CHAPTER IX

LOCAL AND SYSTEMIC ALTERATIONS IN PROTEIN CONTENT AND TRANSAMINASES DURING TAIL REGENERATION IN NORMAL AND ADRENAL SUPPRESSED GEKKONID LIZARDS, HEMIDACTYLUS FLAVIVIRIDIS

Protein metabolism has a crucial significance in vertebrate reparative regeneration, a process which essentially involves localized reactivation of developmental events. Studies on lacertilian tail regeneration have indicated marked fluxes in the metabolism of lipids and carbohydrates. Besides, some of the past studies on vertebrate regeneration have also shown significant alterations in protein content both at the site of regeneration as well as systemically. Since there appears to be a co-ordinate involvement of systemic factors in concert with the local factors, the operation of certain regulatory factors can well be considered possible. Many hormones are known to exert control over metabolic activities of animals, especially the adrenocortical hormones. Knox and Greengard (1965) have reported the stimulatory influence of cortisone on transaminases, especially hepatic GPT. Apart from its importance in regeneration, a general paucity of information on aspects of protein metabolism in reptiles also warrants more studies on this line.

Previous studies from this laboratory had indicated a differential pattern of systemic protein metabolism in response to tail regeneration in Mabuya carinata and Hemidactylus flaviviridis. Whereas Mabuya was shown to depict a positive nitrogen balance in the form of increased hepatic and muscle protein contents, Hemidactylus responded by a negative nitrogen balance denoted by depletion in hepatic and muscle protein contents (Ramachandran et al., 1982; Valsamma, 1982). Importance of systemic protein catabolism as an adaptive response to tail regeneration in H. flaviviridis was further validated by the earlier observation of a reduced response during winter as compared to summer which corresponded well with the decreased regenerative outgrowth occurring during the winter months (Chapter 2). An earlier study on unilateral adrenalectomy conducted primarily to evaluate the regulatory influence of hormonal factors of systemic origin on regeneration had recorded an initial suppression of adaptive systemic protein catabolism with no influence during the later periods which was accredited to compensatory hypertrophy of the intact adrenal (Valsamma, 1982). It was in this context that the effect of complete adrenocortical suppression on tail regeneration was attempted which as recorded earlier depicted a 35% decrement in tail elongation (Chapter 4). The present study is an attempt to find a possible biochemical

correlate to the observed decrement in tail regeneration due to complete adrenocrotical suppression vis-a-vis its influence on in loco and systemic protein contents and transaminase activity, (GOT and GPT) in relation to tail regeneration in the Gekkonid lizards, 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 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 2 segments distal to the vent.

A total of 120 animals were used for the experimental purpose. They were divided into two groups of 60 each. 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 continued thereafter every alternate day till 60 days post-autotomy. The control group

received the vehicle (distilled water) in the same regimes. Lizards were sacrificed at regular time intervals of 3,5,7, 10,15,25,40 and 60 days post-autotomy along with the normal lizards with intact tail. Liver and skeletal muscle were removed quickly along with the regenerating or normal tail, as the case may be and homogenized in ice-cold redistilled water and a 2% homogenate was prepared. The crude homogenate was used for assaying quantitatively the amount of protein by the method of Lowry et al. (1951). A 1% homogenate in KCl solution was prepared for determining the enzyme activity of glutamate-pyruvate and glutamate-oxaloacetate transaminases (GPT and GOT). Activities of GPT and GOT were assayed by using dl-alanine and L+-aspartate as substrates following the method of Bergmeyer and Bernet (1965). The protein content was expressed as mg/100 mg of fresh tissue weight of liver, muscle and tail. The activity of both the transaminases (GPT and GOT) was expressed as Karmin units/mg protein/30 min. and Karmin units/mg protein/ 60 min. respectively.

For each day and each tissue specified, a total of five to seven observations were made. The mean and standard error were obtained and Student's 't' test was used to determine statistical significance.

RESULTS

Protein

Changes in the protein content of the regenerate, liver and muscle during tail regeneration in the control and adrenal suppressed lizards are presented in Figures 1-3 and Tables 1-2. The tissue protein profile indicates definite differences between the control and experimental lizards. In general, the protein content of all the three tissues prior to autotomy was considerably less in adrenal suppressed lizards. Hepatic and muscle protein content were found to be gradually depleted in the controls during the first week post-autotomy with a second phase of depletion during the 25th and 60th days of tail regeneration. The experimental lizards did not however depict such a depletion of hepatic and muscle protein stores. Infact the protein content in both the tissues registered an increase. The only noticeable protein loss occurred between 10th and 15th days in the case of liver and between the 15th and 25th days in the case of muscle. The protein content of tail showed fluctuations during regeneration in the control animals, while in the experimental lizards, the protein content remained in general above normal in relation to the pre-autotomy level.

TABLE-1 : Alterations in tissue protein 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	15.15 ±0.31	14.76 ±0.28	12.66** ±0.24	8.87** ±0.3	9.40** ±0.36	14.04 ±0.69	12.35* ±0.58	13.77* ±0.18	10.47** ±0.38
Muscle	9.28 ±0.26	8.36 ±0.51	8.19* ±0.21	6.79* ±0.49	5.43** ±0.44	9.01 ±0.52	7.15* ±0.61	7.58** ±0.13	6.57* ±0.63
Tail	6.96 ±0.39	5.94 ±0.77	7.66 ±0.32	4.67** ±0.26	5.37* ±0.29	7.34 ±0.18	6.17 ± 0.49	6.42 ±0.24	4.88* ±0.39

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

TABLE-2 : Alterations in tissue protein 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	10.61 ±0.12	11.47 ±0.86	11.27 ±0.31	12.47** ±0.21	14.67** ±0.37	9.99 ±0.95	10.4 ±0.86	13.95** ±0.23	12.00@@ ±0.43
Muscle	7.94 ±0.32	7.07 ±0.95	7.24 ±0.12	8.04 ±0.43	8.8 ±0.39	9.26 ±1.23	7.23 ±0.17	8.09 ±0.25	8.13 ±0.77
Tail	4.96 ±0.37	4.65 ±0.36	6.15@ ±0.29	6.62* ±0.32	6.58@@ ±0.32	5.27 ±0.78	4.87 ±0.39	6.74** ±0.24	7.79@@ ±0.77

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

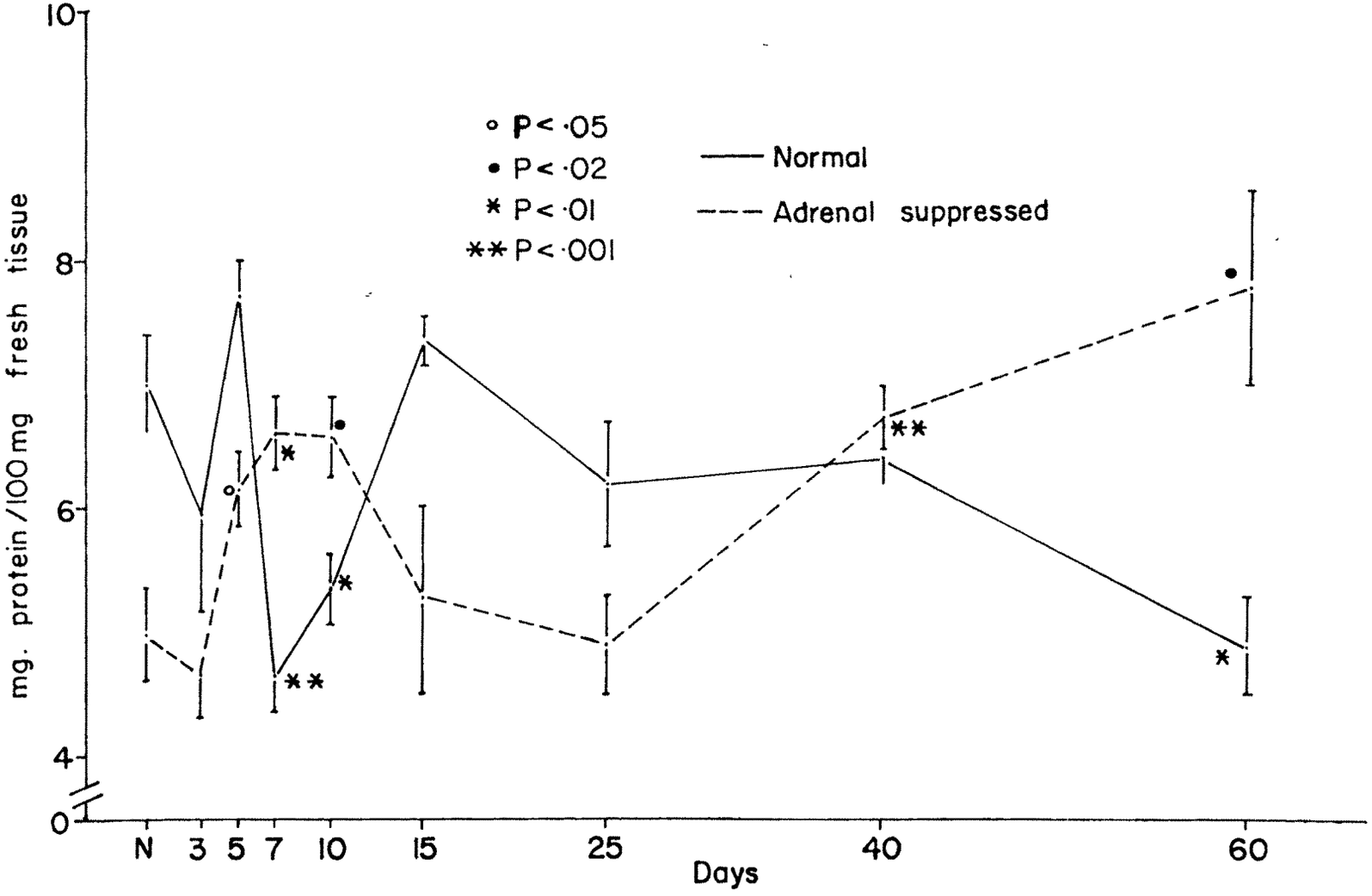

Fig. 1. Changes in the caudal protein content during tail regeneration in normal and adrenal suppressed H. flaviviridis.

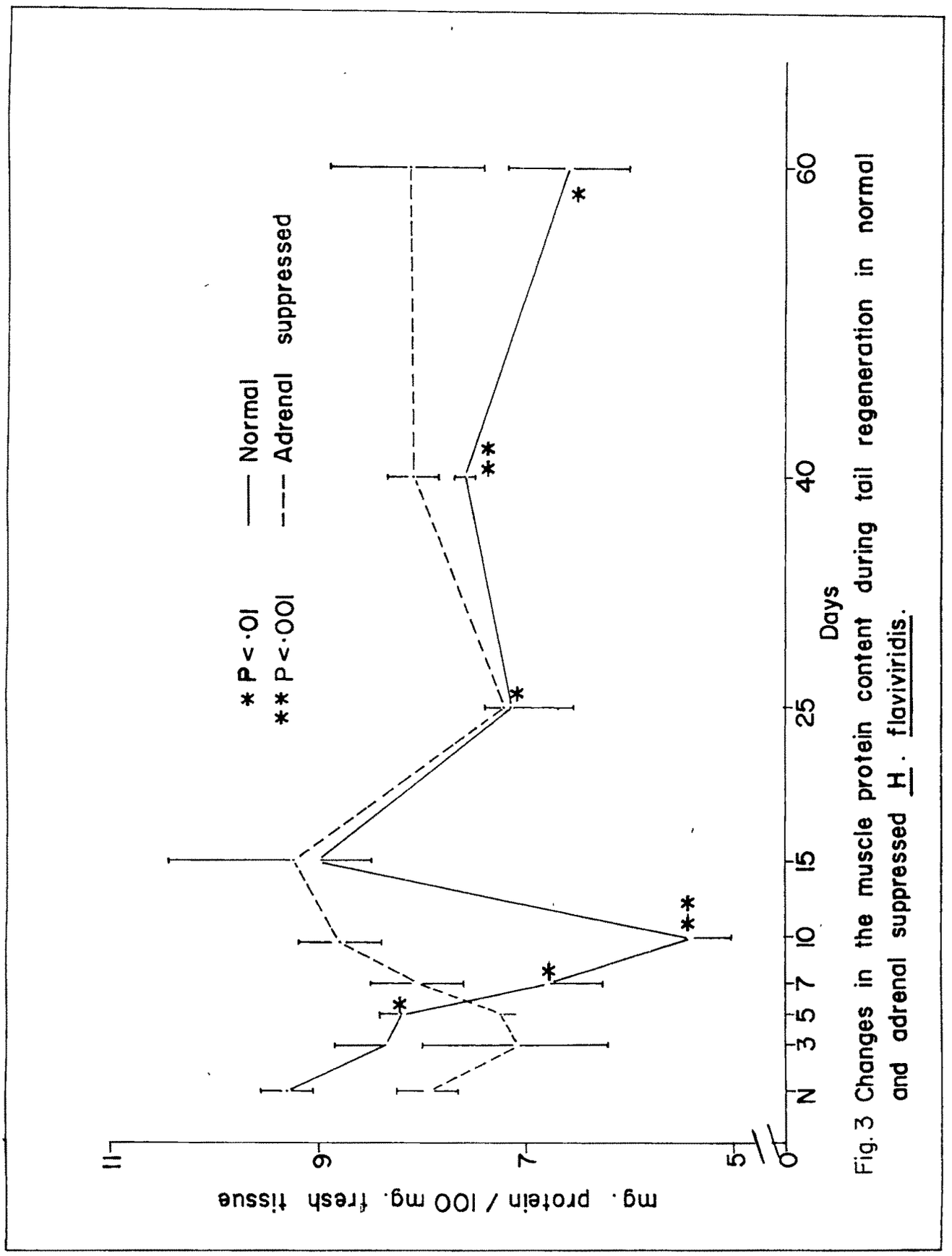

Fig. 3 Changes in the muscle protein content during tail regeneration in normal and adrenal suppressed H. flaviviridis.

TABLE-3 : Alterations in tissue GOT activity (Karmin units/mg protein/60 min.) during tail regeneration in normal H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	128.79 ± 5.36	139.66 ± 7.53	135.06 ± 5.36	160.68@ ±10.02	159.37** ± 3.16	119.46 ± 8.12	121.78 ± 8.19	148.78 ± 7.06	93.98** ± 2.57
Muscle	73.96 ±1.63	59.86* ±3.52	49.46** ±3.45	88.23* ±3.04	92.14 ±8.16	130.30 ±4.89	65.26* ±2.04	91.36* ±3.97	38.63** ±1.52
Tail	38.07 ±2.53	42.85 ±2.74	44.01 ±2.03	42.41 ±3.98	75.11** ±1.92	33.67 ±2.84	32.35 ±1.36	58.09* ±4.17	47.62* ±0.48

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

TABLE-4 : Alterations in tissue GOT activity (Karmin units/mg protein/60 min.) during tail regeneration in adrenal suppressed H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	158.16 ± 8.12	94.74* ±9.88	66.18** ±4.32	49.01** ±2.12	114.93* ±4.07	142.25 ± 5.85	97.34** ±5.64	127.29@ ± 6.85	91.87** ±5.61
Muscle	67.61 ±2.95	46.71* ±4.24	32.6** ±1.1	28.5** ±2.05	51.99@@ ±4.22	63.42 ±5.04	55.34 ±5.23	102.25** ± 3.2	31.95** ±1.59
Tail	60.44 ±4.35	42.88@@ ±3.35	33.65** ±1.70	29.36** ±1.82	39.80* ±2.25	40.59* ±2.32	27.54** ±1.99	45.13@@ ±2.06	41.68* ±2.67

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

TABLE-5 : Alterations in tissue GPT activity (Karmin units/mg protein/30 min.) during tail regeneration in normal H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	36.69 ±2.36	35.36 ±2.37	23.39* ±2.06	24.88* ±1.02	35.78 ±2.97	49.62@@ ±3.09	29.9@ ±1.72	25.36 ±4.23	22.13** ±1.2
Muscle	7.64 ±0.43	4.91* ±0.58	2.39** ±0.35	9.2 ±0.65	12.87* ±1.11	15.05** ±0.99	10.82* ±0.47	2.98** ±0.25	10.65* ±0.37
Tail	7.96 ±0.14	8.48 ±0.61	6.97 ±1.26	9.73 ±1.44	8.15 ±0.28	9.9 ±0.94	8.61@@ ±0.14	2.92** ±0.51	2.15** ±0.21

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

TABLE-6 : Alterations in tissue GPT activity (Karmin units/mg protein/30 min.) during tail regeneration in adrenal suppressed H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	20.33 ±0.66	19.72 ±1.29	12.92** ±0.64	9.86** ±0.58	35.01** ±1.68	51.27** ±2.93	34.75** ±1.45	19.65 ±1.34	16.73* ±2.26
Muscle	4.26 ±0.1	8.19* ±1.09	2.57** ±0.29	2.84** ±0.25	21.94** ±1.16	24.74** ±1.97	6.71** ±0.24	5.47@ ±0.44	8.4* ±1.04
Tail	3.42 ±0.28	8.75* ±0.91	6.81* ±0.61	7.92** ±0.72	22.25** ±0.71	16.64** ±0.21	9.17** ±0.63	2.79 ±0.33	6.76** ±0.33

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

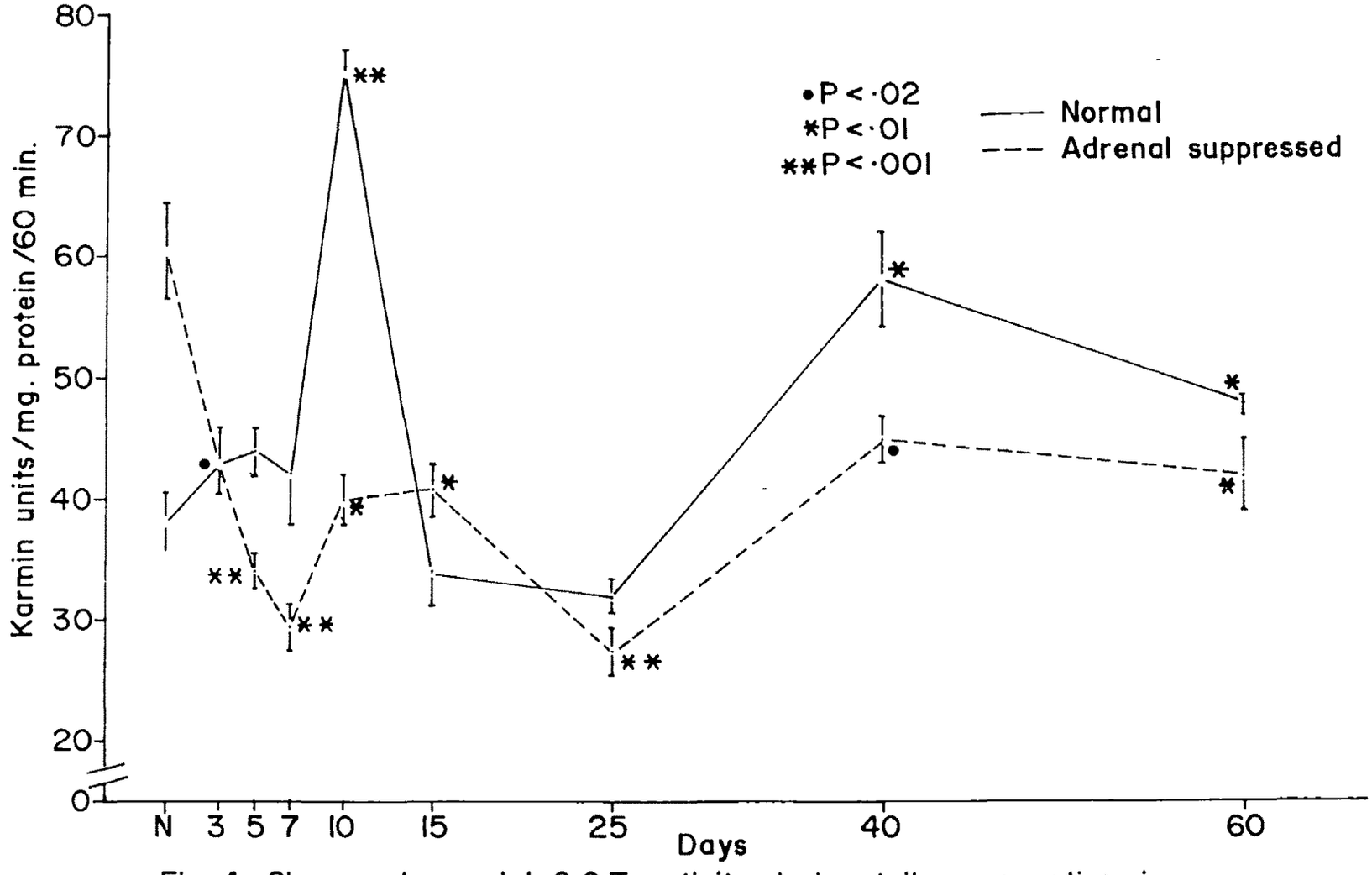

Fig. 4 Changes in caudal G O T activity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

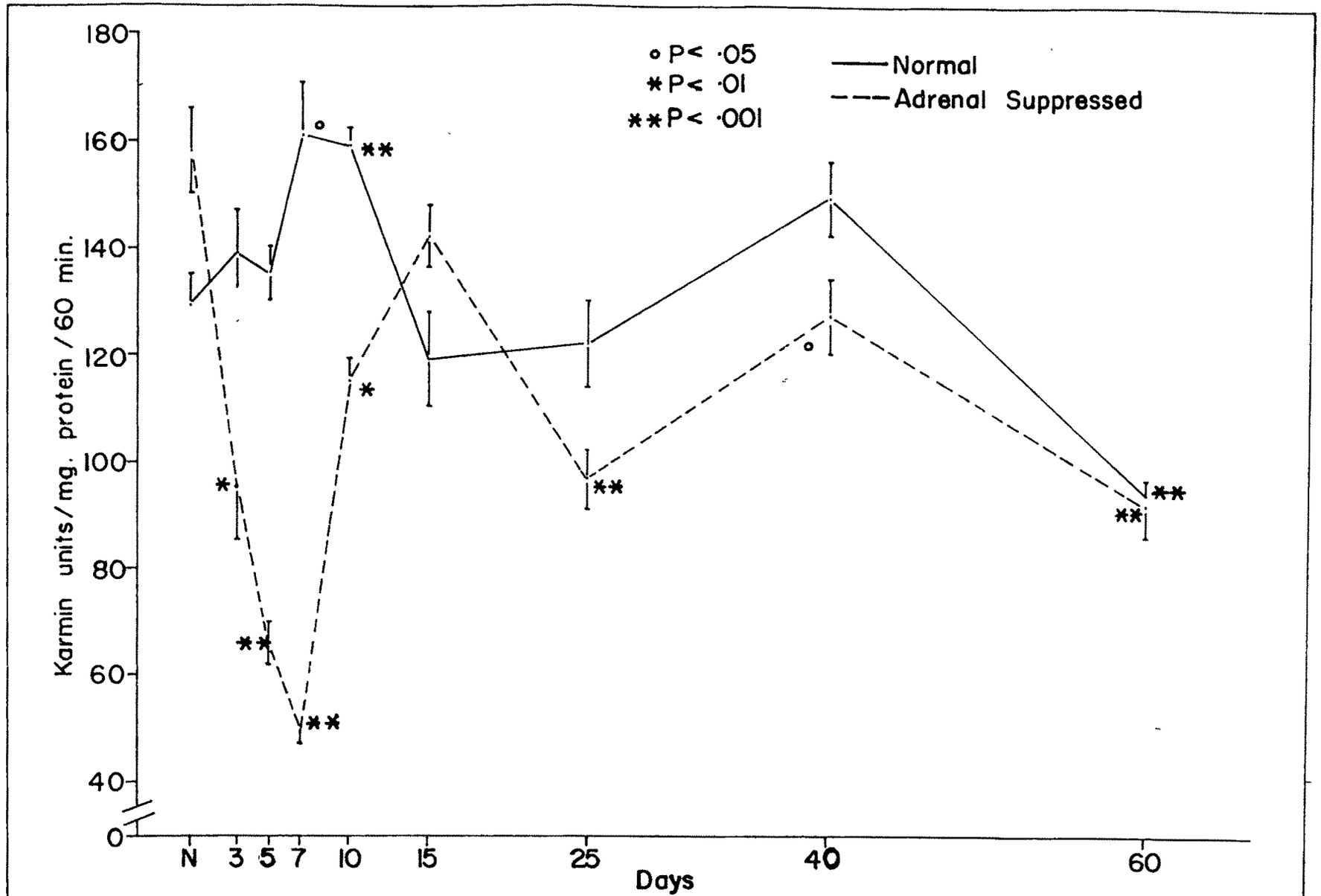

Fig.5. Changes in hepatic GOT activity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

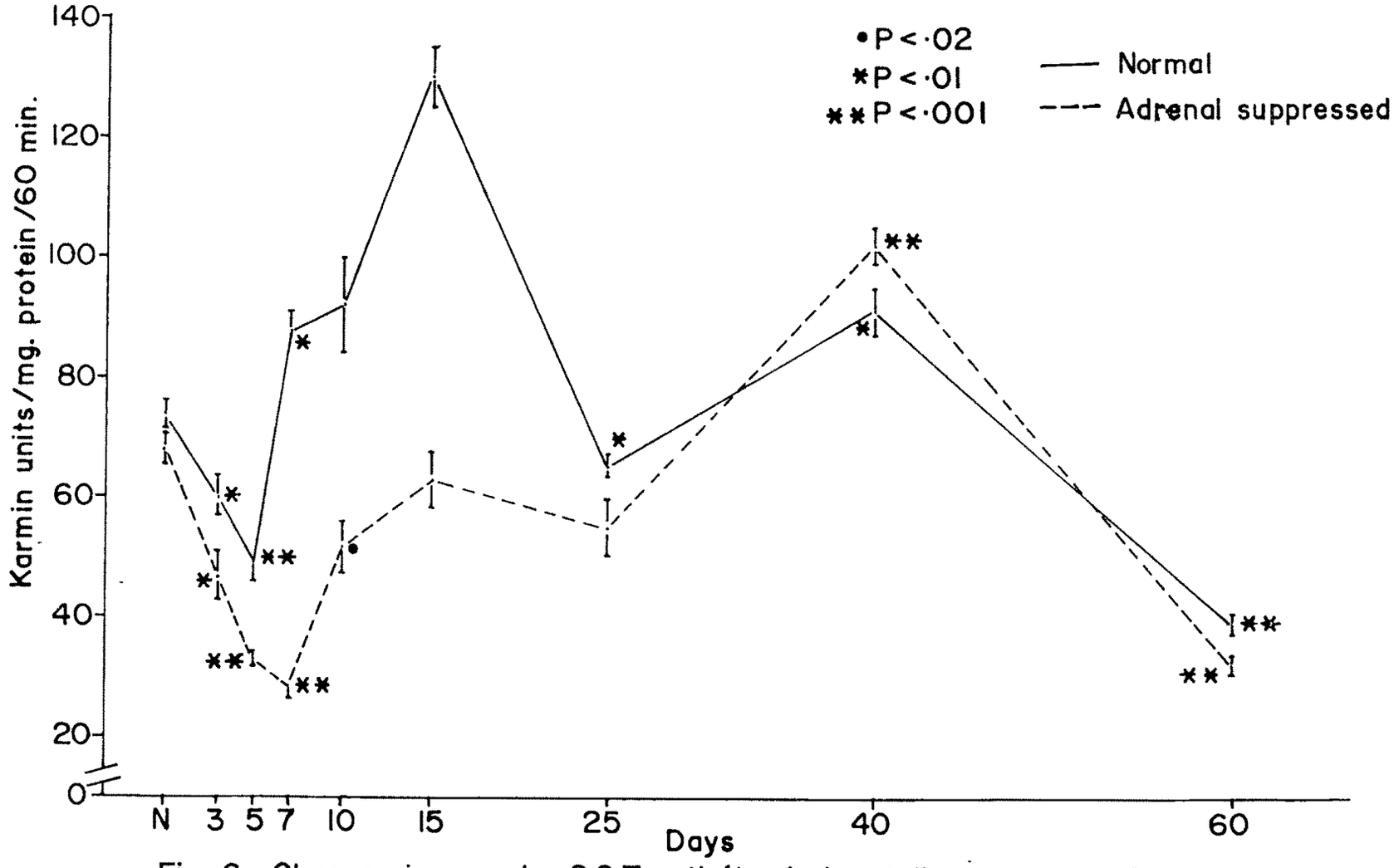

Fig. 6 Changes in muscle GOT activity during tail regeneration in normal and adrenal suppressed H. flaviviridis

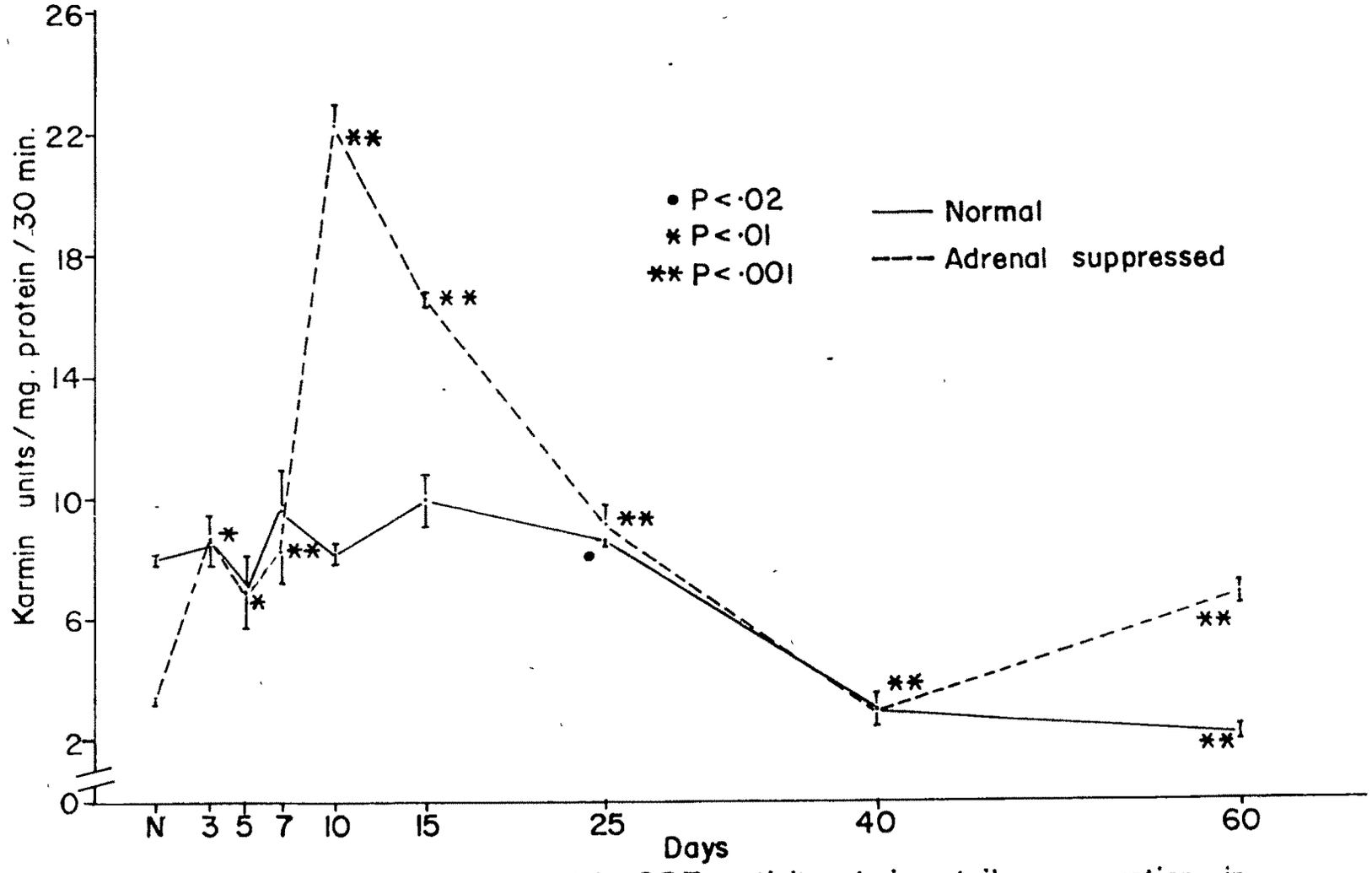

Fig. 7. Changes in the caudal GPT activity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

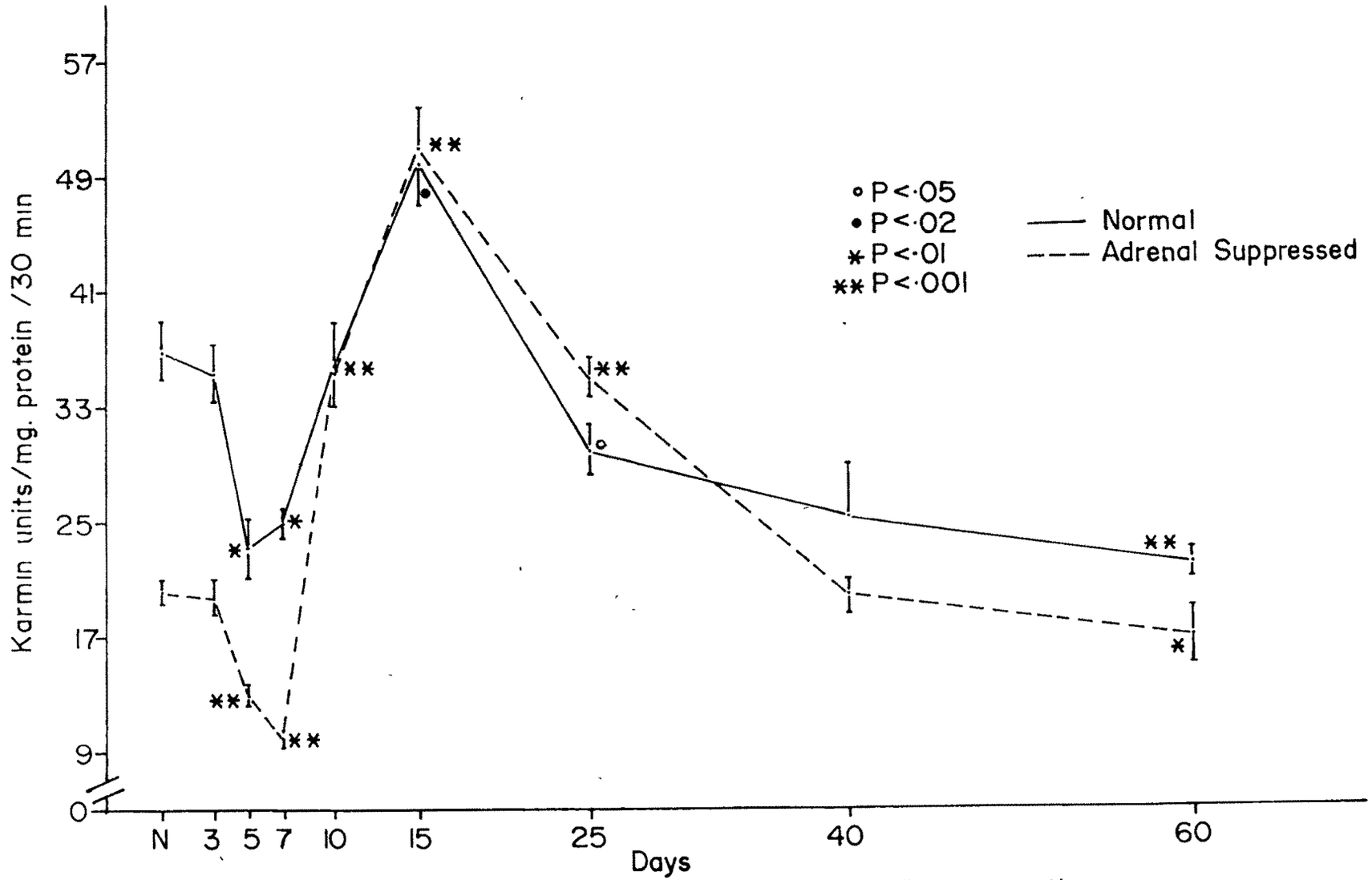

Fig. 8 Changes in the hepatic GPT activity during tail regeneration in normal and adrenal suppressed H flaviviridis.

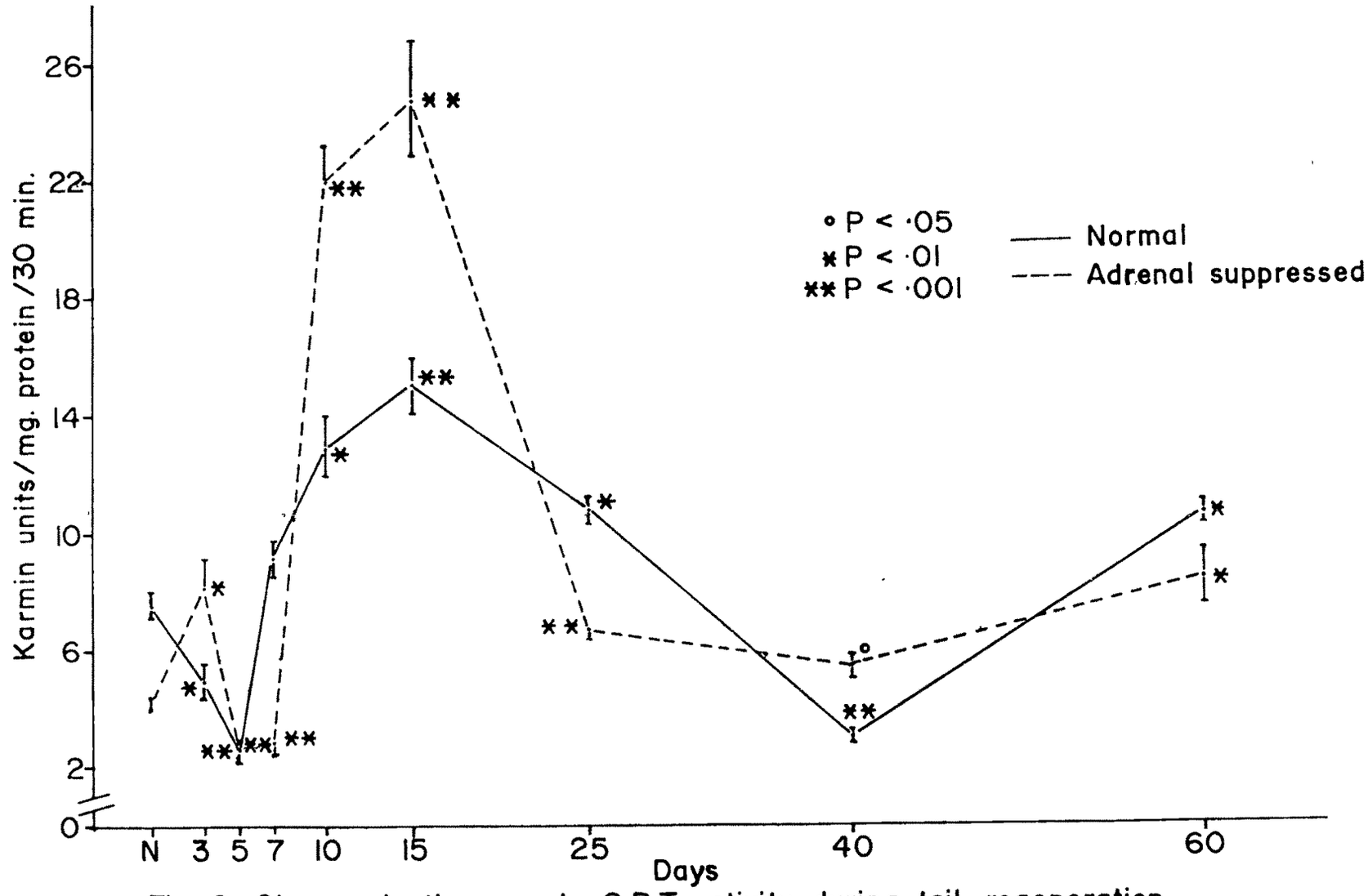

Fig. 9 Changes in the muscle G P T activity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

As compared to GPT activity, GOT activity was seem to be more clearly affected by adrenal suppression. Unlike GPT, the basal level of GOT activity was significantly increased in both liver and tail. Subsequent to autotomy, the GOT activity of all the three tissues decreased and infact remained subnormal for all the periods of regeneration in adrenal suppressed lizards. In contrast, in the control lizards, both hepatic and caudal GOT activity remained above normal during 3rd to 10th days and on the 40th day, while the muscle GOT activity recorded increase between 7-15 days and again on the 40th day. For the remaining periods, the enzyme activity in all the three tissues tended to be subnormal.

DISCUSSION

The present study principally undertaken to evaluate the effect of adreno-cortical insufficiency on tail regeneration in H. flaviviridis vis-a-vis alterations in protein metabolism has revealed definite deviation from the adrenal sufficient control lizards. Adrenal suppressed (DXM) lizards in general showed 30% reduction in the protein content of liver and tail and 15% reduction in muscle protein content in terms of the basal level prior to autotomy. Similar

reduction in the tissue protein contents has also been reported by Valsamma (1982) in unilaterally adrenalectomised lizards. Over and above, the present study has also depicted alterations in the activity levels of tissue transaminases in the form of decreased GPT activity and increased GOT activity.

In the light of the report of Knox and Greengard (1965) of a stimulatory influence of cortisone on GPT, the currently recorded decreased tissue GPT activity in DXM lizards is understandable. However, the concurrent increased levels of GOT activity are inexplicable.

The previous study of Valsamma (1982) on unilateral adrenalectomy and tail regeneration in H. flaviviridis had revealed normal pattern of protein metabolism both in loco as well as systemically except for the initial periods post-caudal autotomy. However, in the present study on near complete adreno-cortical suppression by dexamethasone the deviation in protein metabolism assessed in terms of tissue protein content and activity levels of GPT and GOT, was found to persist all throughout the periods of tail regeneration. When equated with the previous observation of mere retardation on regenerative output (30%) rather than inhibition in DXM lizards, the present findings tend to indicate a permissive influence

rather than a definite regulatory influence of adrenal steroids in saurian caudal regeneration. A general observation of the figures and tables indicate a negative nitrogen balance marked by depletion of hepatic and muscle protein contents coupled with phase specific increase in GPT (more prominently in muscle) and GOT (more prominent in liver) activities in adrenal sufficient control lizards. In comparison, the DXM lizards responded to the stress of autotomy and ensuing regeneration by a more positive nitrogen balance and very attenuated protein depletion from liver and muscle. Interestingly, the DXM lizards also responded by highly pronounced increase in both hepatic and muscle GPT activity (almost all throughout in muscle, and in the post-blastemic phases in liver) and decreased hepatic and muscle GOT activity (during the entire course of regeneration). In loco, though the protein content did not show much difference between the control and experimental lizards, the changes in transaminase activity were more marked. While GOT activity remained subnormal, the GPT activity was significantly elevated at all stages of regeneration. These changes taken as a whole suggest an altered metabolic strategy under adrenal insufficiency with GPT mediated metabolic reactions playing a crucial role. The increased in loco and systemic GOT activity together with reduced GOT activity

post-autotomy in DXM lizards apart from suggesting altered metabolic strategy also suggest the ability of factor(s) (hormonal and/or non-hormonal) other than adrenal steroids in inducing transaminase activity. Presumably the role of corticosteroids in inducing GPT activity at least in reptiles appears more of a permissive influence rather than a definitive one though hitherto reports in mammals have indicated a direct role (Knox and Greengard, 1965). Infact the subnormal level of GOT activity during regeneration inspite of its elevation prior to autotomy in DXM lizards also implies the setting in of differing status of hormonal and physiological homoeostasis both prior to autotomy and post-autotomy.

A very significant feature of the present observations is the reduced protein loss during regeneration in adrenal insufficient lizards. Equated in terms of total length of tail regenerated, the rate of protein loss in the experimental lizards averaged to 0.5 mg%/mm in relation to 0.7 mg%/mm in the control lizards. An apparent decrement in protein availability and/or utilisation under adreno-cortical insufficiency can be presumed and may have some bearing on the recorded retardation in tail regeneration (Chapter 4).

SUMMARY

The effect of induced adrenocortical insufficiency by dexamethasone (DXM) on *in loco* and systemic protein contents and GOT and GPT activities has been assessed during tail regeneration in *Hemidactylus flaviviridis*. DXM treatment brought about decreased tissue protein content and GPT activity and increased GOT activity in the unautotomised condition. Post-autotomy, the control lizards depicted a negative nitrogen balance marked by depletion of hepatic and muscle protein contents coupled with phase specific increase in GPT activity in muscle and GOT activity in liver. In contrast, the DXM treated lizards showed a more positive nitrogen balance and attenuated protein depletion with pronounced systemic GPT activity and decreased GOT activity. *In loco*, the transaminase activity depicted some alteration in the form of increased GPT activity. The present findings are discussed in terms of altered metabolic strategy and tend to suggest only a permissive rather than definitive influence of adrenal corticosteroids in protein metabolism associated with lizard tail regeneration.