CHAPTER VIII

ALTERATIONS IN TISSUE GLYCOGEN CONTENTS AND BLOOD GLUCOSE LEVELS DURING TAIL REGENERATION IN NORMAL AND ADRENAL SUPPRESSED GEKKONID LIZARDS, *HEMIDACTYLUS FLAVIVIRIDIS*

Carbohydrates are generally considered to be instant source of energy for living systems. Schmidt (1960) and Connely *et al.* (1974) have given some insight about the functional significance of carbohydrate metabolism in amphibian limb regeneration. Previous studies from this laboratory have shown the dependence of the process of tail regeneration on hepatic glycogen and blood glucose in the lizards, *Mabuya carinata* and *Hemidactylus flaviviridis* (Shah *et al.*, 1977a,b, 1982a,c; Menon *et al.*, 1981). In fact blood glucose was considered to be the principal source of energy for the blastemal cells, an observation which was further confirmed by the findings of an increased activity of hexokinase in the regeneration blastema (Shah *et al.*, 1980d). When mammalian skin is injured, glycogen accumulates rapidly in the wound epithelium, synthesised from glucose with the catalytic assistance of high uridine diphosphate (UDPG) glycogen transferase activity (Kubo, 1964). Involvement of glycogen

and its metabolism in processes associated with vertebrate appendage regeneration have gained attention in the recent past (Schmidt, 1962; Procaccini et al., 1973).

It has been reported that adrenal steroids can exert marked effect on carbohydrate metabolism (Britton and Silvette, 1932). Adrenalectomy has been shown to bring about decreased glycogenolysis by reducing the responsiveness of phosphorylase to glucagon (Timothy et al., 1979), decreased serum insulin level (Losert et al., 1970), hypoglycaemia and low tissue glycogen content (Long et al., 1940) and reduced absorption of glucose from the intestine (Chin and Wang, 1979). Due to the fact that adrenal hormones exert significant influence on carbohydrate metabolism and that the process of lacertilian tail regeneration is accompanied by in loco and systemic modulations in carbohydrate metabolism, it was thought pertinent to evaluate the influence of adrenocortical suppression on regeneration vis-a-vis carbohydrate metabolism in the Gekkonid lizard, Hemidactylus flaviviridis.

MATERIALS AND METHODS

The lizards, H. flaviviridis 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 acclimatisation to the laboratory conditions. Lizards weighing 10-12 gm and having a snout-vent length of 8-10 cm 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 subjected to chemical adrenocortical suppression by the synthetic corticosteroid dexamethasone (DXM). Injections were given intraperitoneally (15 µg/0.1 ml/day/animal) in the evenings at 17.00 hrs. and was started ten days prior to tail autotomy. Control lizards were injected with the same amount of distilled water. Injections were continued every alternate day even after autotomy till the end of experimentation. Animals with regenerating tail were sacrificed at fixed intervals of 3, 5, 7, 10, 15, 25, 40 and 60 days post-autotomy along with the normal animals with intact tail. Liver, skeletal muscle and the tail (regenerating or normal as the case may be) were removed quickly and were weighed. Estimation of glycogen content in the three tissues was carried out by the anthrone method of Seifter et al. (1950). Blood glucose levels were measured according to the method of Folin and Malmrose (1929).

For each period and each tissue specified, a total of five to seven determinations of blood glucose levels and tissue glycogen contents were made. The mean and standard error were obtained and Student's 't' test was used to determine the statistical significance.

RESULTS

Figures 1 to 4 and Tables 1 to 3 depict the levels of glycogen content and blood glucose levels in the control as well as adrenal suppressed lizards during the various phases of tail regeneration.

In general, adrenal suppression seemed to have a depleting influence on the carbohydrate reserves of the body as the adrenal suppressed lizards depicted a 40% reduction in blood glucose and tissue glycogen when compared with the control lizards prior to caudal autotomy. Post-caudal autotomy, the pattern of regeneration specific changes appeared to be similar in both sets of lizards. However, the changes were very much attenuated both quantitatively as well as in terms of duration in the DXM lizards. In the control lizards, the caudal glycogen content showed an initial depletion of 55% by the 5th day which then gradually increased (by 264%) to a peak level on the 10th day. Subsequently, the glycogen content

again decreased through 15th to 25th day by 56% and then settled to the pre-autotomy level by the 40th day. In the DXM lizards on the other hand the initial glycogen depletion (15%) lasted only till the 3rd day which increased by 76% by the 5th day. Ninety percent of this was depleted gradually between the 7th and 40th day and the pre-autotomy level was ultimately attained by the 60th day. The hepatic glycogen content in the control lizards showed an initial 53% depletion by day 3 post-autotomy which then increased gradually to a maximum level by the 10th day, an increase of 243%. The glycogen content then decreased gradually through 15th to the 25th day by 85% to attain a subnormal level. There was a gradual increase thereafter towards the normal during the 40th and 60th days. In contrast, the DXM lizards depicted the initial glycogen depletion only to the tune of 10% and the following glycogen accumulation lasted only upto the 7th day and the increase was only a mere 150%. The second phase of glycogen depletion (77%) lasted only between the 7th and 15th days whence it was subnormal. Thereafter the hepatic glycogen content increased gradually to the normal pre-autotomy range by the 60th day.

The skeletal muscle glycogen content of control lizards depicted a continuous and gradual increase till the 10th day post-autotomy whence the level was up by

TABLE-1 : Comparative blood glucose levels (mg/dl) during tail regeneration in normal and adrenal suppressed H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Normal	109.96 ±1.59	92.59** ±1.09	83.23** ±2.99	76.08** ±1.16	97.49* ±3.06	90.42** ±1.31	71.11** ±1.69	97.73* ±2.83	98.54* ±3.41
Adrenal suppressed	58.55 ±2.02	45.8** ±1.63	42.91** ±2.41	52.82 ±1.97	35.11** ±1.48	76.98** ±2.22	39.8** ±3.12	59.87 ±0.91	65.89 ±3.11

* $P < 0.01$; ** $P < 0.001$

TABLE-2 : Alterations on tissue glycogen content (mg/100 mg fresh tissue) during tail regeneration in normal H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.58 ±0.03	0.27** ±0.02	0.61 ±0.07	1.14** ±0.08	1.45** ±0.17	0.71 ±0.06	0.22** ±0.02	0.43 ±0.08	0.45@ ±0.04
Muscle	0.17 ±0.01	0.20 ±0.01	0.19 ±0.02	0.28 ±0.05	0.58** ±0.04	0.32* ±0.04	0.16 ±0.01	0.18 ±0.02	0.12 ±0.02
Tail	0.31 ±0.03	0.17* ±0.02	0.14** ±0.01	0.20* ±0.01	0.51** ±0.03	0.41 ±0.04	0.22 ±0.03	0.32 ±0.01	0.27 ±0.01

* P <0.01; ** P <0.001 ; @ P < 0.05

TABLE-3 : Alterations in tissue glycogen content (mg/100 mg fresh tissue) during tail regeneration in adrenal suppressed H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.31 ±0.04	0.28 ±0.05	0.37 ±0.08	0.71* ±0.11	0.31 ±0.03	0.16* ±0.01	0.20@ ±0.01	0.19@ ±0.03	0.26 ±0.02
Muscle	0.10 ±0.01	0.11 ±0.02	0.35** ±0.05	0.11 ±0.003	0.15* ±0.01	0.08 ±0.02	0.19* ±0.02	0.06@@ ±0.01	0.15* ±0.01
Tail	0.20 ±0.03	0.17 ±0.03	0.30 ±0.05	0.15 ±0.02	0.17 ±0.03	0.06* ±0.01	0.05* ±0.01	0.03** ±0.01	0.18 ±0.02

@ P <0.05; @@ P <0.02; * P <0.01; ** P <0.001

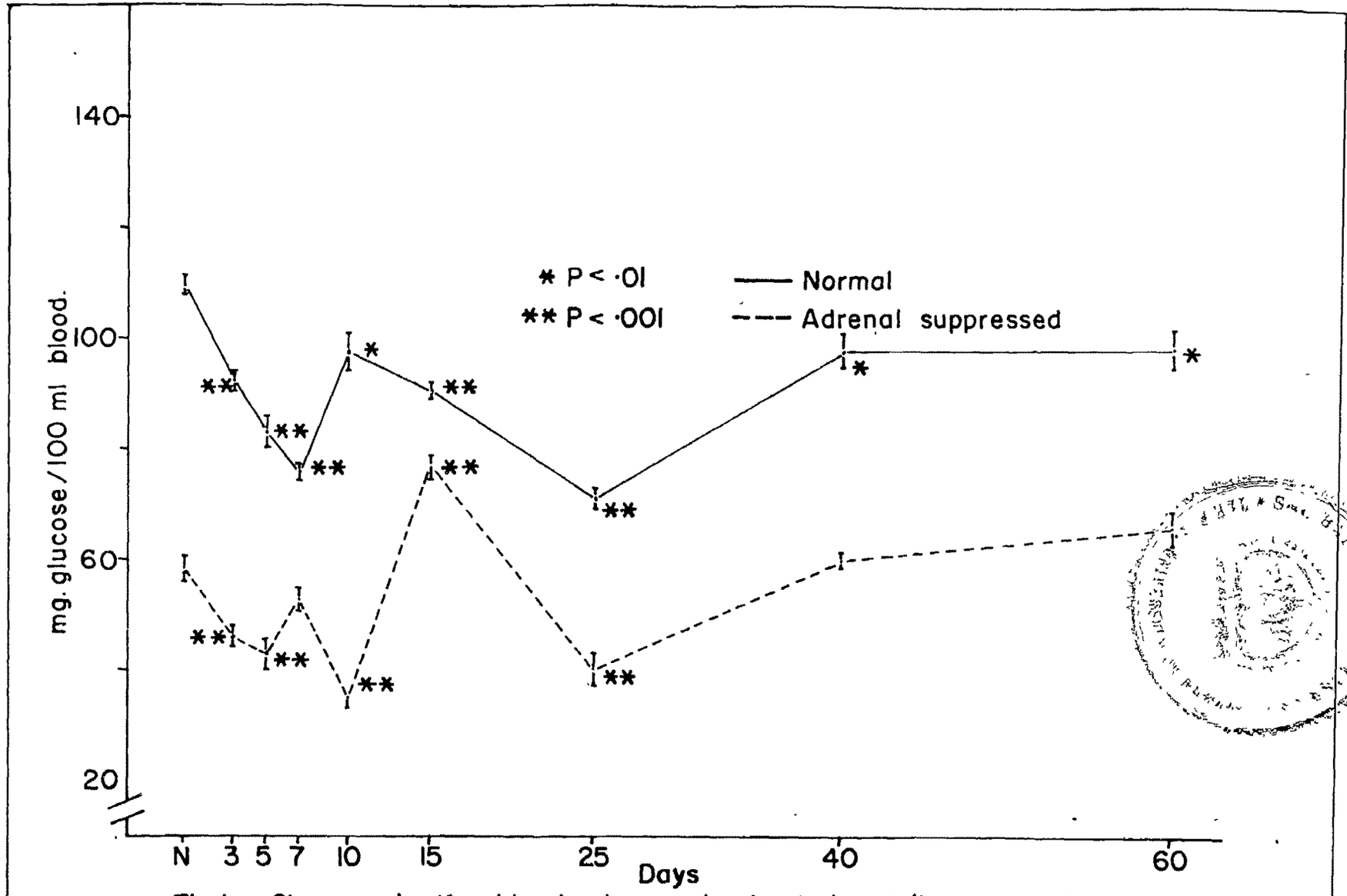

Fig.1. Changes in the blood glucose levels during tail regeneration in nomal and adrenal suppressed H. flaviviridis.

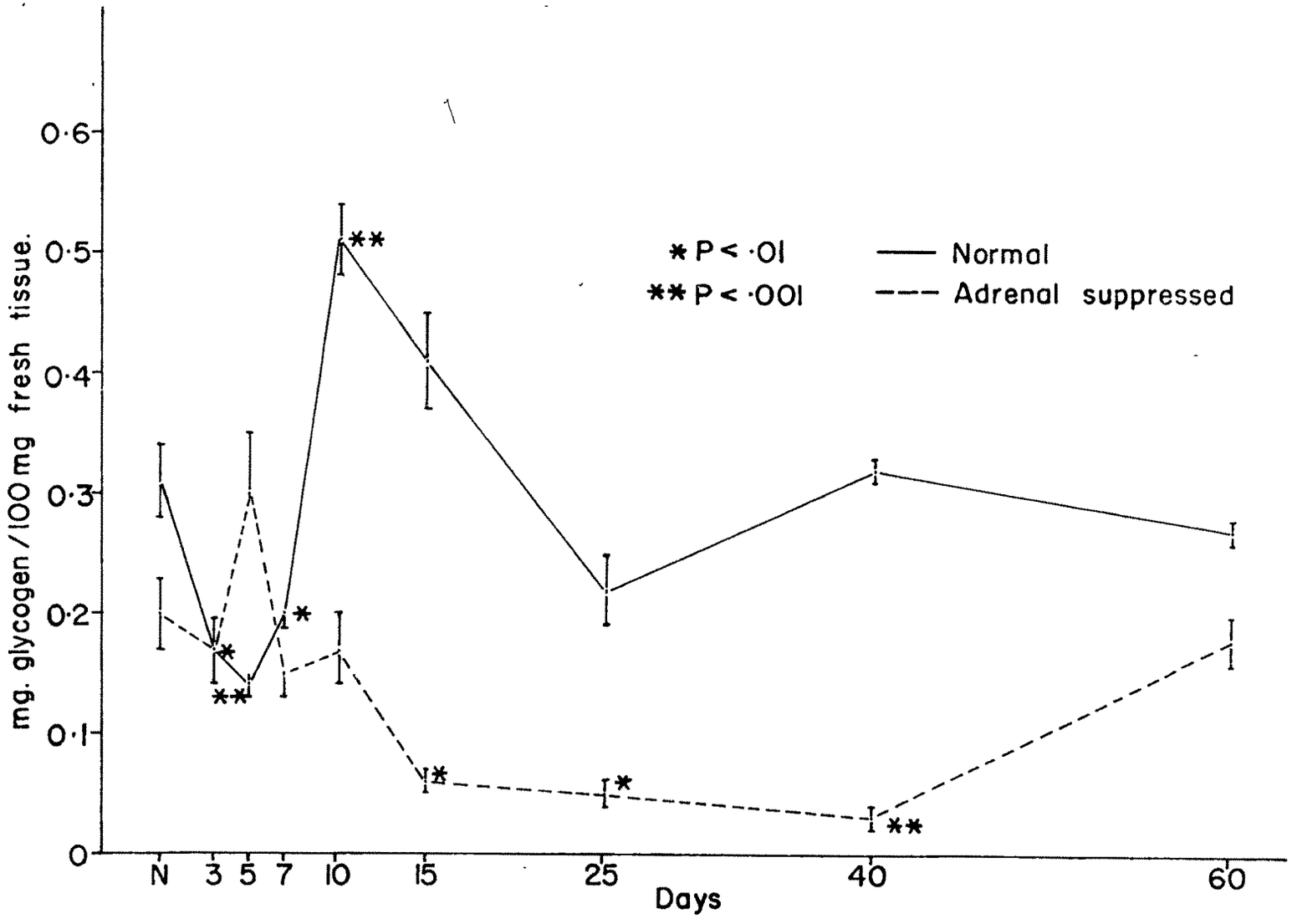

Fig. 2. Changes in tail glycogen content during tail regeneration in normal and adrenal suppressed H. flaviviridis.

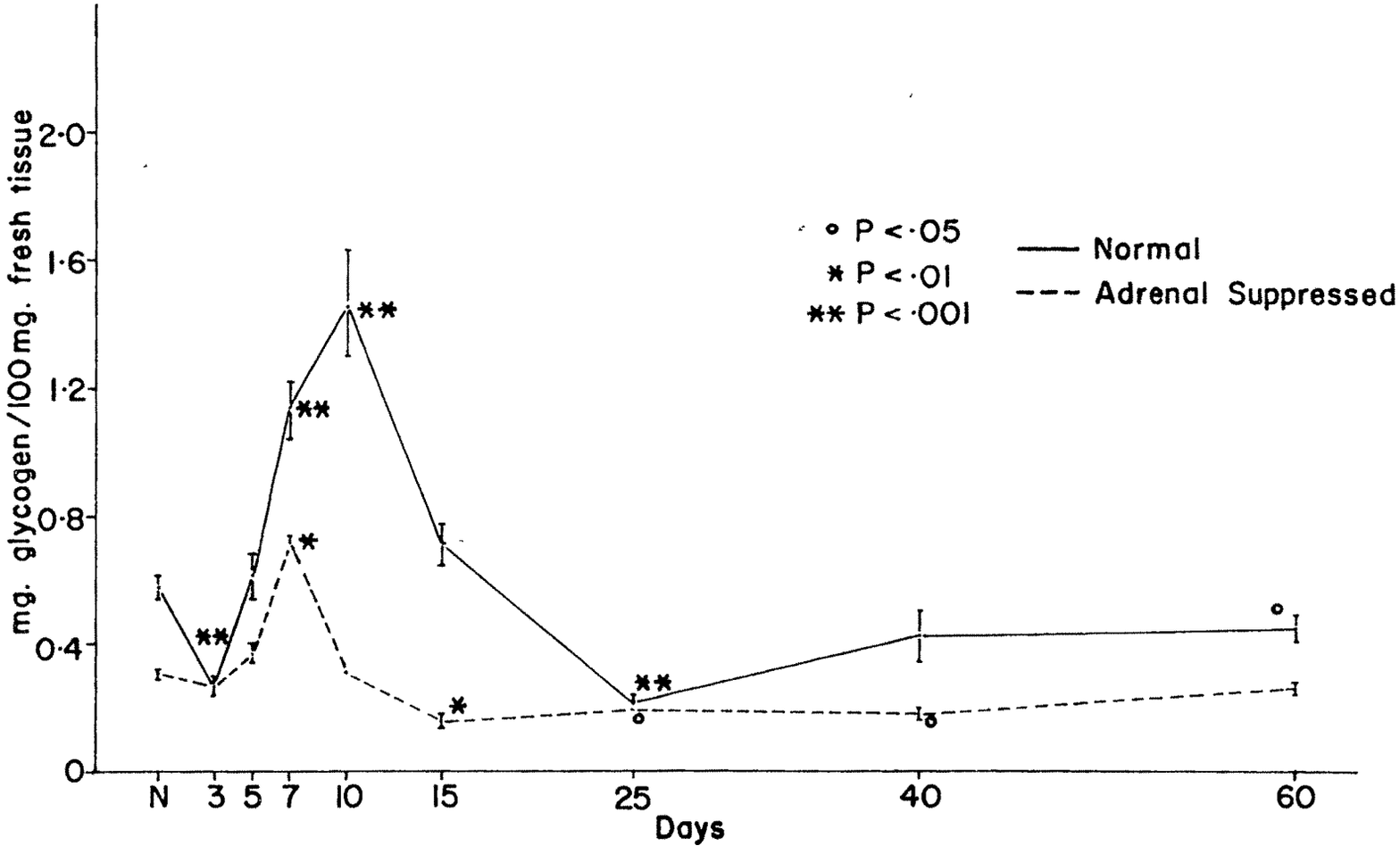

Fig.3. Changes in hepatic glycogen content during tail regeneration in normal and adrenal suppressed H. flaviviridis.

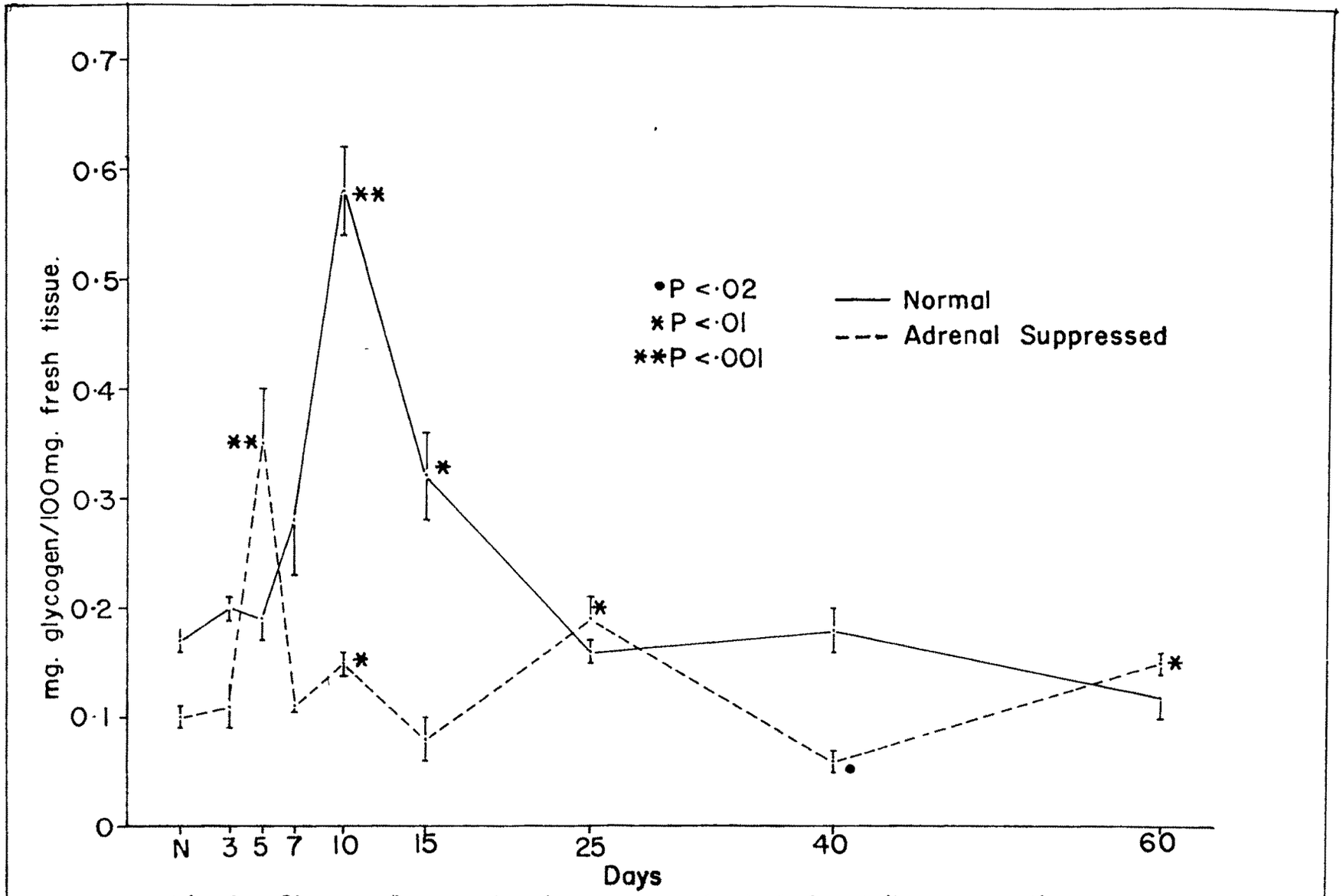

Fig. 4. Changes in muscle glycogen content during tail regeneration in normal and adrenal suppressed H. flaviviridis.

240%. Subsequently the muscle glycogen got depleted through 15th to the 25th day whence the normal pre-autotomy level was attained. In comparison, in the DXM lizards the muscle glycogen content increased to its maximal level by the 5th day post-autotomy and then settled to the normal range by the 7th day itself. Thereafter, the muscle glycogen though depicting slight fluctuations remained more or less in the normal range.

The blood glucose level remained subnormal almost all throughout regeneration with an initial gradual decrease lasting till the 7th day post-autotomy. There was then a sudden increase in the glycaemic level between the 7th and 10th days. Thereafter, there was further decrement in the glycaemic level between the 10th and 25th days followed by an increase to the pre-autotomy level by the 40th day. In the case of DXM lizards, the initial fall in blood glucose lasted till the 10th and then swiftly increased by the 15th day to be followed by a decrement between the 15th and 25th days. Thereafter, the glycaemic level increased to the pre-autotomy level by the 40th day.

DISCUSSION

Previous studies on tail regeneration in *H. flaviviridis* vis-a-vis alterations *in loco* and systemic carbohydrate reserves by Shah *et al.* (1987), a comparative study involving autotomy of a regenerating part (tail) and amputation of a non-regenerating part (limb), and Ramachandran and Chacko (1987a), a comparative study on a seasonal basis, have both revealed a pattern of changes in carbohydrate metabolism which finds identity with the presently observed changes in the control lizards. The pattern that gets established is the biphasic depletion of caudal and hepatic glycogen contents together with blood glucose (corresponding to the regressive and progressive phases of regeneration) with a phase of glycogenesis/gluconeogenesis in between (corresponding to the blastemic phase) and glycogen deposition in skeletal muscle during the first 10 days (regression and blastema phases) and its depletion subsequently during the differentiation phase (10th to 25th days). The utility value of caudal and hepatic glycogen in the many *in loco* mechanisms underlying the process of regeneration and in the associated supportive systemic physiological, biochemical and metabolic adjustments as well as the dependence of blastemal cells on blood glucose have all been discussed thoroughly in the previous reports (Shah *et al.*, 1977a,b; 1987).

The present study principally undertaken to evaluate the influence of the adrenals on carbohydrate metabolism and tail regeneration has succeeded in highlighting a few points of relevance in this context. 1) Adrenocortical suppression reduces the tissue contents of glycogen and induces hypoglycaemia in the unautotomised condition which signifies the important role of adrenals in maintaining tissue glycogen contents and glycaemic status in lizards. 2) The pattern of changes in carbohydrate metabolism post-autotomy and ensuing tail regeneration as outlined above remains essentially unaltered in the lizards with adrenocortical insufficiency relative to the adrenal sufficient lizards. This suggests the relative unimportance of adrenal corticosteroids in the setting in of regeneration specific modulations in carbohydrate metabolism. 3) However, the degree and duration of the modulations are much attenuated in the adrenal insufficient lizards thereby suggesting a permissive influence of the adrenals in regulating regeneration associated carbohydrate metabolism.

Surmisably, the above points of observations indicate the operation of other stronger forces (factors of local origin and/or hormonal agents) in inducing the adaptive modulations in carbohydrate metabolism post-caudal autotomy. However, the participation of adrenal

corticosteroids in the optimisation of the modulations as part of a permissive influence is clearly evident by the observed attenuation of the changes which may bear relevance to the recorded retardation in regenerative outgrowth in DXM lizards (Chapter 4). Some sort of correlation between the poor regenerative growth and the quantitative alteration in carbohydrate metabolism induced by adrenal suppression can be derived from the fact that a calculation of the total depletion in tissue glycogen contents and blood glucose taken separately during the entire course of regeneration, indicates two points of relevance. 1) The amount of glycogen made available for every millimetre of tail regenerated is about 0.073 mg in the adrenal sufficient controls while it was only 0.048 mg in the case of adrenal insufficient experimental lizards. 2) The amount of blood glucose utilised for every millimetre of tail regenerated is about 1.75 mg/100 ml in the control while it is about 2.52 mg/100 ml in the experimental lizards. Taken to their logical conclusion this would indicate a 34% decrement in the availability of glycogen reserves and about 44% over utilization of blood glucose for every millimetre of tail regenerated in the adrenal suppressed lizards. It is quite likely that this attenuation in glycogen depletion is essentially due to the 40% reduction in the tissue glycogen content effected by adrenocortical insufficiency.

In the wake of inadequate glycogen depletion the increased glucose withdrawal from the blood is understandable and represents an uneconomical utilization relative to the adrenal sufficient lizards.

SUMMARY

The involvement of adrenal in tail regeneration in the Gekkonid lizard, H. flaviviridis vis-a-vis carbohydrate metabolism has been evaluated. The pattern of changes in the glycogen content of tail, liver and muscle as well as blood glucose levels remained identical in both adrenal intact as well as adrenal suppressed lizards. The only recorded difference between the two sets of animals was in the degree of decrement or the time scale at which the decrement occurred. The changes observed indicate no alteration in the pattern of carbohydrate metabolism characteristic of regeneration, due to adrenal suppression. However, the recorded difference in the degree of glycogenolysis in the earlier stages and glycogenesis or gluconeogenesis in the later stages of regeneration may be one of the factors responsible for the observed decrement in tail elongation under adrenal suppression.