CHAPTER VII

ELECTROPHORETIC ANALYSIS OF LACTATE DEHYDROGENASE ISOENZYMES DURING TAIL REGENERATION IN EUTHYROIDIC AND HYPOTHYROIDIC GEKKONID LIZARDS, HEMIDACTYLUS FLAVIVIRIDIS

Lactate dehydrogenase is one enzyme that has proved to be particularly amenable to developmental analysis (Markert and Møller, 1959; Flexner et al., 1960; Cahn et al., 1962; Markert and Ursprung, 1962; Vessel et al., 1962). Subsequently, there had been a tremendous proliferation of investigations employing isozyme analyses of ontogeny. Physiological and pathological alterations in isozyme patterns are important for the insights they provide into normal and abnormal metabolism. Changes in the LDH isozyme pattern during early mouse embryogenesis involving high activity of the anodal LDH_1 isozyme during the initial phases prior to implantation and predominance of the cathodal LDH_5 isoenzyme in the post-implantation periods are well documented (Brinster, 1979). These changes in isozyme type have been presumed to have a functional basis and a correlation has been sought between the metabolic requirements of the embryo and its constituent isoenzymes. Though the relationship between the isoenzyme type and metabolic pattern is still controversial, the generalization that the LDH_1 predominates in tissues with

an essentially aerobic metabolism has been held valid. Tissue specific alterations have also been shown to occur during normal ontogeny (Markert and Ursprung, 1962; Fine et al., 1962; Philip and Vessel, 1962). Since regeneration involves a number of local events such as wound healing, dedifferentiation, formation of a blastema, differentiation and growth, with many underlying metabolic modulations, possible changes in isozymic forms are concievable as part of a regulatory mechanism. Previous enzymological studies on tail regeneration in lizards had indicated a metabolic shift from a post-autotomic anaerobiosis to a post-blastemic aerobic and a final shift back to the anaerobic pattern characteristic of the normal tail tissues (Shah and Ramachandran, 1970, 1972, 1973, 1974, 1976; Shah et al., 1982b; Swamy et al., 1982b). Subsequently, the prevalence of an aerobic environment in the body as a whole has also been inferred (Shah et al., 1979c; Shah and Hiradhar, 1978). In this background, a study of LDH isozymic pattern would be a profitable venture for understanding some of the metabolic intricacies involved in the process of regeneration. Moreover, the present study on electrophoretic distribution of LDH isoenzymes in the tail, liver and muscle of Hemidactylus flaviviridis has also been undertaken for having a comparative idea on this aspect as a previous study on the Scincid lizard, Mabuya carinata had revealed certain alterations (Shah et al., 1982d).

In the wake of the previously shown retardative influence of hypothyroidism on tail regeneration in lizards (Shah et al., 1979b; Ramachandran et al., 1984), it was thought pertinent to extend the electrophoretic analysis of LDH isozymes to hypothyroidic lizards as well.

MATERIALS AND METHODS

The lizards, H. flaviviridis, procured from the local animal dealer were maintained in the laboratory on a diet of cockroaches, and were kept for a fortnight for acclimatisation to the laboratory conditions. Lizards weighing 10-12 gms. and having a snout-vent length of 8-10 cms. were taken for the investigation and tail autotomy was done by pinching off the tail 2 segments distal to the vent.

A total of 120 animals were used for the experimentation. They were divided into two groups of 60 each. One group served as the euthyroidic control and the other group was chemically thyroidectomised by force feeding with 0.1 ml of 0.2% 6-propyl, 2-thiouracil (PTU) (obtained from Fluka Chemicals, Switzerland; pH adjusted to 8.0 to 8.2), every alternate day starting 15 days prior to autotomy. PTU feeding was continued even after tail autotomy till the end of the experimentation. The animals were killed at specific time intervals of 3,5,7,10,15,25,40 and 60 days post-autotomy

as well as prior to autotomy. Tissues such as liver, muscle and tail were taken and homogenised in ice-cold redistilled water with a concentration of 50 mg/ml and was cold-centrifuged for 2 minutes and filtered. Hundred µl of the filtrate was used for the running of the samples.

The gels of required thickness (1.5mm) was casted in glass plate moulds to form a gel sandwich of uniform and exact thickness. A gel of 10% acrylamide without SDS in Tris-buffer (pH 8.8) was made use of. Tris-glycine (pH 8.3) was used as the electrode buffer. Gel was prepared and poured into the gel mould and the slot mould was introduced and kept for polymerisation. After polymerisation the sample was introduced with the help of a microsyringe into the various slots and the electrophoretic unit (LKB 2001 vertical electrophoresis) was set up with a power adjustment of 400 volts, 45 milliamperes and 20 watts, with an initial reduced current of 10 m.amps. for 10 minutes and was run for 6 hours, After the running, the gels were removed and incubated in the dark at 37°C in a freshly prepared medium as described by Dietz and Lybrano (1967) for 30 to 40 minutes and finally stored in 7.5% acetic acid.

RESULTS

The zymograms representing alterations in LDH isozymes in the regenerate, liver and muscle during tail

regeneration are represented in Figures 1-6.

All five bands of LDH (1-5) could be discerned in tail, liver and skeletal muscle of both euthyroidic and hypothyroidic animals prior to tail autotomy. However, an additional major band (LDH 'C') with an accompanying minor band ('CB' hybrid) were prominently visible in the tail and muscle while in the liver only the major band of weak intensity could be discerned. The cathodic bands LDH_5, LDH_4 and LDH_3 were the most prominent in the tail and the skeletal muscle with the anodic LDH_1, LDH_2 and LDH 'C' being less prominent. However, in the liver all the 5 bands appeared more or less equally prominent though LDH_5 tended to be slightly darker and LDH_1 slightly lighter. In the hypothyroidic lizards all the LDH forms including LDH 'C' tended to show decreased intensity in the tail and muscle. Additionally, the tail depicted loss of the 'CB' hybrid. The liver pattern remained more or less unaltered in contrast. However, LDH_5, LDH_4 and LDH_1 bands were weakened. On the 3rd day post-autotomy the tail depicted reduced intensity of LDH_1 and LDH_2 which later became very prominent along with the C band as early as the 5th day and remained with even greater intensity till the 25th day of regeneration. The narrow but sharp and dark appearance of these bands on the 10th and 15th days is accredited to the homogeneity in cellular composition that exists in the early to late blastema.

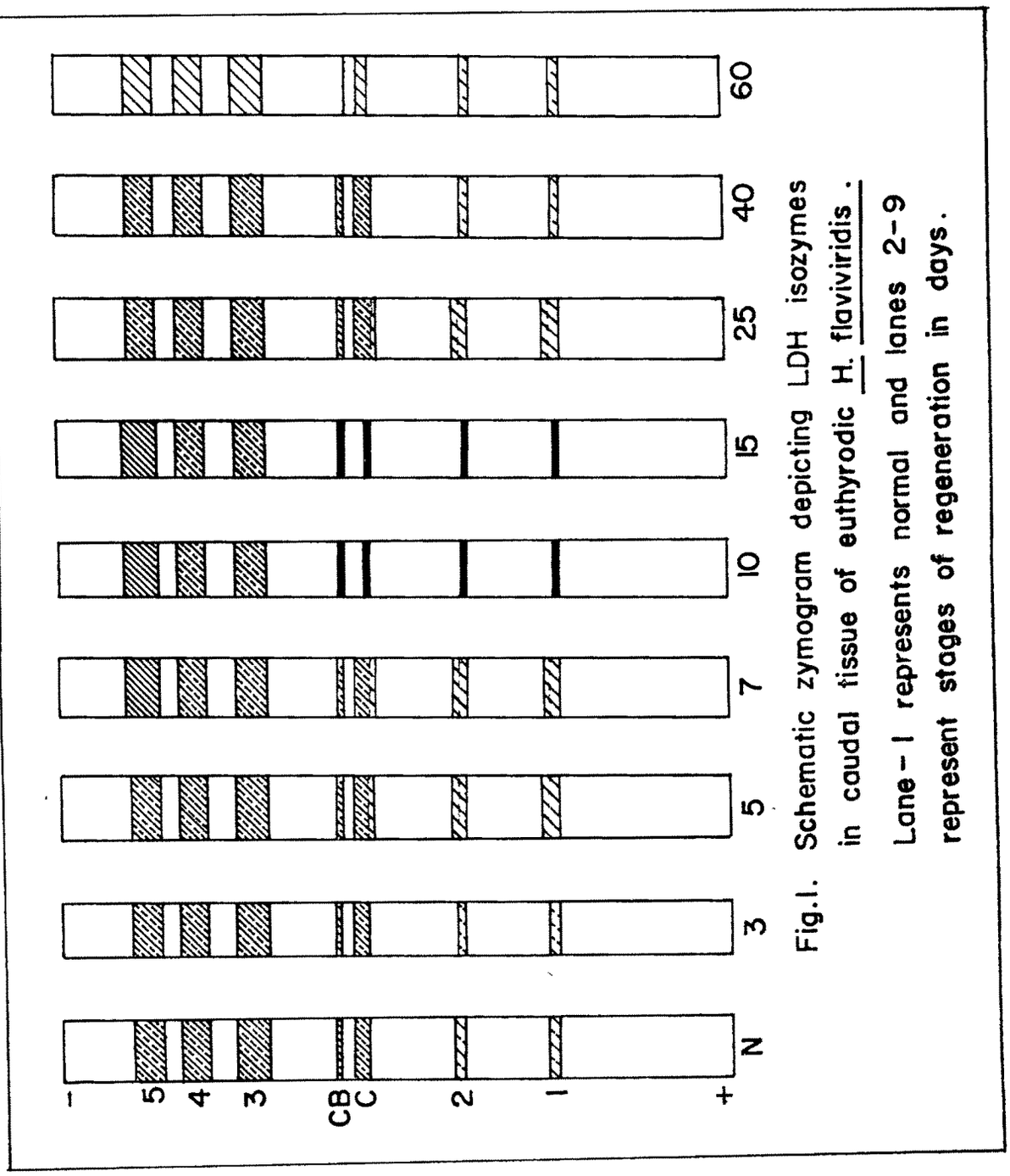

Fig.1. Schematic zymogram depicting LDH isozymes in caudal tissue of euthyrodic H. flaviviridis.

Lane - 1 represents normal and lanes 2-9 represent stages of regeneration in days.

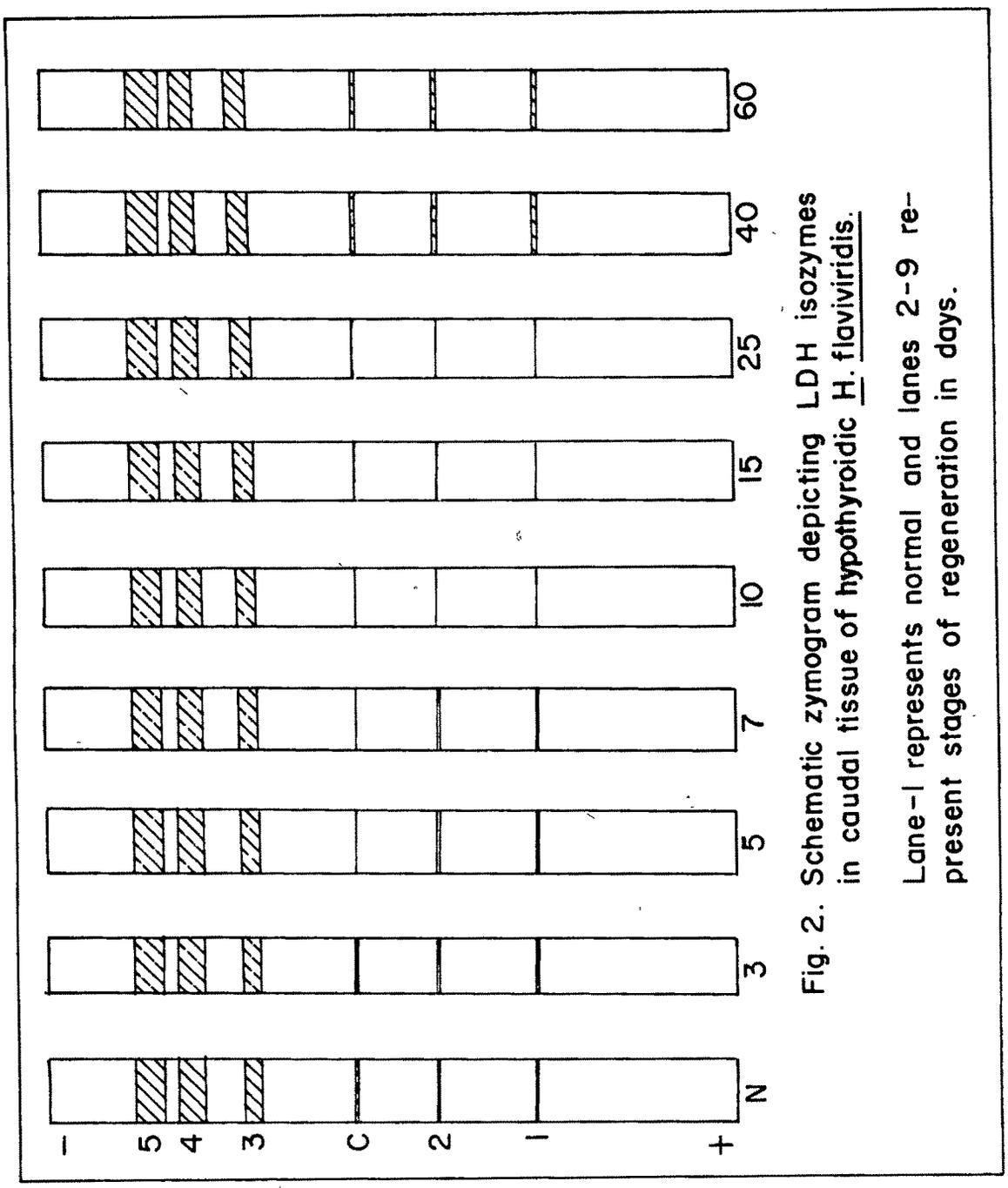

Fig. 2. Schematic zymogram depicting LDH isozymes in caudal tissue of hypothyroidic H. flaviviridis.

Lane-I represents normal and lanes 2-9 represent stages of regeneration in days.

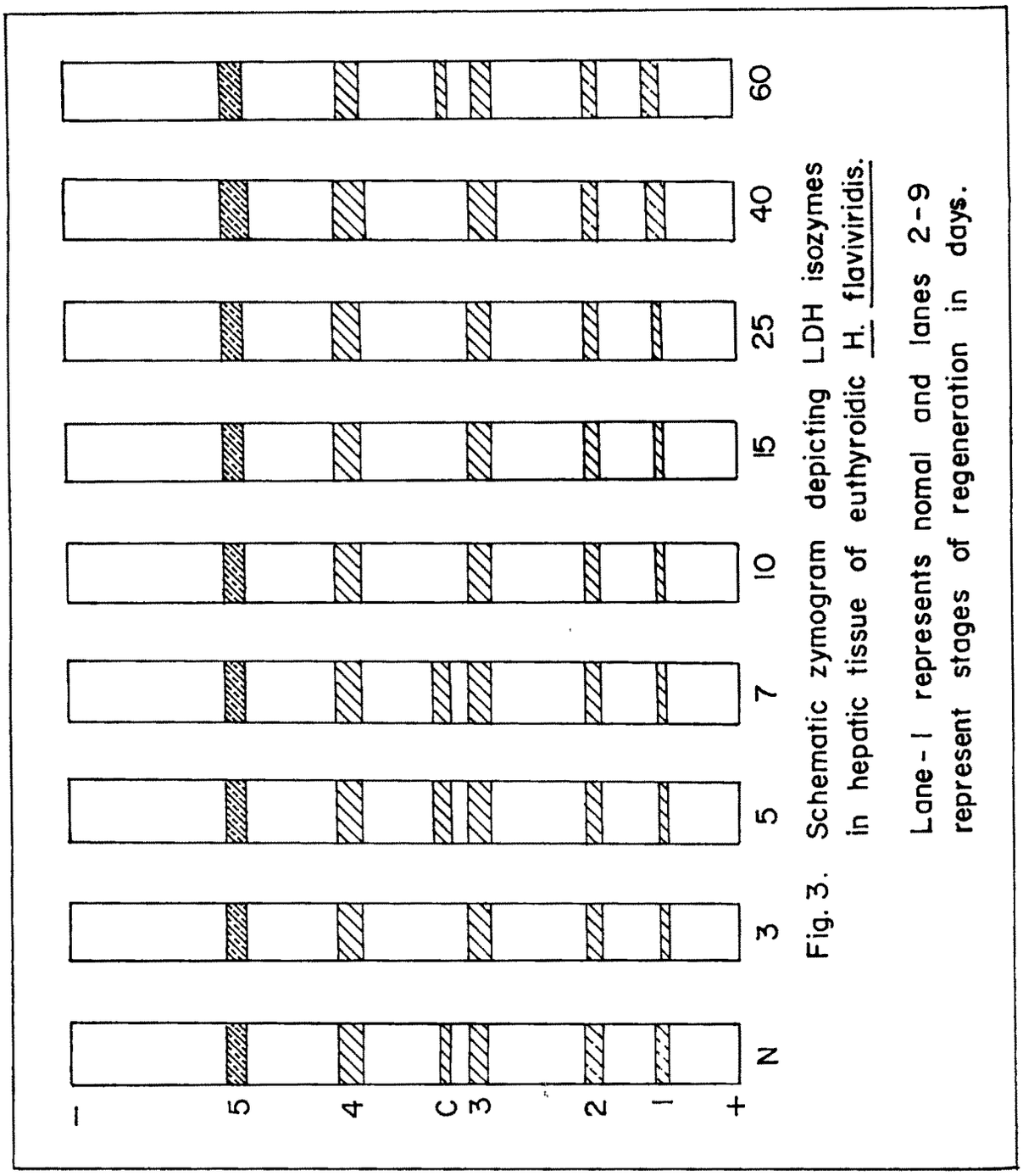

Fig. 3. Schematic zymogram depicting LDH isozymes in hepatic tissue of euthyroidic H. flaviviridis.

Lane - I represents nomal and lanes 2-9 represent stages of regeneration in days.

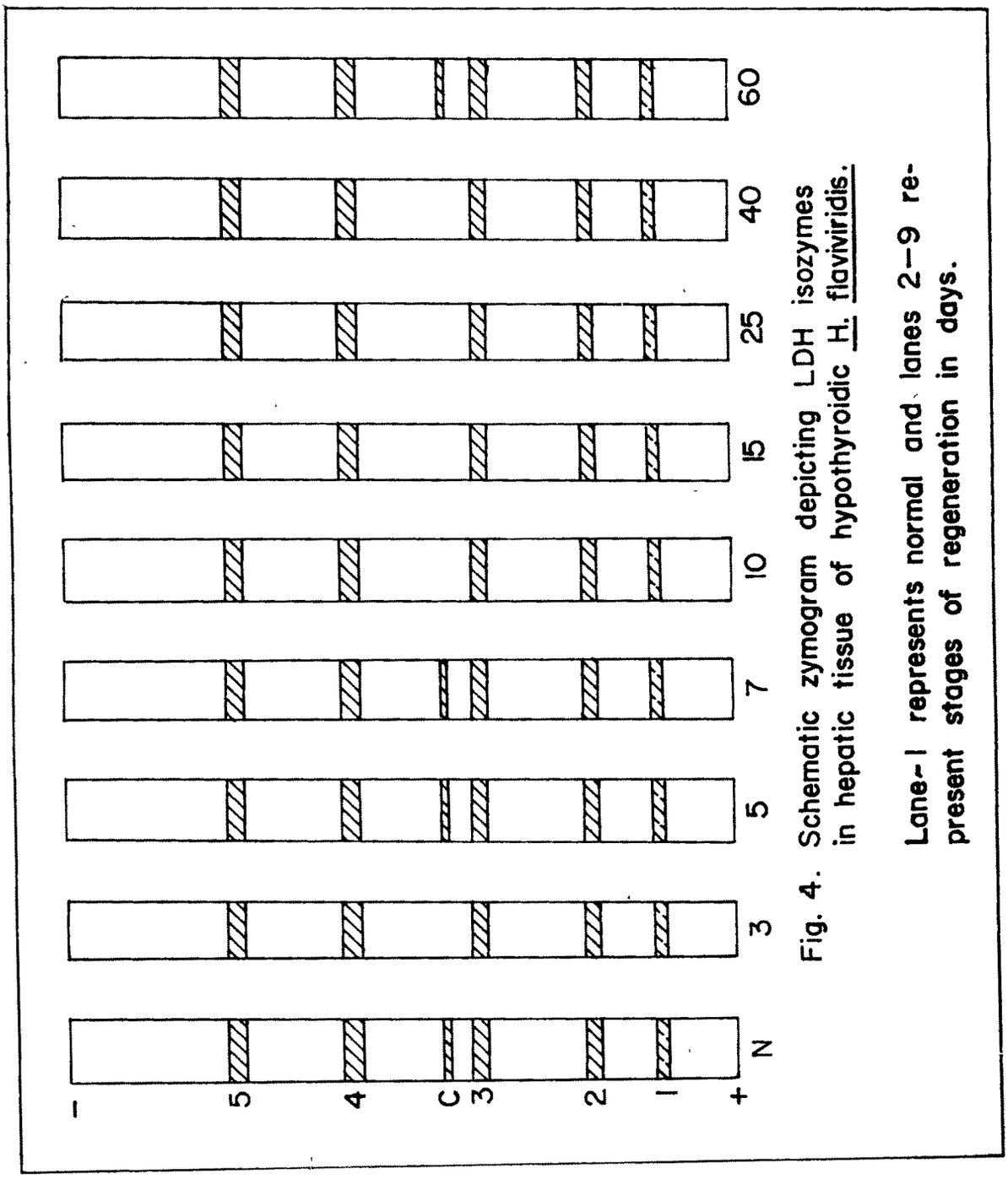

Fig. 4. Schematic zymogram depicting LDH isozymes in hepatic tissue of hypothyroidic H. flaviviridis.

Lane-1 represents normal and lanes 2–9 represent stages of regeneration in days.

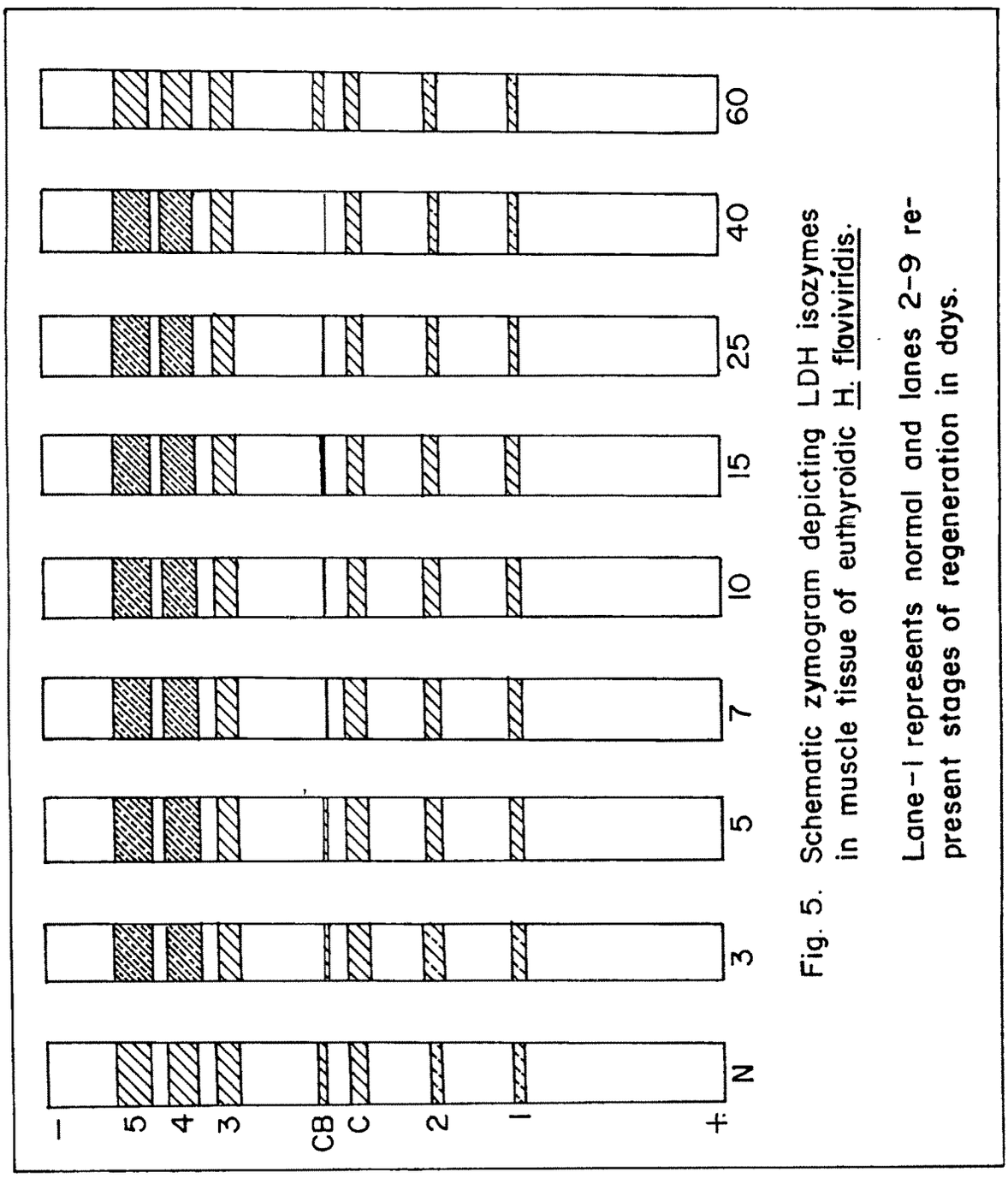

Fig. 5. Schematic zymogram depicting LDH isozymes in muscle tissue of euthyroidic H. flaviviridis.

Lane-1 represents normal and lanes 2-9 represent stages of regeneration in days.

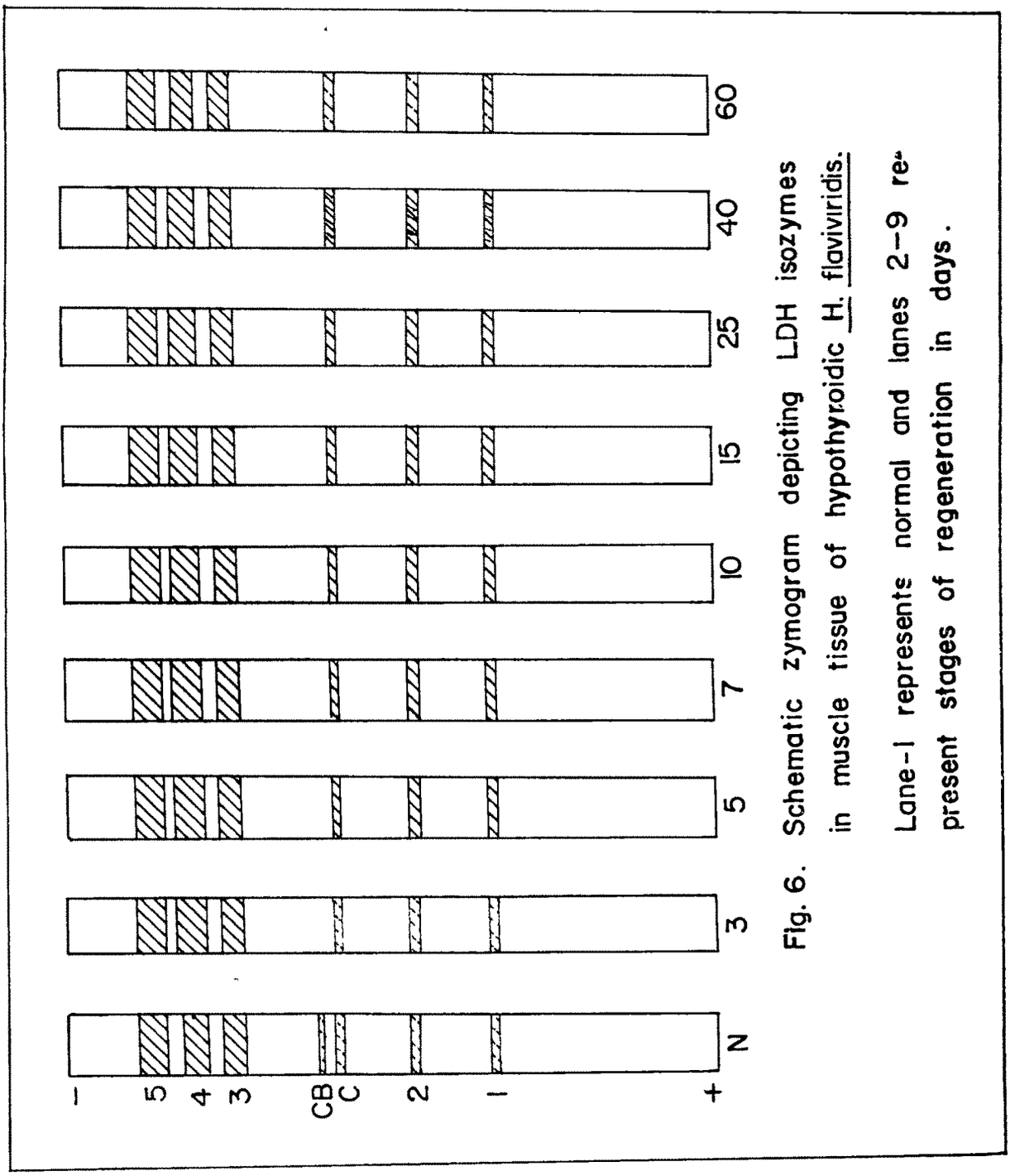

Fig. 6. Schematic zymogram depicting LDH isozymes in muscle tissue of hypothyroidic H. flaviviridis.

Lane-1 represents normal and lanes 2-9 represent stages of regeneration in days.

The cathodic bands 5, 4 and 3 remained prominent all through out with LDH_5 tending to be more intense between 7th and 25th days of regeneration. The hypothyroidic tail regenerate was characterised by weak representation of LDH_1, LDH_2 and LDH 'C' all through out regeneration with no alteration in the cathodic bands. The isozymic pattern of skeletal muscle post-autotomy depicted increased intensity of all the oxidative bands including LDH 'C' right from the 3rd day till the 25th day. Increased intensity of the cathodic bands, specifically bands 5 and 4 was noticeable during the entire course of tail regeneration. In contrast, the hypothyroidic lizards showed loss of CB hybrid band post-autotomy and a generalised decrease in anodic oxidative bands. The anaerobic bands did however show a tendency towards increased prominence during the course of regeneration. The changes in the hepatic isozyme pattern were more or less identical qualitatively as well as quantitatively in both the groups of lizards. The characteristic changes were the loss of LDH C band on 3rd day post-autotomy, its reappearance on 5th and 7th days followed by its disappearance till the 40th day of tail regeneration and increased intensity of all LDH bands except for the LDH_1 band which tended to become weaker during the course of regeneration.

DISCUSSION

The present findings on LDH isozymes in the three tissues studied have given indication of a definite potency for oxidative metabolism in Hemidactylus, which is denoted by the presence of quite prominent anodic LDH_1 and LDH_2 as well as the occurrence of the LDH 'C' bands. Presence of an additional LDH isozyme which has been variously named as LDH X, occurring in the testis of mammals and pigeons (Goldberg, 1963; Blanco and Zinkham, 1963; Blanco et al., 1964), or LDH 'C' (E), occurring in the retinae of teleost fishes (Whitt, 1969), has been by now well established. The isozymic pattern of Hemidactylus is in striking contrast to the principally anaerobic pattern reported previously for Mabuya (Shah et al., 1982b), and the anaerobic pattern of metabolism reviewed for lizard tissues (Shah and Ramachandran, 1970, 1972, 1973, 1974, 1976; Shah et al., 1979, 1982b; Swamy et al., 1982b). The greater incidence of the LDH 'C' in the tail and muscle, even more than in liver, precludes the assignment of any functional correlation and can be looked upon as only tissue specific induction of the 'C' locus and/or differential post-translational assembly. Hypothyroidism reduced the concentration of anodic oxidative LDH isozymes including the 'C' component in tail and muscle but not in liver. Moreover, the CB hybrid heterotetrameric form of LDH was totally lost from the tail while it became weaker in the muscle. Apart from suggesting a differential

response of the tissues to thyroid hormones, the present observations tend to suggest the positive influence of thyroid hormones on transcription of the 'B' and 'C' loci and post-translational assembly, and/or the negative influence on 'B' and 'C' subunit assembly. Early post-autotomy phase lasting upto 3-5 days and corresponding to the wound healing phase has been generally associated with lactate accumulation and increased anaerobiosis at the local site (Okuneff, 1933; Dickens, 1951; Needham, 1952) which is reflected by the herein recorded weakening of the anodic isozymic forms. Similar emphasis on anaerobic mode of metabolism in the liver tissue as well, is denoted by the weakening of LDH_1 and the loss of LDH 'C'. In contrast, the muscle showed increased concentration of LDH_2 and LDH 'C' forms indicating enhanced expression of the oxidative metabolic potential. The changes in the LDH pattern of the three tissues in thyroid suppressed lizards are suggestive of a general inadequacy of the hypothyroidic tissues to raise the metabolic potential in the immediate post-autotomy periods.

An emphasis towards aerobic, mode of metabolic transactions in loco, during the progressive phases of regeneration (preblastema, blastema, and differentiation) is clearly denoted by the representation of prominent anodic LDH isozymes from 7th to 25th days. A generalised increase in total LDH activity is indicated by the concurrent strong

expression of cathodic forms as well. Similar set of changes in the muscle and liver also during the same period signals the remarkable co-ordinate expression of increased metabolic activities systemically as well. Such a co-ordinate expression of LDH isozymic activity was shown to occur during tail regeneration in Mabuya also by Shah et al. (1982b). Increased dependence of the proliferative and differentiative phases of regeneration on oxidative metabolism, as represented by the currently observed alterations in the in loco LDH isozymic pattern, is well corroborated by the recorded changes akin to that of the present ones during early ambryonic development in mouse (Brinster, 1979) and retinal differentiation in teleost fishes (Whitt, 1969). The requirement for co-ordinate systemic changes during lizard tail regeneration is perhaps well exemplified by the many reports emanating from this laboratory implicating the participation of the body as a whole in various physiological, metabolic and haemodynamic adjustments as part of the collective response for a successful process of regeneration (Shah et al., 1977a,b, 1980a,b,c, 1982a,b,c; Ramachandran et al., 1979, 1980, 1982, 1985). However, a comparison of the pattern of changes in LDH isozymes during regeneration in the two lizards, i.e., Mabuya (Shah et al., 1982b) and Hemidactylus, reveals that whereas the changes in Mabuya involved the appearance of fast moving LDH_1 and LDH_2 forms

post-autotomy, from a more or less weak representation in the unautotomised state, the changes outlined herein in Hemidactylus involves only increased expression of these LDH isozymes from a relatively well represented condition in the unautotomised state. Obviously, this bespeaks of a relatively greater potential for oxidative metabolism in Hemidactylus and an inherent difference in metabolic adaptation between the two lizards as has been inferred from many other observations (Chapters 11 and 12). In this context, it can be speculated, that, whereas in Mabuya a stronger stimulus operative at the transcriptional level of 'B' locus may be required, in the case of Hemidactylus, a trigger at the post-translational level involving increased 'B' and 'C' subunit homotetramerisation may suffice. In the hypothyroidic condition, all the three tissues have depicted a generalised incompetence in increasing the oxidative forms of LDH isozymes during the progressive phases of regeneration though the increase in the anaerobic cathodic forms could nevertheless be noted to occur. Apparently, the overall retardation in regenerative tail elongation and the poor quality of the regenerate produced in hypothyroidic lizards as noted earlier (Shah et al., 1979b; Ramachandran et al., 1984) could in all probability be related to the inadequate metabolic competence and/or the inability for enhanced expression of the oxidative metabolic transformations as inferred herein.

However, the ability for increased expression of anaerobic forms of LDH and, the persistence of aerobic forms albeit in slightly reduced intensities, suggest that thyroid hormone per se may not be the regulatory factor for the expression of LDH isozymic forms though it may nevertheless have a permissive influence on the actions of the regulatory factor(s).

Viewed on the whole, the present observations on electrophoretic profile of LDH isozymes during tail regeneration in Hemidactylus flaviviridis have revealed following points of interest. 1) The anodic forms of LDH are adequately represented in the normal tissues thus emphasising the capacity for oxidative metabolism. 2) The 'C' locus of LDH subunit is present in Hemidactylus and 3) Increased incidence of the oxidative forms, including the 'C' form post-caudal autotomy, illustrates the exigency of oxidative reactions in meeting the requirementsof regeneration. Basedon the known differential effects of activators, inhibitors etc. on isoenzyme kinetics, as well as the competence of LDH_1 to catalyse reactions involving homologues of natural substrates such as hydroxy derivatives of butyric, caproic and valeric acids as well as the corresponding oxo-compounds (Rosalki and Wilkinson, 1960; Plummer et al., 1963), the changes noted to occur during tail regeneration in lizards may be considered to confer remarkable flexibility and efficiency

for adaptive utilisation of a wide range of substrates under altered physiological conditions. The correlation by Fine et al. (1963) of the competence of 'B' type LDH subunits to convert fatty acids to glucose in ruminant liver and the suggested occurrence of gluconeogenesis during tail regeneration in lizards (Shah and Ramachandran, 1972, 1973, 1976; Shah et al., 1977a,b) are in this context very relevant.

SUMMARY

Electrophoretic analysis of LDH isozymes in tail, liver and muscle has been carried out during caudal regeneration in euthyroidic and hypothyroidic lizards. All the 5 LDH isozymes were represented in all the three tissues of both groups of lizards in the preautotomy state. An additional LDH 'C' band was discernible in all the three tissues with a 'CB' hybrid form also being present in the tail and muscle tissues. Post-autotomy phase of regeneration were marked by more prominent LDH_1, LDH_2 and LDH 'C' isozymes in the euthyroidic lizards. The hypothyroidic lizards depicted the loss of the hybrid band with weak representation of LDH 'C' together with LDH_1 and LDH_2. These changes in the isozymic pattern suggest a substantial potential for oxidative metabolism in the tissues of Hemidactylus and the expression of this potential to a greater degree during

tail regeneration. Moreover, hypothyroidism, except for reducing the intensities of LDH isozymic forms, do not seem to have any suppressive influence. Apparently, reduced oxidative reactions can be inferred under hypothyroidism.