CHAPTER 2

ALTERATIONS IN CERTAIN LIVER DEHYDROGENASES DURING TAIL REGENERATION IN THE GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

Developmental processes like regeneration wherein large scale restoration of lost tissues occurs, would predictably raise the energy demands of the organisms. Procaccini et al. (1973) have reported a systemic metabolic response of liver to forelimb amputation of newt whereby the hepatic metabolites provide energy to the wounded tissue for repair and regeneration. Our studies on quantitative changes in the concentration of metabolites such as glycogen, lipid, protein (Chapter 1) and ascorbic acid (Chapter 3) in liver have yielded enough evidences to implicate liver as a major participant in the body's overall physiological adjustments to the stress of tail loss and its subsequent restoration in lizard Hemidactylus flaviviridis. Sequential dehydrogenation of the metabolites plays an important role in energy yielding mechanisms in vertebrate tissues. These reactions are mediated by several dehydrogenases, some of which viz., α-glycerophosphate dehydrogenase (α-GPDH) lactate dehydrogenase (LDH), Succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) were chosen for the present study with regard to their localization and distribution in the tissue of liver during different phases of tail regeneration in the house lizard, so as to elucidate their involvement in the altered metabolic pattern of the organ noticeable as an instance of the systemic response and adaptation of the body to the regenerative process. The enzymes, α-GPDH and LDH are involved in anaerobiosis, whereas the enzymes, SDH and MDH operate aerobically. Fluctuations in reactivities of α-GPDH and LDH would provide certain insight into the relative pattern of carbohydrate metabolism in the tissues concerned. Operation of TCA cycle can be inferred from the activities of SDH and MDH. Hence, it was deemed desirable to study the activity pattern of these enzymes histochemically.

MATERIALS AND METHODS

House lizards, *H. flaviviridis* collected from the University campus were maintained in the laboratory on a diet of insects. Autotomy was induced by pinching off the tail leaving two to three basal segments intact. Animals were sacrificed at different stages of tail regeneration as described by Shah and Chakko (1968a). The liver was removed immediately and blotted free of blood and body fluids. Fresh frozen sections of 12 to 18 μ thickness were cut in a cryostat at - 20°C and incubated for 30 minutes at 37°C in respective media for α-GPDH, LDH, SDH and MDH as described by Ogata and Mori (1964). Sections incubated in media containing all the ingredients except specific substrates served as controls.

OBSERVATIONS

All the enzymes studied <u>viz.</u>, α-GPDH, LDH, SDH and MDH were distributed in the parenchymatous cells of hepatic lobules of which α-GPDH and LDH were diffusely localized in cytoplasm while SDH and MDH showed granular, mitochondrial localization.

In the liver of lizards during preautotomy period activity of LDH was higher than those of α-GPDH, SDH and MDH.

After autotomy, during wound healing period, reactivity of α-GPDH and LDH became enhanced and remained so through blastema phase. Reactivities of SDH and MDH also increased during wound healing but were relatively less. Of the two enzymes <u>viz</u>., SDH and MDH, the former exhibited more reactivity than the latter. When blastema

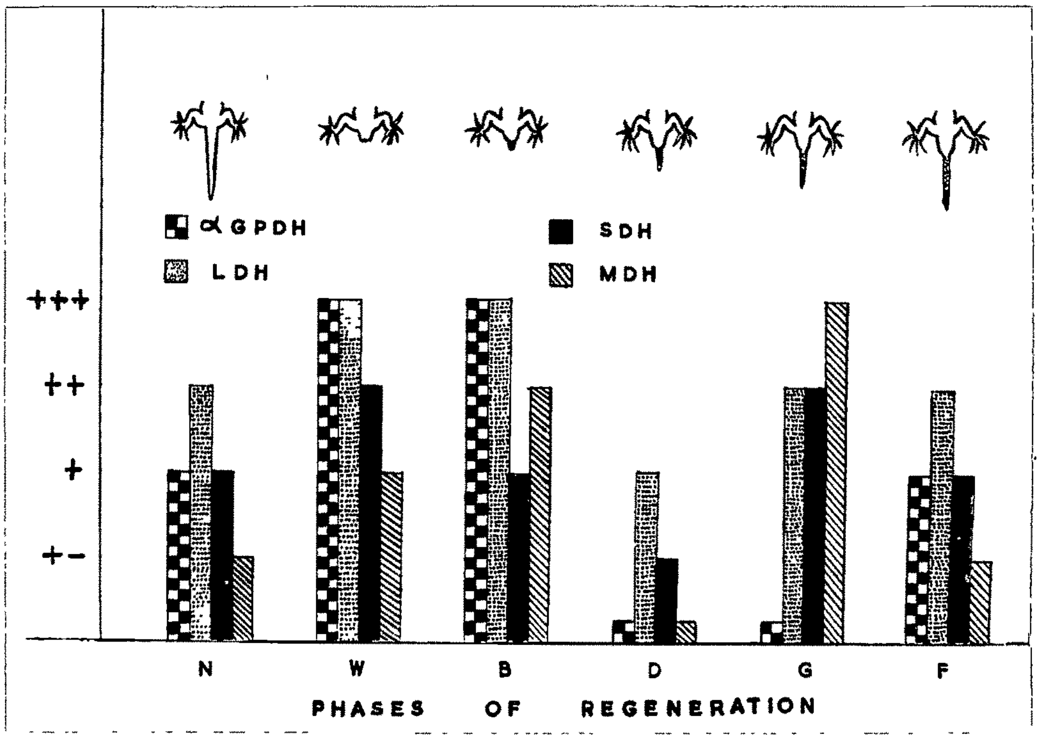

Fig. 1 : Histogram depicting activities of the four dehydrogenases in liver during different phases of tail regeneration in H. flaviviridis.

N - Normal tail
W - Wound healing phase
B - Blastema phase
D - Differentiation phase
G - Growth phase
F - Fully regenerated tail

+- Very low, + Low, ++ Moderate, +++ High.

was formed, reactivity of SDH came down to its preautotomy level, while MDH activity showed a further increase. During the differentiation phase activities of all the four dehydrogenases studied declined to a state which was very much lower than their preautotomy levels. However, during the next phase (growth phase) the activity of α-GPDH remained at a low level as was in the previous phase but that of the other three enzymes mounted considerably. Of these three enzymes, LDH settled to its preautotomy level, whereas SDH and MDH crossed their respective preautotomy levels. Further, of SDH and MDH, the latter had elevated considerably in its reactivity. Once the regenerate attained near full grown state, the activities of all the four dehydrogenases almost settled to their respective preautotomy levels (Fig. 1).

DISCUSSION

From the histochemical data obtained, low levels of SDH and MDH compared to that of LDH in the liver of *H. flaviviridis* during preautotomy period suggest a greater prevalence of anaerobic glycolysis than a functioning of aerobic pathway in the metabolic processes prevalent in the organ. This may be due to the fact that oxygen affinity of lizards is very low (Pough, 1969), and as a result they rely heavily on anaerobic metabolism (Bennet, 1972b; Bennet and Dawson, 1972; 1973; Bennet and Licht, 1972). Bennet (1972a) also reported low activities of aerobic enzymes in the liver of iguanid lizards.

Stress of tail autotomy is reflected in the increased activities of the enzymes, α-GPDH, LDH, SDH and MDH with a concomitant fall in the liver glycogen content, and since only a slight depletion in the hepatic lipid content was observed (Chapter 1), the enzymes of TCA cycle (SDH and MDH) appear to be acting predominantly upon substrates derived from the glycogen catabolism in the liver. Acceleration of liver carbohydrate catabolism following limb amputation of newt has been reported by Procaccini _et_ _al_. (1973). Since strong dehydrogenase activity is restricted to metabolically active sites, presently observed increase in α-GPDH and LDH in liver during the wound healing and blastema phases of tail regeneration with a concomitant depletion of glycogen in the liver could be considered as a response of the organ to meet the energy demands due to autotomy of tail during this phase. The energy demand during these phases of tail regeneration could be for repair of the broken tail tissue, for enhanced haemopoietic activities of bone marrow and spleen (Chapter 4 and 5), for synthesis of ascorbic acid in kidney (Chapter 3) and also for providing metabolic substrates to the proliferating and growing blastema cells (Chapter 1). It is believed that energy required in the initial period of activity in lizards is generated by lactate production (Moberly, 1968; Bennet, 1973), however, the dynamics of lactate production have not been examined in these animals (Bennet and Ruben, 1975).

From the present study on dehydrogenases in the liver of lizards during early phases of tail regeneration, it appears that anaerobic metabolism is in force in the organ to meet energy demands for the healing process and subsequent regeneration. This fact of anaerobic mode of metabolism is well reflected in low RBC count and haemoglobin content which suggests low availability of oxygen at this period (Chapter 4). At this juncture it is interesting to recall the observations made by Shah and Hiradhar (1974) that in the initial phases of tail regeneration, anaerobic metabolism is more apparent in the tail tissue.

The significance of LDH as a regulatory factor in glycolysis has been reported by Somero (1973) and Nelson and Wakefield (1973). The production of energy needed for various synthetic activities, and generation of nicotinamide adenine dinucleotide (NAD) through what has been called α-glycerophosphate (α-GP) shuttle could be one of the major functions of α-GPDH systems (Boxer and Shonk, 1960). Two types of α-GPDH, one NAD linked found in cytoplasm (Baldwin, 1952) and the other which is mitochondrial, reacting through cytochrome system (Kaplan *et al*., 1951; Pette, 1966; Pilstrom and Kiessling, 1973) are found in animal tissues. The importance of α-GPDH in maintaining α-GP shuttle has also been reported in invertebrate muscles by Sacktor (1970), Crabtree and Newsholme (1972), Hochachka *et al*. (1975) and Storey and Hochachka (1975). In this light the presently observed increased α-GPDH activity in the liver during wound healing and blastema phases of the regenerating tail in *H. flaviviridis* could be considered to be contributing towards setting up such metabolic activities in the organ so as to meet with the energy demands of many visceral organs and the regenerate, and also for continuation of EMP pathway of glycolysis through the α-GP shuttle in the organ.

Besides, the increase in the activity of SDH and MDH during wound healing and of MDH during blastema phase indicates the involvement of energy yielding reactions of the TCA cycle in the liver during these phases of regeneration in order to supplement the anaerobic reactions that have already set pace to meet the increased energy demands of the body and the regenerate. Incidence of high activities of SDH and MDH during wound healing and blastema phases respectively were not accompanied by simultaneous high activities of these two enzymes; however, the increase in either SDH or MDH could be considered as an index of increased TCA cycle operation (Ishimura and Fujie, 1970). Similar reports of difference in attainment of peak levels of SDH and MDH during early period of feather development come from the work of Shah and Menon (1973). Presently observed increase in the activities of the enzymes of anaerobic as well as aerobic metabolism in the liver during wound healing and blastema phases of the regenerating tail are quite understandable when overall metabolic activities of the body underlying the process of tail regeneration are geared up.

During differentiation phase of the regenerating tail, the general metabolic picture of hepatic tissue is changed from the one presented during previous phases. The activities of the enzymes <u>viz.</u>, α-GPDH, LDH, SDH and MDH decreased to a subnormal level (lower than the preautotomy level) which is suggestive of a lowered carbohydrate catabolism in the organ. On the other hand, an increased liver glycogen content during the same phase of the regenerating tail has been observed (Chapter 1). Such a change in carbohydrate metabolism in the liver is indicative of low utilization of carbohydrate by the regenerate.

Growth period of the regenerate is associated with greater proliferative and differentiative activities. At this stage, liver becomes an active participant to provide needed energy for the growing tissue. This action of liver becomes evident from the activities of the dehydrogenases, particularly of SDH and MDH. It is known that continuous growth cannot be supported by anaerobic metabolism, hence perhaps in this light the TCA cycle enzymes (SDH and MDH) in liver have become more active during this phase.

With gradual attainment to a fully grown state of the regenerate, the hepatic dehydrogenases assume preautotomy levels of their reactivities heralding normalisation of the systemic metabolic response of the organ to the regenerative process.