CHAPTER XII

HISTOCHEMICAL ALTERATIONS IN LIPIDS, LDH, SDH AND BDH DURING TAIL REGENERATION IN NORMAL AND ADRENAL SUPPRESSED GEKKONID LIZARDS, *HEMIDACTYLUS FLAVIVIRIDIS*

Dehydrogenase catalysed reactions, bring about oxidoreduction of metabolic intermediates in a sequential fashion, constituting well defined metabolic pathways involving the breakdown and synthesis of proteins, lipids and carbohydrates. By their precisely regulated activities they also bring about controlled interconversions between these metabolites and thus play pivotal roles in bringing about adaptive metabolic alterations and energy transformations as per the spatial and temporal requirements of various tissues and organs of a living system. Activities of specific dehydrogenases apart from controlling the operation of anaerobic or aerobic pathways of metabolism, also generate intermediates useful in the interconversions of various metabolites like carbohydrates and proteins and lipids during periods of stress.

Lipids serve as an efficient source of energy, which is required for varied cell functions in a number of tissues (Fredrickson and Gordon, 1958; Rossiter and Strickland, 1966).

They serve as major reserves of oxidisable substrate in all living systems. However, sparseness of literature regarding their role in the regenerating tissues has been felt for quite some time. Relevant studies in this field include those of Hess (1959) in the regenerating tadpole tail of <u>Xenopus laevis</u> and of Schmidt (1966b,c,d) in the regenerating limb of <u>Diemictylus viridescens</u>. Similar histochemical studies on the localization and distribution of lipids in the regenerating reptilian tail have been carried out in this laboratory by previous workers (Chakko, 1967; Magon, 1970; Shah and Hiradhar, 1977, 1978). The activities of the degradative enzymes of lipids <u>viz</u>., lipase, esterase and BDH were accentuated during tail regeneration (Chakko, 1967; Magon, 1970; Shah and Ramachandran, 1975; Radhakrishnan and Shah, 1985). BDH and SDH have also been histochemically localised, and based on the changes in their activity patterns, the importance of these enzymes in the metabolic adaptations underlying the process of tail regeneration in lizards has been established (Shah and Ramachandran, 1970, 1975, 1976; Shah <u>et al</u>., 1979). However, the hormonal regulation if any on these <u>in loco</u> and systemic metabolic profiles by specific alterations in enzyme activity has not been looked into. It was therefore decided to study the histochemical distribution of lipids and LDH, BDH and SDH in the regenerating tail, and liver under induced adrenocortical insufficiency during tail regeneration in the Gekkonid lizards, <u>Hemidactylus flaviviridis.</u>

MATERIALS AND METHODS

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

A total of 120 animals were used for the experimental purpose. They were divided into two groups. One group served as the control and the other group was chemically adrenalectomised using the synthetic corticosteroid dexamethasone~~.~~ (DXM). Injections were given intraperitoneally (15 µg/0.1 ml/day/animal) in the evenings at 17.00 hrs. starting 10 days prior to tail autotomy and were continued post-autotomy every alternate day till the end of experimentation. Controls received an identical amount of the vehicle (distilled water). Lizards from both the groups were sacrificed at regular time intervals of 3,5,7,10,15,25,40 and 60 days post-autotomy along with the normal animals with intact tail. Liver and tail were taken and blotted free off blood and tissue fluids and kept in a cold microtome maintained at -20°C. Sections of 10 μ thickness were cut and processed for the histochemical localisation of total and neutral lipids and LDH, BDH and SDH. Neutral lipids and total lipids were histochemically stained by the method of Pearse (1968) using Fettrot 7B and Sudan black B respectively. LDH and BDH were localised by the methods described by Ogatta and Morri (1964), and SDH was localised by the method of Nachlus et al. (1957).

OBSERVATIONS

A subjective grading of intensity of localisation and distribution of lipids, LDH, SDH and BDH in the liver and tail during tail regeneration is represented in table 1.

Normal tail

Of the three dehydrogenases, LDH showed highest activity in the tail tissues as compared to SDH and BDH. All the three enzymes showed intense activity in the epidermal and subepidermal regions, the muscle, and the spinal cord. Both diffuse cytoplasmic as well as mitochondrial localisation were discernible in the case of LDH, while mere mitochondrial localisation was the feature for both SDH and BDH. In general, adrenal suppressed lizards depicted reduced reactivity for all the three enzymes with the activity of LDH being low and that of SDH and BDH negligible.

Regenerating tail

During the wound healing phase which corresponds to the first five days post-autotomy, LDH activity was noticeably increased at the cut end of the stump tissues. The wound epithelium also depicted appreciable enzyme activity. However, SDH and BDH activities remained more or less in the pre-autotomy range. In the experimental lizards, though LDH tended to show a slightly increased activity, both SDH and LDH activity remained negligible. The blastemic and differentiation phases of regeneration (7-25 days) were marked by increased peak level activity of all the three dehydrogenases in the control lizards. The activity of the enzymes could be localised in the epithelium and mesenchymal cells of the blastema, and differentiating epidermis, muscle, cartilaginous neural canal and ependyma of the differentiating tail. In the experimental lizards, though the LDH activity tended to show a slight increase, both SDH and BDH activities more or less remained unchanged during the corresponding phases of regeneration. Through late differentiation and growth phases to the fully regenerated condition, the activity of the three dehydrogenases declined gradually to the original pre-autotomy levels in the case of control lizards which was not however necessary in the adrenal suppressed lizards.

Changes in the liver : In the liver, the enzymes were distributed in the parenchaymatous cells of the lobules. Whereas LDH showed a diffuse cytoplasmic localisation, both SDH and BDH depicted a granular mitochondrial localisation. All the three dehydrogenases showed a more or less high intense localisation prior to caudal autotomy, while in experimental lizards activities of all the three dehydrogenases were noticeably decreased; more prominently that of SDH and BDH. Post-autotomy, the first seven days were marked by increased activity of all the three dehydrogenases in the control lizards. After the 10th day, whereas the activities of LDH and SDH settled to the pre-autotomy levels, BDH activity tended to remain significantly reduced between 10th and 25th days.
In contrast, the DXM treated lizards showed a more or less unaltered level of activity all throughout except for LDH and BDH which tended to show a slightly increased activity by about the 25th day.

Table-1 : Histochemical distribution of lipids and dehy

			0	3	5
Neutral lipids	Normal	Liver	+++	++	+
		Tail	+ ±	+	+
	Adrenal suppressed	Liver	+++	++	+
		Tail	+ ±	+	+
Total lipids	Normal	Liver	+++	++	+
		Tail	++	+ ±	+ ±
	Adrenal suppressed	Liver	+++	++±	++
		Tail	++	+ ±	+ ±
LDH	Normal	Liver	+++	++++	++++
		Tail	++	+++	+++
	Adrenal suppressed	Liver	++	+	±
		Tail	+	+±	+±
SDH	Normal	Liver	+++	++++	++++
		Tail	+	+	+ ±
	Adrenal suppressed	Liver	+	+	+
		Tail	±	±	±
	Normal	Liver	+++	+++	+++

Lipids in normal and regenerating tail

In general, presence of lipids was not very remarkable in the normal tail tissues except for the subcutaneous and submuscular adipose tissues and the marrow cells of the vertebrae. Prior to autotomy, the DXM treated lizards did not depict any alteration in lipid localisation in the tail tissues in relation to the controls. The first five days post-autotomy, corresponding to the wound healing period, revealed a slightly decreased lipid localisation in both the control and experimental lizards. Later periods of regeneration were marked by increased above normal content of Sudanophilic lipids between 7th and 40th days and of neutral lipids between 7 to 15 days. Comparable increase in lipid localisation could not be discerned clearly in the adrenal suppressed lizards.

Hepatic lipids

Sudanophilic and Fettrot positive lipids showed high localisation in both control and experimental lizards prior to caudal autotomy. Both total and neutral lipid contents were reduced in the liver of control lizards in the immediate post-autotomy periods corresponding to the wound healing phase (5 days). The adrenal suppressed lizards during the corresponding period showed similar depletion of neutral lipids while the total lipid content remained more or less unchanged. Periods after the 7th day of tail regeneration were marked by differential changes with respect to total and neutral lipids in the control animals. Whereas the neutral lipid content increased and remained high to very high between 7th and 15th days, the total lipid content remained low in the controls. During 15th-25th days, the hepatic neutral lipids were depleted to a low level; and then increased towards the pre-autotomy level; while the total lipid content remained low till the 15th day and then increased to pre-autotomy level by 25th day and remained so thereafter. The DXM suppressed lizards depicted decreased neutral lipid content throughout while the total lipid content was very much reduced after the 10th day.

DISCUSSION

Low oxygen affinity and marked anaerobic metabolism together with low level of anaerobic enzymes have been reported for lizards (Pough, 1969; Bennet, 1972; Bennet and Dawson, 1972, 1973; Bennet and Licht, 1972). This is well reflected in the presently observed low lipid contents and more intense localisation of LDH as compared to SDH and BDH in the normal tail tissue of _H. flaviviridis._ In fact, the dependence of normal tail tissues of lizards principally on anaerobic mode of metabolism for energy requirement has been stressed by a number of previous studies from this laboratory (Shah and Ramachandran, 1970; Shah et al., 1979c; 1982d; Swamy et al., 1982b). However, the herein observed relatively rich lipid content and appreciable SDH and BDH activities along with LDH in the hepatic tissue bespeak of the adequate competence of this organ to undertake oxidative metabolism. Strong evidence in this connection comes from the equally well represented aerobic and anaerobic LDH isozymic bands in the liver (Chapter 7). Chemical adrenalectomy did not seem to have any significant influence with reference to histochemically detectable lipid contents either in the tail or in the liver prior to autotomy. But a reduced potential for oxidative metabolism can be inferred from the slightly reduced LDH activity and significantly reduced SDH and BDH activities in both the organs.

Marginal reduction in lipid content together with unchanged SDH and BDH activities in the tail during the first five days post-autotomy corresponding to the wound healing phase indicate the insignificance of lipid metabolism in loco in the initial periods. However, the hepatic tissue seems to contribute substantially to the overall increased energy requirements for the preparative events associated with the initiation of regeneration as can be inferred from the noticeable lipid depletion and enhanced activities of all the three dehydrogenases occurring during this period. Similar findings have also been made previously by Shah et al. (1979c). The crucial role of liver in the altered metabolic adjustments and energy equilibrium is further emphasised by the depletion of its glycogen and protein reserves as well (Chapters 1 & 2). The identical decline in hepatic and caudal lipids in the DXM lizards during the same period in the wake of reduced dehydrogenase activity appears surprising and the possibility of incomplete lipid degradation unaccompanied by oxidation seems very much in order. The preblastemic and blastemic phases of regeneration (7-15 days) were marked by increasing total and neutral lipid contents in the regenerate. Whereas the total lipid content remained elevated till the 40th day, the neutral lipid content declined between 15th and 40th days. These periods of regeneration are marked by concomitant increasing levels of activity of SDH and BDH. These set of changes tend to portray enhanced lipid oxidation through the TCA cycle in keeping with the high metabolic necessities and extra energy requirements of the actively proliferating and differentiating cells of the regenerate. Such an exigency of altered metabolic status has been clearly brought out by some of the previous studies on metabolites and enzymes during lizard tail regeneration (Shah et al., 1979a, 1980a,b,c, 1982a,b; Ramachandran et al., 1979, 1980, 1982, 1983, 1985; Swamy et al., 1982a,b, 1983). Parallel changes in the neutral lipid content and the maintenance of moderately high to high levels of SDH activity in the hepatic tissue indicate the active participation of oxidative reactions in this organ along with the regenerate, probably to aid in the increased energy demands of the body in general and the regenerate in particular. The increased total lipid content noticeable in the tail regenerate till about the 40th day of regeneration is correlatable with the requirement of the actively proliferating and differentiating cells for structural lipid components like phospholipids and cholesterol. The significant hepatic lipid depletion noted in the present study in H. flaviviridis underscores the importance of liver as a principal systemic source of lipid moieties as the visceral fat bodies of H. flaviviridis have been reported to be insensitive to the stress of autotomy and the ensuing process of regeneration (Kothari, 1977). This is very much in contrast to the picture in Mabuya in which there is substantial loss of lipids from the visceral fat bodies (Kinariwala et al., 1978).

The adrenal suppressed lizards did not reveal the characteristic lipid depletion occurring in both liver and tail during the late blastemic and differentiation phases as the lipid accumulation noted to occur during the preblastemic and blastemic phases in the control lizards was more or less unnoticeable in the experimental lizards. Moreover, even the activities of SDH and BDH tended to remain repressed.

In general, it is presumable from the present observations that lipid metabolism *per se* is highly restricted in the adrenocortical suppressed lizards. The poor quality of the regenerate produced in the adrenal suppressed lizards in this context (Chapter 4) may be reflective of this decreased potential for systemic and *in loco* lipid metabolism.

SUMMARY

Dehydrogenases like LDH, SDH and BDH and neutral and total lipids were histochemically localised in the adrenal sufficient and insufficient lizards. The normal tail tissues depicted low lipid content and more intense localisation of LDH as compared to SDH and BDH. But the hepatic tissue tended to show rich lipid content and considerably high SDH and BDH activities along with LDH. Chemical adrenalectomy did not seem to have any significant influence with reference to histochemically detectable lipid contents either in the tail or the liver prior to

caudal autotomy. In the hepatic tissue all the three dehydrogenases showed a more or less high intense localization prior to caudal autotomy while in the experimentals the activities were noticeably decreased more prominently that of SDH and BDH. Post-caudal autotomy, during the course of regeneration, substantial alterations in lipid metabolism could be discerned in the regenerate and liver of control lizards. However, based on the localisation of lipids and LDH, SDH and BDH, a low profile of lipid metabolism was the feature in the adrenal suppressed lizards. The poor quality of the regenerate produced by the adrenal suppressed lizards may be correlated with this decreased potential for systemic and in loco lipid metabolism.