease in the state 3 respiration rate. Effects of T_4, on the other hand, were apparent only at the higher

Table 4. Effect of thyrotoxicosis on oxidative phosphorylation in rat heart mitochondria with glutamate as substrate.

Treatment	ADP/O ratio	Respiration rate (nmol O_2/min/mg protein)		ADP-phosphorylation rate (nmol/min/mg protein)
		+ADP	−ADP	
Control (8)	3·0 ± 0·12	72·8 ± 5·0	9·8 ± 1·2	424·0 ± 17·1
0·5 μg T_3 (8)	2·6 ± 0·11	17·4 ± 1·3***	7·6 ± 0·6^{NS}	95·4 ± 3·3***
1·0 μg T_3 (8)	3·1 ± 0·05	21·6 ± 1·3***	5·6 ± 0·3*	133·2 ± 12·1***
0·5 μg T_4 (10)	3·1 ± 0·09	73·4 ± 4·6^{NS}	8·5 ± 1·2^{NS}	448·6 ± 19·8^{NS}
1·0 μg T_4 (8)	3·1 ± 0·08	48·8 ± 5·3*	4·6 ± 0·6**	294·6 ± 27·8**

Measurements of respiration rates were carried out at 25°C with a Clark-type oxygen electrode, employing a medium (total volume 1·3 ml) containing 225 mM sucrose, 20 mM KCl, 1 mM EGTA, 10 mM MOPS, pH 7·4 and 5 mM potassium phosphate buffer, pH 7·4. Calculations of state 3 and state 4 respiration rates, ADP/O ratio and ADP-phosphorylation rates were as described in the text.
Results are given as mean ± SEM of the number of observations indicated in the parentheses.
*P<0·01, **P<0·002 and ***P<0·001 compared with control.
NS, Not significant.

Table 5. Effect of thyrotoxicosis on oxidative phosphorylation in rat heart mitochondria using pyruvate + malate as the substrate.

Treatment	ADP/O	Respiration rate (nmol O_2/min/mg protein)		ADP-phosphorylation rate (nmol/min/mg protein)
		+ADP	−ADP	
Control (10)	3·0 ± 0·10	105·1 ± 5·9	19·1 ± 1·1	630·9 ± 42·0
0·5 μg T_3 (8)	3·1 ± 0·07	48·3 ± 3·1***	13·8 ± 0·2***	297·0 ± 22·9***
1·0 μg T_3 (8)	3·3 ± 0·06	56·0 ± 2·1***	10·0 ± 0·5***	371·4 ± 20·4***
0·5 μg T_4 (10)	3·0 ± 0·07	74·0 ± 4·1***	12·5 ± 0·9***	441·6 ± 29·7**
1·0 μg T_4 (8)	3·0 ± 0·06	76·1 ± 3·3***	11·8 ± 0·8***	457·7 ± 23·6*

Experimental details are as described in tables 1 and 4.
Results are given as mean ± SEM of the number of observations indicated in the parentheses. *P<0·01, **P< 0·002 and ***P< 0·001 compared with control.
NS, Not significant.

dose of 1 μg with a 33% decrease in the state 3 respiration rate. The state 4 respiration rates also decreased correspondingly but to a lesser extent following T_3 treatment so that the values of respiratory control ratios (RCR) were low in T_3-treated rats. By contrast, these values were somewhat higher for/T_4-treated animals (data not given). The ADP/O ratios were in normal range but ADP-phosphorylation rates decreased from 30-78% which is a reflection of decreased state 3 respiration rates.

When pyruvate + malate was employed as the substrate pair (table 5), the state 3 respiration rates decreased from 48–54% with T_3 treatment and from 28–30% with T_4 treatment respectively with corresponding decreases in state 4 respiration rates. Consequently, the RCR values were more or less unchanged (data not given). Thus the effects of thyrotoxicosis seemed to be different on pyruvate and malate than those seen for glutamate above (table 4). The ADP/O ratios were not altered.

Table 6. Effect of thyrotoxicosis on oxidative phosphorylation in rat heart mitochondria using succinate as the substrate.

Treatment	ADP/O ratio	Respiration rate (nmol O_2/min/mg protein)		ADP-phosphorylation rate (nmol/min/mg protein)
		+ADP	−ADP	
Control (10)	1·3 ± 0·08	245·1 ± 8·5	136·0 ± 12·1	614·1 ± 37·8
0·5 μg T_3 (10)	1·1 ± 0·07	155·6 ± 3·3***	104·2 ± 5·6*	318·0 ± 22·6***
1·0 μg T_3 (10)	1·2 ± 0·10	132·1 ± 4·2***	85·8 ± 5·7**	304·5 ± 28·5***
0·5 μg T_4 (10)	1·4 ± 0·06	222·8 ± 9·8^{NS}	108·6 ± 10·5^{NS}	637·5 ± 23·4^{NS}
1·0 μg T_4 (8)	1·3 ± 0·07	156·8 ± 9·8***	78·7 ± 7·7**	398·5 ± 28·3***

Experimental details are as described in tables 1 and 4.
Results are given as mean ± SEM of the number of observations indicated in the parentheses.
*P< 0·05, **P< 0·002 and ***P< 0·001 compared with control.
NS, Not significant.

Table 7. Effect of thyrotoxicosis on oxidative phosphorylation in rat heart mitochondria using ascorbate + TMPD as the substrate.

Treatment	ADP/O ratio	Respiration rate (nmol O_2/min/mg protein)		ADP-phosphorylation rate (nmol/min/mg protein)
		+ADP	−ADP	
Control (10)	0·33 ± 0·004	370·5 ± 14·6	179·6 ± 8·3	249·3 ± 9·6
0·5 μg T_3 (8)	0·32 ± 0·007	127·9 ± 7·8*	92·6 ± 3·2*	81·4 ± 5·5
1·0 μg T_3 (8)	0·33 ± 0·017	145·4 ± 9·2*	96·2 ± 8·0*	94·8 ± 6·2*
0·5 μg T_4 (10)	0·37 ± 0·050	254·6 ± 7·5*	114·8 ± 4·8*	191·3 ± 5·8*
1·0 μg T_4 (8)	0·37 ± 0·009	137·5 ± 3·3*	95·8 ± 6·7*	100·3 ± 2·9*

Experimental details are as described in tables 1 and 4.
Results are given as mean ± SEM of the number of observations indicated in the parentheses.
*P<0·001 compared with control.

However, as in the case of glutamate, the ADP-phosphorylation rates decreased significantly from 28–53%, the effects being more pronounced with T_3 treatment.

With succinate as the respiratory substrate, the state 3 respiration rates decreased by 37–46% and 9–36% respectively with T_3 and T_4 in a dose-dependant manner. Similar pattern was also noted for the state 4 respiration rates and the extent of decrease was 23–37% and 20–42% respectively (table 6).

For ascorbate + TMPD (table 7), T_3-toxicosis caused 61–66% decrease in the state 3 respiration rates with a concomitant 46–48% decrease in the state 4 respiration rates. Interestingly, with T_4, both state 3 and state 4 respiration rates decreased in a dose-dependant manner by 31–63% and 36–42% respectively. The general effects of thyrotoxicosis therefore seem to be decreased state 3 respiration which was substrate specific and thyroid hormone dose-dependant which was also reflected in terms of the ADP-phosphorylation. The ADP/O ratios and respiratory control ratios (data not given) were generally not affected by thyrotoxicosis.

The contents of mitochondrial cytochromes as affected by thyrotoxicosis are given in table 8. It may be noted that treatment with T_3 as well as T_4 caused 20-29% decrease in the cytochrome aa3 content. The contents of cytochromes c + c_1 also decreased somewhat following treatment with T_3 and higher doses of

Table 8. Effect of thyrotoxicosis on the mitochondrial cytochrome contents.

Treatment	Cytochrome content, nmol/mg mitochondrial protein		
	aa_3	b	$c + c_1$
Control (10)	0·624 ± 0·024	0·384 ± 0·024	0·854 ± 0·023
0·5 μg T_3 (8)	0·442 ± 0·016***	0·345 ± 0·027*	0·758 ± 0·027**
1·0 μg T_3 (8)	0·499 ± 0·015***	0·455 ± 0·018**	0·765 ± 0·025**
0·5 μg T_4 (6)	0·448 ± 0·030***	0·390 ± 0·046^{NS}	0·907 ± 0·058^{NS}
1·0 μg T_4 (10)	0·451 ± 0·017***	0·361 ± 0·029^{NS}	0·653 ± 0·045***

Experimental details are as given in table 1 and described in the text. Contents of cytochromes were calculated from the difference spectra as described in the text. Results are given as mean ± SEM of number of observations indicated in the parentheses.

*P<0·05, **P<0·02 and ***P<0·01 compared with control.

NS, Not significant.

T_4 (11–24% decrease). Cytochrome b content, however, increased at 1 μg T_3 dose by about 19 %, while registering a decrease at a low dose of T_3 (10% decrease). Treatment with T_4 had no effect on cytochrome b content.

Discussion

These studies assume importance because in human patients thyrotoxic condition is known to cause severe cardiac dysfunctions including tachycardia, anxiety, palpitation, loss of weight and general muscular weakness (Williams and Bakke, 1982). Effect of thyrotoxicosis on mitochondrial functions have, been investigated earlier by other researchers in various tissues. Some of these workers, however, have employed excessively higher doses of either T_3- or T_4- (Maley and Lardy, 1953; Hoch and Lipmann, 1954). Gustafsson *et al.* (1965) using hypothyroid rats concluded that thyroid hormones were required for maintaining the synchrony of turnover of mitochondrial protein components in the skeletal muscle. Holloszy and co-workers (Booth and Holloszy, 1975; Winder *et al.,* 1975; Winder and Holloszy, 1977) have concluded that thyrotoxicosis did not cause impairment of energy metabolism in the skeletal muscle, although turnover of cytochrome c was significantly influenced by thyroid-hormone-status. Stocker *et al.* (1968) concluded that thyrotoxicosis in dogs did not significantly influence energy metabolism in skeletal muscle mitochondria. Dow (1967) also did not find any abberation in the function of skeletal muscle mitochondria from thyrotoxic rats.

There are, not many reports in literature on the thyrotoxicosis effects on mitochondrial function of heart which comprises of specialized muscle tissue. Most of the investigators have directed their efforts to investigate effects of thyrotoxicosis on sarcolemmal and myosin ATPase activities (Goodkind *et al.,* 1974; Edelman and Ismail-Beigi, 1974; Banerjee *et al.,* 1976; Lo and Edelman, 1976; Curfman *et al.,* 1977; Litten *et al.,* 1981; Chizzonite *et al.,* 1982; Dillmann, 1982, 1985; Sinha *et al.,* 1982).

The investigations on energy metabolism reported in literature, were carried out employing either T_3- or T_4-thyrotoxicosis, at widely varying dose levels (Hoch and Lipmann, 1954; Dow, 1967; Booth and Holloszy, 1975; Winder *et al.,* 1975). In the

present investigation, an attempt has been made to examine systematically the dose-dependant effects of T_3- and T_4-thyrotoxicosis. These studies assume relevance since in human patients, cases of T_3-, T_4- and combined T_3- T_4- thyrotoxicosis have been reported (Nikkila and Pitkanen, 1959; Lin and Nankin, 1980).

The present studies had to be restricted to a lower dose of the hormones since higher doses led to excessive hyperthermia, tachyacardia and increased mortality rate. Nevertheless, the present studies have brought forth the significant observations that toxicosis induced by T_3-resulted in a severe impairement of active state 3 respiration in the cardiac mitochondria than did T_4-thyrotoxicosis, with most of the substrates examined excepting ascorbate + TMPD. Interestingly the effects were substrate specific and depended on the dose of T_3 or T_4 (tables 4–7). The net result was impaired rate of ATP synthesis with all the substrates employed. The enhanced contractile response of the hyperthyroid heart referred to above and the impaired rate of ATP synthesis as observed in the present studies may explain the fatigue in the cardiac muscle leading to the congestive heart failure. Another interesting feature was, unlike in case of other tissues such as liver, kidney and brain (Hoch and Lipmann, 1954; Satav and Katyare, 1982), heart mitochondria were not uncoupled *i.e.*, their ADP/O-ratios did not decrease with any of the substrates studied. This is similar to reported observations for skeletal muscle mitochondria (Dow, 1967; Stocker *et al.,* 1968). The impairement or dysfunction therefore seems to be localized to the electron transport system rather than the phosphorylating machinery of the mitochondria.

The decreased contents of cytochromes aa_3 and $c + c_1$ can but only partly explain the decreased respiration rates. In this connection, it is interesting to note that T_3-thyrotoxicosis resulted in a generalized decrease in the contents of cytochromes aa_3 and b with a concomitant increase in the $c + c_1$ component in liver and kidney mitochondria (Winder *et al.,* 1975; Winder and Holloszy, 1977; Satav and Katyare, 1982). Impaired primary dehydrogenase functions would therefore seem to be an interesting possibility to explore. It may however be pointed out here that thyroidectomy did not result in any significant changes in the primary dehydrogenases levels in tissues such as liver, kidney and brain (Katyare *et al.,* 1977). However, Dave *et al.* (1989) have shown that the kinetic properties of succinate oxidase and ATPase change significantly in thyrotoxicosis.

Examination of T_4 and T_3 content in serum (table 2) revealed that T_3 treatment, on the one hand, suppressed serum T_4 levels while elevating the T_3 levels. T_4 treatment, on the other hand, resulted in an overall increase in the serum levels of T_3 and T_4. However, the increase in T_3 level was not of the same magnitude as in the case of T_3 treatment. These observations are in agreement with those on respiration effects, and point out to an inverse relationship between serum T_3 level and the respiratory activity. Serum T_4 levels would therefore seem to play a less significant role in influencing the respiratory activity. Interestingly, in other studies it has been observed that T_4 rather than T_3 plays a prominent role in influencing the respiratory activity in rat brain (Katyare, S. S., unpublished results). It is interesting to note in this connection that recently it has been shown that tissue levels of T_3 and T_4 are important for tissue functions (Obregon *et al.,* 1981). It is possible that in thyrotoxicosis the heart tissue may accumulate disproportionately high amounts of T_3 and T_4 leading to mitochondrial dysfunction. Interestingly however, no catabolic influence (Winder *et al.,* 1975; Winder and Holloszy, 1977;

Carter *et al.,* 1981; Satav and Katyare, 1981; Carter *et al.,* 1982; Cote′ and Boulet, 1985) on tissue protein synthesis was seen since increased protein content and cardiac hypertrophy were already evident under these conditions (table 2).

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