# CHAPTER 2

## EFFECT OF HYPOPHYSECTOMY ON TISSUE GLYCOGEN AND BLOOD GLUCOSE LEVELS IN THE HOUSE LIZARD, HEMIDACTYLUS FLAVIVIRIDIS DURING TAIL REGENERATION

Importance of glycogen as a major energy yielder for metabolic activities in animal tissues during development has been well recognized (Borghese, 1957; O'Connor, 1957; Engel, 1961; Falin, 1961). Studies on amphibian regeneration (Schmidt, 1960; 1962; Procaccini et al., 1973; Connelly et al., 1974) and those on reptilian tail regeneration (Shah and Chakko, 1967b; Radhakrishnan and Shah, 1973; Shah and Hiradhar, 1974; Shah et al., 1977a,b) have contributed some basic information regarding importance of glycogen in the process of repair and regeneration. It is well-known that the pituitary hormones have a regulatory influence on carbohydrate metabolism (Brown, 1978) and the removal of hypophysis in reptiles causes hypoglycemia and depletion of glycogen from liver and muscle (Rangneker and Sabnis, 1966; Rangneker and Padgaonkar, 1972).

Earlier studies from our laboratory have shown that the tissues of the normal and regenerating tail of the lizard,

Hemidactylus flaviviridis, utilize glycogen either through Embden Meyerhof Pathway (EMP) or Hexose monophosphate Shunt (HMP) for energy procurement (Shah and Hiradhar, 1978). Removal of hypophysis in H. flaviviridis caused a marked reduction in the growth rate and structure of the tail regenerate (Chapter 1). Relative levels of glycogen in the tail regenerate, liver and thigh muscles, and the levels of glucose in the blood have been estimated during different phases of tail regeneration in the hypophysectomized and sham operated lizards, H. flaviviridis in order to evaluate the influence of hypophysial hormone/s on the carbohydrate metabolism during reptilian tail regeneration.

## MATERIALS AND METHODS

The house lizards, H.flaviviridis, were collected from the university campus and maintained in the laboratory on a diet of insects. The animals were kept in the laboratory for a fortnight to get them acclimatized to the laboratory conditions. Adult lizards (weighing 10-12 gms) were selected for hypophysectomy and sham operation, and were operated upon as described in Chapter 1. Tail autotomy was induced 10 days after operation. Fifty lizards in each case viz., hypophysectomized and sham operated were used for the present

experiment. At specific intervals in accordance with the various phases of tail regeneration, 6 to 8 animals per phase were sacrificed.

Estimation of glycogen contents in the tail regenerate, liver and thigh muscles, and glucose levels in blood in hypophysectomized and sham operated lizards during different phases of their tail regeneration were carried out by Anthrone method of Seifter et al. (1950) and micro-method of Folin and Malmros (1929) respectively.

## RESULTS

The data on glycogen contents in tail regenerates, liver and muscles and glucose in the blood obtained at various stages of tail regeneration are presented in Tables 1,2,3, & 4 and Figs. 1,2,3 & 4.

### Tail Tissue:

On hypophysectomy there was a considerable fall in the glycogen content of tail tissue which further declines as autotomized tail begins to regenerate. Continuous but gradual fall in the glycogen level in tail regenerate was observed till blastema was fully formed. Thereafter, a

Table 1

Levels of glycogen in the tail of hypophysectomized and sham operated lizard, H.flaviviridis, during tail regeneration.

| Stages | Hypophysectomized (a) | Sham operated (b) | $\frac{a}{b}$ x 100 |
|---|---|---|---|
| 10 days after operation | 0.09278 ± 0.0034 ($P < .005$) | 0.23569 ± 0.0869. | 39.36 |
| Phases of tail regeneration | | | |
| Wound healing | 0.06896 ± 0.0086 ($P < .0025$) | 0.04816 ± 0.0057 | 143.18 |
| Blastema | 0.06840 ± 0.0123 ( NS ) | 0.07713 ± 0.0042 | 88.68 |
| Differentiation | 0.08637 ± 0.0198 ($P < .025$) | 0.17057 ± 0.0620 | 50.63 |
| Growth (30 days) | 0.17523 ± 0.0276 ($P < .0025$) | 0.43750 ± 0.1449 | 40.05 |
| Growth (50 days) | 0.18641 ± 0.0211 ($P < .0005$) | 0.6209 ± 0.1570 | 30.02 |

P values in parentheses were obtained in comparison with sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in g/100g fresh tissue as mean ± S.D.

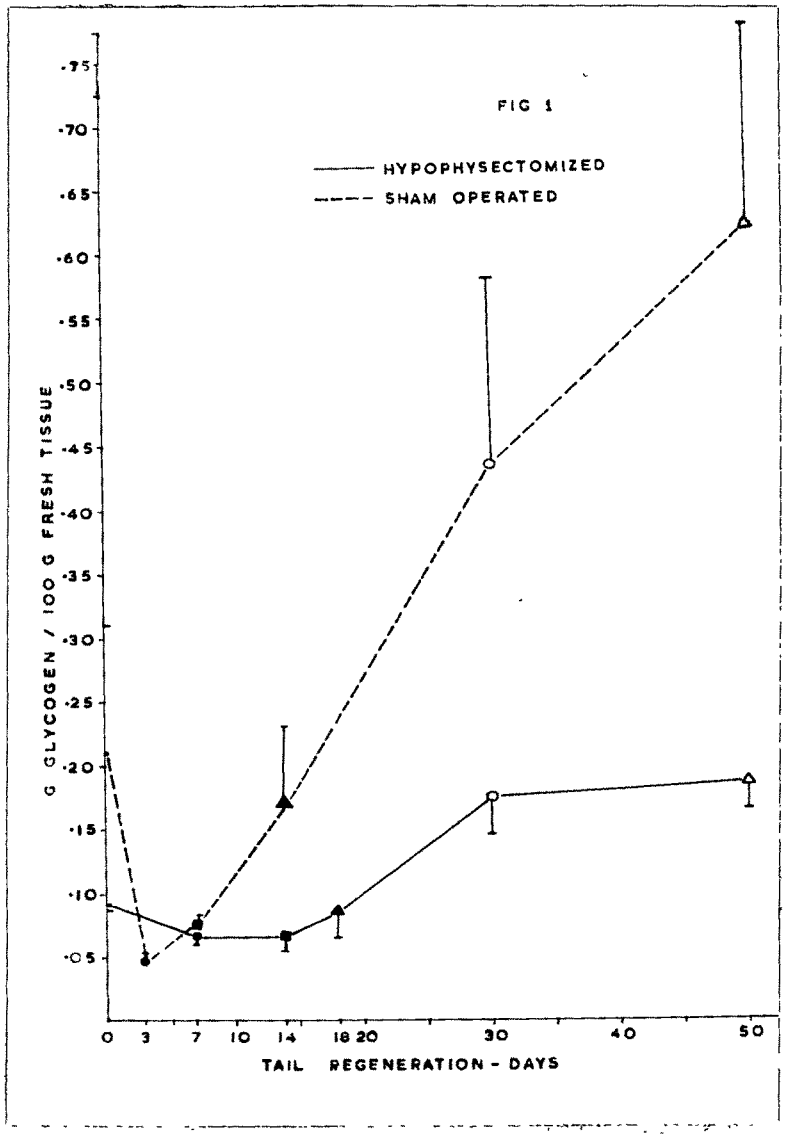


Fig. 1 : Graphic representation of levels of glycogen in normal and regenerating tail, during different phases of tail regeneration in hypophysectomized and sham operated lizards, H. flaviviridis.

● Wound healing phase
■ Blastema phase
▲ Differentiation phase
○ Growth phase (30 days after autotomy)
△ Growth phase (50 days after autotomy)

Table 2

Levels of glycogen in the liver of hypophysectomized and sham operated lizard, H.flaviviridis, during tail regeneration.

| Stages | Hypophysectomized (a) | Sham operated (b) | $\frac{a}{b}$ x 100 |
|---|---|---|---|
| 10 days after operation | 0.53088 ± 0.1261 (P < .0005) | 1.34012 ± 0.2798 | 39.61 |
| Phases of tail regeneration: | | | |
| Wound healing | 0.12939 ± 0.0167 (P < .0005) | 2.2619 ± 0.3236 | 5.72 |
| Blastema | 0.06377 ± 0.0301 (P < .0005) | 2.5071 ± 0.2667 | 2.54 |
| Differentiation | 0.11977 ± 0.0577 (P < .0005) | 0.43353 ± 0.0360 | 27.62 |
| Growth (30 days) | 0.15582 ± 0.0932 (P < .0005) | 0.9737 ± 0.2190 | 16.002 |
| Growth (50 days) | 0.14025 ± 0.0856 (P < .0025) | 1.2675 ± 0.5104 | 11.06 |

P values in parentheses were obtained in comparison with sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in g/100g fresh tissue as mean ± S.D.

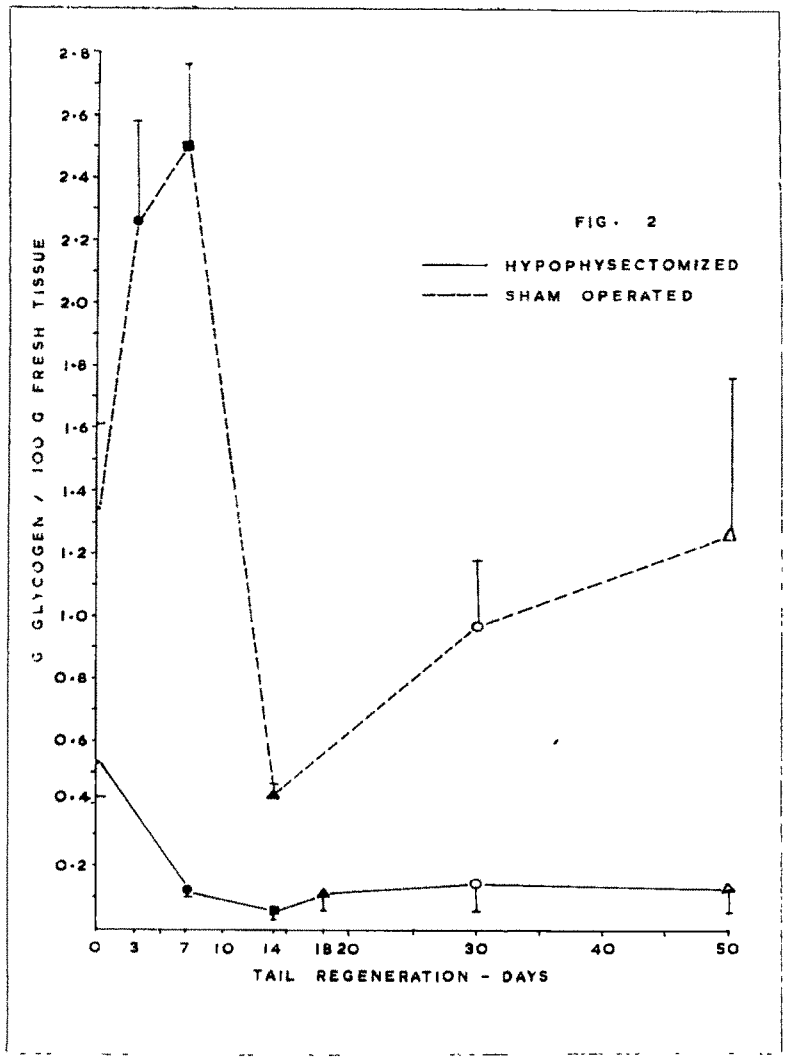


Fig. 2 : Graphic representation of levels of glycogen in liver, during different phases of tail regeneration in hypophysectomized and sham operated lizards, H. flaviviridis.

- ● Wound healing phase
- ■ Blastema phase
- ▲ Differentiation phase
- ○ Growth phase (30 days after autotomy)
- △ Growth phase (50 days after autotomy)

gradual increase in this carbohydrate content occurred during differentiation and growth phase. From the data it became evident that 50 day old tail regenerate in a hypophysectomized lizard had far low level of glycogen compared to that noticed in the intact normal tail of the control (sham operated) animals. In sham operated lizards, there was a fall in glycogen level in the tail regenerate during early phases and a rise during later phases of regeneration.

Liver:

Liver also, in the hypophysectomized animals, showed low level of glycogen content prior to autotomy. During wound healing liver glycogen level declined considerably, which further dipped during blastema formation when autotomized tail was regenerating. As the differentiation began, the glycogen level in the liver ascended and that continued till the end of the experiment when the regenerate had become 50 days old. However, the glycogen content of the liver in the lizard with 50 day old regenerate was much less than that was observed in the liver of the lizard prior to tail autotomy.

In the sham operated lizards the liver glycogen increased during wound healing and became two-fold in the

Table 3

Levels of glycogen in the thigh muscle of hypophysectomized and sham operated lizard, H.flaviviridis, during tail regeneration.

| Stages | Hypophysectomized (a) | Sham operated (b) | $\frac{a}{b}$ x 100 |
|---|---|---|---|
| 10 days after operation | 0.05236 ± 0.0217 ($P < .0005$) | 0.14350 ± 0.0214 | 36.48 |
| Phases of tail regeneration: | | | |
| Wound healing | 0.13120 ± 0.0274 ($P < .05$) | 0.18410 ± 0.046 | 71.26 |
| Blastema | 0.04216 ± 0.0239 ($P < .0005$) | 0.13967 ± 0.0250 | 30.18 |
| Differentiation | 0.04262 ± 0.0176 ($P < .001$) | 0.10327 ± 0.0260 | 41.27 |
| Growth (30 days) | 0.14042 ± 0.0206 ($P < .01$) | 0.18181 ± 0.0223 | 77.23 |
| Growth (50 days) | 0.12558 ± 0.0252 ( NS ) | 0.16025 ± 0.0420 | 78.36 |

P values in parentheses were obtained in comparison with sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in g/100g fresh tissue as mean ± S.D.

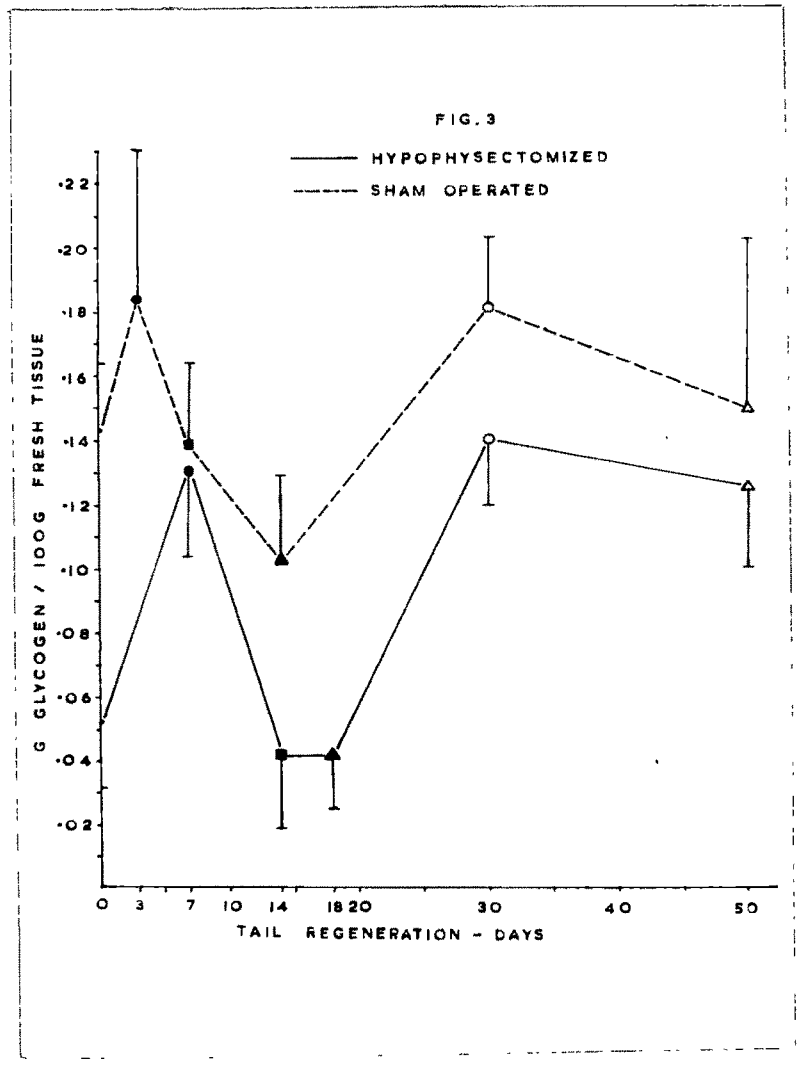


Fig. 3 : Graphic representation of levels of glycogen in thigh muscles, during different phases of tail regeneration in hypophysectomized and sham operated lizards, H. flaviviridis.

- ● Wound healing phase
- ■ Blastema phase
- ▲ Differentiation phase
- ○ Growth phase (30 days after autotomy)
- △ Growth phase (50 days after autotomy)

Table 4

Levels of glucose in blood of the hypophysectomized and sham operated lizard, H.flaviviridis,during tail regeneration.

| Stages | Hypophysectomized (a) | Sham operated (b) | $\frac{a}{b}$ x 100 |
|---|---|---|---|
| 10 days after operation | 135.56 ± 19.579 (P < .0005) | 245.16 ± 19.567 | 55.29 |
| Phases of tail regeneration: | | | |
| Wound healing | 132.36 ± 12.957 (P < .0005) | 160.64 ± 11.026 | 82.39 |
| Blastema | 101.39 ± 19.249 (P < .0005) | 152.93 ± 15.447 | 66.30 |
| Differentiation | 107.56 ± 16.700 (P < .0005) | 163.70 ± 10.654 | 65.70 |
| Growth (30 days) | 93.91 ± 20.096 (P < .0005) | 193.77 ± 17.187 | 48.46 |
| Growth (50 days) | 99.17 ± 7.303 (P < .0005) | 241.10 ± 22.657 | 41.13 |

P values in parentheses were obtained in comparison with sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in mg/100 ml of blood as mean ± S.D.

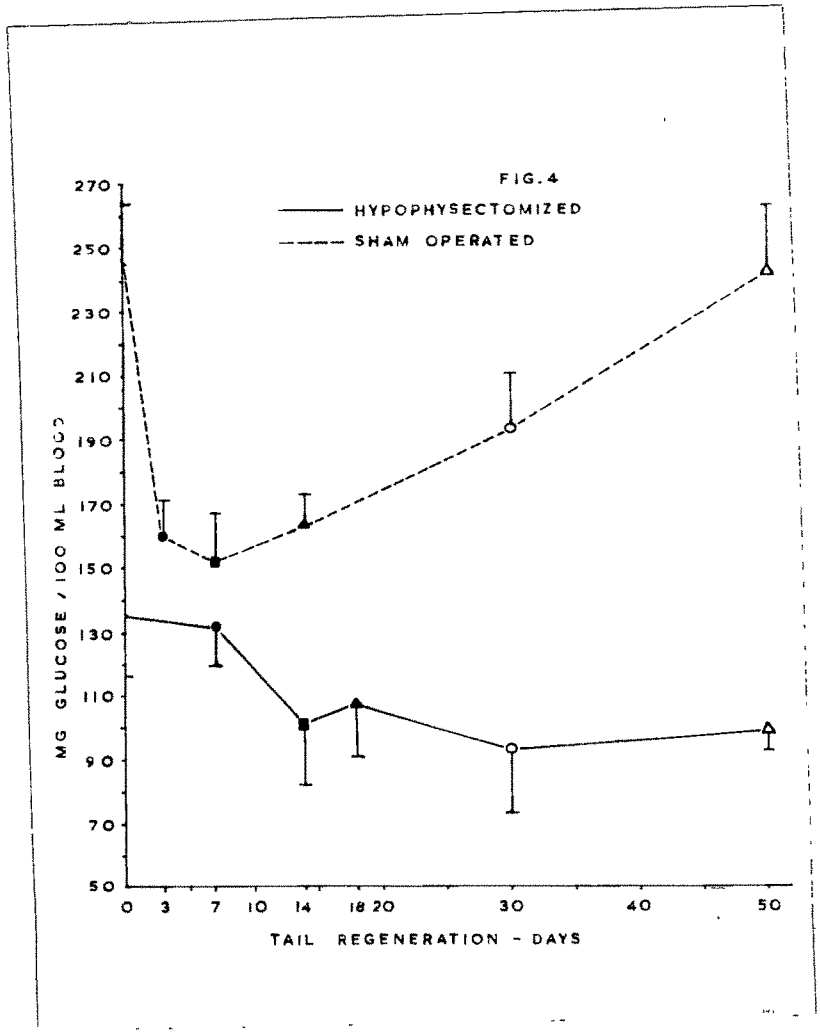


Fig. 4 : Graphic representation of levels of glucose in blood, during different phases of tail regeneration in hypophysectomized and sham operated lizards, H. flaviviridis.

- ● Wound healing phase
- ■ Blastema phase
- ▲ Differentiation phase
- ○ Growth phase (30 days after autotomy)
- △ Growth phase (50 days after autotomy)

blastemal phase, compared to the preautotomy glycogen level. However, during differentiation phase it considerably declined but again began to rise during the growth period and attained more or less the same level on 50th day, which was observed prior to tail autotomy.

Thigh Muscles:

Hypophysectomy produced a marked reduction in the glycogen content of thigh muscles as compared to that observed in the sham operated lizards. In both the experimental as well as control animals during wound healing phase the muscle glycogen increased but again declined during the blastema and remained so during differentiation phase. However, it rose again during the growth phase of the regenerate. When regenerate became 50 days old, the thigh muscle glycogen in hypophysectomized and sham operated lizards did not show any significant difference.

Blood Glucose:

Compared to the sham operated lizards blood glucose level in hypophysectomized animals, at 10th day after hypophysectomy was found to be quite low. During the

wound healing, blastema, differentiation and early growth phase (30 days) the blood glucose level in the sham operated as well as in hypophysectomized lizard remained at a lower level than that recorded during preautotomy period. But by 50th day in the sham operated lizards, the level of glucose reached the preautotomy value, and in the hypophysectomized lizards it remained considerably low which was lower than the preautotomy value.

## DISCUSSION

The results of the present study show that the hypophysectomy has a significant effect on glycogen contents of the tail tissue, liver and muscle, and also on the blood glucose in the house lizard. Effect of hypophysectomy on carbohydrate metabolism in lizards has been reported (Rangneker and Sabnis, 1966; Suryawanshi and Rangneker, 1974). In the present study glycogen content of the original tail of the hypophysectomized lizards showed a significantly low level when compared with that in the sham operated lizards. Hence the delay in wound healing and blastema formation (Chapter 1) observed in the hypophysectomized lizard, could be due to

the reduced availability of carbohydrates. Utilization of glycogen from the stump of the tail after autotomy during dedifferentiation phase has been observed during lizard tail regeneration (Shah and Chakko, 1967b; Radhakrishnan and Shah, 1973; Shah and Hiradhar, 1974). Similar observation has also been made during amphibian limb regeneration (Schmidt, 1962).

Wound healing and blastemal phases depicted remarkably low levels of glycogen in the sham operated and hypophysectomized lizards when compared to those observed during all the subsequent stages of tail regeneration. Considerable paucity of glycogen in the blastema may be due to lack of its synthesis (Shah and Hiradhar, 1974). Whatever little glycogen could be detected was possibly due to the phagocytic properties of wound epithelium and the scattered glycogen droplets in the extracellular spaces among the blastemal cells (Singer and Salpeter, 1961; Norman and Schmidt, 1967).

With the onset of differentiation of the mesenchymal cells, the overall metabolic activities of the regenerate are considerably geared up (Shah and Hiradhar, 1978). Involvement of carbohydrates in the formation of the structural elements and their role in energy supply have

been discussed in the earlier studies during reptilian tail regeneration (Radhakrishnan and Shah, 1973; Shah and Hiradhar, 1974). The present study on glycogen content in the regenerating tail of the hypophysectomized lizards during differentiation and growth phases (See Table 1) revealed a significantly low level of the metabolite as compared to that in the sham operated lizards. Such low level of glycogen in the regenerate is probably due to a reduction in enzyme/s activities concerned with carbohydrate metabolism following the removal of hypophysis (Weber and MacDonald, 1961; Bornstein, 1972). The reduction in rate of growth and the poorly developed structure of the regenerate in the hypophysectomized lizard (Chapter 1) indicate either inability of the cells engaged in the process of regeneration to utilize the available glucose (Martin, 1976) or a direct effect on the metabolism of these cells resulting from the absence of pituitary hormone (Sato and Inoue, 1973). Inhibition of limb regeneration in Diemictylus viridescens by somatostatin, a hypophysial factor, which inhibits the production of thyroid stimulating hormone, prolactin, growth hormone, insulin and glucagon is also found to lower the blood glucose and glycogen levels (Vethamany-Globus et al., 1977). Such possibility in the present context cannot be ruled out.

The presently observed fluctuations in levels of glycogen in liver and thigh muscles, and glucose in blood during different phases of tail regeneration in the sham operated lizards clearly indicate mobilization of this metabolite from the distant organs like liver and muscles. Reduction in blood glucose during wound healing from pre-autotomy value suggests utilization of circulating glucose moieties. However, during wound healing and blastema formation the hepatic glycogen content showed two-fold increase in the sham operated lizard. This increase could be due to either active glycogenesis or low glycolysis in the hepatic tissue. The persisting low level of blood glucose during blastemal phase could perhaps be due to the utilization of glucose molecules by the regenerate through HMP shunt which is significantly associated with synthesis of nucleotides and lipids in the regenerating reptilian tail (Magon, 1970; Shah and Chakko, 1972; Shah and Ramachandran, 1973; Radhakrishnan, 1972).

In the sham operated animals during differentiation and early growth phase the liver glycogen showed a significant reduction. However, the consequent rise in the blood glucose level, though observed, was not above the preautotomy level. The increased breakdown of liver

glycogen and the persisting low level of blood glucose could be correlated with active uptake of glucose by differentiating and growing cells of the regenerate. Presently observed increase in glycogen contents of the regenerate during these phases supports the above contention.

It has been shown that in most of the vertebrate species, there is a reduction in liver glycogen and the blood glucose following hypophysectomy. Hypophysectomy in lizard, Varanus monitor (Rangneker and Sabnis, 1966) produced a severe hypoglycemia but in another species Eumeces obsoletus (Miller and Wurster, 1958) only a mild hypoglycemia ensued in the so operated animals. On the other hand in the lizard, Uromastix hardwickii (Suryawanshi and Rangneker, 1974) hypophysectomy has no effect on the blood glucose level. In the present study the hypophysectomized H.flaviviridis showed a significant decrease in liver glycogen and a progressive decrease of the blood glucose level. Since the liver glycogen and the blood glucose are involved in providing the extra energy during repair and regeneration (Shah et al.,1977a,b), the alterations in carbohydrate metabolism following hypophysectomy (Rangneker and Sabnis, 1966; Rangneker and

Padgaonkar, 1972) could interfere with contribution of required energy to the regenerating system. Hence, the observed delay in regenerating process and poor growth of the regenerate in the hypophysectomized lizards could be well correlated with the alterations in the carbohydrate metabolism due to absence of hypophysial hormone/s.

The glycogen in the skeletal muscle is not normally utilized for the support of general body metabolism, but it is rather held in reserve for production of ATP through glycolysis during muscular activity (Packard and Randall, 1975). Participation of muscle glycogen in event of an excessive need is reported during breeding in amphibians (Gourley et al., 1969). The fluctuations in the levels of muscle glycogen in sham operated and hypophysectomized lizards during different phases of their tail regeneration probably reflect participation of body muscle glycogen in the energetics of the regenerate. The hypophysectomized lizards showed a significant reduction in the muscle glycogen compared to that in the sham operated lizards. Such reduction in muscle glycogen is characteristic of hypophysial insufficiency (Russell, 1936; 1938; Rangneker and Padgaonkar, 1972). Thus, the presently observed reduced rate of growth and poor development of the




regenerate structure in the hypophysectomized lizards could be well correlated with inadequate supply of carbohydrate associated with deranged carbohydrate metabolism in the absence of pituitary hormones.