# CHAPTER IX

## LOCAL AND SYSTEMIC ALTERATIONS IN SUCCINATE DEHYDROGENASE ACTIVITY IN RELATION TO ALTERED THYROID FUNCTIONING AND TAIL REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA.

Spatial and temporal shifts in energy balances and differential synthesis of macromolecules are quite often the result of synchronized and controlled activities of various dehydrogenases in a living system. Succinate:INT Oxido-reductase (EC 1.3.99.1) (SDH) is quite often studied as an important mitochondrial marker enzyme to evaluate the energy competence of tissues and processes. Our previous studies have indicated the involvement of geared up *in loco* TCA cycle oxidation during blastemic and differentiation phases of tail regeneration in *Mabuya carinata* (Shah and Ramachandran, 1970, 1976). Besides, alterations in hepatic metabolites and enzymes too are recorded to occur during tail regeneration in lacertilians (Shah *et al.*, 1977 a,b; Shah *et al.*, 1979 a; Chapters II-VII). Thyroxine is shown to have a positive influence on both glycolytic as well as TCA cycle enzymes (Lardy, 1954; Tishler, 1963; Tarentino *et al.*, 1966; Hoch, 1974; Milette *et al.*, 1981) especially $\alpha$-GPDH, SDH and MDH. In the

present chapter an attempt is made to evaluate the influence of varying functional status of thyroid on quantitative levels of SDH activity in the regenerate, liver and thigh muscle of the Scincid lizard, Mabuya carinata and correlate these alterations with the quantitative and qualitative changes affecting the process of tail regeneration thereof.

## MATERIALS AND METHODS

Healthy Mabuyas of both sexes obtained from Hyderabad, India and allowed to get acclimated to the laboratory conditions were kept on insect diet. Animals were subjected to chemical thyroidectomy and thyroxine replacement therapy as described in Chapter I. Tails were autotomized by pinching off at about 1.5-2.0 cms distal to the vent. Animals were then sacrificed under mild anaesthesia at fixed intervals of 3, 5, 7, 10, 12, 15, 25, 40 and 60 days post-autotomy.

Liver, skeletal muscle and normal or regenerating tail, as the case may be, were quickly removed and homogenized in ice-cold redistilled water. A 2% homogenate was prepared for liver and skeletal muscle whereas in the case of tail a 4% homogenate was found satisfactory. Quantitative estimation of activity of succinate dehydrogenase (SDH) and protein content were done according to the methods described in Chapter III.

For each day and each tissue specified a total of five to seven estimations were made. The mean and standard deviation were obtained and students' 't' test was used to determine statistical significance.

## RESULTS AND DISCUSSION

The changes in the activity levels of SDH in all the three tissues under hypothyroidism, T4 replacement as well as normal euthyroidic condition are depicted in Table 1, Figs. 1-3. The data recorded therein show a clearcut gradation of the SDH activity all throughout regeneration in all the three tissues in the form of subnormal and supranormal patterns respectively in the two experimental groups of animals (B and C), as compared to the control group (A). Functional status of thyroid seems to affect the tail and liver tissues to a greater extent than the muscle. This becomes well evident from the Table 2 which depicts the percentage difference in SDH activity in the three groups of animals during tail regeneration, Obviously, the regenerate appears to be more sensitive to thyroid hormone, as from 7th day post-autotomy onwards SDH activity was found to be subnormal with the maximal reduction of about 70% being attained by 25th day. Similarly T4 replacement is noted to have a pronounced effect in the regenerate with maximum increase of 150% occurring on the 10th day in tail regenerate.

Table 1. Quantitative levels of SDH in the regenerate, liver and skeletal muscle during tail regeneration under euthyroidic, hypothyroidic and T4 replaced conditions in M. carinata.
(Values are expressed as μMoles of INT reduced/mg protein/min.)

| Periods of tail regeneration in days | TAIL | | | LIVER | | | MUSCLE | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control Group (A) | PTU Group (B) | PTU + T4 Group (C) | Control Group (A) | PTU Group (B) | PTU + T4 Group (C) | Control Group (A) | PTU Group (B) | PTU + T4 Group (C) |
| N | 2.17 ±0.49 | 1.71 ±0.39 | 2.27 ±0.31 | 14.81 ±1.08 | 12.78 ±0.67 | 13.48 ±1.06 | 3.42 ±0.36 | 3.27 ±0.46 | 4.38 ±0.61 |
| 3 | 2.34 ±0.43 | 1.51 ±0.17 | 1.91 ±0.21 | 14.88 ±1.47 | 12.95 ±1.11 | 13.72 ±0.72 | 4.23 ±1.01 | 2.58 ±0.27 | 4.52 ±0.52 |
| 5 | 2.64* ±0.21 | 2.26 ±0.17 | 3.48@ ±0.51 | 16.76 ±1.46 | 12.71 ±1.23 | 17.73*@ ±0.91 | 5.31@ ±0.52 | 4.05 ±0.27 | 5.36 ±0.54 |
| 7 | 3.52@ ±0.49 | 1.51 ±0.14 | 4.01*@ ±0.25 | 16.96 ±1.81 | 15.08*@ ±0.87 | 15.71* ±0.69 | 5.42*@ ±0.77 | 4.19 ±0.32 | 6.86* ±1.03 |
| 10 | 3.09@ ±0.19 | 1.18@ ±0.11 | 5.73*@ ±0.47 | 15.12 ±1.83 | 10.26@ ±0.35 | 18.48*@ ±0.39 | 5.45* ±0.63 | 2.86 ±0.35 | 5.64* ±0.01 |
| 12 | 2.96@ ±0.16 | 1.29@ ±0.17 | 4.82*@ ±0.48 | 12.08@ ±0.58 | 13.59 ±0.67 | 19.13*@ ±0.71 | 4.89@ ±0.44 | 2.63 ±0.21 | 6.65@ ±0.51 |
| 15 | 2.12 ±0.28 | 1.16@ ±0.14 | 3.08* ±0.21 | 15.52 ±0.61 | 14.24* ±0.39 | 17.77*@ ±0.55 | 6.16*@ ±0.81 | 4.21 ±0.54 | 7.14*@ ±0.45 |
| 25 | 2.86@ ±0.12 | 0.51*@ ±0.18 | 2.74 ±0.28 | 14.51 ±0.88 | 9.72*@ ±0.93 | 20.42*@ ±1.91 | 5.40*@ ±0.65 | 3.81 ±1.02 | 5.45 ±0.47 |
| 40 | 2.64* ±0.08 | 1.69 ±0.16 | 4.44*@ ±0.82 | 13.82 ±1.09 | 9.81*@ ±0.93 | 21.58*@ ±1.29 | 5.87@ ±0.71 | 5.15* ±0.46 | 10.01 ±0.46 |
| 60 | 2.41 ±0.33 | 2.18 ±0.22 | 4.06@ ±0.48 | 17.41 ±0.73 | 14.43 ±0.73 | 21.70*@ ±0.73 | 5.03* ±0.48 | 5.59* ±0.29 | 9.35 ±0.29 |

* P < 0.01; * P < 0.005; @ P < 0.0025; @ P < 0.001; *@ P < 0.0005

N - Normal (Pre-autotomy state)

Table 2. Percentage modulations in SDH activity in the regenerate, liver and skeletal muscle during tail regeneration under euthyroidic, hypothyroidic and T4 replaced conditions in M. carinata.

(Values are calculated against corresponding normal values).

| Phases of tail regeneration in days | TAIL | | | LIVER | | | MUSCLE | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control Group (A) | PTI Group (B) | PTU + T4 Group (C) | Control Group (A) | PTU Group (A) | PTU + T4 Group (C) | Control Group (A) | PTU Group (B) | PTU + T4 Group (C) |
| N | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| 3 | +8% | -11% | -16% | +1% | +4% | +2% | +24% | +21% | +3% |
| 5 | +22% | +33% | +53% | +13% | -1% | +32% | +55% | +24% | +23% |
| 7 | +62% | -11% | +77% | +15% | +17% | +17% | +59% | +28% | +57% |
| 10 | +42% | -31% | +153% | +2% | -20% | +37% | +59% | -13% | +29% |
| 12 | +36% | -24% | +112% | -18% | +6% | +42% | +43% | -20% | +52% |
| 15 | -2% | -32% | +36% | +5% | +11% | +32% | +80% | +28% | +63% |
| 25 | +32% | -70% | +21% | -2% | -24% | +66% | +58% | +16% | +24% |
| 40 | +21% | -22% | +95% | -7% | -23% | +60% | +72% | +58% | +128% |

N - Normal (Pre-autotomy state)

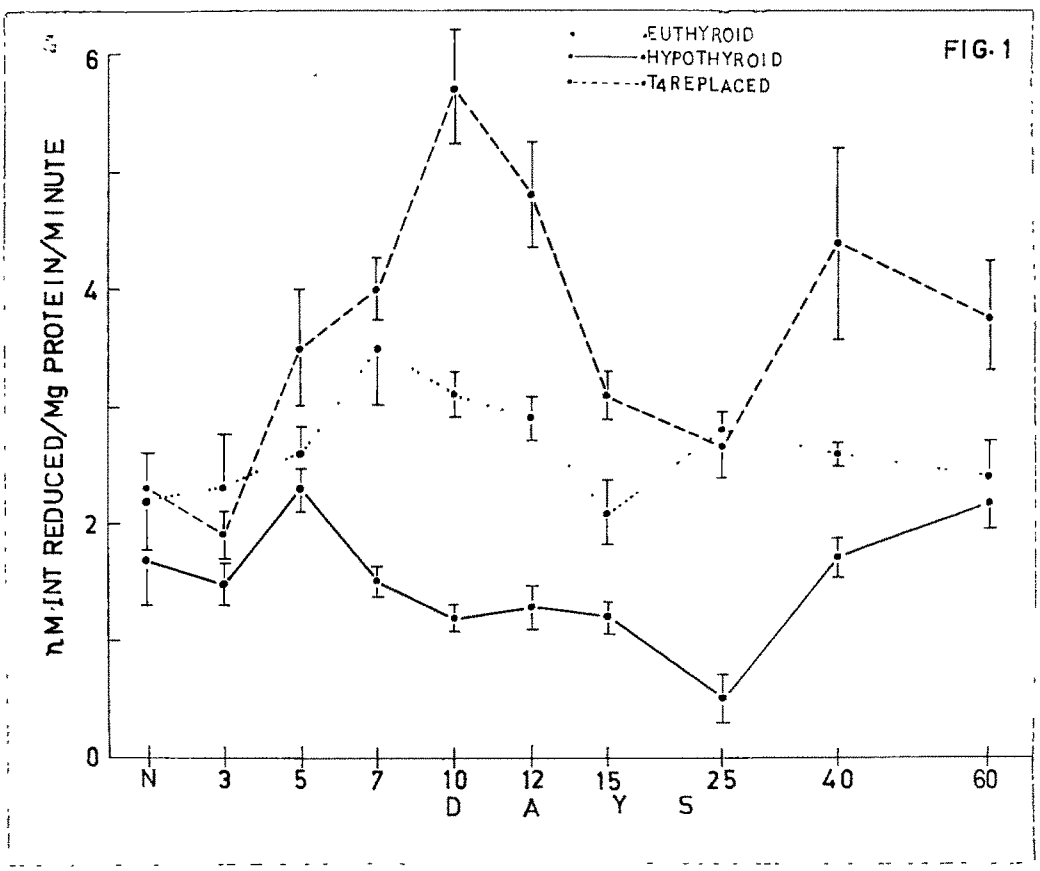


Fig. 1. Changes in succinate : INT reductase (SDH) activity in the regenerate during tail regeneration under control euthyroidic, PTU induced hypothyroidic and T4 replaced conditions in M. carinata.

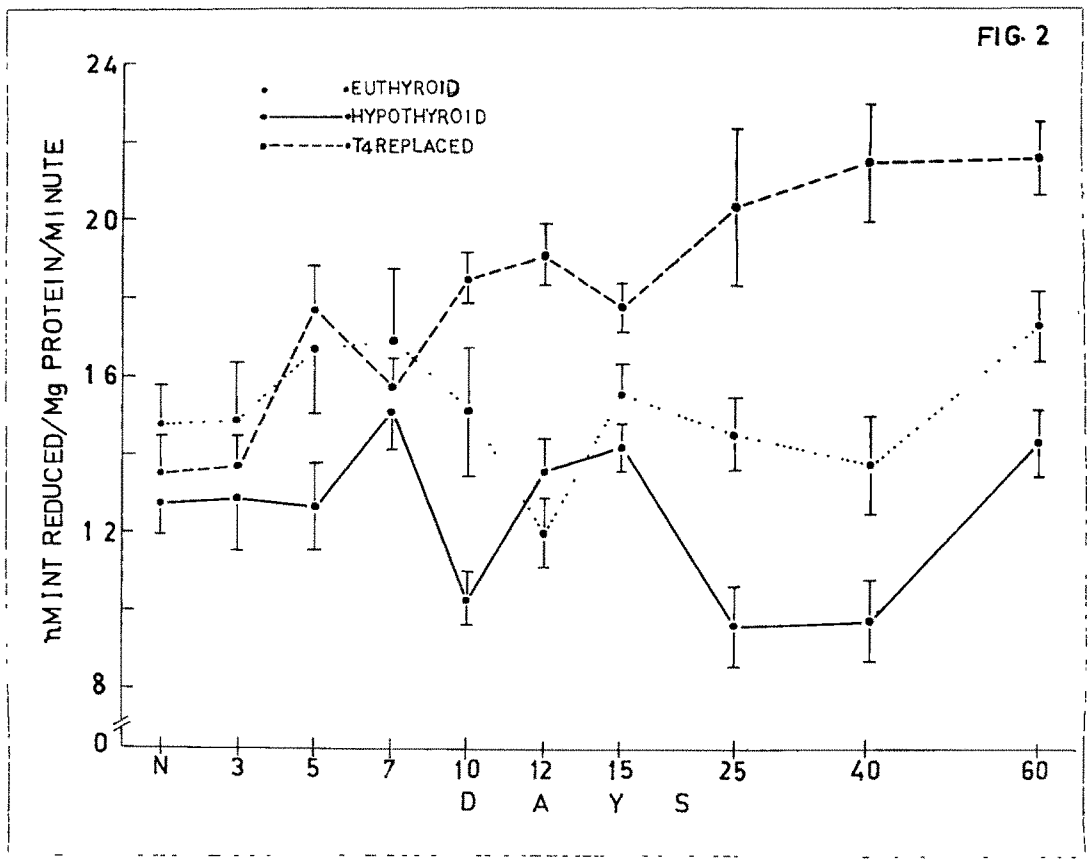


Fig. 2. Changes in succinate : INT reductase (SDH) activity in liver during tail regeneration under control euthyroidic, PTU induced hypothyroidic and T4 replaced conditions in M. carinata.

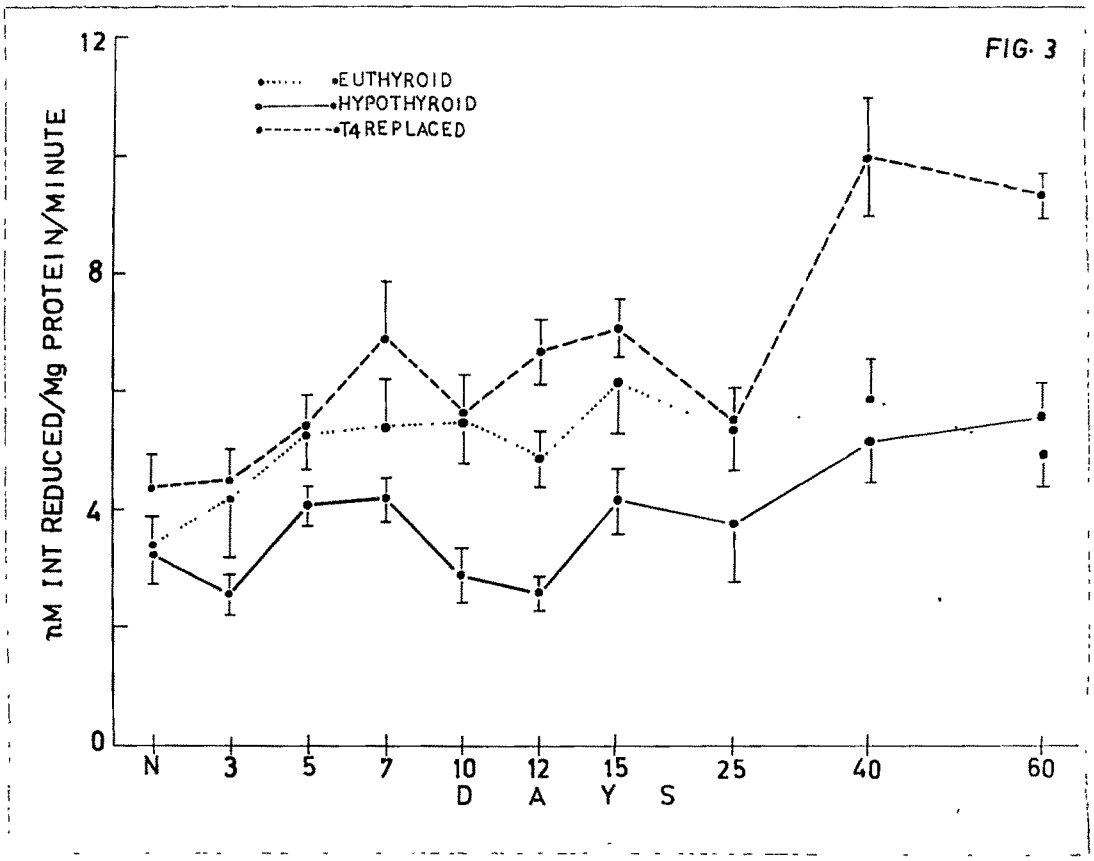


Fig. 3. Changes in succinate : INT reductase (SDH) activity in muscle during tail regeneration under control euthyroidic, PTU induced hypothyroidic and T4 replaced conditions in M. carinata.

Concurrent hyperresponsiveness of the hepatic tissue too was evident with continuous T4 replacement (group C) and the enzyme activity after depicting a continuous increase all throughout touched a plateau at 60% above the normal value. However, in the muscle the SDH activity was more or less above normal in all the three groups of animals with the muscle tissue not depicting the hypersensitivity recorded by the regenerate and liver under replacement treatment, uptill 25th day of tail regeneration. There was however, an hyper-induction of the enzyme in the muscular tissue to about 128% by about 40th day and the level thereafter stood at 116% above normal on 60th day of tail regeneration. These observations probably indicate : (1) The inductive action of thyroxine on succino-oxidase system especially in the regenerate which is constituted of pluripotential mesenchymal cells (akin to the embryonic mesenchymal cells) and the hepatic tissue, and (2) the comparative insensitiveness of the skeletal muscle SDH towards exogenous thyroxine. Reports of Maher (1964), Wolff and Wolff (1964) and Hulbert et al. (1976) bear relevance to the present inferences.

Since the Saurian tail is principally constituted of muscle, the increased incidence of SDH activity observed in the C group of animals during 40th and 60th days of tail regeneration in both the regenerate (where the process of myogenesis is complete) and the skeletal muscle may suggest

a delayed response under continuous T4 stimulation. The similarity in the modulations of hepatic SDH activity as well as the nearness of the values of percentage variations during the various periods of tail regeneration between the hypothyroidic and euthyroidic group of animals tend to underscore the existence of multiple controls of enzyme activity. This coupled with the recorded elevation in SDH activity in group C animals under continuous T4 replacement therapy, probably indicate the ability to induce/activate the succino-oxidase system either by acting at the nuclear level or by affecting the mitochondrial structure and function (Hulbert et al., 1976) without however, having any direct sole control on basal levels of the enzyme in the adult tissues. However, in the skeletal muscle the enzyme activity in group B animals tended to be markedly lowered as compared to the euthyroidic controls; this might reflect the permissive role of thyroxine in maintaining normal SDH levels. In addition the inability of group C animals to attain levels of enzyme activity characteristic of group A animals might also be probably due to the comparatively more effective depressive action of PTU on T4 to T3 conversion in the skeletal muscle (Escobar del Rey et al., 1962; Yamada et al., 1974; Kodding and Hoffken, 1978; Lauerberg, 1978). Finally, significant subnormal levels of SDH activity

observed in the regenerate of hypothyroidic animals is the cause or effect of the recorded inhibition of tail regeneration is difficult to ascertain. Moreover, the highly elevated SDH activity observable in the regenerate of group C animals might also denote the probable hyper-responsiveness of the undifferentiated mesenchymal cells towards thyroid hormone.

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