J. Biosci., Vol. 14, Number 4, December 1989, pp. 341–349. © Printed in India.

Altered kinetic properties of rat heart mitochondrial enzymes following experimental-thyrotoxicosis

B. N. DAVE, F. R. BILLIMORIA[§] and S. S. KATYARE*†

Department of Biochemistry, L. T. M. Medical College and L. T. M. G. Hospital, Sion, Bombay 400 022, India

*Biochemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India

†Present address: Department of Biochemistry, Faculty of Science, M. S. University of Baroda, Baroda 390 002, India

MS received 26 October 1988; revised 7 August 1989

Abstract. Effects of T_3- and T_4-induced thyrotoxicosis on temperature-dependent Arrhenius kinetics of succinate oxidase and Mg^{2+}- and Mg^{2+} + 2,4-dinitrophenol-dependent ATPase activities in rat heart mitochondria were examined, For succinate oxidase system, treatment with T_3 and T_4 caused increase in the energy of activation in high temperature range in a dose-dependent manner. For low temperature range, increase in energy of activation was apparent only with higher doses of the hormones; with low doses a small but reproducible decrease was evinced. The phase transition temperature decreased significantly under these conditions. For the Mg^{2+}- and Mg^{2+}+2,4-dintrophenol-dependent-ATPase activities, the activation energy values in high temperature range decreased in general. Activation energy values in low temperature range recorded a generalized increase in the Mg^{2+}-ATPase enzyme system while the value did not change significantly for the Mg^{2+} + 2,4-dinitrophenol-ATPase; phase transition temperature registered a small but reproducible decrease under these conditions. The results are suggestive of increased membrane fluidization possibly through increased proportion of unsaturated fatty acids. The differential effects seen for succinate oxidase and ATPase systems are consistent with different lipid protein domains of these enzyme systems.

Keywords. Thyrotoxicosis; Arrhenius kinetics; ATPase, succinate oxidase.

Introduction

Regulation of mitochondrial membrane function by thyroid harmones is well documented (Tata *et al.,* 1963; Tata, 1964; Gustafsson *et al.,* 1965; Rajwade *et al.,* 1975; Satav *et al.,* 1976; Katyare *et al.,* 1977). These hormones, when administered in physiologic doses restore mitochondrial function in hypothyroid animals (Tata *et al.,* 1963; Tata, 1964; Gustafsson *et al.,* 1965), while hyperthyroidism, by contrast, leads to membrane dysfunction (Maley and Lardy, 1953; Hoch and Lipmann, 1954; Satav and Katyare, 1982; Katyare and Billimoria, 1989). Studies from our laboratory have shown that 3,3′, 5-triiodo-L-thyronine (T_3)- and L-thyroxine (T_4)-induced toxicosis resulted in impairment in energy coupling in heart mitochondria from experimental animals (Katyare and Billimoria, 1989). Studies by other research workers have shown that thyroid-hormone-status and hyperthyroidism also lead to membrane lipid alterations (Chen and Hoch, 1976; Hoch *et al.,* 1976, 1977; Hoch, 1977; Gnoni *et al.,* 1980; Pasquini *et al.,* 1980; Fass and Carter, 1981,

[§]To whom all correspondence should be addressed.

Abbreviations used: SDS, Sodium dodecyl sulphate; DNP, 2,4-dinitrophenol; T_t, phase transition temperature; E_1, high temperature range; E_2, low temperature range; T_3, 3,3′, 5-triiodo-L-thyronine; T_4, L-thyroxine.

1982; Hoch *et al.,* 1981; Ruggiero *et al.,* 1984). These membrane associated changes can be easily assessed in terms of possible alterations in temperature-dependent changes in enzyme kinetics (Raison *et al.,* 1971; Raison, 1972; Watson *et al.,* 1975a, b; Chen and Hoch, 1976, 1977; Hulbert, 1978). Our findings on such altered membrane characteristics of cardiac mitochondria following T_3- and T_4-thyrotoxicosis are described in the present communication. The observations reported here relate to examination of temperature-dependent changes in the activities of two enzyme systems of the respiratory assembly of heart mitochondria *viz.,* succinate oxidase and Mg^{2+}-, and Mg^{2+}+ 2,4-dinitrophenol (DNP)-dependent ATPases. The data have been analyzed in terms of Arrhenius plots (Raison *et al.,* 1971; Raison, 1972; Watson *et al.,* 1975a, b) and indicate that changes in lipid milieu may occur in the electron transport system following T_3- and T_4- induced thyrotoxicosis.

Materials and methods

Vanadium free ATP, sodium dodecyl sulphate (SDS), sodium succinate and T_3 and T_4 were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. DNP was purchased from the British Drug Houses, Dorset, Poole, UK and was recrystallized from benzene before use. Other chemicals used were of analytical-reagent grade.

Treatment of animals with T_3 and T_4 and isolation of heart mitochondria was according to the procedures already described (Katyare and Billimoria, 1989).

Succinate oxidase activity

Succinate oxidase activity was measured with a Clark-type oxygen electrode in a medium (total volume 1·3 ml) containing 67 mM potassium phosphate buffer pH 7·4, 0·4 mM $CaCl_2$ and 0·4 mM $A1C1_3$ essentially as described by Katyare *et al.* (1971). Mitochondria were incubated in the reaction medium for 2–5 min for maximum activation of the enzyme (Thorn, 1962; Katyare *et al.,* 1971) and respiration was then initiated with addition of 10 mM sodium succinate.

ATPase activity

The Mg^{2+}-, and Mg^{2+}+ DNP-dependent ATPase activities (Pullman and Penefsky, 1963) were measured in a medium (1ml) containing: 50 mM Tris-acetate buffer, pH 7·4, 2 mM $MgCl_2$ and 50–180 μg of mitochondrial proteins; 0·1 mM DNP was included wherever indicated. After incubation for 2–5 min, the reaction was started by adding 2 mM ATP (adjusted to pH 7·4 with Tris base). The reaction was terminated at the end of 10 min with 0·1 ml of 10% (w/v) SDS (Shallom and Katyare, 1985) and the inorganic phosphate liberated was estimated according to the method of Fiske and Subba Row (1925).

Linearity of the reaction with time under the assay condition was ascertained in separate experiments (data not given).

Protein was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

The analytical treatment of the data was essentially as described (Raison *et al.,* 1971; Raison, 1972; Watson *et al.,* 1975a, b). The log of specific activity of the

enzyme was plotted against reciprocal of absolute temperature to obtain Arrhenius plots and the values of energies of activation and phase transition temperature (T_t) were determined.

Results

Figure 1 represents the temperature-dependent changes in succinate oxidase activity in heart mitochondria as influenced by T_3- and T_4-induced thyrotoxicosis. It can be

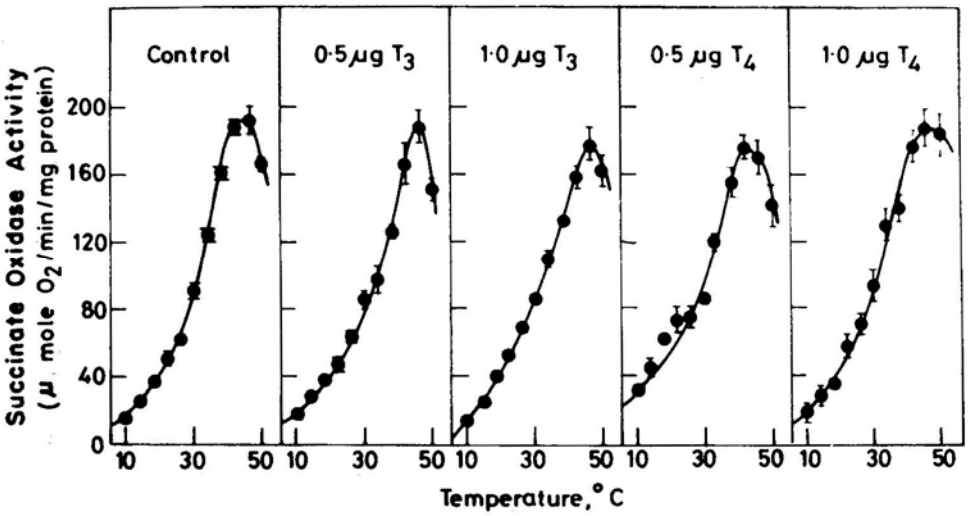

Figure 1. Temperature-dependent changes in succinate oxidase activity in rat heart mitochondria following T_3 and T_4 treatment, Male rats were given either T_3 or T_4 (0·5 or 1 μg hormone/g body weight) for 4 days. Succinate oxidase activity was measured with a Clark-type oxygen electrode.

Results are given as mean ± SEM of 4 animals.

noted that the maximum activation of the succinate oxidase activity occurred between 42–46°C. Nevertheless, the extent of activation was different for the various experimental groups compared to the control. It may be seen that the Arrhenius plots (figure 2) indeed reflect the changes induced by thyrotoxicosis with respect to the energies of activation in the high and low temperature ranges and T_t. The representative values for energies of activation and T_t are shown on the plots and mean values calculated from replicate observations are given in table 1. Thus it can be noted that energies of activation on the high temperature range (E_1) increased progressively with increasing doses of T_3 and T_4. Similar pattern was also noted for the energy of activation at low temperature range (E_2) particularly at the higher doses of T_3 and T_4. This agrees well with earlier observations on decreased succinate oxidation rates (Katyare and Billimoria, 1989). The T_t also showed progressive decrease with increasing doses of T_3 and T_4 and in fact at higher dose of the hormones, the T_t had decreased by almost 20°C.

The results on Mg^{2+}-ATPase activities as influenced by T_3- and T_4- treatment are shown in figure 3. The differential effect of thyrotoxicosis is once again apparent

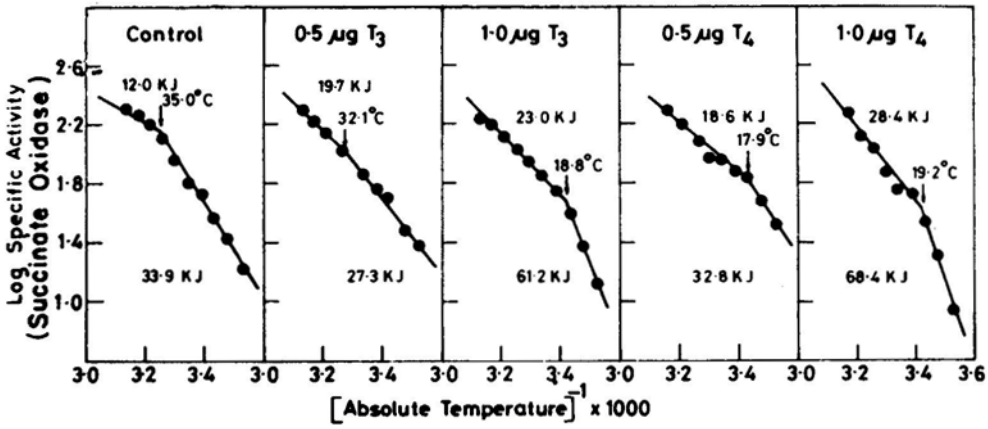

Figure 2. Arrhenius plots of log succinate oxidase activity against reciprocal of absolute temperature in rat heart mitochondira following T_3 and T_4 treatment.

The experimental details are as given in figure 1. Results are typical of 4 independent animals. Calculation of E_1, E_2 and T_t, were as detailed in the text.

Table 1. Effect of thyrotoxicosis on succinate oxidase activity in rat heart mitochondria

	Energy of activation (KJ/mol)		
Treatment	E_1	E_2	T_t
Control	9·1 ± 1·74	34·1 ± 1·42	38·0 ± 0·16
0·5 μg T_3	20·2 ± 1·04*****	29·5 ± 0·77*	34·4 ± 1·25*
1·0 μg T_3	24·6 ± 0·55*****	55·8 ± 4·95***	18·7 ± 0·34*****
0·5 μg T_4	18·9 ± 1·21***	27·9 ± 1·30**	20·4 ± 0·51*****
1·0 μg T_4	26·2 ± 2·81****	57·1 ± 5·13***	19·4 ± 1·40*****

Animals were injected with doses of T_3 or T_4 as indicated/g body weight for 4 consecutive days.
Other experimental details are as described in the text.
Results are given as mean ± SEM of 4 independent experiments.
*P <0·05, **P <0·02, ***P<0·01, ****P<0·002 and *****P<0·001 compared with control.

and is also reflected in Arrhenius plots shown in figure 4. The observed changes relating to energies of activation and T_t are summarized in table 2. Thus, it can be noted that in contrast to the succinate oxidase system, the values of E_1 decreased particularly at higher doses of T_3 and T_4 while the E_2 values increased in general except for 1 μg T_4 group. The value of T_t also decreased but to a lesser extent except for the 1 μg T_4 group, where no change in T_t was evident.

The data for Mg^{2+} + DNP-ATPase activities are shown in figures 5 and 6 respectively, and the values of E_1, E_2 and T_t are summarized in table 3. It can be noted that once again as in the case of Mg^{2+}-ATPase activity the values of E_1 decreased particularly very significantly at the higher doses of the hormones while those for E_2 showed a generalized increase. Decrease in T_t values was also recorded but this was of lesser magnitude, compared to the succinate oxidase system.

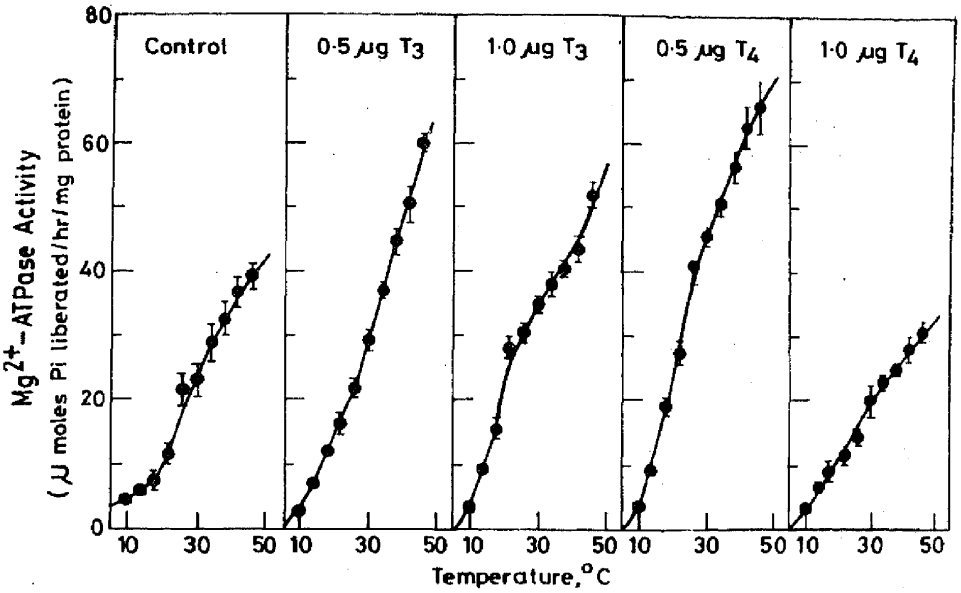

Figure 3. Temperature-dependent changes in Mg^{2+} -ATPase activity in rat heart mitochondria following T_3 and T_4 treatment.
Results are given as mean ± SEM of 4 independent animals.

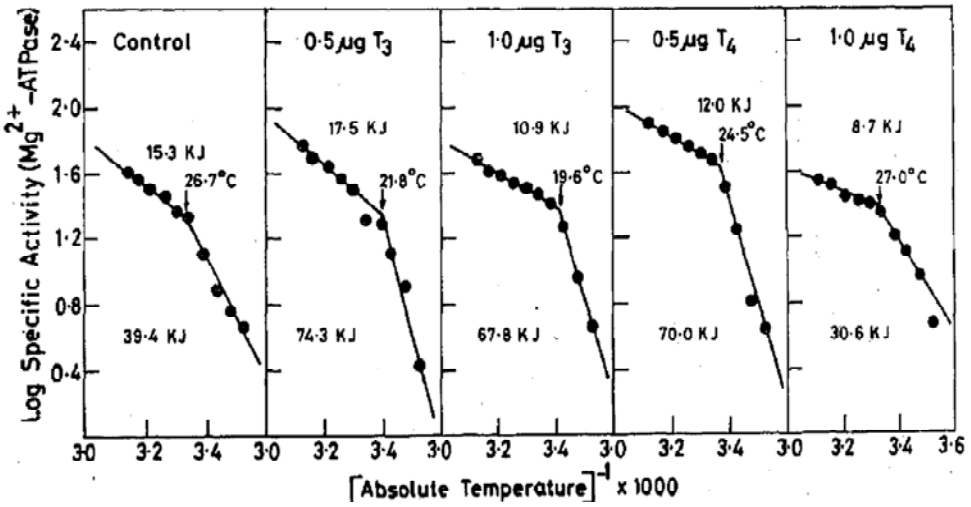

Figure 4. Arrhenius plots of log Mg^{2+}-ATPase activity against reciprocal of absolute temperature in rat heart mitochondria following T_3 and T_4 treatment.
Results are typical of 4 independent animals.

Discussion

The present investigations were undertaken to examine lipid-dependent perturbations in the membrane functions of rat heart mitochondria following treatment with T_3 or T_4. The results have clearly shown that the energies of activation for succinate oxidase increased in general, following hormone treatment whereas the T_t

Table 2. Effect of thyrotoxicosis on Mg^{2+}-ATPase activity in rat heart mitochondria.

Treatment	Energy of activation (KJ/mol)		
	E_1	E_2	T_t
Control	15·6 ± 1·44	37·7 ± 2·73	28·5 ± 1·08
0·5 μg T_3	19·1 ± 1·30^{NS}	63·7 ± 6·78**	24·1 ± 1·05*
1·0 μg T_3	11·3 ± 0·70*	69·2 ± 10·51*	21·6 ± 0·009****
0·5 μg T_4	13·1 ± 0·77^{NS}	68·3 ± 3·23***	23·3 ± 0·40***
1·0 μg T_4	9·8 ± 0·45**	30·9 ± 1·13^{NS}	28·7 ± 1·06^{NS}

Experimental details are as given in table 1.
Results are given as mean ± SEM of 4 independent experiments.
*P< 0·05, **P<0·01 and ***P<0·002 and ****P <0·001 compared with control.
NS, Not significant.

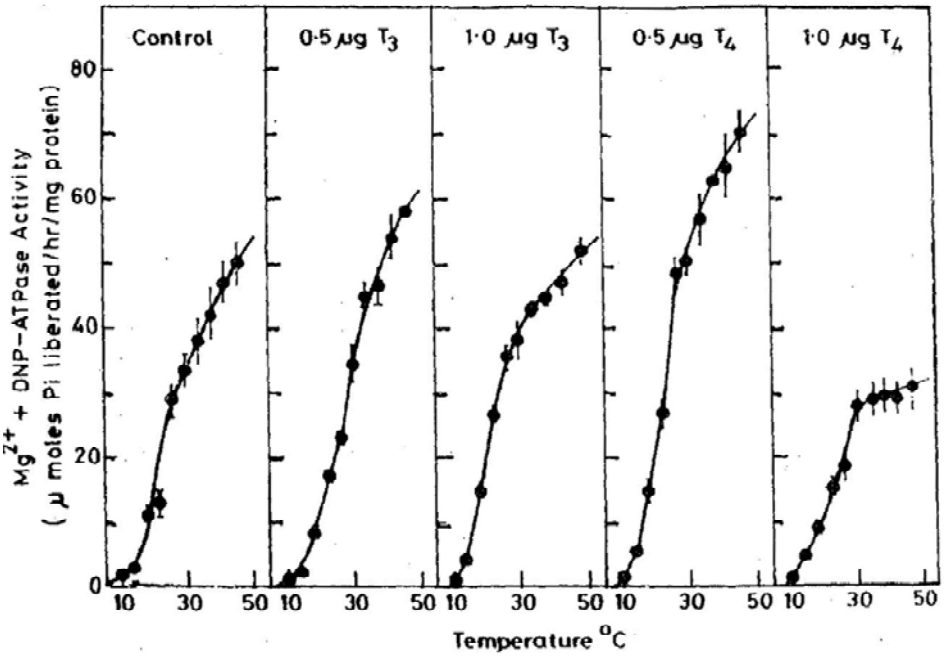

Figure 5. Temperature-dependent changes in Mg^{2+} + DNP-ATPase activity in rat heart mitochondria following T_3 and T_4 treatment.
Results are given as mean ± SEM of 4 independent animals.

decreased significantly (almost 20°C decrease). For the Mg^{2+}- and Mg^{2+} + DNP-dependent ATPase, the picture is somewhat different, *i. e.,* the energies of activation E_1 decreased in general following hormone treatment except in case of 0·5 μg T_3 and T_4 treatment. The values of E_2 increased only for the Mg^2 +-ATPase activity. The T_t recorded consistently a small but reproducible decrease amounting to 5–7°C. The observed changes are therefore indicative of differential alterations in the lipid environments of the two enzyme systems. Decrease in the T_t is taken as indicative of the increased degree of unsaturation in the fatty acids (Raison *et al.,*

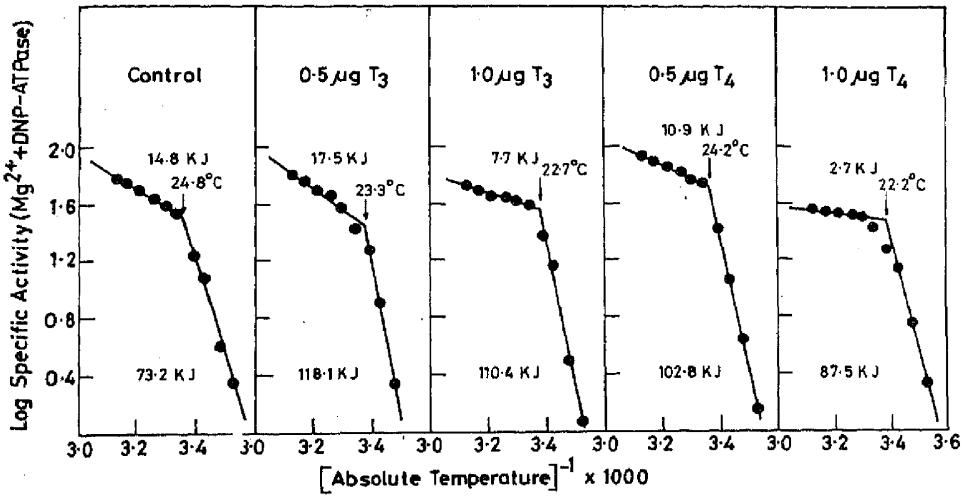

Figure 6. Arrhenius plots of log Mg^{2+} +DNP-ATPase activity against reciprocal of absolute temperature in rat heart mitochondria following T_3 and T_4 treatment.
Results are typical of 4 independent animals.

Table 3. Effect of thyrotoxicosis on Mg^{2+} + DNP-ATPase activity in rat heart mitochondria.

	Energy of activation (KJ/mol)		
Treatment	E_1	E_2	T_t
Control	15·0 ± 1·13	88·8 ± 10·19	27·1 ± 0·58
0·5 μg T_3	18·0 ± 0·55*	117·3 ± 9·44^{NS}	22·7 ± 0·44*
1·0 μg T_3	7·2 ± 0·26***	116·7 ± 12·55^{NS}	21·9 ± 0·62*
0·5 μg T_4	9·0 ± 1·13**	92·9 ± 3·06^{NS}	24·2 ± 0·56*
1·0 μg T_4	2·1 ± 0·34***	103·1 ± 12·53^{NS}	22·1 ± 0·38*

The experimental details are as given in table 1 and in the text.
Results are given as mean ± SEM of 4 independent experiments.
*P<0·05, **P<0·01 and ***P< 0·001 compared with control.
NS, Not significant.

1971; Raison, 1972; Watson *et al.,* 1975a, b). In this connection, it is interesting to note that in hypothyrodism total unsaturation of phospholipid fatty acid in rat liver mitochondrial inner membrane decreased significantly by 10% (Chen and Hoch, 1976). Abnormal Arrhenius profile for adenine nucleotide carrier of rat liver mitochondria in hypothyroid rats has been reported which was corrected after a single injection of L-T_4 (Hoch, 1977). The polyunsaturated fatty acid contents of the inner mitochondrial membrane were also similarly corrected (Chen and Hoch, 1976; Hoch *et al.,* 1976). The desaturation of essential polyenoic fatty acids is also accelerated by thyroid hormones *in vitro* (Fass *et al.,* 1972). Thyroid-hormone-dependent changes in fatty acid composition of microsomal and mitochondrial lipids in rat liver have also been reported by other research workers (Fass and Carter, 1981, 1982; Hoch *et al.,* 1981; Ruggiero *et al.,* 1984).

It is of interest, to note that the effects were differential for the succinate oxidase

and the ATPase systems. The succinate oxidase spans almost completely the entire respiratory chain and therefore several components of the electron transport system are involved. It would be obvious that the temperature-dependent changes observed for succinate oxidase system represent a composite and complex process involving several respiratory chain components. By contrast, for the ATPase system, the lipid-protein interactions pertain only to the single enzyme domain.

It may be mentioned here that the specific dependency of mitochondria ATPase on linoleoyl cardiolipin has been shown (Lopez-Moratalla *et al.,* 1973). Lipid-dependency of other mitochondrial enzymes such as *β*-hydroxybutyrate dehydrogenase, succinate dehydrogenase and cytochrome oxidase has also been reported (Vik and Capaldi, 1977; Abuirmeileh and Elsen, 1980; Ashraf *et al.,* 1980; Fry and Green, 1980, 1981). Stimulation of fatty acid biosynthesis in the liver of thyrotoxic rat and decreased phospholipid content and choline and CDP-choline incorporation in phosphatidyl choline in mitochondrial fraction have been reported (Gnoni *et al.,* 1980; Pasquini *et al.,* 1980). It is possible that the observed changes in profiles of succinate oxidase and ATPase systems may correlate with the observed decrease in state 3 respiration rates and ADP-phosphorylation rates (Katyare and Billimoria, 1989).

References

Abuirmeileh, N. M. and Elsen, G. E. (1980) *Lipids,* **15,** 925.

Ashraf, J., Somasundaram, T. and Jayaraman, J. (1980) *Biochem. Biophys. Res. Commun.,* **97,** 263.

Chen, Y. -D. I. and Hoch, F. L. (1976) *Arch. Biochem. Biophys.,* **172,** 741.

Chen, Y. -D. I. and Hoch, F. L. (1977) *Arch. Biochem. Biophys.,* **181,** 470.

Fass, F. H., Carter, W. J. and Wynn, J. (1972) *Endocrinology,* **91,** 1481.

Fass, F. H. and Carter, W. J. (1981) *Biochem. J.,* **193,** 845.

Fass, F. H. and Carter, W. J. (1982) *Biochem. J.,* **207,** 29.

Fiske, C. H. and Subba Row, Y. (1925) *J. Biol. Chem.,* **66,** 375.

Fry, M. M. and Green, D. E. (1980) *Biochem. Biophys. Res. Commun.,* **95,** 1238.

Fry, M. and Green, D. E. (1981) *J. Biol. Chem.,* **256,** 1874.

Gnoni, G. V. Landriscina, C. and Quagliariello, E. (1980) *FEBS Lett.,* **122,** 27.

Gustafsson, R., Tata, J. R., Linberg, O. and Ernster, L. (1965) *J. Cell Bilo..,* **26,** 555.

Hoch, F. *U* (1977) *Arch. Biochem. Biophys.,* **178,** 535.

Hoch, F. L. and Lipmann, F. (1954) *Proc. Natl Acad. Sci. USA,* **40,** 909.

Hoch, F. L., Chen, Y. -D. L, Evano, T. C, Shaw, M. J. and Neymark, M. A. (1976) in *Proc. Seventh International Thyroid Conference,* Boston, 1975 (eds J. Robbins and L. E. Braverman) (Amsterdam: Excerpta Medica), p. 347,

Hoch, F. L., Subramanian, C, Dhopeshwarkar, G. A. and Mead, J. F. (1981) *Lipids,* **16,** 328.

Hulbert, A. J. (1978) *J. Theor. Biol.,* **73,** 81.

Katyare, S. S., Fatterpaker, P. and Sreenivasan, A. (1971) *Indian J. Biochem. Biophys.,* **8,** 138.

Katyare, S. S., Joshi, M. V., Faterpaker, P. and Sreenivasan, A. (1977) *Arch. Biochem. Biophys.,* **182,** 155.

Katyare, S. S. and Billimoria, F. R. (1989) *J. Biosci.,* **14,** 329.

Lopez-Moratala, N., Segovia, J. L. and Santiago, E. (1973) *Rev. Esp. Fisiol.,* **29,** 329.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265.

Maley, G. F. and Lardy, H. A. (1953) *J. Biol. Chem.,* **204,** 435.

Pasquini, J. M., Faryana de Raveglia, I., Capitman, N. and Soto, E. F. (1980) *Biochem. J.,* **186,** 127.

Pullman, M. E. and Penefsky, H. S. (1963) *Methods Enzymol.,* **6,** 277.

Raison, J. K. (1972) *Bioenergetics,* **4,** 285.

Raison, J. K., Lysons, J. M. and Thomson, W. W. (1971) *Arch. Biochem. Biophys.,* **142,** 83.

Rajwade, M. S., Katyare, S. S., Fatterpaker, P. and Sreenivasan, A. (1975) *Biochem. J.,* **152,** 379.

Ruggiero, F. M., Landriscrina, C, Gnoni, G. V. and Quagliariello, E. (1984) *Lipids,* **19,** 171.

Satav, J. G. and Katyare, S. S. (1982) *Mol. Cell. Endocrinol.,* **28,** 173.

Satav, J. G., Katyare, S. S., Fatterpaker, P. and Sreenivasan, A. (1976) *Biochim. Biophys. Acta.,* **451,** 92.
Shallom, J. M. and Katyare, S. S. (1985) *Biochem. Pharmacol.,* **34,** 3445.
Tata, J. R. (1964) in *Action of hormones on molecular processes* (eds G. Litwack and D. Kritschevsky) (New York: Wiley), p. 58.
Tata, J. R., Ernster, L., Lindberg, O., Arrhenius, E., Pedersen, S. and Hedman, R. (1963) *Biochem. J.,* **86,** 408.
Thorn, M. B. (1962) *Biochem. J.,* **85,** 116.
Vik, S. B. and Capaldi, R. A. (1977) *Biochemistry,* **16**, 5755.
Watson, K., Bertoli, E. and Griffiths, D. E. (1975a) *Biochem. J.,* **146,** 401.
Watson, K., Haughton, R. L, Bertoli, E. and Griffiths, D. E. (1975b) *Biochem. J.,* **146,** 409.