CHAPTER II

LOCAL AND SYSTEMIC ALTERATIONS IN GLYCOGEN CONTENT AND PHOSPHORYLASE ACTIVITY DURING TAIL REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA

Most of the previous studies on vertebrate appendage regeneration have been essentially restricted to the local site itself. There was hardly any attempt made in understanding the possible metabolic and other alterations occurring in the body in response to the demands of regeneration. In this wake, studies were initiated in this laboratory for monitoring such changes, if any, mainly through histological, histochemical and physiological alterations. One such investigation showed involvement of hepatic glycogen and blood glucose during tail regeneration in Mabuya carinata (Shah et al., 1977b). Since reserve stores of carbohydrates serve as the primary source of energy in diverse biological 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, were deemed in the fitness of the context. Hence quantitative changes of glycogen content and phosphorylase activity of the two principal carbohydrate stores of the body i.e. liver and skeletal muscle were

undertaken. Though histochemical alterations in phosphorylase activity and glycogen content in the regenerating tail of Mabuya carinata during morphologically delineated arbitrary stages of tail regeneration are available (Radhakrishnan and Shah, 1973), to have a complete picture, especially with respect to the present study wherein precisely time bound phases of regeneration have been selected, studies on quantitative alterations in the glycogen content and phosphorylase (EC 2.4.1.1) activity in the regenerating tail were also undertaken.

MATERIALS AND METHODS

Healthy Mabuyas of both sexes weighing around 20-24 gms and obtained from Hyderabad, India were maintained under laboratory conditions, on a diet of insects. Autotomy was performed by pinching off the tail at about 1.5-2.0 cms distal to vent. The animals were sacrificed on fixed intervals of 3, 5, 7, 10, 12, 15, 25, 40 and 60 days post-autotomy along with normal animals with intact tails. Liver and skeletal muscle were removed quickly along with the regenerating tail as well as intact one, and homogenized in icecold redistilled water. A 2% homogenate was prepared for liver and skeletal muscle whereas a 4% homogenate was found satisfactory in the case of tail tissue. The crude homogenate was used for

assaying quantitatively the amount of glycogen, as per the method of Seifter et al. (1950). Phosphorylase (EC 2.4.1.1) activity was measured by the method of Cahil et al. (1957), and inorganic phosphate thus released due to the enzymatic action on the substrate (Glucose-1-phosphate; obtained from Sigma Chemicals, U.S.A.) 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 whereas the specific activity of the enzyme phosphorylase was expressed as μmoles of phosphate released/mg protein/min.

The protein content in all the three 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 of glycogen content and phosphorylase activity were made. The mean and standard deviation were obtained and students' 't' test was used to determine statistical significance.

RESULTS

The prevailing levels of glycogen content and phosphorylase activity in liver, skeletal muscle and tail of the normal animals with unautotomized intact tail are shown in Table 1 and Figs. 1-3. Following tail autotomy all the three

Table 1 : Quantitative levels of glycogen and phosphorylase in the regenerate, liver and skeletal muscle during tail regeneration of M. carinata.

(Values expressed as : Glycogen - mg/100 mg of tissue wt.

Phosphorylase - µg phosphorus released/mg protein)

Periods of tail regeneration in days	Tail		Liver		Muscle	
	Glycogen	Phosphorylase	Glycogen	Phosphorylase	Glycogen	Phosphorylase
N	0.061 ±0.008	10.48 ±1.47	2.44 ±0.18	8.32 ±0.54	0.88 ±0.09	8.17 ±1.61
3	0.039*@ ±0.003	7.57** ±0.95	1.51*@ ±0.13	15.98*@ ±1.43	0.52*@ ±0.04	12.38** ±1.21
5	0.105*@ ±0.009	5.84*@ ±0.64	0.77*@ ±0.08	19.62*@ ±1.39	0.32*@ ±0.02	20.77*@ ±1.67
7	0.171*@ ±0.015	3.74*@ ±0.79	2.09** ±0.13	8.12 ±0.56	0.25*@ ±0.03	31.48*@ ±2.51
10	0.220*@ ±0.015	4.58*@ ±0.44	2.03** ±0.16	11.68** ±1.47	0.23*@ ±0.01	28.42*@ ±3.89
12	0.271*@ ±0.025	9.94 ±1.17	1.55*@ ±0.16	15.69*@ ±1.59	0.37*@ ±0.03	36.34*@ ±4.94
15	0.314*@ ±0.030	13.87** ±1.55	1.30*@ ±0.09	14.41*@ ±1.39	0.36*@ ±0.02	35.72*@ ±3.22
25	0.265*@ ±0.019	18.81*@ ±1.64	2.38 ±0.24	8.63 ±0.82	0.44*@ ±0.04	31.48*@ ±2.52
40	0.171*@ ±0.016	9.59 ±0.95	2.76* ±0.23	5.42*@ ±0.56	0.54*@ ±0.06	21.5*@ ±1.45
60	0.104** ±0.019	8.97* ±0.93	2.56 ±0.21	8.63* ±0.93	0.64** ±0.05	18.73*@ ±1.97

* $P < 0.01$; ** $P < 0.005$; *@ $P < 0.0005$

N - Normal (Pre-autotomy state)

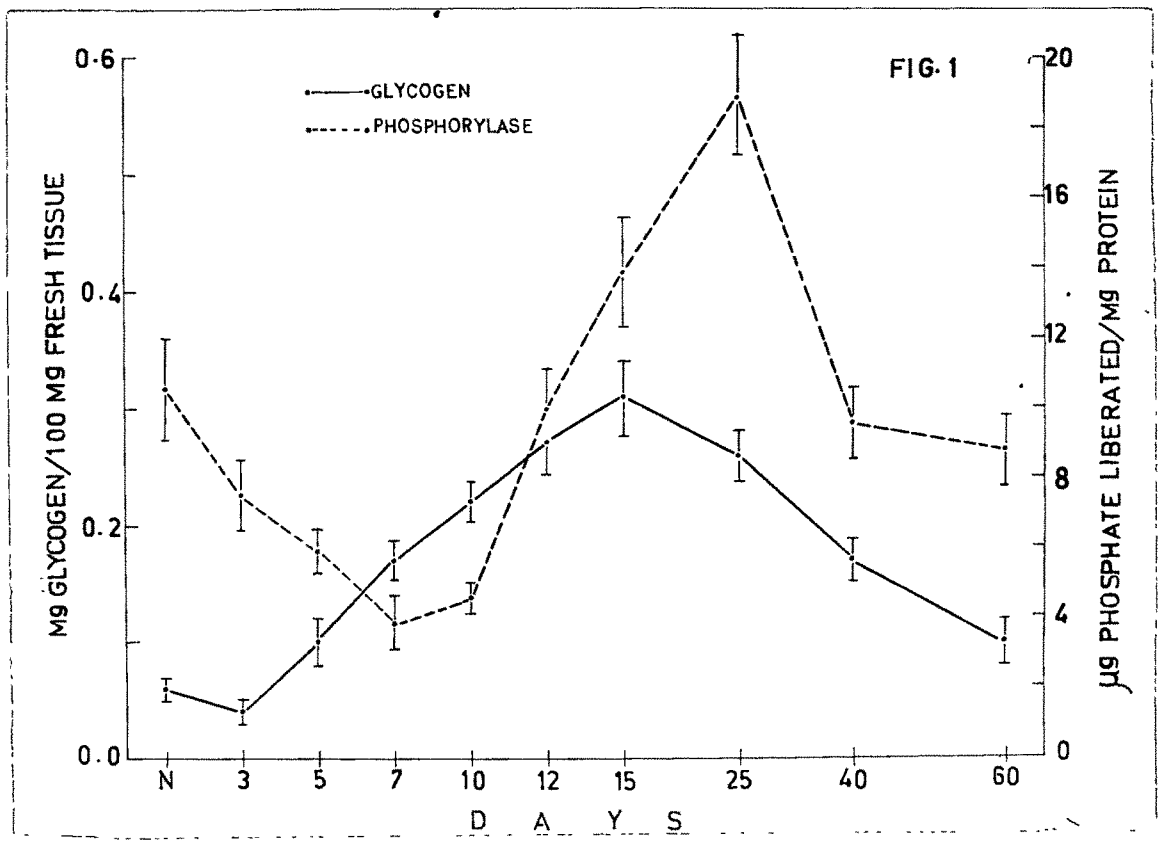

Fig. 1. Changes in glycogen content and phosphorylase activity in the regenerate during tail regeneration in M. carinata.

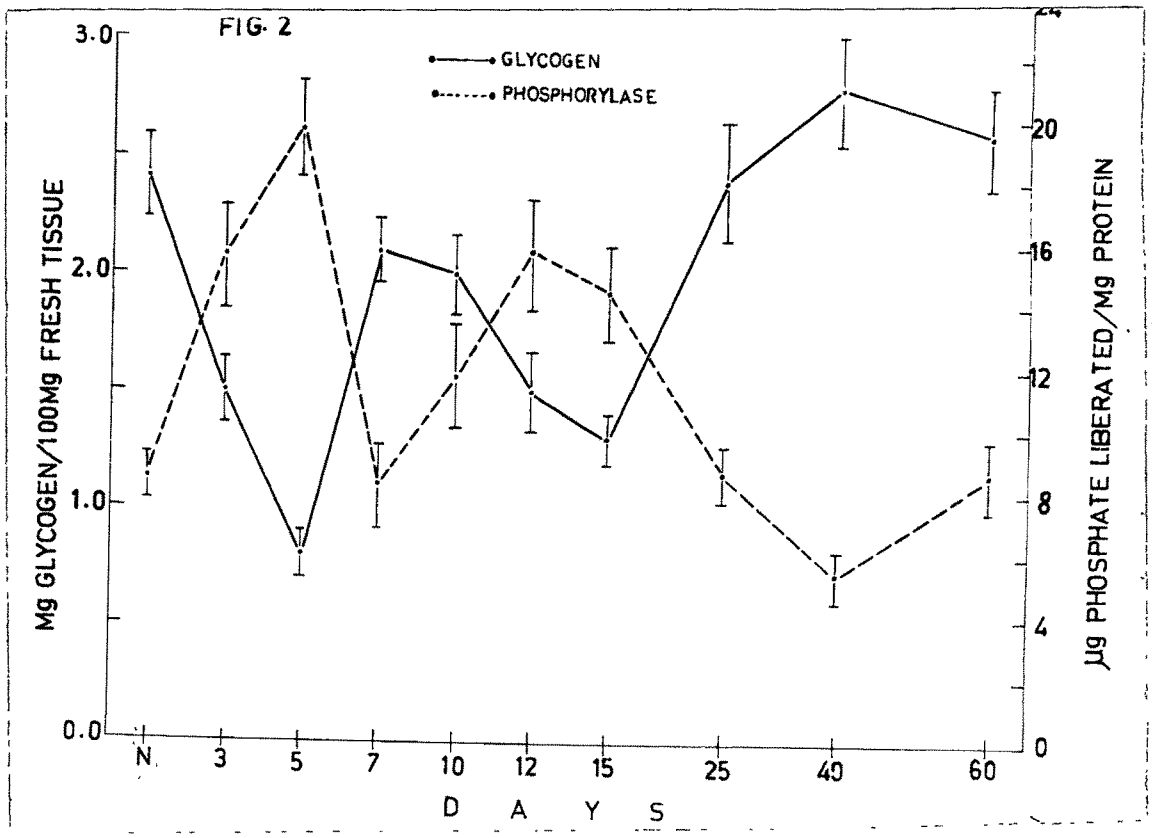

Fig. 2. Changes in glycogen content and phosphorylase activity in liver during tail regeneration in M. carinata.

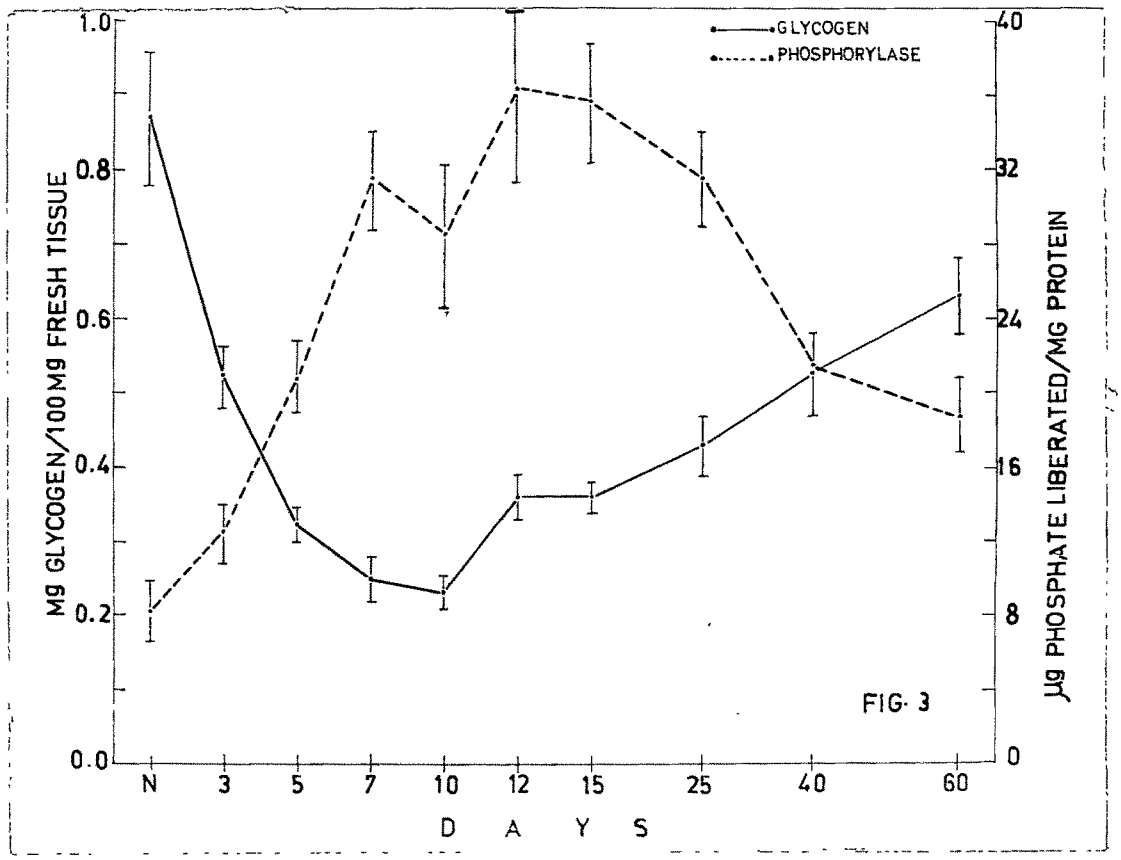

Fig. 3. Changes in glycogen content and phosphorylase activity in muscle during tail regeneration in M. carinata.

tissues (viz., liver, skeletal muscle and regenerate) depicted glycogen depletion by 3rd day post-autotomy. Since then the caudal glycogen content started increasing gradually through 5th, 7th, 10th and 12th days to reach the maximal level on the 15th day. Thereafter, the glycogen content of the regenerating tail started declining through 25th and 40th days to reach a near normal level on 60th day. At the same time the caudal phosphorylase activity showed a gradual decline continuously post-autotomy, till the lowest level of its activity was recorded on the 7th day. Thereafter, by gradual increment through 10th, 12th and 15th days the enzyme activity reached a peak level on the 25th day. By 40th day the enzyme activity regressed significantly to a more or less normal level and then to a slight subnormal level by 60th day.

Both liver and skeletal muscle showed pronounced glycogen depletion and increased phosphorylase activity in the period immediately after tail autotomy. Both these organs, more or less, depicted a 'mirror image' pattern of changes with respect to glycogen content and phosphorylase activity. Though the increase in the phosphorylase activity and decrease in glycogen content lasted initially for 5 days post-autotomy in the case of liver, muscle depicted continuous increase in phosphorylase activity and depletion in glycogen content till about 12th and 15th days, respectively. Since then there was a gradual decline in phosphorylase activity and increase in

glycogen content of the muscle during 15th, 25th and 40th days post-autotomy. On the 60th day the glycogen content was still subnormal and phosphorylase activity was still above normal. After showing an initial depletion in glycogen content and increase in phosphorylase activity in the first 5 days, the hepatic tissue depicted a sudden reverse trend between 5th and 7th days post-autotomy leading to near normal levels of glycogen content and phosphorylase activity. Subsequently there was a second phase of depletion in glycogen content and increase in phosphorylase activity during 10th, 12th and 15th days post-autotomy. Thereafter, the glycogen content of the liver increased to a slightly above normal level on 40th day with a near normal level on 25th day. Correspondingly the phosphorylase activity too fell down to subnormal level on 40th day from a near normal level on 25th day. Finally on day 60, both the levels returned to more or less the normal condition.

DISCUSSION

The results obtained in the present study indicate the irrefutable involvement of systemic carbohydrate source in meeting the exigeneies of caudal reparative regeneration in Mabuya carinata. Though the initial depletion of glycogen shown by all the three tissues immediately subsequent to tail autotomy on the 3rd day may be looked upon as a stress

induced response, the persisting depletion of the metabolite observed in the liver and skeletal muscle even after the 3rd day in the wake of the reversed trend in the caudal tissue signifies a definite adaptive response. The depleting glycogen content of the liver and skeletal muscle is well paralleled by the observed increase of phosphorylase activity. The previously observed ∝-cell degranulation in the pancreatic islets of M. carinata in the first fortnight of tail regeneration (Ramachandran et al., 1980 a) is very relevant in the present context as glucagon is known to activate hepatic phosphorylase. Since glucagon is known to be incapable of activating muscle phosphorylase (Cohen, 1976), and as muscle phosphorylase is noted to show an increased activity subsequent to tail autotomy, adrenaline that could be predictably expected to be released due to the stress of autotomy may be considered to be of significance in inducing muscle glycogenolysis (Cohen, 1976). The elevated level of cAMP as denoted by the inhibition of phosphodiesterase activity in the skeletal muscle in the first week of tail regeneration (Chapter VI) may bear testemony to the present observations. The importance of hepatic glycogen in meeting the autotomy induced stress as well as events associated with wound healing becomes evident by the observed 68% depletion of glycogen from this organ which is paralleled by the 138% increase in phosphorylase activity by 5th day of caudal autotomy. Dependence of the healing tissues of the tail

on a systemic source of carbohydrate becomes clear when viewed in the light of decreasing levels of phosphorylase activity in the tail stump during first 7 days of post-autotomy. The interesting paradoxical feature of increasing glycogen content in the tail stump from the 3rd day post-autotomy onwards concurrent to decreasing phosphorylase activity point towards a gradual deposition of glycogen in the stump tissues for its ready utilization by the regenerate after the establishment of a blastema. Attainment of maximum supranormal level of glycogen content on the 15th day and that of phosphorylase only by 25th day are indicative of the insufficiency, inadequacy and inability of the local store of glycogen to participate in the inherent energetics of the healing mechanics as well as the blastemal cell metabolism. However, the changes cited above do indicate the active participation of caudal carbohydrate store in the progressive phases of regeneration commencing from the time of early differentiation. Convincing evidences in favour of this come from the reported paucity of glycogen and low level of phosphorylase activity in the newly developing tissues of the tail regenerate of both M. carinata and H. flaviviridis upto about early differentiation (Radhakrishnan and Shah, 1973; Shah and Chakko, 1967; Shah and Hiradhar, 1974a). Further, based on one of the previous studies, Shah et al. (1977) had suggested the dependence of the process of wound healing as well as the

blastemal cells on blood glucose (contributed by hepatic glycogenolysis) for meeting their metabolic requirements. A very interesting observation of the present study is the sudden and swift replenishment of the hepatic glycogen store from the lowest level on the 5th day to a near normal level on the 7th day with concomitant decrease in phosphorylase activity. Worth recalling here in this context is the identical observations of Shah et al. (1977b) in M. carinata itself. The specificity of the time factor in the two studies as well as the repeatability of the observations suggest a definite and precisely synchronized and highly adaptive metabolic alteration tilted towards hepatic glycogenesis and/or gluconeogenesis. Both the availability of necessary precursor moieties, as well as existance of the regulatory factor which promotes glycogenesis/gluconeogenesis at this specific period of tail regeneration in M. carinata seem easily surmisable. For instance, the suggested involvement of glucagon in the metabolic intricacies underlying the process of tail regeneration in the first fortnight (Shah et al., 1977b; Ramachandran et al., 1980 a) as well as the reported role of glucagon in gluconeoganesis (Exton, 1960; Huibregtsea, 1977; Cook et al., 1979) account for the latter requirement. At the same time as reported earlier by Shah et al. (1977b) the increased accumulation of lactic acid and protein catabolite products at the wound site during wound healing and dedifferentiation seem to

meet the former requirement. Further, the currently observed muscle glycogen depletion in the light of the known inability of muscle glycogen to serve as a direct source of blood glucose (Cohen, 1976) and the increased anaerobic activity associated with skeletal muscle during this period of tail regeneration (Chapter IV) seem to serve as an efficient mechinery for generating and supplying the necessary lactate and pyruvate molecules which could be efficiently utilized for hepatic glycogenesis and gluconeogenesis.

A second phase of steady depletion of hepatic glycogen during 10th, 12th and 15th days post-autotomy together with the depletion of glycogen content from the caudal tissues from the 12th day of tail regeneration onwards with corresponding increasing activities of phosphorylase in both these tissues are indicative of increased energy demands associated with the process of histodifferentiation and growth occurring at the local site of regeneration and probably other metabolic requirements of the body as a whole. The utility value of glycogen at the local site at this phase could be partly to serve as an energy source by undergoing oxidation and partly for the synthesis and store of lipids as submuscular adipose tissue in the differentiated region of the tail regenerate. Systemic requirements of this metabolite could also be to serve as an efficient energy source not only for the active hepatocytes which are involved in many biochemical alterations

as well as other visceral organs which are also engaged in meeting the challenges of the regenerative process (Shah et al., 1980 a, 1980 b; Ramachandran et al., 1981) and/or even in the replenishment of the depleted stores of lipids from the fat body (Ramachandran et al., 1980 b). Munro et al. (1961) have shown loss of liver glycogen after administration of proteins or amino acids in rats which they have correlated with the extra energy expended during absorption and assimilation of the amino acids. In this light the loss of liver glycogen noted in the present study during 12th and 15th days of tail regeneration which is well paralleled by a protein anabolic influence affecting the regenerate, serum, muscle and liver (Chapter V) could be very tentative and rather tempting to be drawn towards a similar conclusion. Excepting for the tail glycogen which showed continuous depletion all throughout the progressive phases of regeneration right from the 12th day uptill the 60th day, the glycogen stores of liver and muscle are noted to show an increasing trend by about late differentiation phase (25th day) and early differentiation phase (12th day), respectively. This might indicate the emancipation of muscle glycogen from the demands placed upon it by the regenerative process as early as early differentiation phase itself. Apparently the muscle glycogen could be considered to play important supplementary and supportive role albeit an indirect one by probably contributing towards hepatic glycogenesis and well as generalised protein and

amino acid synthesis (Chapter V) during the first fortnight of tail regeneration. Similarly the hepatic tissue also appears to be relieved of its commitments by 25th day as denoted by the replenishment of the glycogen store. Increased glycogenesis seems to occur in liver and muscle from about 15th and 10th days of tail regeneration, respectively; and the declining levels of phosphorylase activity in these two tissues from about 12th day post-autotomy along with that of the tail from 25th day onwards. are all evidences in favour of a common regulatory influence in carbohydrate metabolism. In this connection the reported degranulation changes of the β cells of pancreas between 12th and 25th days of tail regeneration in Mabuya (Ramachandran et al., 1980 a) may indicate the release of insulin and its anabolic influence on carbohydrates by its positive and negative influences on glycogen synthetase and protein kinase respectively (Walkemback et al., 1980). Finally, it may be presumed from the present observations that the systemic carbohydrate metabolism does play a pivotal role in meeting the exigencies of tail regeneration in Mabuya carinata.

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