

INTRODUCTION

To quote Waddington (1935), "the fundamental fact about cancerous tissue is that it has escaped from the normal growth controlling agents of the body". These "growth controlling" agents called individuation fields, are highly potent in animals that are capable of regeneration. During regeneration, the primitive cells initially multiply rapidly with a certain abandon resembling that of a cancerous growth. But unlike a cancer, which would continue to grow in a disorderly fashion, the regenerating tissue eventually grows at a slower rate and gradually takes the form of a functional organ. Obviously there is some practical interest in the possibility that human beings might some day be able to regrow tissues and organs which they are presently unable to. But apart from such possibilities regeneration holds both theoretical and experimental interest for the biologist, because it demonstrates that developmental processes are not entirely absent in the adult animal. The adult organism "remembers" some of its developmental history and repeats it, albeit in a limited and sometimes devious way. And regeneration is essentially an attainment of more or less a complete substitute for the original form of the structure that is lost.

Regeneration is customarily distinguished into two types viz., physiological regeneration and reparative regeneration. Physiological regeneration is the restoration of the lost elements of a living organism, during its normal day to day activity (eg. epithelial and RBC regeneration). Physiological regeneration is a universal phenomenon characteristic of tissues and cells of animals, plants and microorganisms; and it is not lost during phylogenesis and ontogenesis. On the contrary, reparative regeneration is restoration of lost parts caused by trauma or damage, and is restricted to lower animals, while in higher animals it is rather limited to such an extent that in man even minor wounds often leave scars. Wound healing in higher vertebrates, especially man, is far from ideal, and regenerative capacity is highly restricted. Studies now underway in different laboratories on this aspect, attempt to minimize or even eliminate scarring, expedite the healing process and possibly activate regeneration. Although answers are still not available, nor are unequivocal explanations as yet forthcoming, the remarkable regenerative capacity of lower vertebrates is an undeniable fact. It is believed that the key to improving man's lot in the repair and regeneration of lost structures and organs lies in the molecular activity that is part of and directs regeneration in lower vertebrates. Maderson and Salthe (1969) have suggested that poorly controlled wound healing is a good factor for regeneration.

Regeneration like other biological processes is susceptible to analysis at various levels of organizations. It occurs in varying degrees in plants and animals. Goss (1969) in his treatise on "Principles of regeneration" has opined that if there were no regeneration, there could be no life, if everything regenerated there could be no death. And the process of regeneration of an organ can be considered as an extension on a grander scale of the process of wound healing (Bullough and Laurence, 1966). According to Morgan (1901) regeneration is the resumption of temporarily arrested growth. However, the numerous studies that followed in the subsequent decades showed that regeneration is something more than mere growth; it includes transformation, differentiation and quantitative as well as qualitative changes in metabolic profile to restore the lost parts of an organism. Therefore it appears that the capacity to regenerate a lost appendage in a vertebrate would depend on both the local potentiality as well as the ability to evoke specific, synergistic, supportive and permissive systemic responses (Valsamma, 1982).

During the last few decades, studies have been carried out to reveal the factors that either suppress or entirely inhibit repair and regenerative processes among vertebrates. Such inhibition has been accomplished by a wide array of physicochemical means, including application of X-rays, ultraviolet irradiation, antimetabolites and other toxic

agents as well as induced lack or excess of endocrine hormones.

The development of the regenerate of an animal occurs in structural association with the fully differentiated animal body and probably even in functional association. Though the inbuilt genetic programme, may be considered to be reactivated during reparative regeneration for reconstitution of a more or less exact replica of the part lost, the structural and functional association with the body suggests a definite systemic involvement (Swamy, 1981). There is a primary requisite for building up the necessary energy source, information molecules etc., and a precise, conducive set-up for the reactivation of the developmental events. A vertebrate body is known to respond to injuries at the site as well as systemically wherein circulating metabolites and the distant organs get involved. Obviously invoking of such systemic responses would require the mediatory influence of hormonal factors. This conception has provided motivation for explorations on endocrine participation on lizard tail regeneration. A regenerating system, such as the lizard's tail provides a unique opportunity to evaluate hormonal influences in an adult organism in which metabolic shifts and extensive developmental events can be studied simultaneously. Data on circulating and, stored metabolites in some tissues in lizards during tail regeneration

are available from our laboratory. Voluminous literature has been compiled from such studies making use of the lizards M. carinata and H. flaviviridis. The present investigations on H. flaviviridis have been designed to examine these in the light of endocrine involvement and to comprehend the extent of influence of hormone/s on the lizard tail regeneration.

The influence of external factors like temperature, humidity, light and electric current on appendage regeneration has been well documented (Becker, 1961a,b; Bodemer, 1964; Maderson and Licht, 1968; Magon, 1970; Schauble, 1972; Smith, 1974; Schauble and Nentwig, 1974; Borgens et al., 1977, 1979). Long day photoperiod was found to enhance regenerative growth of the tail in Anolis carolinensis (Turner and Tipton, 1972) and of the limb of Notophthalmus viridescens, while total darkness was found to depress the growth (Maier and Singer, 1977). Earlier studies from this laboratory have shown that there is a seasonal variation in the rate of regeneration with maximal growth occurring during the summer months (Chakko, 1967; Magon, 1970). To unravel the metabolic and/or biochemical correlates if any for the observed seasonal difference in growth rate during the seasons (i.e. summer and winter), studies were undertaken and the adaptive systemic metabolic alterations evaluated with relation to lacertilian tail regeneration. The morphologic changes occurring during various phases of

regeneration are undoubtedly reflected in the metabolic manifestations of the tissues involved. Seasonal changes are believed to have definite influence in the metabolic activities of certain animals. Since liver and skeletal muscle are thought to be organs of immense importance in carbohydrate metabolism, and since regeneration is a biological process which requires high energy output, it was deemed fit to study the quantitative changes in carbohydrate stores like glycogen and blood glucose with respect to these two organs along with the regenerate in order to understand *the* relative systemic and in loco responses. Hepatic glycogen phosphorylase also was studied (Chapter 1). The seasonal difference in regenerative growth was noted to have little biochemical correlation in terms of pattern of changes in local and systemic carbohydrate stores, as the temporal changes in carbohydrate metabolism were more or less identical in both the seasons subsequent to tail autotomy. However, the observable reduced regenerative outgrowth during the winter months is correlated with the seasonal difference in turnover of carbohydrate reserves.

Development of an organ demands greater synthesis of macromolecules such as proteins, carbohydrates and lipids. Synthesis of protein is particularly important to a regenerating system during histodifferentiation of tissues forming the new regenerate. Apart from their significance in protein

synthesis, amino acids also contribute to general metabolism by acting as either oxidative substrates or as raw materials for lipid and carbohydrate synthesis. Since regeneration has been found to induce alterations with respect to carbohydrate, lipid as well as protein metabolisms (Shah et al., 1977a,b, 1979a, 1980a, 1982a; Ramachandran et al., 1979, 1980, 1982), a quantitative evaluation of the total protein content in liver, muscle and tail was undertaken during various stages of tail regeneration. Besides, aspartate and alanine aminotransferases (GOT and GPT), the two enzymes which are important in linking amino acid metabolism with TCA cycle oxidations were also thought fit to be investigated (Chapter 2). These investigations have revealed a pronounced seasonal difference in tissue protein content. Summer season showed a high protein content in tail whereas in the winter season the protein content was considerably less. On a seasonal basis the degree of protein depletion from liver and muscle was more evident during the summer months than the winter months. The results denote a general inertness by the systemic tissues in releasing their labile pool of protein store, for ready utilization during regeneration in the winter months which is well reflected in the pattern of changes in the activity of the transaminases. The transaminases showed phase specific variations in enzyme activity throughout the various stages of regeneration. From the

activity levels of these two transaminases in the tail it appears, that these two enzymes are playing insignificant role in directing the TCA cycle intermediates towards amino acid synthesis.

Cyclic nucleotides are believed to be involved in cell division (Thomas et al., 1973; Berridge, 1975; Carlone and Foret, 1979), cell differentiation (Friedman, 1976; Miller, 1977; Taban and Cathieni, 1978; Kosher and Savage, 1980), macromolecule synthesis (Sharma and Talwar, 1970; MacManus et al., 1972, 1973; Babich and Foret, 1973; Dokas, 1973; Foret and Babich, 1973; Short et al., 1975) and metabolic alterations (Rall et al., 1957; Drummond et al., 1969; Beriz et al., 1977; Ishibashi and Catton, 1978). In the present investigation, functionally correlatable alterations in cAMP level were evaluated in relation to the process of tail regeneration. Levels of cAMP in liver, muscle and regenerating tail, throughout the various phases of tail regeneration have been evaluated indirectly by assaying the activity of cAMP phosphodiesterase, the only known degradatory enzyme of cAMP (Chapter 3). Alterations in enzyme activity were observed in all the three tissues, but the regenerating tail exhibited maximum variation. The changes in phosphodiesterase activity tend to show elevated cAMP levels during the 3rd, 7th, 25th and 40th days of regeneration which correspond to early wound healing,

preblastema and differentiation phases. Apparently, cAMP mediated processes are thought to have an important role in the various in loco cellular events and metabolic adaptations underlying these phases of tail regeneration.

The vertebrate body is known to respond to physical trauma with characteristic physiological changes in the region of injury, and systemically. Involved in the latter response are the body tissues, organs and specifically the endocrine glands. Although considerable interest has been directed towards the study of hormonal control of regeneration, specific role(s) of endocrine glands is yet to be clearly elucidated. Presently, two important hormones, the thyroid hormone and the adrenocortical hormone were selected for studying their role in lizard tail regeneration and to this end chemical suppression of both the glands by thiouracil and dexamethasone^(DXM) respectively was employed as the technique of choice (Chapter 4). The thyroid suppressed lizards showed a 60% retardation in the regenerative tail elongation in the initial stages by the 10th day which however got minimised to only about 40% by the 60th day post-autotomy and the regenerates were qualitatively and quantitatively poor with a shrunken and stunted appearance. The adrenal suppressed animals also revealed an overall retardation in the rate of tail regeneration as well as the final length attained. A measurable growth of 1 mm was attained by the controls by

the 7th day while in the experimentals this was attained only by the 10th day. The percentage retardation in regeneration was about 30% by the 60th day. In this case too the regenerates were of poor quality. A disturbance observed in the morphogenetic events of lizard tail regeneration as a result of altered functional status of these two endocrine glands, amounting to extension of the initial phases (a delay in wound healing and blastema formation), and defective differentiation of several regenerate tissues leading to retarded growth, called forth for an examination of the causative factors in terms of metabolic profile.

Thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) appear to be of immense importance to vertebrates in general due to their role in tissue metabolism and differentiation. Korneluk and Liversage (1978) have studied the circulating levels of both these thyroid hormones during forelimb regeneration in adult newts. And Kar and Chandola (1985) have worked out the T_3 and T_4 levels in Calotes versicolor, a non-regenerating reptile. To this end it was thought worth studying the T_3 and T_4 levels in H. flaviviridis during the process of tail regeneration. The results accrued indicate lower T_3 levels in comparison to T_4 . The study has also revealed definite, phase-specific variations in the



circulating levels of these hormones in the form of increased T_4 levels during the initial phases and increased T_3 levels during the later phases of tail regeneration which are correlated with the associated in loco and systemic changes.

Importance of proteins in mammalian wound repair has been recognised for several years (Williamson, 1956). It is well realised that injury induced trauma elicits a remarkable fluctuation in the protein profile of an animal body. To ascertain qualitative differences in the proteins of normal and regenerating tissues, Schmidt (1966^a) had separated soluble proteins of regenerating tissues on polyacrylamide gels. It is evident from such data that, not only are there quantitative differences during the process of regeneration, but that 'new' proteins are synthesised. The present study was undertaken to elucidate the possible involvement of such regeneration specific proteins as well as isoenzymes that may play crucial roles in the molecular mechanisms underlying the process of tail regeneration in lizards. Operation of certain regulatory factors such as hormones in this process is well documented, especially with regard to the role of thyroid hormones. In this wake, it was thought appropriate to study the electrophoretically separable proteins and the LDH isoenzymes in the control as well as experimental (thyroid suppressed) animals during the various stages of

regeneration, to see the changes if any, in the protein profile and LDH isoenzymic pattern throughout the process of regeneration (Chapters 6 and 7). A striking aspect of this electrophoretic separation of proteins was, the appearance of four 'new' protein bands in the tail tissue of which one was permanent and three were transitory. The permanent protein acquisition appeared as early as the 3rd day. Of the three transitory proteins, one was very short-living and was induced as early as the 3rd day and remained only till the 7th day. The other two were comparatively long living and made their appearance gradually one by one between the 3rd and 5th days and remained till the 25th day. In the thyroid suppressed lizards, though there was a generalised decrease in the electrophoretically separable proteins in liver, muscle, and tail, the two long living regeneration specific protein bands did appear in the regenerate. The electrophoretic separation of LDH isozymes too revealed appearance of oxidative bands during regeneration in intact lizards which was suppressed to a greater degree by thiouracil induced thyroid inhibition. Moreover, the presence of a 'C' locus for LDH, as presumed to occur in some fishes, has been identified as two additional bands could be visualised in all the tissues. In the light of the above results it could be concluded that the thyroid hormone influences regeneration indirectly by controlling biochemical transformations in an adaptive manner.

It has been reported, that adrenal steroid hormones exert marked effect on carbohydrate metabolism (Britton and Silvette, 1932). And since carbohydrate metabolism was thought to have a prominent role in meeting the energy requirements during tail regeneration, the levels of blood glucose and tissue glycogen contents were evaluated under adrenal sufficient and insufficient conditions in relation to the process of regeneration (Chapter 8). The results obtained indicate that the general pattern of changes in the glycogen content of tail, liver and muscle as well as blood glucose remained more or less identical in both adrenal intact as well as adrenal suppressed lizards. But it was observed that the depletion in the local and systemic stores of glycogen during the early stages, and ~~the~~ subsequent build-up for utilisation in the later phases of regeneration were both of low magnitude in DXM treated lizards. The blood glucose level remained subnormal all throughout regeneration in both the groups of animals, with a more pronounced hypoglycaemia being characteristic of the adrenal suppressed animals. The above changes indicate no alteration in the pattern of carbohydrate metabolism characteristic of regeneration in lizards due to adrenal suppression. However, the inadequate glycogen depletion and the increased withdrawal^a of glucose from the blood in the experimentals show an uneconomical utilization as compared to the adrenal sufficient lizards.

Since protein metabolism is of vital significance in developmental processes and since there is lack of information on transaminases in normal physiological processes associated with the process of regeneration, an analysis of total protein content together with an assay of glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) have been undertaken during tail regeneration under altered adrenal functioning (Chapter 9). In the present study, it was observed that the tissue protein profile does indicate a definite difference between the control and experimental lizards. Both the systemic protein stores were depleted in control lizards in the initial stages of regeneration. However, no such change could be observed in the adrenal suppressed lizards denoting comparative resistance towards systemic protein depletion in the experimental animals. The alterations noticed in the systemic protein profile, could be one of the factors that could be implicated in the slow rate of regeneration. As far as transaminases were concerned, most of the stages of regeneration depicted more or less reduced levels of enzyme activity in the experimentals. This reduced enzyme activity also might be contributing to the reduced rate of regeneration in the experimentals.

Vitamin C deficiency interferes with collagen synthesis (Wolbach, 1933; Gouldⁱ, 1963) and thus in the repair of wounds in mammals including man (Hunt, 1941; Bartlett et al., 1942a, b; Hartzell and Stone, 1942). Ryvkina (1940) had studied the involvement of ascorbic acid (AA) in amphibian regeneration and suggested that Vitamin C might be contributing to the synthesis of collagen in the differentiation phase of regeneration. Apart from a few reports (Thomas, 1980; Valsamma, 1982), endocrine regulation of this metabolite in relation to the process of regeneration has not received much attention. To this end it was thought worth studying the quantitative alterations in the in loco and systemic AA contents during the process of tail regeneration under DXM treatment. The results obtained suggest that the characteristic modulations in AA turnover occurring during tail regeneration are not much affected by adrenal suppression. However, in general the metabolite level was reduced in all the three tissues in the experimentals.

Dehydrogenase catalysed reactions bring about oxido-reduction of metabolic substrates involving the breakdown and synthesis of proteins, lipids and carbohydrates. Lactate dehydrogenase, the enzyme which catalyses the reversible reaction between pyruvate and

lactate is found in cells actively engaged in anaerobic glycolysis. Since the process of regeneration in lizards is known to be dependent on anaerobic glycolysis for meeting the energy requirements, it was thought feasible to study the effect of adrenocortical hormones if any. In this respect, the enzyme activity was evaluated in the controls as well as DXM treated animals. In general, all the three tissues recorded reduced level of enzyme activity under DXM treatment and the retarded regenerative growth observable in the experimental animals are being correlated with reduced energy output caused by the subnormal operation of the glycolytic pathway.

Histochemical localisation of lipids and dehydrogenases like LDH, BDH and SDH were also carried out in both the control and experimental lizards to see the possible alterations if any under altered adrenal functioning during regeneration. Sudanophilic and ^tFetrot positive lipids showed noticeably reduced localisation in DXM treated lizards. However, the pattern of changes in lipid distribution in both liver and the regenerate depicted no significant difference between the experimentals and controls. Histochemical distribution of *the* three dehydrogenases viz., LDH, BDH and SDH showed in general a decrement in adrenal suppressed lizards, more specifically in the hepatic tissue than the tail. The post-autotomy

induced alterations were more significantly depressed in the liver and to a minor extent in the tail of adrenal suppressed lizards.

Even though the precise mechanism by which the neuronal contribution affects regeneration is not known, neurotrophic influence on increase in volume and mitotic activity of the early regenerate have been demonstrated (Dresden, 1969; Lebowitz and Singer, 1970; Singer and Caston, 1972; Morzlock and Stocum, 1972; Bantle and Tassava, 1974). Major studies on these lines have been restricted to amphibian limb regeneration. A former study by Ramachandran et al. (1981^b) in the Scincid lizard Mabuaya carinata had shown the involvement of AChE in regenerative mechanics of the lizard. In the present investigation, this neurochemical substance has been studied in relation to tail regeneration in H. flaviviridis under adrenal suppressed condition. DXM suppressed animals depicted an increased AChE activity in all the three tissues prior to autotomy. However, post-autotomy stages were marked by reduced levels of AChE activity in both liver and muscle all throughout while in the case of the regenerate the enzyme activity did depict modulations characteristic of regeneration exhibited by the control lizards. These changes indicate an altered systemic modulation in AChE activity due to adrenal

suppression. However, the in loco response of ^{the}tail regenerate seemed to show an independence in bringing about modulations in enzyme activity irrespective of the adrenal status and may bear relevance in the context of no observable inhibition of tail regeneration in H. flaviviridis under adrenocortical suppression.

CHAPTER I

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

Polysaccharides have been recognised since long as versatile compounds in a variety of biological systems. Special importance is given to the role of polysaccharides in such regeneration affiliated fields, as wound healing (Jackson, 1958), tissue interactions and growth (Grobstein, 1955). Since reserve stores of carbohydrates serve as the primary source of energy in diverse metabolic processes, and as the process of regeneration is known to be a highly energy oriented one, a detailed analysis of the changes in the carbohydrate stores of the body together with the degradatory enzyme, glycogen phosphorylase, was deemed fit to be investigated. Previous studies on amphibian limb regeneration (Schmidt, 1960, 1962; Procaccini et al., 1973; Connely et al., 1974) and on reptilian tail regeneration (Shah and Chakko, 1967; Radhakrishnan and Shah, 1973; Shah and Hiradhar, 1974; Shah et al., 1977a, b) have revealed the importance of glycogen in reparative regeneration. Earlier studies from this laboratory have indicated that there is a definite

systemic response in the form of hepatic glycogenolysis and altered glycaemic levels during tail regeneration in lizards (Shah et al., 1977b; Valsamma, 1982).

Seasonal changes are believed to have definite influence on the metabolic activities of certain animals. Earlier studies from this laboratory have also shown that there is a seasonal variation in the rate of regeneration with maximal growth occurring during the summer months (Chakko, 1967; Magon, 1970). Apparently, the ambient temperature has an influence on the regenerative tail elongation. This aspect has also been stressed by Maderson and Licht (1968) in Anolis carolinensis, Schauble (1972) and Schauble and Nentwig (1974) in Notophthalmus viridescens. Though the influence of temperature as a factor in regeneration has been demonstrated on a morphological level, no biochemical or metabolic correlation has ever been attempted. Hence it was thought appropriate to study the changes in the carbohydrate stores on a seasonal basis in the Gekkonid lizard, Hemidactylus flaviviridis. A comparative evaluation of the levels of these metabolites, viz., glucose and glycogen, and the degradatory enzyme glycogen phosphorylase, has been undertaken during the winter and summer months so as to reveal metabolic and/or biochemical correlates for the observed seasonal difference in growth rate.

MATERIALS AND METHODS

The lizards, H. flaviviridis were procured from the local animal dealer and 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 to 12 gms and having a snout-vent length of 8 to 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 were sacrificed at fixed intervals of 3, 5, 7, 10, 15, 25, 40 and 60 days post-autotomy along with normal ones with intact (unautotomized) tails. Liver and skeletal muscle (femoral) as well as the tail (regenerating or normal as the case may be) were quickly removed and 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 by the method of Folin and Malmros (1929). A 2% tissue homogenate was used for the quantitative assay of liver phosphorylase as per the method of Cahil et al. (1957) and the inorganic phosphate released due to the enzymatic action on the substrate

(Glucose-1-phosphate; obtained from Sigma Chemicals, USA) was measured by the method described by Fiske and Subbarow (1925). The amount of glycogen in the tissues was expressed as percentage with respect to the wet tissue weight and the specific activity of the enzyme phosphorylase was expressed as μg phosphorous released/mg protein.

The protein content of the tissues was estimated by the method of Lowry et al. (1951).

For each day and each tissue specified, a total of five to seven 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.

RESULTS

The results of the experiments are depicted in Tables 1-4 and Figures 1-5.

Blood glucose - The whole period of regeneration was marked by a hypoglycaemic condition during both the seasons. In general, the pattern of changes in the glycaemic level occurring during tail regeneration was identical in the two seasons. Immediate post-autotomy phase lasting upto 7 days was marked by a hypoglycaemic condition with the blood glucose level showing a decrement of 40%. Whereas this 40% decrement was attained by the 3rd day itself in the summer, the same occurred only by the 5th day in the winter. Blood glucose

TABLE-1 : Comparative levels of blood glucose (mg/dl) during tail regeneration in summer and winter months in H. flaviviridis. (\pm SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Summer	114.14 ± 1.46	69.8 ^{**} ± 0.60	72.42 ^{**} ± 2.34	68.1 ^{**} ± 1.22	92.91 ^{**} ± 2.24	91.74 ^{**} ± 1.65	73.74 ^{**} ± 2.31	81.20 ^{**} ± 0.82	95.93 ^{**} ± 1.42
Winter	112.19 ± 2.55	79.8 ^{**} ± 2.77	66.93 ^{**} ± 1.87	68.3 ^{**} ± 2.07	95.97 [*] ± 1.6	95.73 ^{**} ± 1.35	76.11 ^{**} ± 2.49	89.08 ^{**} ± 1.64	97.98 [*] ± 1.54

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

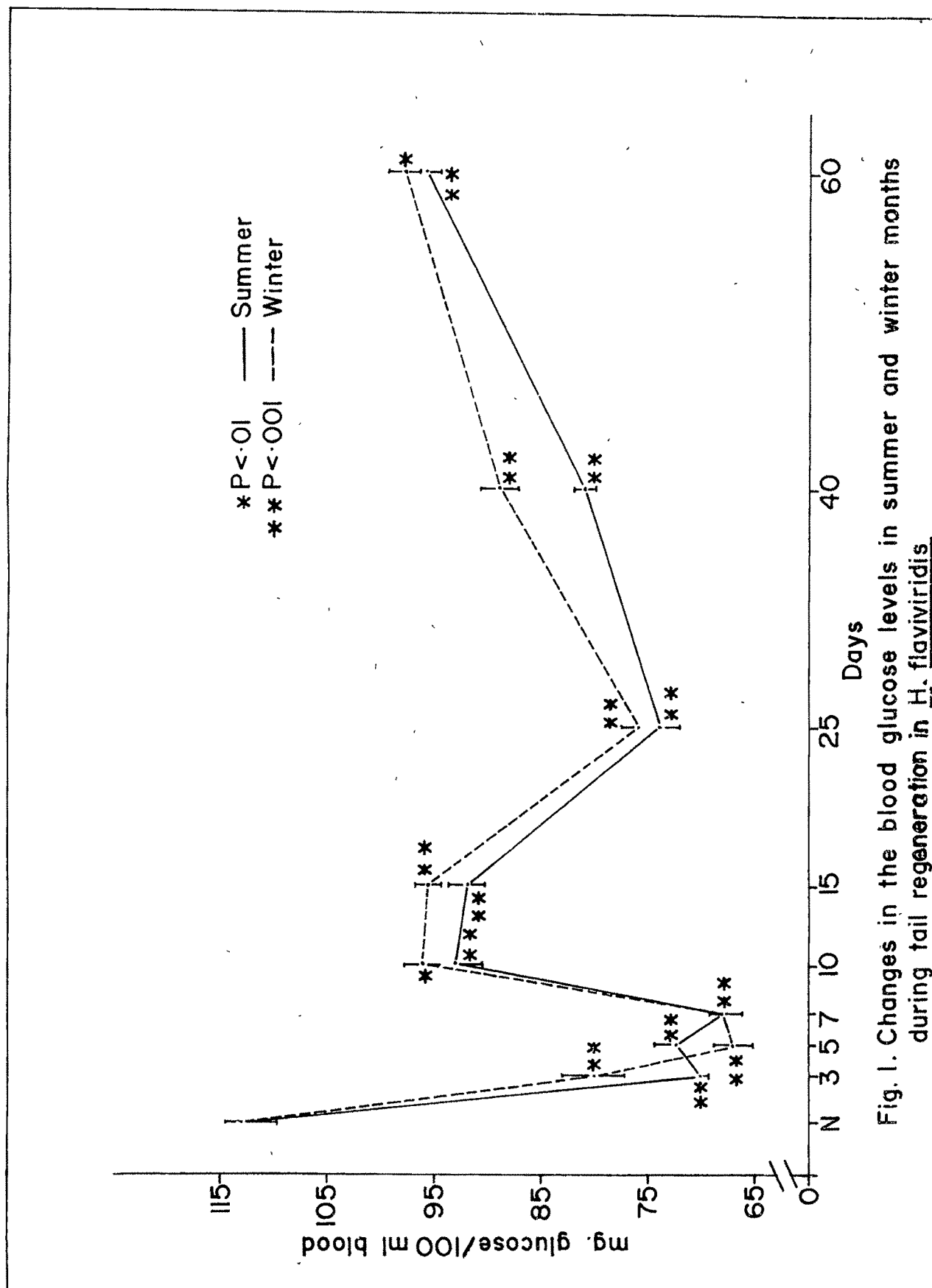


Fig. 1. Changes in the blood glucose levels in summer and winter months during tail regeneration in H. flaviviridis.

level recorded an increment towards the normal during the 10th and 15th days to be followed by a second phase of decrement (20%) between 15th and 25th days during both seasons. Since then the glycaemic level gradually started increasing towards the normal during 40th and 60th days post-autotomy.

Tissue glycogen contents - Liver, tail and skeletal muscle had comparatively higher glycogen content in that order during the winter season than in the summer season. The trend of changes in the tissue glycogen contents post-autotomy was identical in both the seasons. Both the caudal and hepatic glycogen contents recorded a significant depletion by the 3rd day post-autotomy which was slightly more pronounced in the winter than in the summer. Thereafter, the glycogen reserve of both the tissues increased to an above normal level, the peak of which was reached by 7th day in liver and by 10th day in the case of tail. On a comparative basis, this glycogenic effect was more pronounced during summer than during winter. Thereafter the caudal glycogen store decreased gradually to reach the control level by the 60th day, while in the case of liver, there was a second phase of glycogen depletion (85%) between the 10th and 25th days to be followed by a gradual increase to normal through 40th to 60th days. In contrast, muscle glycogen content depicted continuous increase from 3rd day post-autotomy till 10th day, whence maximal level was registered (600% in summer and 350% in winter). Thereafter, the muscle glycogen reserve gradually depleted to the normal level by the 60th day.

2. Phosphorylase - Changes in phosphorylase activity were inversely related to that of glycogen content. Accordingly, the

TABLE-2 : Alterations in tissue glycogen content (mg/100 mg fresh tissue) during tail regeneration in the summer months in H. flaviviridis. (\pm SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.43 ± 0.01	** 0.23 ± 0.02	* 0.55 ± 0.02	** 1.86 ± 0.04	** 1.7 ± 0.02	** 0.91 ± 0.01	** 0.28 ± 0.01	* 0.37 ± 0.01	0.46 ± 0.02
Muscle	0.09 ± 0.002	** 0.13 ± 0.003	** 0.14 ± 0.002	** 0.24 ± 0.01	** 0.64 ± 0.01	** 0.31 ± 0.02	** 0.14 ± 0.002	** 0.13 ± 0.002	0.09 ± 0.002
Tail	0.32 ± 0.01	** 0.16 ± 0.002	** 0.21 ± 0.004	** 0.24 ± 0.01	** 0.62 ± 0.01	** 0.53 ± 0.01	* 0.38 ± 0.01	0.33 ± 0.01	0.31 ± 0.002

* $P \leq 0.01$; ** $P \leq 0.001$

TABLE-3 : Alterations in tissue glycogen content (mg/100 mg fresh tissue) during tail regeneration in the winter months in H. flaviviridis. (\pm SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.50 ± 0.02	0.21 ^{**} ± 0.02	0.55 ± 0.02	1.73 ^{**} ± 0.18	1.42 ^{**} ± 0.18	1.10 ^{**} ± 0.05	0.22 ^{**} ± 0.02	0.36 ^{**} ± 0.01	0.53 ± 0.03
Muscle	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.23 [*] ± 0.03	0.49 ^{**} ± 0.02	0.18 ^{@@} ± 0.02	0.14 ± 0.02	0.13 ± 0.01	0.06 [*] ± 0.01
Tail	0.44 ± 0.03	0.15 ^{**} ± 0.01	0.18 ^{**} ± 0.02	0.23 ^{**} ± 0.02	0.58 [@] ± 0.05	0.51 ± 0.04	0.35 ± 0.03	0.31 [*] ± 0.02	0.42 ± 0.01

@ P \leq 0.05; @@ P \leq 0.02; * P \leq 0.01; ** P \leq 0.001.

TABLE-4 : Alterations in hepatic phosphorylase activity (μg phosphorous released/mg protein) during tail regeneration in H. flaviviridis. (\pm SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	13.63 ± 1.00	21.27* ± 1.75	12.42 ± 0.56	11.20 ± 0.69	7.00** ± 0.42	12.92 ± 1.12	17.84@ ± 0.43	15.76 ± 1.21	16.68@ ± 0.72

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

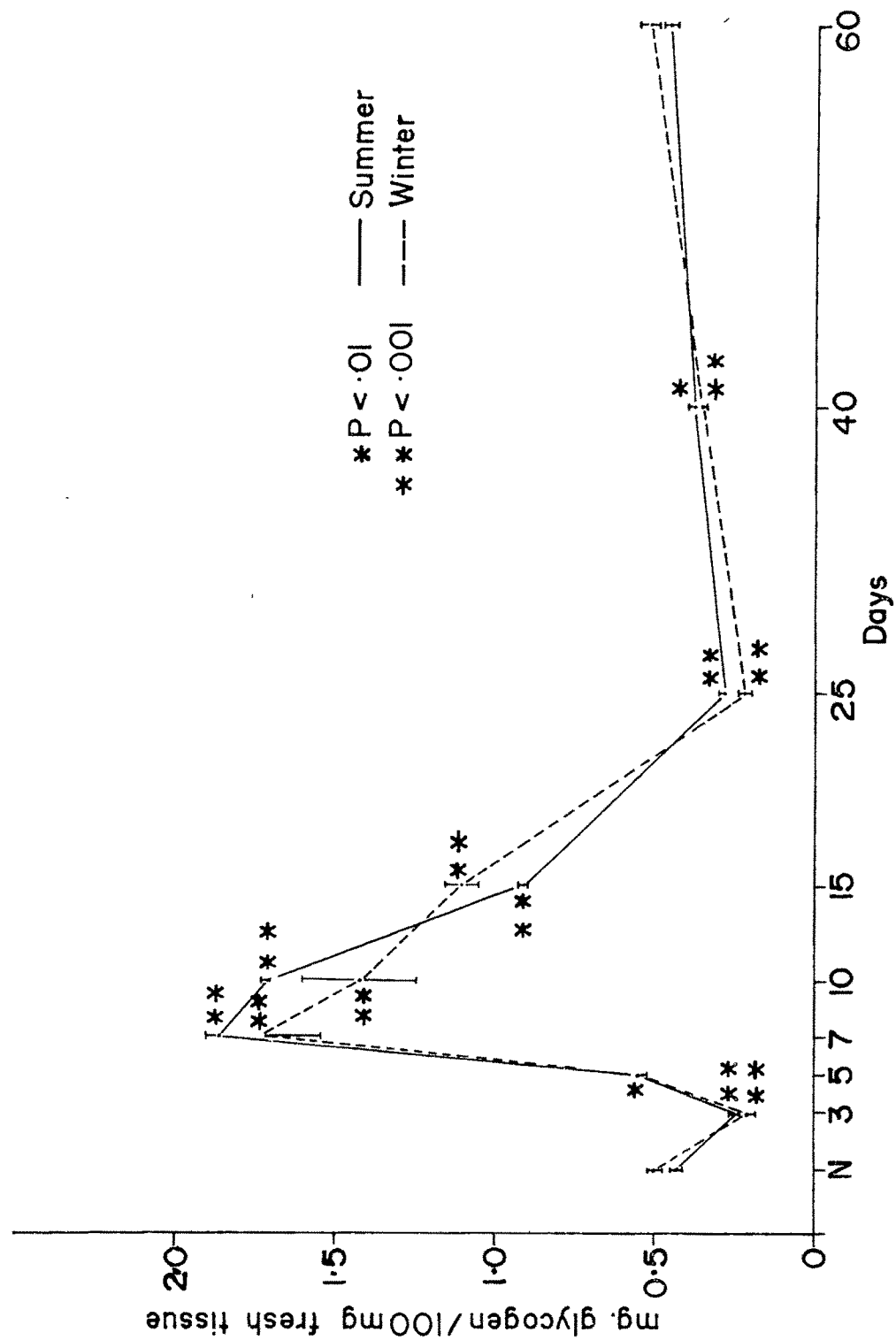


Fig. 2. Changes in hepatic glycogen content in summer and winter months during tail regeneration in H. flaviviridis

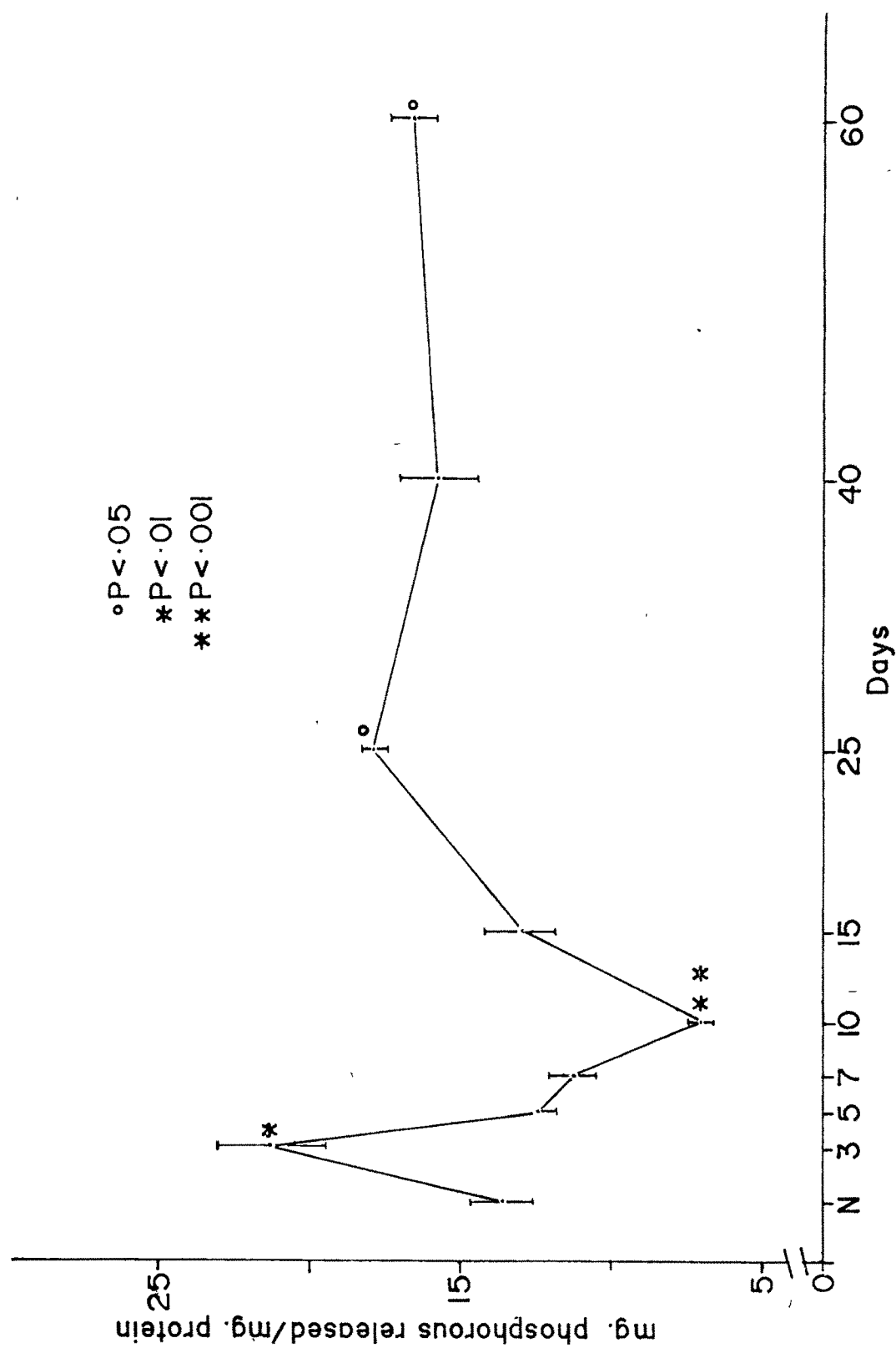


Fig. 3. Changes in hepatic phosphorylase activity during tail regeneration in H. flaviviridis.

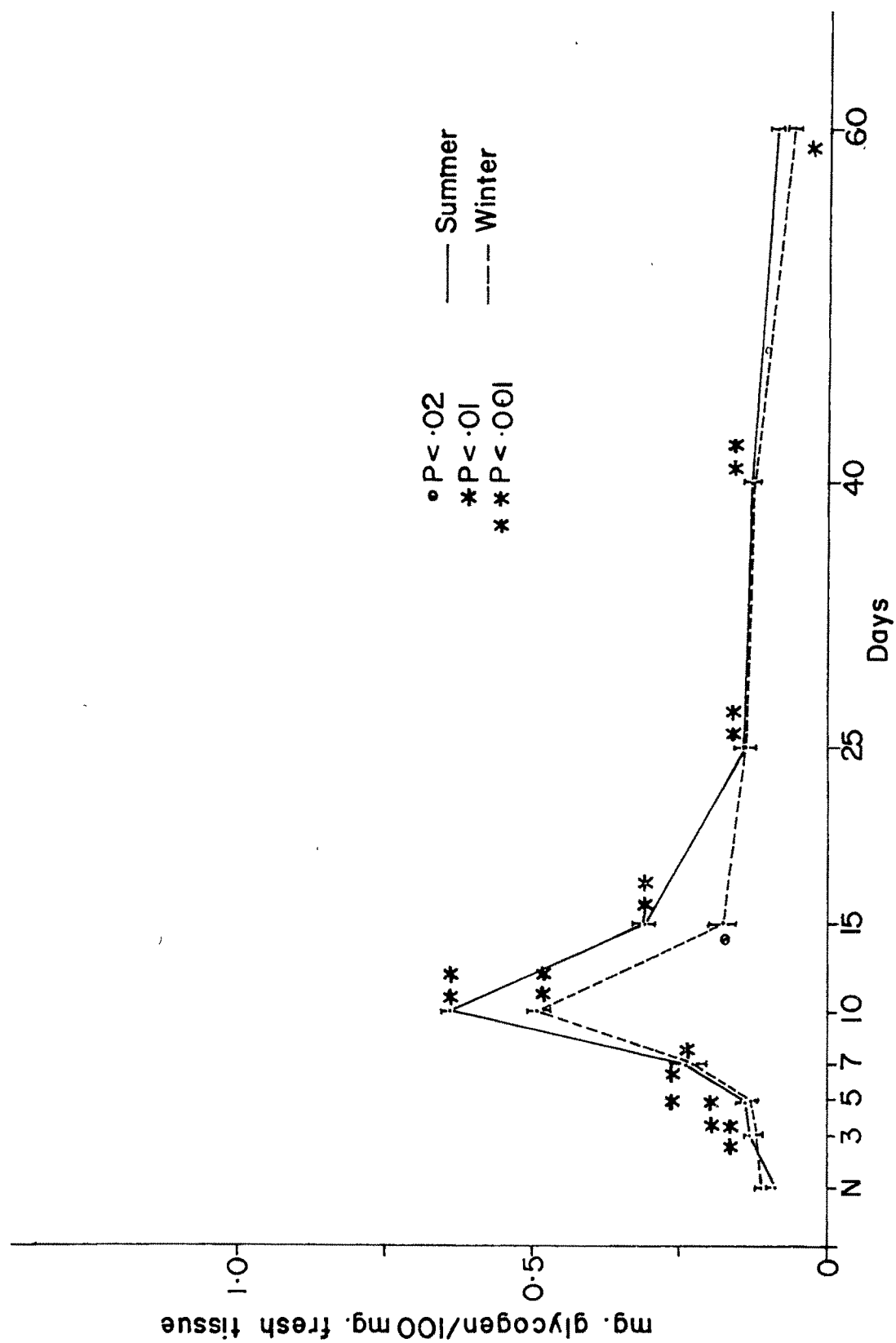


Fig. 4. Changes in muscle glycogen content in summer and winter months during tail regeneration in H. flaviviridis.

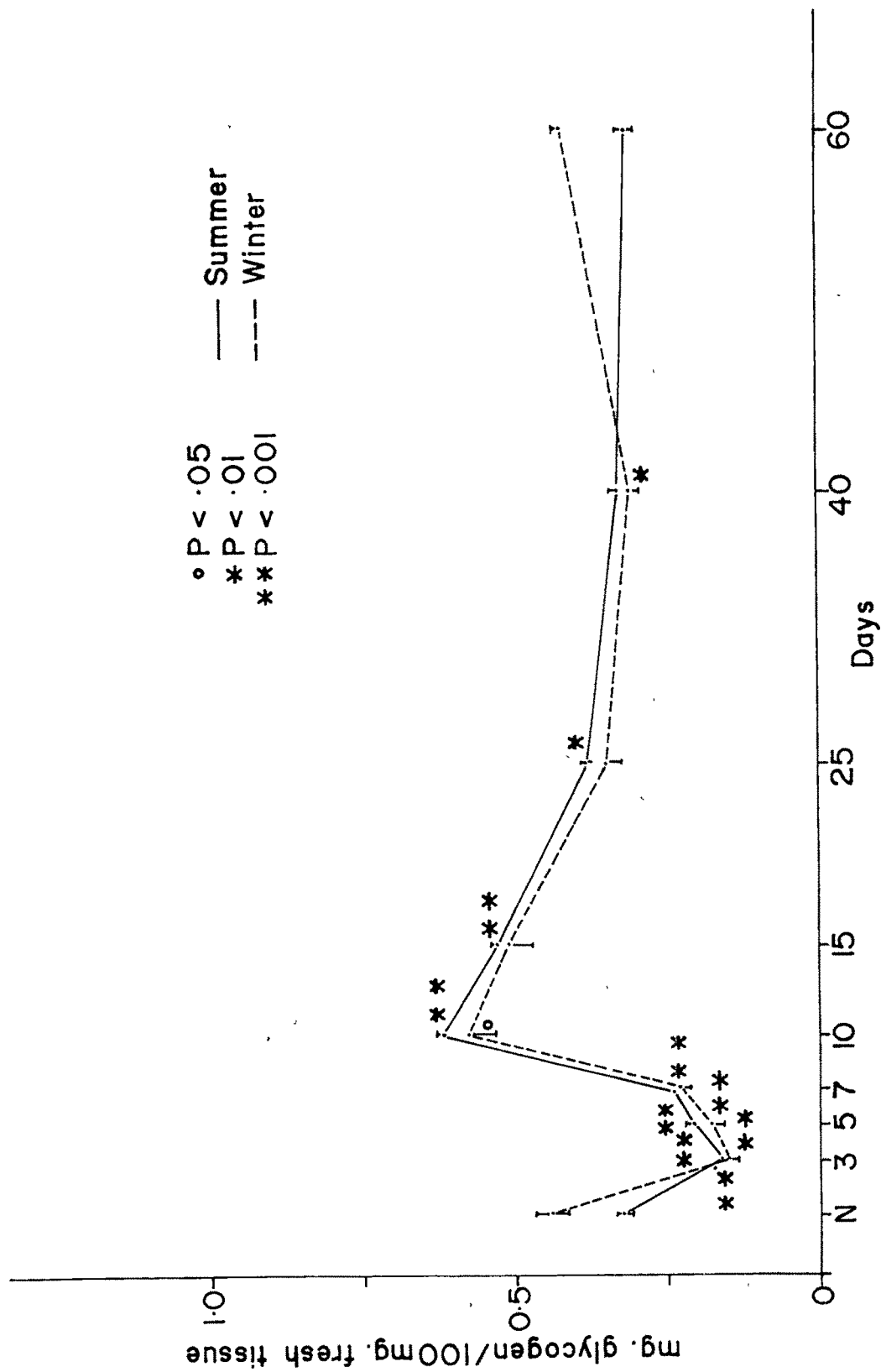


Fig. 5. Changes in caudal glycogen content in summer and winter months during tail regeneration in H. flaviviridis.

enzyme activity increased on the 3rd day, decreased to a normal level by 10th day and then again increased by the 25th day.

DISCUSSION

The results accrued from the present evaluation indicate in loco (tail) as well as systemic (liver) glycogen depletion immediately subsequent to caudal autotomy, corresponding to the wound healing phase. The dedifferentiative pre-blastemic phase (7th day) was marked by supra-normal hepatic glycogen deposition which was followed by a second phase of glycogen depletion lasting between 10th and 25th days. This biphasic hepatic glycogen depletion coupled with the initial caudal glycogen depletion is indicative of the increased energy demands associated with the process of regeneration both at the local site as well as in the lizard body as a whole, which is in keeping with the pronounced systemic participation highlighted by the earlier studies from this laboratory (Kinariwala et al., 1978; Ramachandran et al., 1979, 1980, 1982, 1985; Shah et al., 1980a,b, 1982a). Similar changes affecting the hepatic and caudal glycogen stores have also been shown to occur during regeneration in the Scincid lizard, Mabuya carinata (Shah et al., 1977b, 1982a). Apparently, Hemidactylus with an indoor habitat and nocturnal habits, and Mabuya with an outdoor habitat and diurnal habits respond identically to the stress of caudal autotomy and the ensuing process of regeneration

with reference to hepatic glycogen reserve. However, the changes shown by the muscle glycogen content and the glycaemic status of blood are of differential nature in the two lizards. Whereas *Mabuya* responded to the stress of autotomy by hyperglycaemia and muscle glycogen depletion (Shah et al., 1982a), *Hemidactylus* showed hypoglycaemia and increased muscle glycogen content. This differential response has been accredited to the different metabolic adaptations characteristic of the two lizards as has been previously discussed by Valsamma (1982).

The present study, principally undertaken to evaluate the seasonal influence if any on carbohydrate metabolism in relation to the differential regenerative growth rates in *H. flaviviridis* during summer and winter months (Magon, 1970), has apparently not revealed any variation. However, the percentage changes in tissue glycogen contents provide some interesting observations on a comparative basis between the summer and winter months. Interestingly, the initial hepatic and caudal glycogen depletions subsequent to caudal autotomy are greater during winter than during summer. But the muscle glycogen deposition that is characteristically exhibited during the first 10 days post-autotomy is significantly greater during summer than during winter. In this context, it is presumable that during the prevailing phase of glycogenesis (between the 5th and 10th days), also marked by a

hypoglycaemic state, more of the sugar taken up by the skeletal muscle is converted to glycogen during summer than during winter. This fact together with the observable greater glycogen depletion from liver and tail in the immediate post-autotomy period as well as the 45% subnormal muscle glycogen content at the end of the regenerative period during the winter months indicate more utilisation of glycogen reserves during the latter season in response to the regenerative stress. A calculation of the total amount of glycogen depleted from liver, tail and muscle in terms of the total length of tail regenerated during the two seasons reveals a utilisation rate of 0.07 mg % + during summer as opposed to 0.13 mg% during winter for 1 mm of tail regenerated. Hence it can be surmised that during the winter months *Hemidactylus* would require double the amount of glycogen reserve to bring about regenerative growth equal to that of summer. This could be accredited to the metabolic lethargy prevailing during winter leading to inefficient utilisation of the energy reserves of the body which may have a bearing on the near 50% less regenerative outgrowth occurring during this period.

SUMMARY

Seasonal changes in carbohydrate metabolism in relation to tail regeneration was evaluated during summer and winter seasons. Analysis of the two main carbohydrate stores viz., blood glucose and tissue glycogen was undertaken. Regeneration specific changes in these parameters appeared to be more or less of an identical nature in the two seasons. Changes of reciprocal nature were apparent with reference to hepatic glycogen content and phosphorylase activity. The whole period of regeneration was marked by a hypoglycaemic condition. The results show no difference in the pattern of carbohydrate metabolism induced by autotomy during the two seasons. However, the observable reduced regenerative outgrowth during the winter months is correlated with the seasonal difference in turnover of carbohydrate reserves.