CHAPTER 1

ALTERATIONS IN CERTAIN METABOLITES DURING TAIL REGENERATION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Most studies on metabolic aspects of regeneration in vertebrates have been confined to the alterations at the local site during wound healing and regeneration of the organs or appendages. It is logical to believe that regenerative process might evoke overall physiological and even histomorphological changes in a number of organs of the animal body which could be considered as their adaptive response in an attempt to restore the lost part. An isolated attempt to evaluate alterations in metabolic pattern in a distant organ, *viz.*, liver, has been made during regeneration of amphibian appendage by Procaccini *et al.* (1971, 1973). No such studies on any visceral organs in reptiles have been made so far. Considerable work on histology, histochemistry and physiology of regenerating tail of the wall lizard, *Hemidactylus flaviviridis*, has been done in this laboratory (Shah, 1975). With such basic information on regenerating tail in the house lizard at hand, it was deemed worthwhile to investigate the alterations in metabolic patterns in certain visceral organs of this reptile during its tail regeneration. The present investigation

is aimed to report the changes occurring in the levels of certain metabolites in blood, liver, tail, skeletal muscle and adipose tissue of H. flaviviridis during different phases of its tail regeneration.

MATERIALS AND METHODS

The house lizards, H. flaviviridis, were collected from the University campus and were maintained in the laboratory on a diet of insects. Autotomy was induced by pinching off the normal, original tail leaving two or three basal segments intact. Adult lizards with intact original tail, as well as those at various phases of tail regeneration (Shah and Chakko, 1968a) were used for the following studies:

Glucose estimation in blood : Blood was collected by cardiac puncture from the animals under hypothermic anaesthesia. Estimation of glucose in blood was carried out employing the micromethod of Folin and Malmros (1929).

Liver, thigh muscle, adipose tissue and original tail or the regenerate (as the case may be) were excised immediately, blotted free of blood and tissue fluids, weighed and utilised for the following investigations.

Glycogen: Quantitative estimation of glycogen in liver, and thigh muscle of the experimental lizards with intact original tail and of those with regenerating tail at different phases was carried out using the method of Seifter *et al*. (1950).

Lipid: Liver and adipose tissue of the animals with intact original tail and of those with regenerating tail at different stages were weighed, dried in an air oven maintained at 80°C and weighed until constant weights were obtained. The total lipid contents of both these tissues were estimated gravimetrically (Folch *et al*., 1957).

Protein: Total protein content in the liver, original tail (with all its components combined) and the regenerate at different stages of its growth was estimated by Folin-phenol method of Lowry *et al*. (1951).

RESULTS

The data of quantitative estimation of glucose in blood, glycogen in the liver and muscle, lipid in liver and adipose tissue and protein in tail and liver have been presented in the tables 1, 2 and 3 and figures 1, 2 and 3 respectively.

The value of glucose in blood of lizards with intact original tail was 78.06 ± 9.20 (mg/100 ml). However, its highest concentration i.e., 113.40 ± 19.1 mg/100 ml was noted during the wound healing and beginning of blastema formation. Once the blastema was formed, glucose concentration in the blood fell to a subnormal level; however, during differentiation phase, it rose slightly above the normal value followed by attainment of the pre-autotomy level when the regenerate reached the fully grown state (Table 1, Fig. 1).

Liver glycogen was reduced by a little more than half its normal value during wound healing phase and remained almost at the same level till the blastema was fully formed. However, after this period the glycogen level in the liver rose till the differentiation phase, and thereafter gradually reached the preautotomy level when the regenerate reached the full grown state (Table 1, Fig. 1).

The glycogen content of thigh muscle (taken as a representative of the body muscle) had reduced to half its normal value during the wound healing phase and then increased gradually during the further course of

Table 1 : Levels of glucose in blood, glycogen in liver, muscle and tail* during tail regeneration in the house lizard, Hemidactylus flaviviridis

Normal tail and different phases of tail regeneration	Blood glucose (mg/100 ml)	Glycogen (gm/100 gm fresh tissue)		
		Liver	Muscle	Tail
Normal tail	78.06± 9.20[@] (24)	3.09±0.01[@](15)	0.78±0.01[@](15)	0.30±0.03[@](10)
Regenerating tail[$]				
Wound healing	113.40±19.1 (21)	1.29±0.82 (15)	0.34±0.02 (15)	
Blastema	70.08± 7.37 (27)	1.34±0.12 (17)	0.40±0.05 (17)	0.50±0.06 (8)
Differentiation	82.43± 9.89 (19)	2.47±0.11 (18)	0.59±0.02 (18)	0.96±0.14 (10)
Growth	79.23± 8.76 (21)	2.80±0.03 (14)	0.67±0.03 (14)	0.68±0.10 (9)
Fully regenerated	77.49±12.9 (23)	2.91±0.05 (15)	0.78±0.03 (15)	0.28±0.06 (10)

Figures in the parentheses indicate the number of experiments done.

[@]Mean ± S.D.

[$]The phases of regeneration are arbitarily chosen for purpose of discussion.

*Data after Shah and Hiradhar (1974)

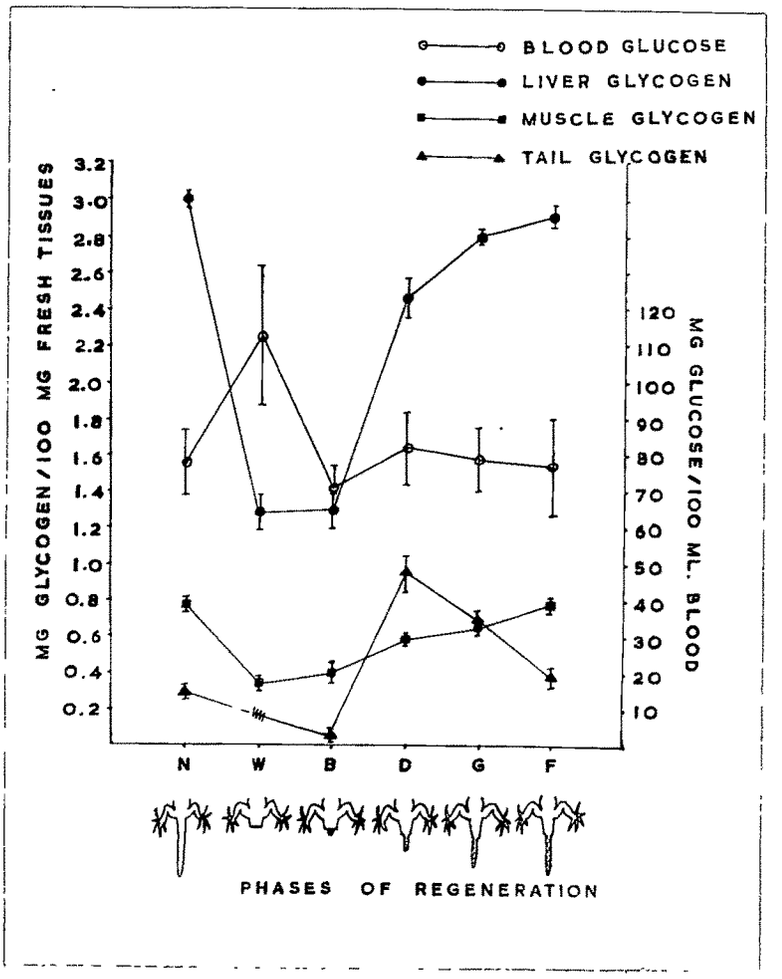

Fig. 1 : Graphic representation of levels of glucose in blood; glycogen in liver, muscle and tail during different phases of tail regeneration in H. flaviviridis.

N - Normal tail

W - Wound healing phase

B - Blastema phase

D - Differentiation phase

G - Growth phase

F - Fully regenerated tail.

regeneration reaching almost its normal value when the animals acquired full grown state of their tail (Table 1, Fig. 1).

Liver lipids did not show notable fluctuations during the entire process of tail regeneration. With regard to lipid levels in the abdominal adipose tissue during tail regeneration, the changes were quite drastic during differentiation and growth phases of the regenerate. A significant decrease in the lipid content of adipose tissue was observed during differentiation phase, which slightly increased during growth phase; nevertheless, the value observed during growth phase was less than its preautotomy level. In fully grown regenerate, adipose tissue had almost regained its preautotomy level (Table 2, Fig. 2).

In the normal lizards, the total protein content in the liver was about 19.20 $\pm$ 2.02 mg/100 mg and that of the original tail (with all its component tissues) was 4.802 $\pm$ 0.55 mg/100 mg. During wound healing phase, there was no change in the protein content of either of these two tissues. The protein content in the blastema and the regenerate during its differentiation phase had risen to

Table 2 : Levels of lipids in liver, adipose tissue and tail* during tail regeneration in the house lizard, Hemidactylus flaviviridis

Normal tail and different phases of tail regeneration	Total lipids (gm/100 gm fresh tissue)		
	Liver	Adipose tissue	Tail
Normal tail	17.23±1.76[@](19)	27.50±3.14[@](21)	24.49±5.93[@](15)
Regenerating tail[$]			
Wound healing	15.77±0.84 (21)	25.30±1.97 (20)	
Blastema	16.07±1.50 (24)	26.79±2.56 (21)	5.020±0.56 (16)
Differentiation	17.83±1.60 (21)	10.50±1.973(19)	4.302±0.72 (14)
Growth	16.59±1.23 (20)	18.16±3.91 (20)	12.56±4.76 (14)
Fully regenerated	16.46±0.70 (23)	24.94±2.97 (23)	38.52±7.47 (13)

Figures in the parentheses indicate the number of experiments done.

[@]Mean±S.D.

[$]The phases of regeneration are arbitrarily chosen for the purpose of discussion.

*Data after Shah and Hiradhar (1977)

Table 3 : Levels of proteins in liver and tail during tail regeneration in the house lizard, Hemidactylus flaviviridis

Normal tail and different phases of tail regeneration	Total protein (mg/100 mg fresh tissue)	
	Liver	Tail
Normal tail	19.20 ± 2.02[@] (13)	4.802 ± 0.55[@] (13)
Regenerating tail[$]		
Wound healing	20.52 ± 2.83 (15)	4.735 ± 0.76 (15)
Blastema	18.90 ± 1.88 (14)	10.71 ± 1.37 (14)
Differentiation	10.90 ± 1.46 (13)	10.02 ± 0.20 (12)
Growth	17.76 ± 0.96 (12)	5.558 ± 1.013 (12)
Fully regenerated	18.93 ± 1.03 (11)	4.912 ± 0.63 (11)

Figures in the parentheses indicate the number of experiments done.

[$]The phases of regeneration are arbitrarily chosen for the purpose of discussion.

[@]Mean ± S.D.

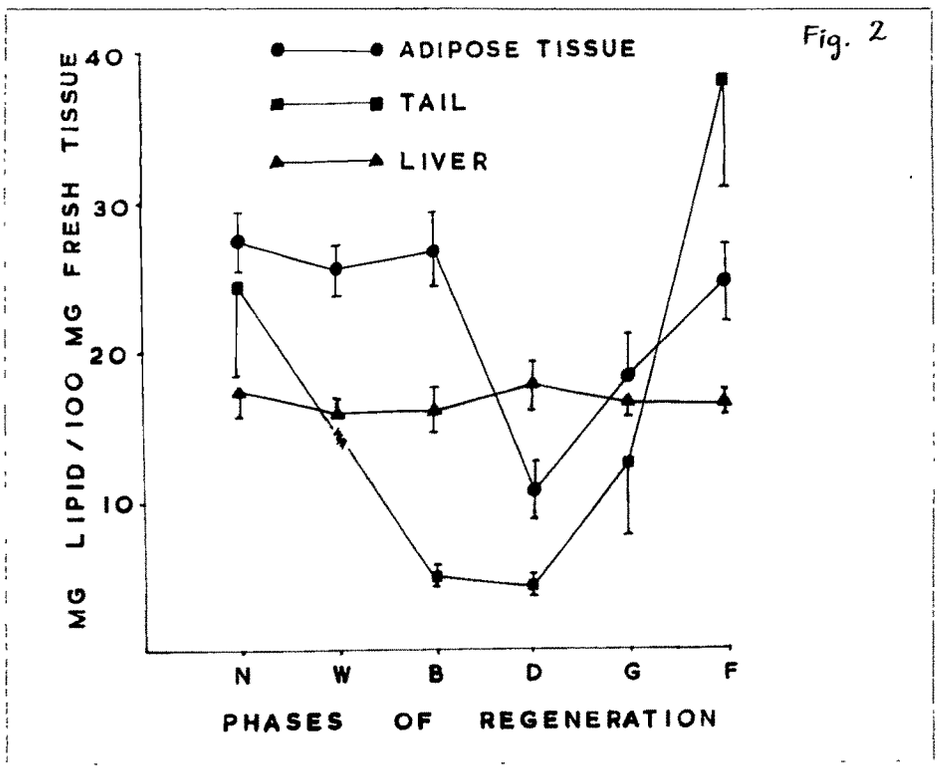
ADIPOSE TISSUE
TAIL
LIVER
MG LIPID / 100 MG FRESH TISSUE
40
30
20
10
N
W
B
D
G
F
PHASES OF REGENERATION

Fig. 2

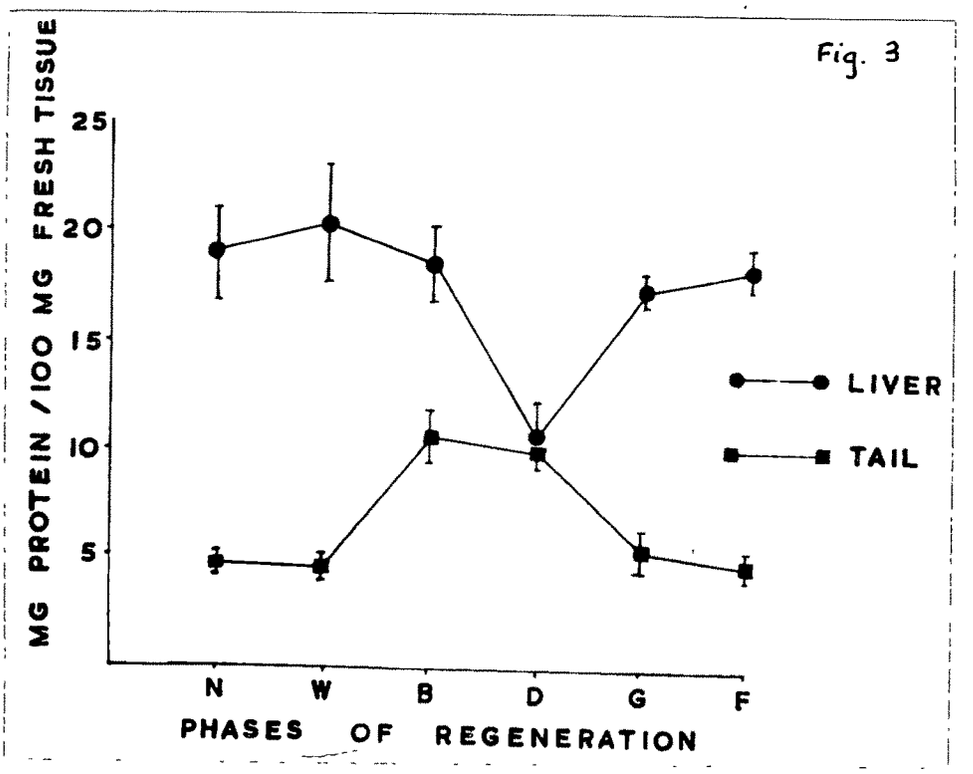
MG PROTEIN / 100 MG FRESH TISSUE
25
20
15
10
5
LIVER
TAIL
N
W
B
D
G
F
PHASES OF REGENERATION

Fig. 3

Fig. 2 : Graphic representation of levels of total lipids in adipose tissue, tail and liver during different phases of tail regeneration in H. flaviviridis.

Fig. 3 : Graphic representation of levels of total protein in liver and tail 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.

almost double its value (10.71 ± 1.37 and 10.02 ± 0.20 respectively) for the normal tail; while hepatic protein content during the differentiation phase showed a decrease (10.90 ± 1.46), but thereafter these organs almost regained their preautotomy levels when the regenerate was fully grown (Table 3, Fig. 3).

DISCUSSION

The present study indicates that in H. flaviviridis, immediately after autotomy of the tail, physiological homeostasis of the body gets altered sufficiently so as to adapt towards the extra energy demands of the broken tail stump tissue for repair and subsequent regeneration. The reduction in liver and muscle glycogen to half their normal values during wound healing phase is indicative of such an adaptive response. Importance of glycogen as one of the energy yielders in animal tissues is well known. Tassava (1969) while studying amphibian limb regeneration reported that energy derived from sources other than the stump tissue increases the survival chance and regenerative capacity of the animal. Shah and Chakko (1967) and Shah and Hiradhar (1974) have reported depletion in the glycogen content of the broken tissues of the tail stump in H. flaviviridis during wound healing phase. Presently noted

parallel depletion of glycogen in the liver and muscle which is reflected in rise in the blood glucose level may primarily be for meeting the stress condition in the initial stages; the energy demands for the wounded tissue for the repair; for high proliferative activities of the wound epithelium and also of haemopoietic tissues such as spleen and bone marrow (Chapter 4). Similar reports are available from the work of Procaccini et al. (1973) who observed depletion of hepatic glycogen during wound healing phase of amphibian limb regeneration. Since the level of glucose in blood is inversely related to the level of hepatic glycogen; and as muscle glycogen cannot directly serve as a source of blood glucose (Bullman et al., 1925), it appears that liver is the single major source of carbohydrate supply for the body which is so geared to meet the high energy requirements during regeneration at this stage. It is known that muscle glycogen can only be converted to glycogen in the liver via blood lactate, hence, it is possible that muscle glycogen may participate in energetics of tail regeneration in this indirect manner. Contribution of muscle glycogen in event of an excessive energy need as during breeding season in amphibians is suggested by Gourley et al. (1969).

During blastemic phase, the liver and muscle glycogen remained almost at the same low level as was during wound healing period, but the blood glucose content during the corresponding period declined to a subnormal level from its previously highly elevated state. From these observations, it could be surmised that possibly a balance is struck between the synthetic and catabolic activities with regard to glycogen in liver and muscle; and the release of glucose into blood is not effected as is seen from a fall in blood glucose level during this period. From these observations and reported paucity of glycogen as well as the absence of adequate machinery for its utilization in the blastema cells of amphibian limb and reptilian tail regenerates (Schmidt, 1968; Radhakrishnan and Shah, 1973; Shah and Hiradhar, 1974), one can conclude that glycogen is not the preferred metabolite for energy production during blastema phase. The reduction in blood glucose level during this period could possibly be due to diversion of glucose moieties to hexose monophosphate (HMP) pathway (Magon, 1970; Shah and Ramachandran, 1973) which is significantly associated with nucleotide and lipid synthesis in the regenerating lacertilian tail (Magon, 1970; Shah and Chakko, 1972; Shah and Ramachandran, 1973; Radhakrishnan, 1973; Chakko, 1967).

During differentiation of the regenerate, the liver and muscle glycogen levels increased, nevertheless, they were below the preautotomy levels. At this stage, the blood glucose concentration rose a little above the normal level. These observations indicate that glucose from blood is being made available to the differentiating tail tissue where glycogen synthesis was also observed at a greater pace (Shah and Hiradhar, 1974). Besides, it could also be said that the blood glucose at this stage is being taken up by liver and muscle to replenish their earlier depleted glycogen levels.

During the next phase, i.e. growth, and thereafter muscle and liver glycogen levels took a gradual ascending course and reached almost their respective normal (pre-autotomy) levels when the regenerate attained fully grown state by which time the blood glucose level also settled to, its normal value.

No drastic fluctuations in total lipid content of liver were noticed during the course of regeneration. The total lipid content of abdominal adipose tissue did not show any significant changes in the initial phases of regeneration viz., wound healing and blastema, but during differentiation and growth phases of the regenerate,

its lipid content had decreased. The decrease in lipid content of adipose tissue at this stage can be well accounted for considering the possibility of increased lipid mobilisation from this tissue for meeting the tremendous energy demands for synthetic activities of the differentiating tail tissues. Similar suggestion on lipid transport from distant organs of the body during amphibian limb regeneration has been made by Procaccini et al. (1973). In animals with fully regenerated tail, abdominal adipose tissue had regained its preautotomy level.

Since no change in total protein content of the liver as well as the tail was noticed during wound healing phase, it may be assumed that protein metabolism in these organs is not yet accelerated. However, during blastema phase, protein metabolism in the regenerate assumes an important role as evidenced by two fold increase in its contents. This high content of protein in the tail regenerate was maintained during differentiation phase too. During blastema formation, cell proliferation and cell growth are quite high, this can well account for the rise in the protein content of the regenerate. Since differentiation involves synthesis of tissue specific proteins, the above mentioned observations of high protein

content of the regenerate are self explanatory. However, the presently noted depletion in total protein content of the liver during differentiation phase of the regenerate possibly contributes to the amino acid reserves for the protein synthesis during histodifferentiation of the regenerate, and/or protein being used as the precursor of glucose which may be made available to the regenerate. The latter contention finds support in the work of Tassava (1969) on amphibian limb regeneration wherein he has suggested the role of proteins as a precursor of glucose in later phases of regeneration. Thus, it appears that hepatic catabolism centered around glycogen during wound healing phase is shifted to protein catabolism during differentiation phase of the regenerating tail of H. flaviviridis.

In animals with fully regenerated tail, the levels of metabolites viz., glycogen, lipid and protein in liver and tail, glycogen in thigh muscle and lipids in adipose tissue showed almost preautotomy levels denoting the normalisation of the upset metabolic pattern of visceral organs and tail tissues which had occurred in response to tail regeneration.