CHAPTER II

PROTEIN METABOLISM IN RELATION TO TAIL REGENERATION IN THE GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS : A SEASONAL EVALUATION

The importance of proteins to repair processes in mammalian wounds has been recognized for several years. Many of the early studies have been reviewed by Williamson (1956). One striking example of the effect of trauma on the body is the negative nitrogen balance that follows injury. The negative nitrogen balance is largely the result of catabolic depletion of proteins and an impaired anabolic mechanism (Moore, 1959a; Cuthbertson, 1960). The most important biochemical process representing growth, is the biosynthesis of proteins; and the nucleic acids (DNA and RNA) have an essential role in this process (Rappaport and Fritz, 1972). Thornton and Bromley (1973) opined that protein metabolism gets extensively geared up in accordance with regressive (dedifferentiation) as much with the progressive phases (blastema formation, differentiation and growth) of regeneration.

Regeneration under conditions of different environmental temperatures and different seasons has so far received only sporadic attention. Barfurth (1891) found that tadpole tail regeneration proceeded two to two and one half times more rapidly at 28°C than at 14°C. Temperature studies on other vertebrates have also shown some correlation between wound repair and environmental temperature. Campbell and Cuthbertson (1966) reported briefly that rats repaired skin wounds at a rate significantly faster at an ambient temperature of 30°C than at 20°C. Seasonal changes which occur during breeding and non-breeding periods are also found to influence the process of regeneration. The effects of altered temperature and seasons may be due to their interference on enzyme function, by inactivating critical temperature sensitive catalysts (Schmidt, 1968). Previous studies from this laboratory had shown a definite seasonal variation in regenerative outgrowth, with a summer maximum and winter minimum (Magon, 1970). Since such seasonal morphological studies are not backed up with correlatable biochemical evaluations, it was thought pertinent presently to analyse alterations in protein metabolism if any, on a seasonal basis. In this respect, changes in in loco as well as systemic protein contents together with the activity levels of glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) - the two key transaminases which by their strategic positioning in the metabolic pathway are pivotal in linking the metabolism of carbohydrates and lipids with that of proteins, were quantitatively assayed during caudal regeneration in Hemidactylus flaviviridis during summer and winter months.

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 sixty animals were used for the study during each season i.e. summer (breeding) and winter (non-breeding). The 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 tails. Liver and skeletal muscle (femoral) as well as the tail (regenerating or normal as the case may be) were taken and weighed. The protein content of the tissues was estimated by the method of Lowry et al. (1951) using a 2% homogenate prepared in ice-cold redistilled water. For the estimation of GPT and GOT activities, a 1% homogenate in KCl was prepared and the activity of the enzymes was assayed by making use of dl-alanine and L-aspartate respectively as the substrates as per the method of Bergmeyer and Bernet (1965). The amount of protein was expressed as mg/100 mg of fresh tissue weight of liver, muscle and tail while the activitye of both the transaminases(GPT and GOT)was expressed as Karmin units/mg protein/30 mins and Karmin units/mg protein/60 mins respectively.

For each day and each tissue specified, a total of 5 to 7 determinations were made for each parameter. The mean and standard error were calculated and Student's 't' test was used to determine the statistical significance.

TABLE-1 : Alterations in tissue proteins (mg/100 mg fresh tissue) during tail regeneration in the summer months in <u>H. flaviviridis.</u> (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	27.35 ±0.96	26.03$^{@}$ ±1.27	23.74* ±1.03	21.85* ±0.77	20.11* ±0.68	15.89** ± 0.4	17.74* ±0.33	16.95* ± 0.2	20.72 ± 0.27
Muscle	23.78 ±0.34	18.92** ± 0.5	19.88** ±0.12	25.42* ±0.18	18.85** ±0.21	8.49** ± 0.2	9.33** ±0.28	13.88** ±0.22	11.01** ±0.09
Tail	7.01 ±0.02	10.63** ±0.14	8.27** ±0.14	7.35$^{@}$ ±0.14	8.3* ±0.28	8.61** ±0.29	6.78 ±0.11	9.35** ±0.22	8.68* ±0.43

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

TABLE-2 : Alterations in tissue protein content (mg/100 mg fresh tissue) during tail regeneration in the winter months in <u>H. flaviviridis.</u> (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	14.44 ±0.62	13.17 ±0.35	12.24 ±1.39	15.74 ±0.58	14.90 ±0.41	12.94 ±0.38	17.20$^{@}$ ±0.69	11.89$^{@}$ ±0.81	11.86 ±0.57
Muscle	10.37 ±0.20	10.86 ±0.61	8.43* ±0.40	9.96 ±0.44	9.71 ±0.79	8.13* ±0.48	8.98 ±0.64	7.10* ±0.66	8.71* ±0.41
Tail	5.97 ±0.73	6.45 ±0.58	6.28 ±0.58	7.52 ±0.39	5.64 ±0.22	5.83 ±0.29	8.33$^{@}$ ±0.52	5.77 ±0.37	8.09$^{@}$ ±0.14

$^{@}$ $P < 0.05$; * $P < 0.01$

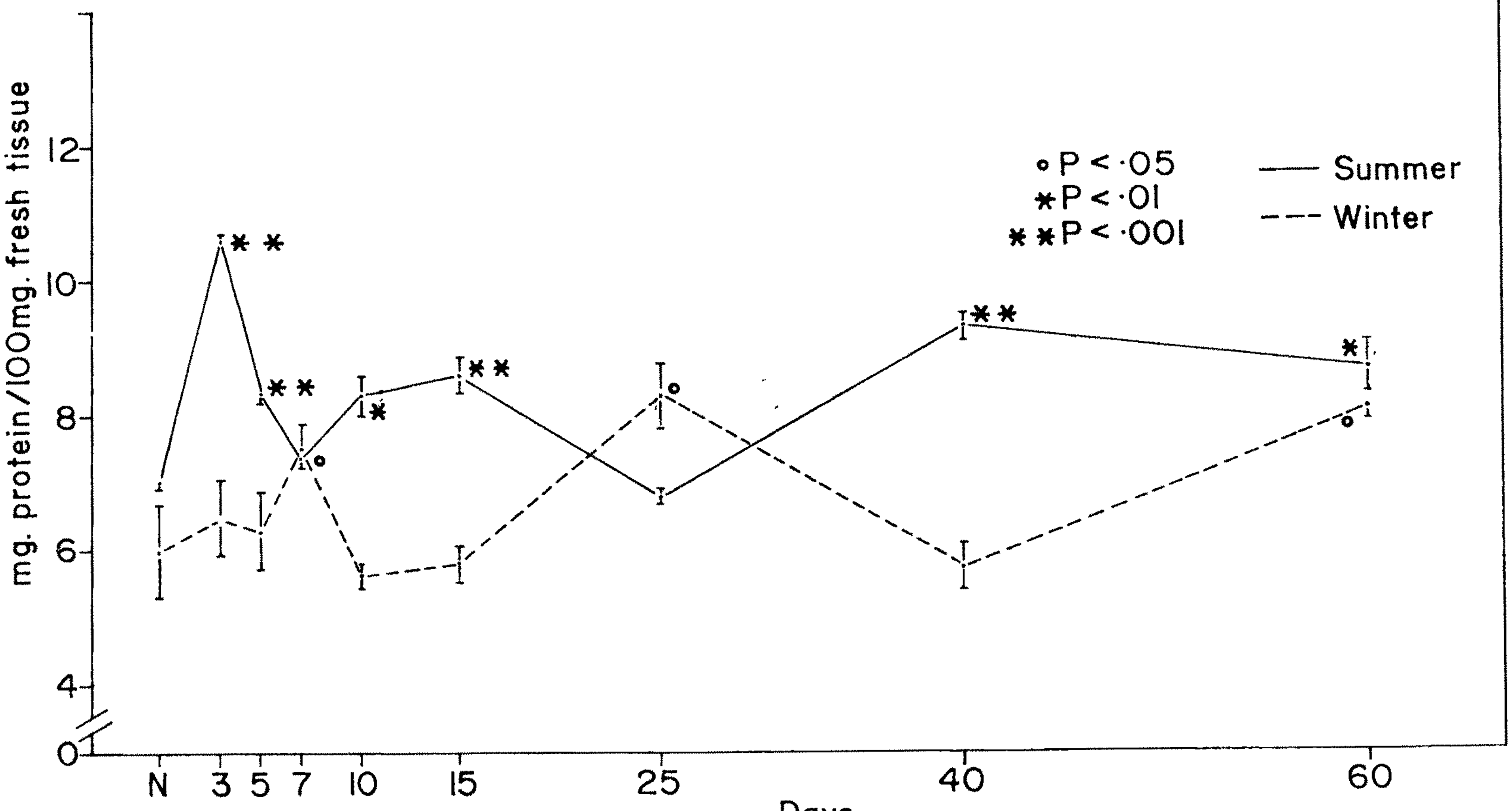

Fig. 1. Changes in caudal protein content in summer and winter months during tail regeneration in <u>H. flaviviridis.</u>

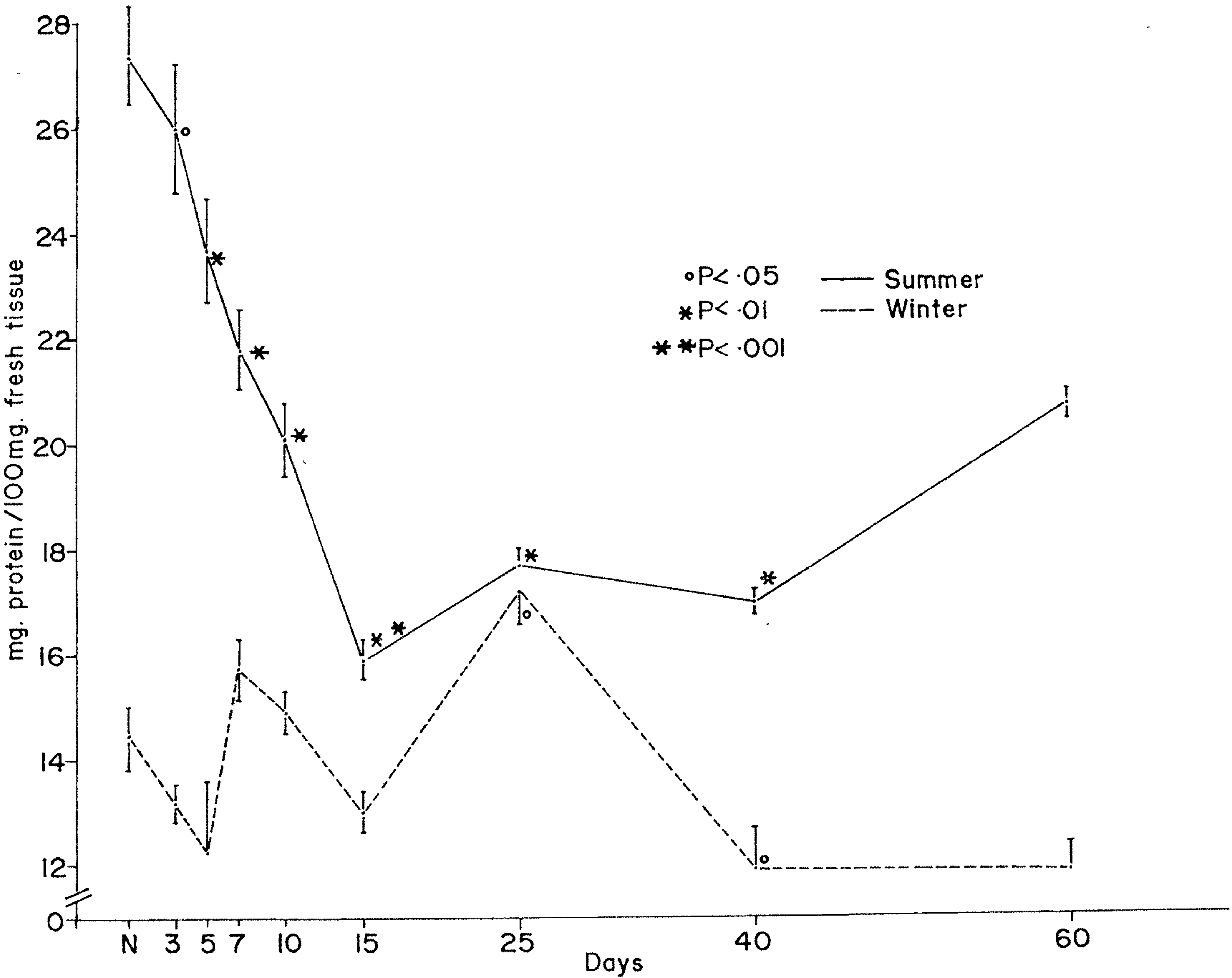

Fig 2. Changes in the hepatic protein content in the summer and winter months during tail regeneration in <u>H. flaviviridis</u>.

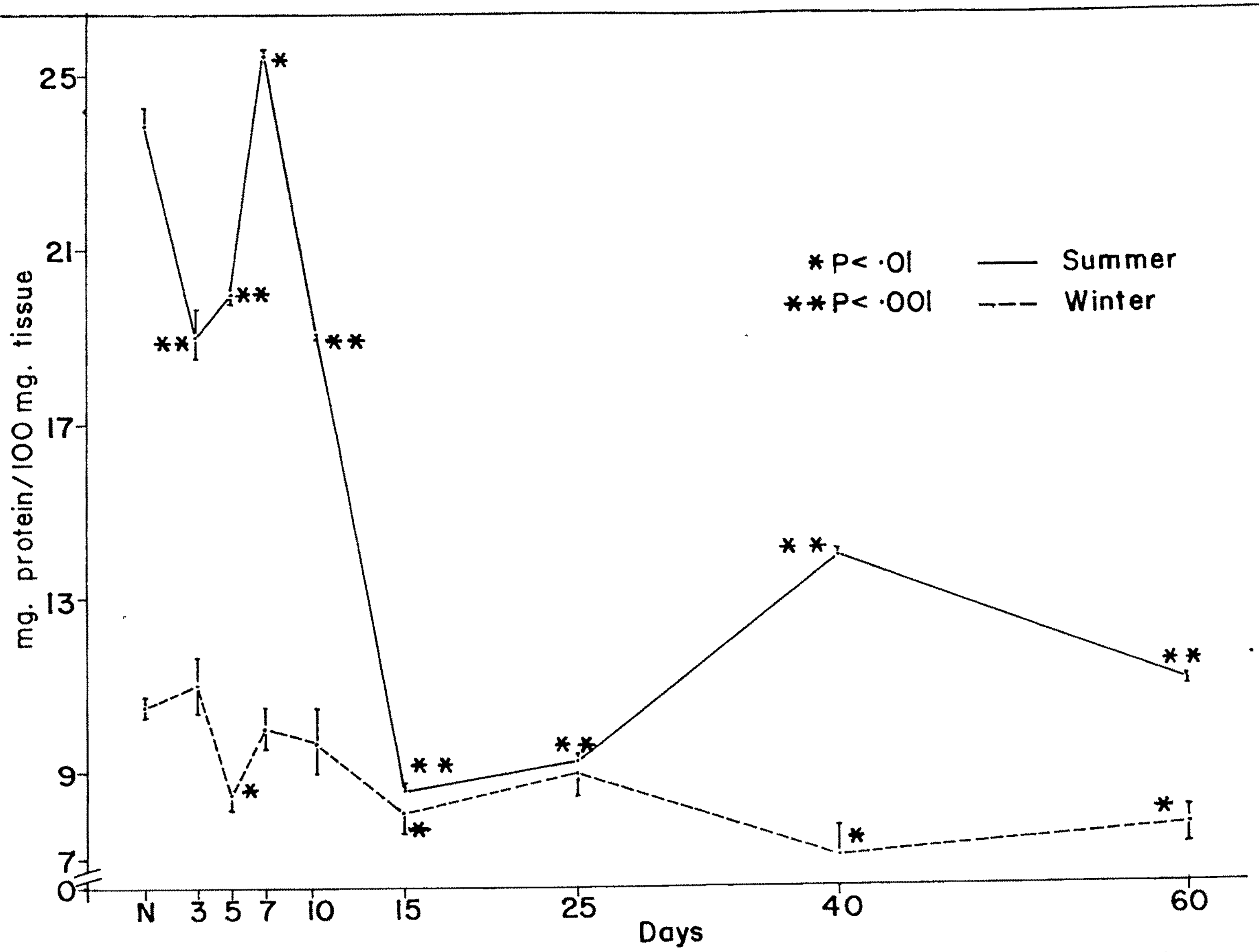

Fig. 3. Changes in muscle protein content in summer and winter months during tail regeneration in H. flaviviridis.

significant depletion of muscle protein in the summer. The depletion tended to be biphasic with an initial depletion during the first week and a second one between the 10th and 25th days. Though a similar tendency of muscle protein depletion could be noted during winter too, it was very much attenuated in quantitative terms thus depicting a sluggishness in the release of muscle protein.

GOT activity (Figs. 4,5,6; Tables 3 & 4)

The GOT activity did not depict any seasonal difference, with the liver, muscle and tail depicting enzyme activity in that order. In general, the changes in GOT activity in all the three organs were similar in the two seasons though the degree of changes was less marked during the winter. Accordingly, the caudal GOT activity recorded a biphasic increment during the 10th and 40th days post-autotomy. Though the increment recorded on the 40th day was of a similar degree in both the seasons, that recorded on the 10th day was attenuated in the winter. Moreover, during the first five days post-autotomy there was a tendency for decreased enzyme activity in the winter.

The hepatic GOT activity also registered a biphasic increase with an initial increase during 10th to 15th days and a later increase on the 40th day, the increments being

TABLE-3 : Alterations in tissue glutamate-oxaloacetate transaminase levels (Karmin units/mg protein/60 min) during tail regeneration in the summer months in *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	158.78 ± 6.38	93.98** ± 2.57
Muscle	73.96 ± 1.63	59.86* ±3.52	49.46** ±3.45	88.23* ±3.04	92.14* ±3.16	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 Glutamate-oxaloacetate transaminase levels (Karmin units/mg protein/60 min.) during tail regeneration in the winter months in *H. flaviviridis*. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	122.04 ± 5.38	99.16$^{@}$ ±9.41	68.08* ±10.82	133.86 ± 7.40	150.86* ± 5.49	66.39* ±14.46	66.35** ±3.74	170.66 ±20.26	127.72 ±4.47
Muscle	64.1 ±6.55	56.56 ± 5.97	35.25* ±1.88	43.63$^{@}$ ±5.14	66.12 ±4.66	71.99 ± 7.43	50.51$^{@}$ ±6.15	87.22$^{@}$ ±9.04	46.77 ±7.33
Tail	41.47 ±4.10	40.08 ±6.17	27.53$^{@@}$ ±2.74	32.17 ±4.64	50.89$^{@}$ ±4.7	30.11$^{@}$ ±0.54	38.83 ±2.85	61.11** ±3.68	41.03 ±1.91

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

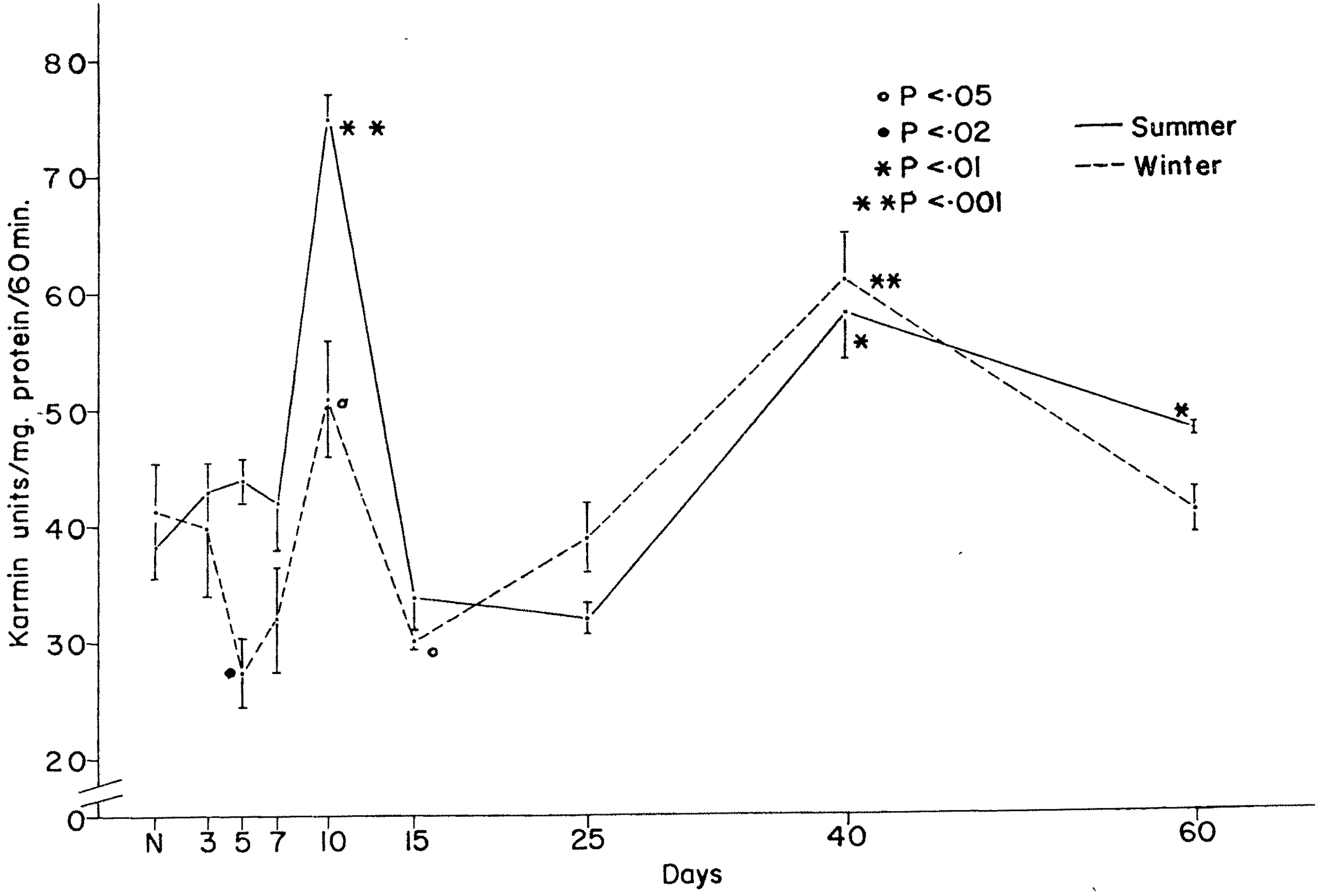

Fig.4 . Changes in caudal GOT activity in summer and winter months during tail regeneration in <u>H. flaviviridis.</u>

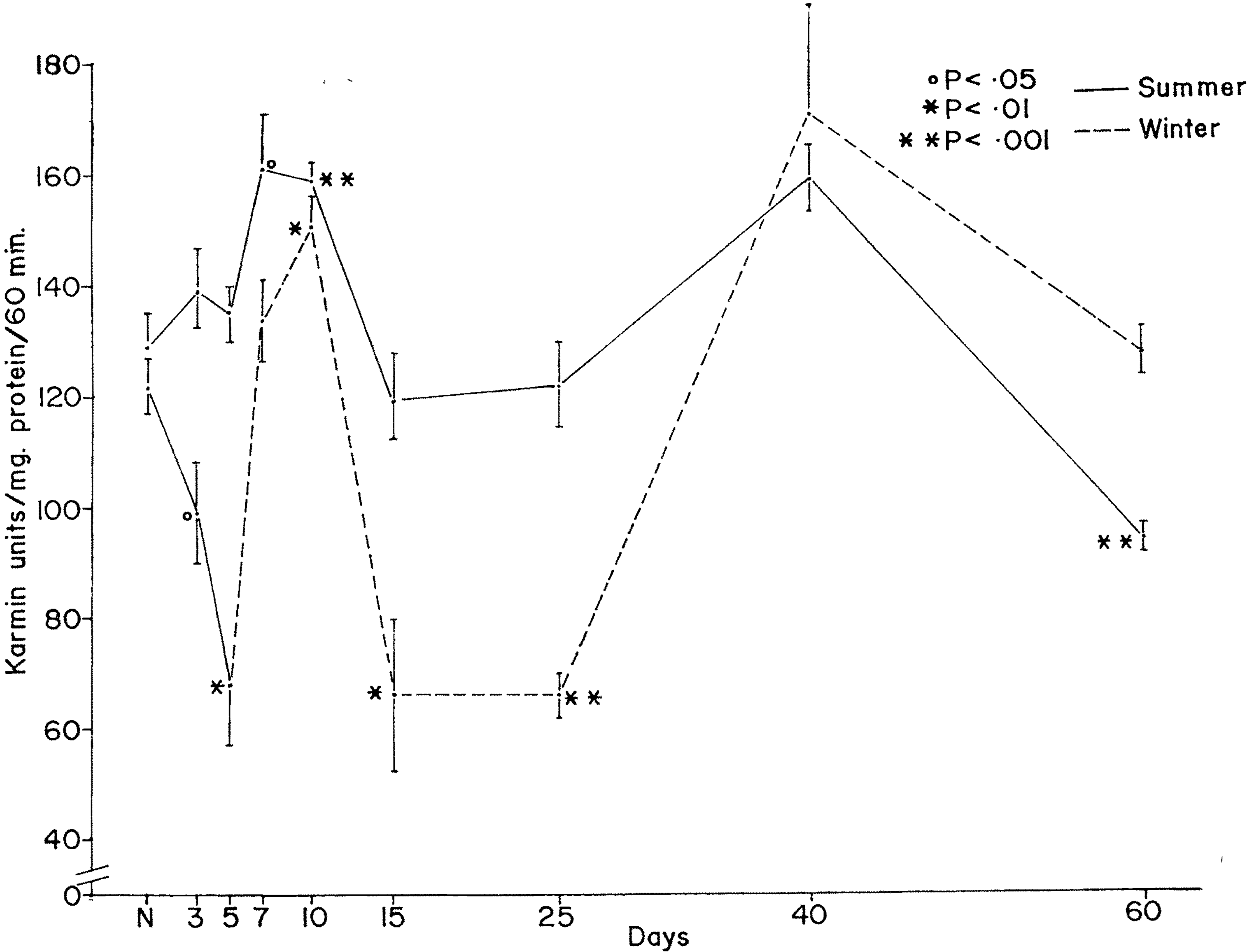

Fig. 5. Changes in hepatic GOT activity in winter and summer months during tail regeneration in H. flaviviridis.

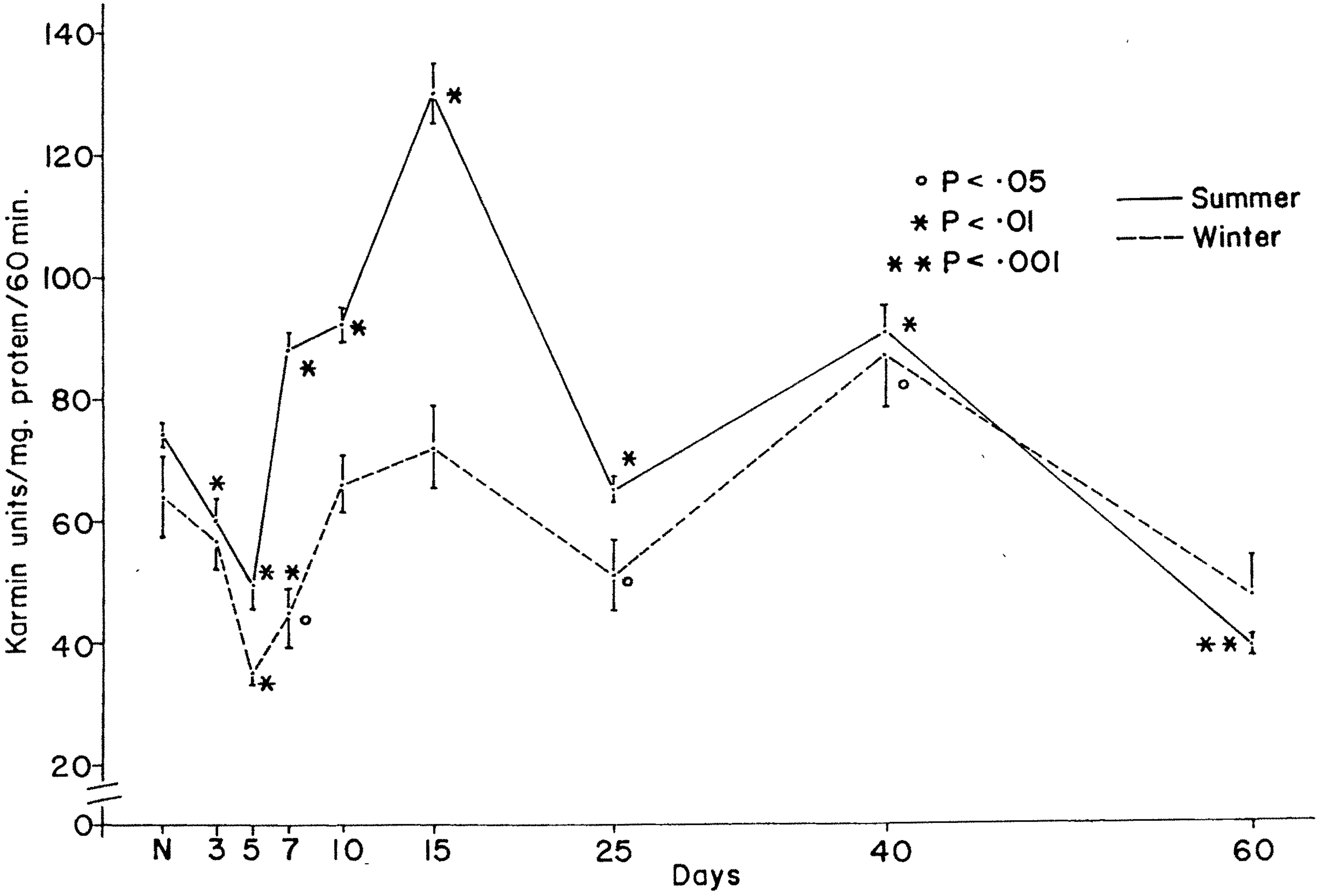

Fig. 6 . Changes in muscle GOT activity in summer and winter months during tail regeneration in *H. flaviviridis*.

of similar degree in both summer and winter. However, during the winter these two phases of increased enzyme activity was accompanied by significant subnormal levels in between which was not observable in summer.

The muscle GOT activity showed a decrement during the first five days post-autotomy which increased to above normal levels by the 15th day. This increment in enzyme activity was however pronounced during summer and was attained by the 7th day itself. In both the seasons, the enzyme activity decreased between 15th and 25th days and again increased to supranormal levels on the 40th day. Another interesting observation was the significant subnormal level of GOT activity in both liver and muscle on the 60th day which was not shown in winter.

GPT activity (Figs. 7, 8, 9; Tables 5 & 6)

Levels of GPT activity in liver, muscle and tail were identical in both the seasons. Subsequent to caudal autotomy, changes in GPT activity were nearly identical in both the seasons. In summer, except for the terminal period of regeneration (40th to 60th days) whence the enzyme activity remained significantly subnormal, all the earlier periods of regeneration were marked by maintenance of normal level of enzyme activity with a tendency for increased

TABLE-5 : Alterations in tissue Glutamate-pyruvate transaminase levels (Karmin units/mg protein/30 min.) during tail regeneration in the summer months in <u>H. flaviviridis</u>. (± 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 Glutamate-pyruvate transaminase levels (Karmin units/mg protein/30 min.) during tail regeneration in the winter months *H. flaviviridis.* (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	35.29 ±2.94	29.46 ±4.72	29.03 ±2.27	20.45** ±0.86	38.69 ±2.24	20.78** ±1.1	33.6 ±7.67	39.65 ±4.04	31.8 ±2.68
Muscle	8.62 ±1.57	5.56 ±0.25	2.87@@ ±0.36	5.72 ±0.69	5.64 ±0.92	7.45 ±0.71	5.86 ±1.95	8.69 ±0.81	5.64 ±0.78
Tail	8.23 ±1.09	5.03 ±1.14	5.73 ±1.03	8.43 ±1.42	10.95 ±2.23	9.02 ±0.75	7.89 ±0.18	8.1 ±2.59	5.37 ±0.4

@@ $P < 0.02$; ** $P < 0.001$.

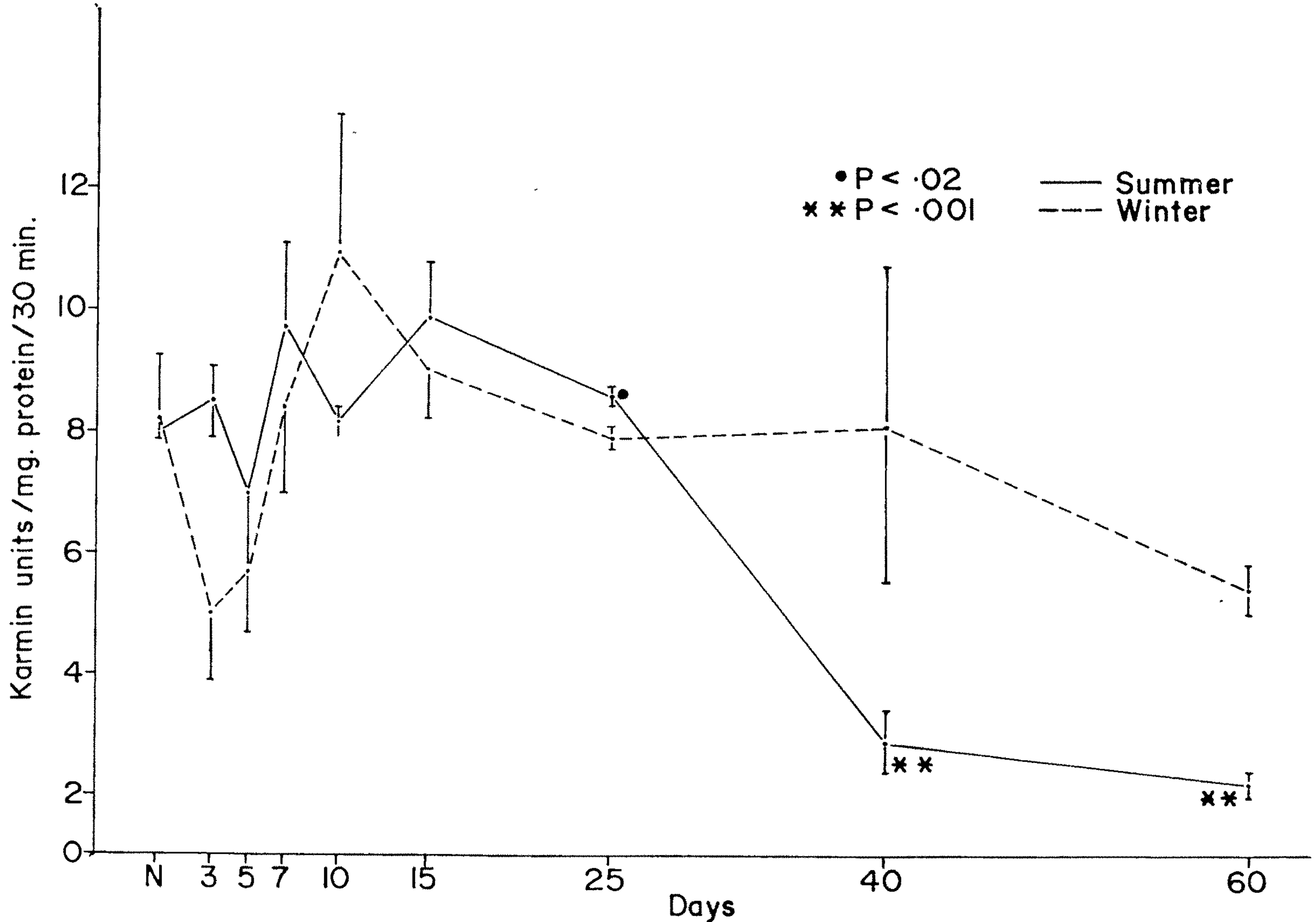

Fig. 7 . Changes in caudal GPT activity in summer and winter during tail regeneration in <u>H. flaviviridis</u>.

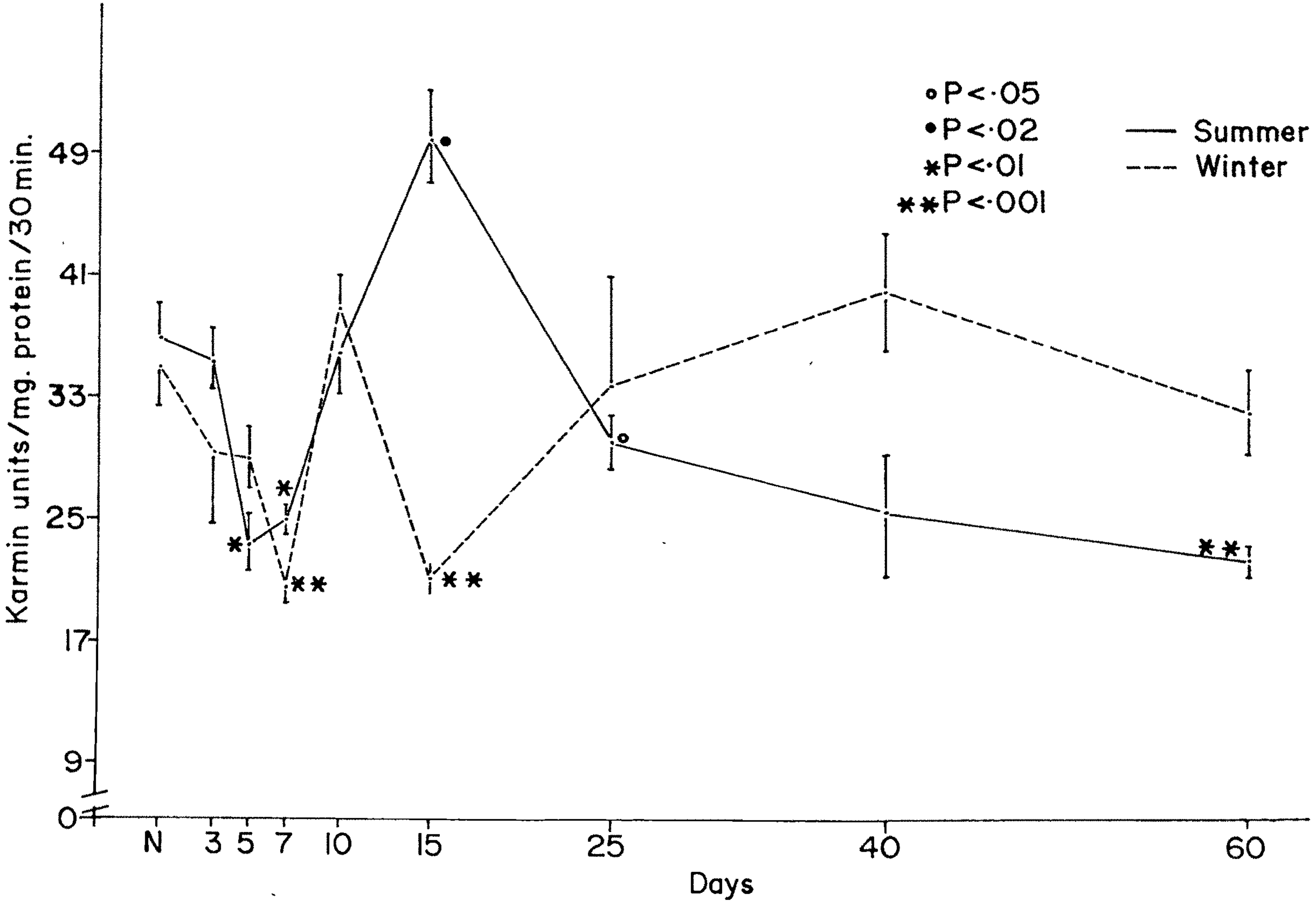

Fig. 8. Changes in hepatic GPT activity in summer and winter months during tail regeneration in <u>H. flaviviridis.</u>

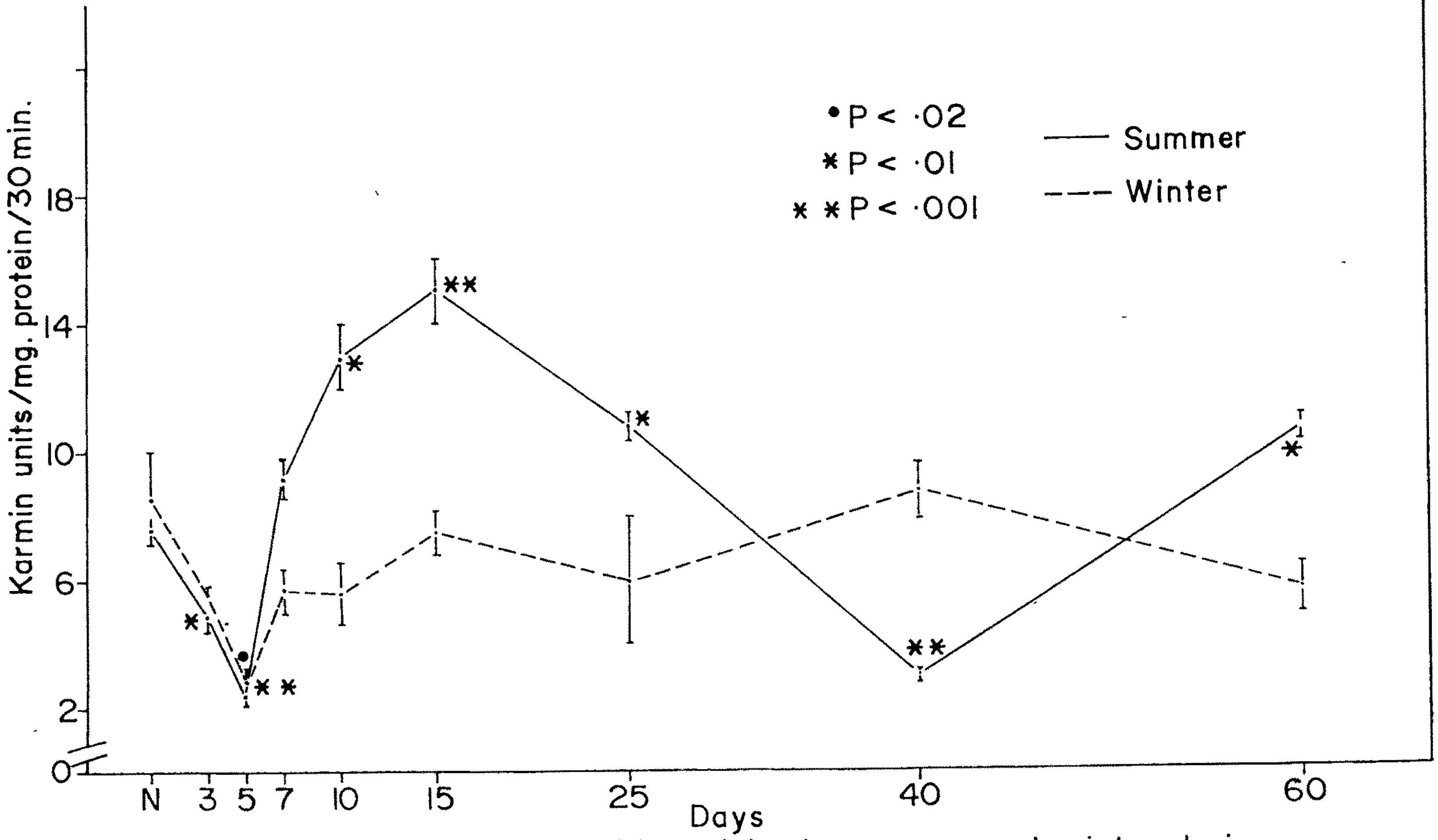

Fig 9. Changes in muscle GPT activity in summer and winter during tail regeneration in <u>H. flaviviridis</u>.

above normal levels between 7th and 25th days. Though a similar pattern was obtained during the winter season as well, GPT activity tended to be subnormal even during 3rd and 5th days post-autotomy.

The changes in hepatic GPT activity which were not very remarkable tended to be more or less similar in the two seasons. In both the seasons, the enzyme activity showed a gradually decreasing trend during the first seven days. Thereafter, the enzyme activity remained in the normal range all throughout except for the 15th day in summer whence the activity was significantly supranormal.

The changes in muscle GPT activity was significant and of differential nature in the two seasons. In the summer the enzyme activity decreased significantly during the 3rd and 5th days in that order and then reached significantly above normal levels during 7th, 10th and 15th days. The enzyme activity remained supranormal for the remainder of the periods except for the 40th day whence there was a significantly reduced level. In contrast, during the winter season, the muscle GPT activity remained subnormal till the 10th day post-autotomy (with a significantly low level on the 5th day) and then remained in the normal range thereafter.

DISCUSSION

Epimorphic regeneration as exemplified by the lizard tail represents an extensive process of morphogenesis involving a conglomerate of events. Predictably, such a process would demand apart from energetic considerations, a heavy protein turnover which will have to be met by the animal. Previous studies from this laboratory have shown extensive metabolic and haemodynamic adjustments as part of a systemic response to the stress of autotomy and the ensuing process of regeneration (Shah et al., 1980a,b,c, 1982,a,b; Ramachandran et al., 1980, 1983, 1985). Moreover, Valsamma (1982) in her doctoral study, has demonstrated inconclusively the existence of a definite adaptive systemic response in lizards (Hemidactylus) endowed with the ability of regeneration of a lost part. The absence of such a response in lizards (Calotes) not endowed with the power of regeneration was also documented (Kinariwala, 1977). The in loco requirement of proteins during regeneration is an integral aspect which can be considered to be of prime importance. Even minor tissue repair processes are known to be characterised by protein anabolism at the sites of repair. A recent labelled incorporation study of phenylalanine in regenerating skeletal muscle (Schwartz et al., 1986) has demonstrated significantly elevated protein turnover occurring during muscle graft regeneration in rats. In this light, the degree and extent of protein turnover required to meet the exigencies of tail regeneration can be really staggering. The systemic endeavours in meeting this requirement has been shown to be met differentially by Mabuya carinata, a Scincid lizard, and Hemidactylus flaviviridis, a Gekkonid lizard, in keeping with their differing habits and habitats and the underlying metabolic adaptations. Whereas Mabuya was shown to depend upon muscle glycogenolysis and transamination of the glycolytic intermediates to generate amino acids (Shah et al., 1982a; Ramachandran et al., 1982), Hemidactylus was considered to make use of hepatic and muscle protein catabolism (Valsamma, 1982).

The present seasonal evaluation has also depicted systemic protein catabolism as an important systemic response to the regenerative stress. However, on a comparative basis, the degree and extent of protein loss occurring in loco as well as systemically was noticeably less in winter than in summer. This was particularly marked in the case of skeletal muscle. This seasonal difference is well reflected on the nearly 50% reduction in regenerative outgrowth recorded during winter (Ndukuba and Ramachandran, unpublished). This decrement in regenerative outgrowth during winter is paralleled by a near 50% decrease in protein loss from the three tissues (under study) taken as a whole. The amount of protein loss equated in terms of total length of tail regenerated in the two seasons has interestingly revealed an identical rate of 1 mg% protein loss/mm of tail regeneration. Apparently, the rate of protein loss remaining identical, the reduced regenerative potential exhibited by *Hemidactylus* during the winter months could be attributed to the relative sluggishness in releasing systemic stores of protein. This aspect is emphasized by the pattern of changes in GOT and GPT activity of the three tissues. Tail regeneration during summer was marked by elevated hepatic and muscle GOT and GPT activities during blastemic and post-blastemic periods (7th - 15th day) as well as the growth phase (40th day), which in general was of an attenuated nature in the winter and hence the observed difference in systemic protein loss. The subnormal or normal levels of caudal GOT and GPT activities during winter and summer respectively for most of the periods of regeneration barring the above-normal levels during 10th to 15th days and the 40th day, bespeak of the inability of diverting metabolic intermediates towards amino acid biogenesis by way of transamination reactions *in loco*, during lacertilian tail regeneration.

SUMMARY

A seasonal evaluation of alterations in in loco and systemic protein content and GPT and GOT activities has been carried out during tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis. In general, the protein content was higher in summer, and the degree of protein depletion and alterations in enzymes' activity occurring in response to caudal autotomy and ensuing regeneration were more pronounced during summer than during winter. The changes recorded are taken to indicate the relative sluggishness in releasing systemic store of proteins and has been correlated with the reduced regenerative outgrowth occurring during the winter months.