

CHAPTER X

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

Besides its roles in metabolic and other molecular events of adult organisms, ascorbic acid (AA) is also known to influence the process of development (Barnett and Bourne, 1942; Mazur et al., 1961; Gauldi, 1963; Barnes and Kodicek, 1972). As regeneration is a major biological phenomenon entailing a reactivation of developmental and morphogenetic events, and being associated with many intricate biochemical and metabolic aspects, the role of AA during tail regeneration in lizards has been investigated in this laboratory (Shah et al., 1976, 1980b). Although ample literature on the biological actions of AA in animal systems is available, barring a few reports (Stubbs and Mekernan, 1967; Stubbs et al., 1967; DeNicola et al., 1968; Dieter, 1969; Biswas, 1969; Ishi et al., 1970; Padhi and Patnaik, 1979), endocrine regulation of the synthesis, storage and release of this vitamin has received scant attention. Adrenalectomy is reported to

cause reduced synthesis and increased degradation of AA in the liver and kidney of rats (Nathani et al., 1971). An assessment of alterations in hepatic and renal ascorbic acid contents in relation to tail regeneration under adrenal suppressed condition was deemed of interest. Such a study apart from providing information on the functional relation between adrenals, ascorbic acid and tail regeneration, may also be useful in understanding the possible role, if any, of adrenocortical hormones in AA turnover.

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 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 two segments distal to the vent.

A total of 120 lizards were used for the experimental purpose. They were divided into two groups of 60 each. One group served as the control and the other was chemically adrenalectomised by intraperitoneal injection of the

synthetic corticosteroid dexamethasone (DXM). The injections were given ($15 \mu\text{g}/.1 \text{ ml/day/animal}$) in the evenings at 17.00 hrs. and was started 10 days prior to tail autotomy and were continued thereafter every alternate day post-autotomy till the end of experimentation. Controls received the same amount of distilled water. Lizards from both the groups were sacrificed at regular time intervals of 3,5,7,10, 15,25,40 and 60 days post-autotomy along with the normal animals with intact tail. Liver, kidney and tail tissues were used for the estimation of ascorbic acid by the method of Roe (1954).

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

RESULTS

The AA content in liver, kidney and tail prior to caudal autotomy as well as post-autotomy during the various periods of tail regeneration in control and adrenal suppressed lizards are given in Tables 1 and 2 and Figures 1-3.

In general, the AA content in all the three tissues was less in the DXM group relative to the control group

prior to autotomy and amounted to 15% in the liver, 26% in the kidney and 40% in the tail. Subsequently to autotomy there was a differential pattern of changes in the three tissues of the control group. The least change was shown by the hepatic tissue which after an initial depletion by the 3rd day post-autotomy maintained its ascorbic acid content in the pre-autotomy level for the remaining periods of regeneration. The kidney AA content depleted gradually after caudal autotomy to register a minimum level on the 10th day. Subsequently the renal AA content increased to the pre-autotomy level by the 15th day itself and was followed by a second depletion by the 25th day and lasted till the 40th day. By the 60th day the renal AA content had however returned to the pre-autotomy level. The caudal AA content in contrast increased gradually during the first 7 days post-autotomy and reached a double the normal level on the 10th day. However, by the 10th day the AA content fell to a slightly above normal level and remained so till the 15th day. Thereafter there was a second phase of AA accumulation on the 25th day followed by a decrement to the pre-autotomy level by the 40th day itself.

The DXM group of lizards depicted a pattern of changes quite distinct from that of the control group. Unlike the control lizards, DXM lizards depicted a gradual depletion

TABLE-1 : Alterations in tissue ascorbic acid content during tail regeneration in normal H. flaviviridis. Values are expressed as mg/100 mg tissue \pm SE.

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.027 ± 0.002	0.014* ± 0.002	0.025 ± 0.002	0.037 ± 0.004	0.024 ± 0.002	0.023 ± 0.001	0.026 ± 0.002	0.033 ± 0.003	0.027 ± 0.001
Kidney	0.023 ± 0.001	0.022 ± 0.0005	0.017* ± 0.001	0.016* ± 0.002	0.015** ± 0.001	0.029** ± 0.002	0.018@@ ± 0.001	0.018* ± 0.0004	0.026 ± 0.001
Tail	0.010 ± 0.001	0.012@ ± 0.0004	0.018** ± 0.0004	0.020** ± 0.0006	0.013 ± 0.001	0.013 ± 0.001	0.029** ± 0.0002	0.009 ± 0.0005	0.008 ± 0.0006

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

TABLE-2 : Alterations in tissue ascorbic acid content during tail regeneration in adrenal suppressed H. flaviviridis. Values are expressed as mg/100 mg tissue \pm SE.

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.023 ± 0.002	0.02 ± 0.002	0.017 [@] ± 0.001	0.013 [*] ± 0.002	0.016 [@] ± 0.002	0.017 [@] ± 0.001	0.023 ± 0.001	0.025 ± 0.0004	0.015 [@] ± 0.002
Kidney	0.017 ± 0.001	0.015 ± 0.003	0.017 ± 0.001	0.021 ± 0.003	0.008 [*] ± 0.002	0.011 ^{**} ± 0.001	0.013 [@] ± 0.001	0.007 ^{**} ± 0.001	0.005 ^{**} ± 0.0006
Tail	0.006 ± 0.0005	0.006 ± 0.0006	0.01 [*] ± 0.001	0.012 [*] ± 0.001	0.002 ^{**} ± 0.0003	0.007 ± 0.0004	0.006 ± 0.001	0.003 [@] ± 0.001	0.002 ^{**} ± 0.0004

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

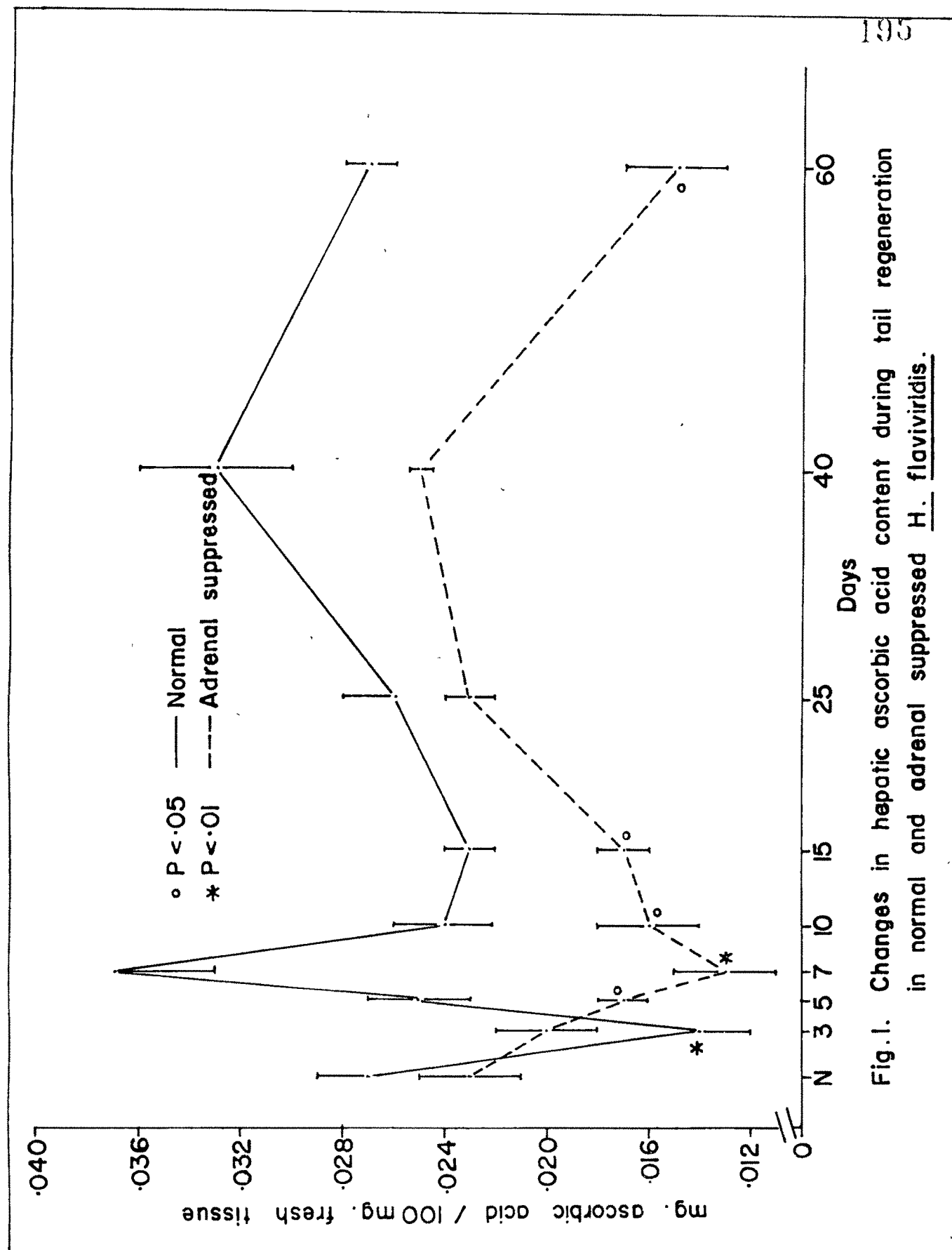


Fig.1. Changes in hepatic ascorbic acid content during tail regeneration in normal and adrenal suppressed H. flavivirdis.

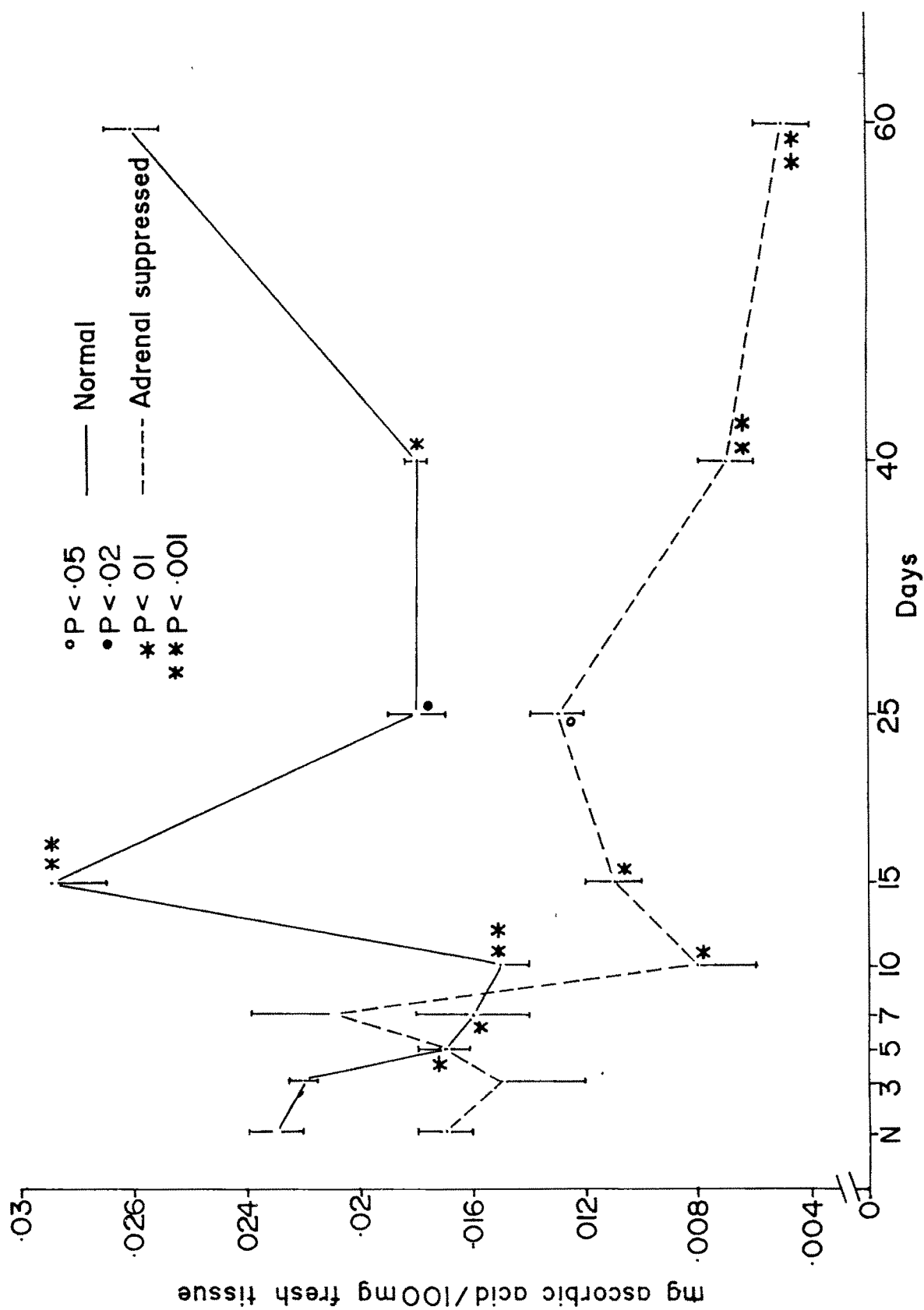


Fig. 2 Changes in the renal ascorbic acid content during tail regeneration in normal and adrenal suppressed H. flaviviridis.

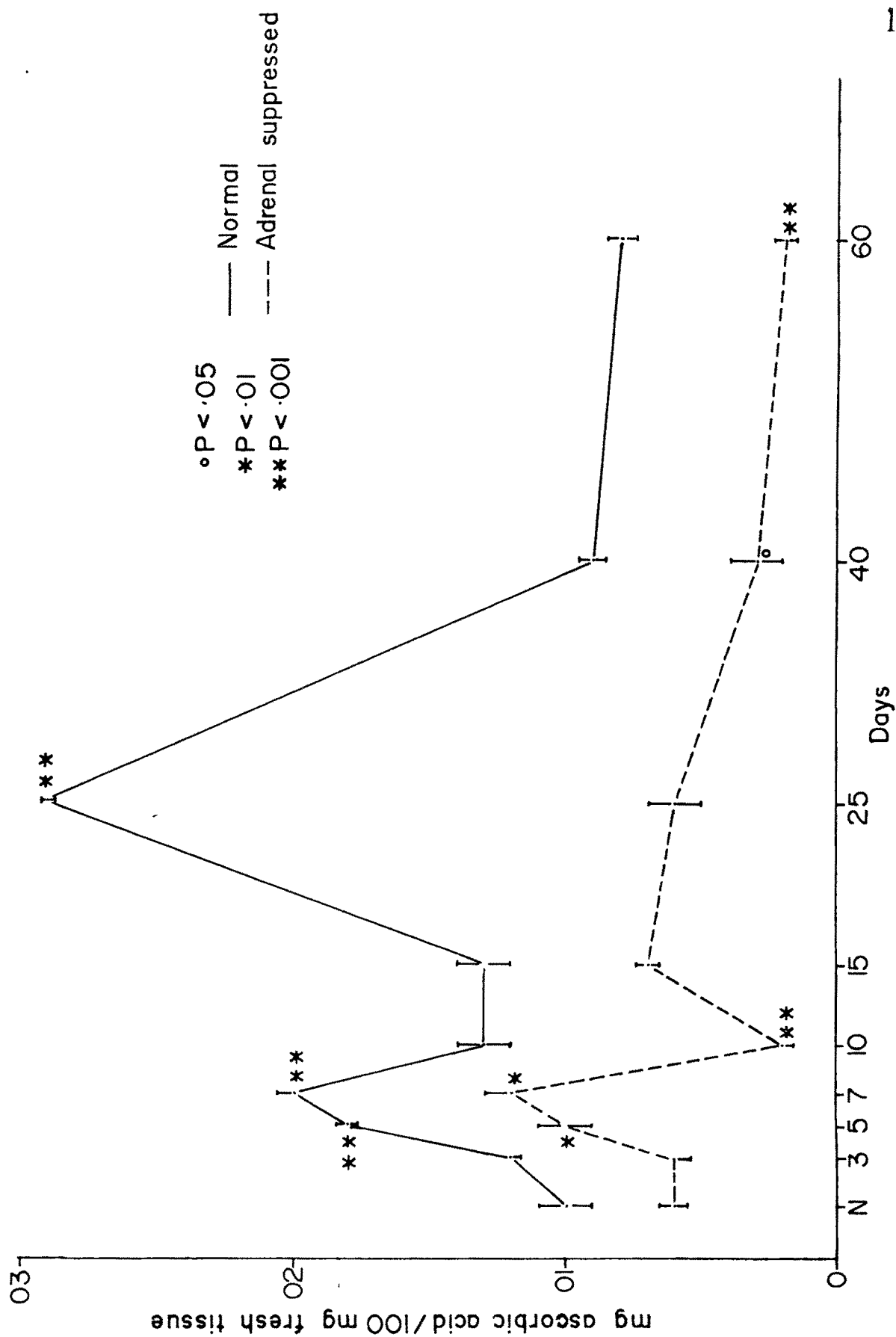


Fig. 3 Changes in caudal ascorbic acid content during tail regeneration in normal and adrenal suppressed *H. flaviviridis*.

of hepatic AA content during the first week post-autotomy while the renal AA content remained steady during this period. Subsequently, the hepatic AA content started increasing gradually from the 10th day to reach the pre-autotomy level by the 25th day, while the renal AA content decreased quickly on the 10th day and then increased gradually towards the normal level during 15th and 25th days of regeneration. Thereafter the renal AA content remained depleted on the 40th and 60th days. The hepatic AA content also was depleted between the 40th and 60th days. The caudal AA content in DXM lizards showed the initial increase during the first week post-autotomy followed by decrease by the 10th day as in the controls. However, the increase in caudal AA content occurring during the 15th and 25th days was only to the normal range in the adrenal suppressed lizards, unlike the controls, in which the AA accumulation during the same period was to a level three times the normal. Like the renal AA content, the caudal AA content too remained depleted during the 40th and 60th days in the DXM group of lizards.

DISCUSSION

The three tissues in which AA content has been assayed involved the organ of synthesis (kidney), the

organ of storage (liver) and the organ of utilisation (tail). The control lizards have depicted marked changes in the AA content of kidney and tail during tail regeneration. Except for a sudden initial depletion, probably as part of a generalised response to the stress of autotomy (Shah et al., 1980b), the hepatic AA content has remained unchanged. The renal AA content in contrast showed continuous depletion lasting ten days post-autotomy while the caudal AA content showed an increase during the first seven days. Since this period corresponds to wound healing and dedifferentiative activities in loco at the autotomised tail site, the increased AA can be functionally correlated with the mechanisms of wound healing and dedifferentiation as has been inferred by the previous workers (Shah et al., 1971; Ramachandran et al., 1975). The simultaneous depletion in renal AA content in this context is indicative of the source of mobilisation for caudal AA. The reduced requirement for AA in the blastemic phase is denoted by the decreased caudal AA content coupled with increased renal AA content between the 10th and 15th days post-autotomy. The involvement of AA during peak histodifferentiation as has been inferred earlier (Shah et al., 1971; Ramachandran et al., 1975) is indicated by the tremendous increase in the tail regenerate on the 25th day corresponding to a second

phase of renal AA depletion. These set of changes suggest a close and effective liaison between the renal tissue and tail, during its regeneration.

A previous study by Valsamma (1982) on tail regeneration in unilaterally adrenalectomised Hemidactylus flaviviridis had shown no significant deviation in the pattern of changes in AA content either in loco or systemically from that of the controls. The present investigation involving more complete adreno-cortical suppression has however revealed some significant changes in the experimental lizards. Apart from a generalised reduction in the AA content of all the three tissues prior to caudal autotomy itself, the post-autotomy induced changes were also somewhat different from those of the controls. Unlike the control lizards which showed two phases of AA accumulation in the tail after its autotomy, the DXM treated lizards showed only the initial accumulation occurring first week post-autotomy. The later periods of regeneration were marked by subnormal levels of AA. The hepatic and renal AA contents also showed changes which were at variance from that of the controls. Whereas the hepatic AA content showed a gradual decrease in ^{the} first week post-autotomy, the renal AA showed a sudden depletion only on ^{the} 10th day. Though

the AA content on 15th and 25th days were in the normal range, the terminal periods of regeneration were marked by significantly subnormal levels in both the tissues. Obviously, adreno-cortical insufficiency has somehow induced increased AA depletion from liver and resisted the regeneration specific renal AA depletion in the initial periods. Moreover, the terminal phases of regeneration were marked by significantly depleted levels of AA in all the three tissues. These observations lend credence to the report of Nathani et al. (1971) of reduced synthesis and increased degradation of AA in both liver and kidney of adrenalectomised rats.

A comparison of the overall AA turnover during regeneration in control and adrenal suppressed lizards reveals a net negative AA balance under adrenal suppressed condition. This is made obvious by the fact that whereas the control group registered an overall positive AA balance to the tune of 5% at the end of regeneration, the adrenal suppressed group registered a net negative AA balance to the tune of 30%. Moreover, the overall AA deficit of 23% recorded prior to caudal autotomy in the adrenal insufficient group of lizards increased to 64% towards the end of tail regeneration. When equated with the 30% less regenerative output in adrenal suppressed lizards