CHAPTER 1

COMPARATIVE EVALUATION OF IN LOCO AND SYSTEMIC CARBOHYDRATE METABOLISM DURING CAUDAL AUTOTOMY AND FORE-LIMB AMPUTATION IN HEMIDACTYLUS FLAVIVIRIDIS

The carbohydrates of the body represent the chief metabolite sources which serve as fuels to be oxidised to provide energy for the many endergonic metabolic processes. Special importance is given to the role of polysaccharides, in such regeneration affiliated fields, as wound healing (Jackson, 1958), induction (Grobstein, 1955) and growth (Harris, 1958) among others. Previous studies on amphibian limb regeneration (Schmidt, 1960, 1962; Procaccini et al., 1973; Connelly et al., 1974) and on reptilian tail regeneration (Shah and Hiradhar, 1974; Shah and Chakko, 1967b;Radhakrishnan and Shah, 1973; 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 to regenerative activities in the form of glycogenolysis and altered glycemic conditions. To prove that these changes occurred due to regenerative process only, a need for comparable studies during non-regenerative wound healing in the same animal is logical. Information on these aspects in relation to wound healing in animals in general and reptiles in particular, is inadequate. Vertebrates are known to respond to stress by catabolism of food reserves for energy production (Seyle and Stone, 1950), changes in liver glycogen content and lactate dehydrogenase activity during wound healing and repair of pigeon liver (Asnani et al., 1972) and changes in glycogen content during normal and diabetic wound healing in liver and skin of rat (Kishnani, 1976; Kathuria, 1976) are some of the relevant reports in this context. Apart from the above studies, no other relevant information is available on this topic. Kinariwala (1979) concluded that the non-regenerative wound healing of Calotes tail takes place in a low tone metabolic activity involving less metabolic intricacies and adaptations. The above mentioned report was a comparative account of the systemic responses during tail regeneration in Mabuya carinata and tail wound healing in Calotes versicolar. Though in the above study, a non-regenerating lizard was taken as a control to the regenerating type, it would be appropriate and more valid to study the responses during regeneration, of a part endowed with regenerative potential as opposed to that during wound healing of another part with no regenerative potential in the same animal. It was in this context that comparative studies on carbohydrate metabolism during post-caudal autotomy (regeneration) and post-limb amputation (non-regenerating) have been carried out in the Gekkonid lizard, *Hemidactylus flaviviridis*.

MATERIALS AND METHODS

The house lizards, *H. flaviviridis*, collected from Baroda were kept on a diet of insects in the laboratory for a fortnight to get them acclimatized to the laboratory conditions. The adult animals were divided into two groups, group I - animals in this group were used to study the changes during tail regeneration. The tails of these animals were autotomized two to three segments away from the vent. Group II - animals in this group were used to study the changes during non-regenerative limb wound healing. One of the fore-limbs was amputated with a sharp blade from the distal end of the humerus (Stinson, 1964).

The animals were later sacrifised at regular time intervals of 1,2,3,5,7,10,20,25,40 and 60th days post-autotomy/amputation. Estimation of glycogen was carried out in liver, muscle, tail and limb tissues as per the method of Seifter *et al.*(1950) and that of blood glucose by the method of Folin and Malmros (1929).

RESULTS

The sub-normal levels of blood glucose which were noticed in both the cases throughout, showed a more or less similar pattern with slight change in the peak level. The tail regenerating animals showed two peak levels, one on the 2nd day and the other on 10th day. The limb amputated lizards showed only one peak, that on the 10th day. The hepatic glycogen content of the tail regenerating lizards showed a sub-normal level on 3rd day and an above normal level from 5th to 20th days with the maximum peak level on the 7th day. Glycogen content of the liver of the limb amputated lizards showed an above normal level from the 1st day till the 10th day with a peak on the 5th day and another on the 25th day. Muscle glycogen of the lizards with regenerating tail showed one peak on the 10th day, while the limb amputated lizards showed two peaks, one smaller one on the 7th, and a larger one on the 20th day. In both the cases, the level remained above normal.

Glycogen content of the tail regenerate showed sub-normal levels on the 3rd, 5th and 7th days with the maximum peak value on the 10th day. Tail glycogen of the limb amputated lizards remained more or less in the normal range till the 7th day, thereonwards showed an increase with the peak value being attained on the 25th day. Limb glycogen of the tail regenerating lizards showed two peak levels, one smaller one on the 10th day and a higher one on the 25th day. Limb glycogen of the limb amputated lizards

Table 1. Comparative levels of blood glucose (mg/100 ml blood) post-limb amputation and post-caudal autotomy in *H. flaviviridis*.

Periods of regeneration in days	N	1	2	3	5	7	10	20	25	40	60
Post-caudal autotomy	114.00 ±8.28	68.36 ±10.33 0.0005*	96.978 ±5.95	71.17 ±10.44	67.89 ±11.35 0.0005*	65.23 ±8.65	97.46 ±5.82	93.51 ±10.58	78.00 ±8.32	81.001 ±12.35	98.00 ±4.88
Post-limb amputation	114.00 ±8.28	92.00 ±5.437 0.0005*	92.00 ±12.36	79.55 ±8.54	81.00 ±4.87 0.001*	76.00 ±11.24	91.5 ±13.05	90.5 ±9.66	89.00 ±7.37	83.00 ±5.65	75.00 ±10.33

± S. D.

* P value

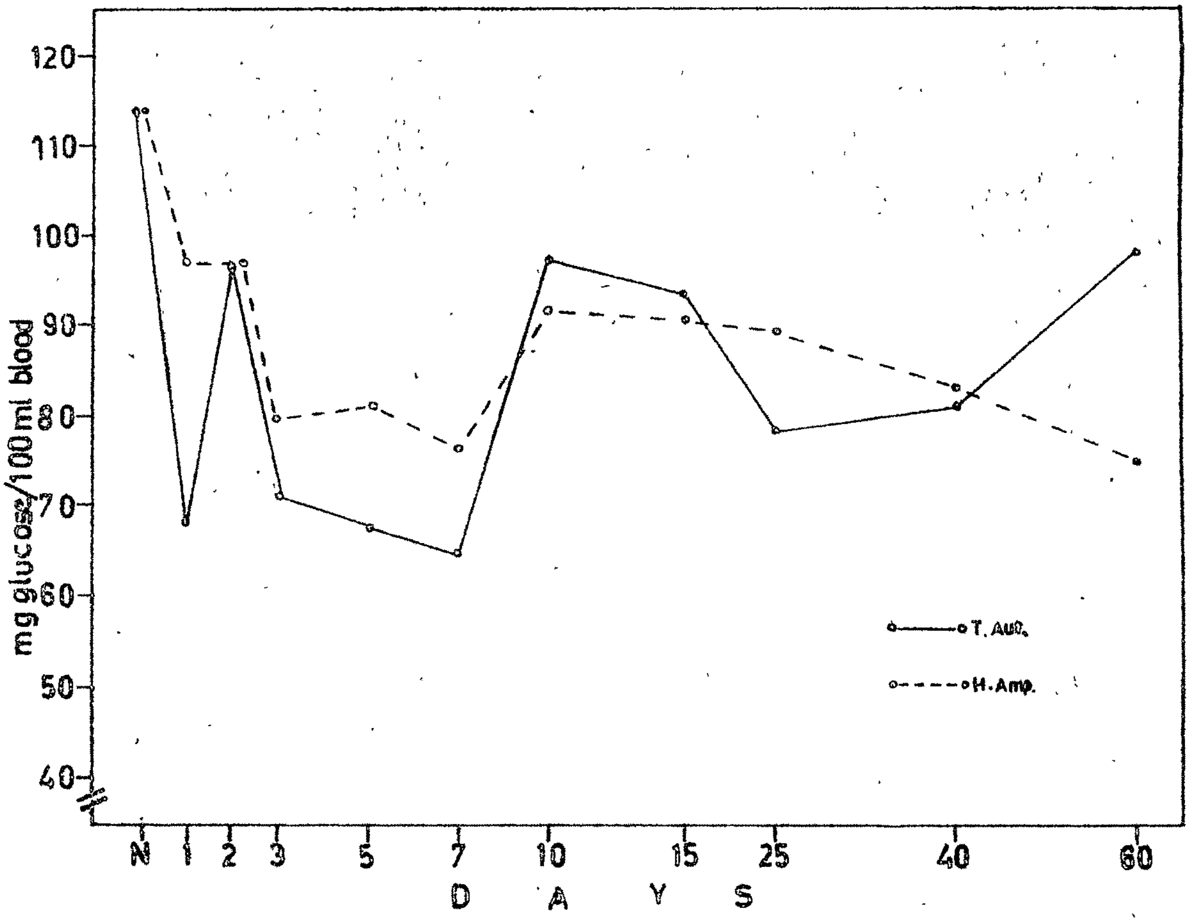

Fig. 1 : Graphic representation of the levels of blood glucose in the limb amputated and tail autotomised lizards, *H. flaviviridis*

Table 2. Alterations in the levels of tissue glycogen content (mg/100 mg fresh tissue) during tail regeneration in *H. flaviviridis*.

Periods of regeneration in days	N	1	2	3	5	7	10	20	25	40	60
Liver	0.4556 ±0.104	0.454 ±0.122	0.4676 ±0.048	0.2033 ±0.013	0.5263 ±0.172	1.828 ±0.43 0.01*	1.735 ±0.72	0.908 ±0.47	0.288 ±0.005	0.360 ±0.031	0.467 ±0.145
Muscle	0.0922 ±0.0175	0.154 ±0.007	0.0945 ±0.0053	0.1229 ±0.013	0.1457 ±0.047	0.2437 ±0.07	0.640 ±0.053 0.001*	0.283 ±0.013	0.133 ±0.0067	0.120 ±0.004	0.868 ±0.215
Tail	0.3066 ±0.084	0.4217 ±0.147	0.384 ±0.21	0.15736 ±0.046	0.2012 ±0.006	0.2338 ±0.01	0.624 ±0.024	0.53 ±0.13	0.38 ±0.056	0.332 ±0.009	0.318 ±0.017
Limb	0.1607 ±0.008	0.1914 ±0.0078	0.0948 ±0.0069	0.0987 ±0.0057	0.214 ±0.0013	0.177 ±0.012	0.502 ±0.10 0.001*	0.408 ±0.14	0.638 ±0.23	0.210 ±0.11	0.183 ±0.04

± S. D.

* P value

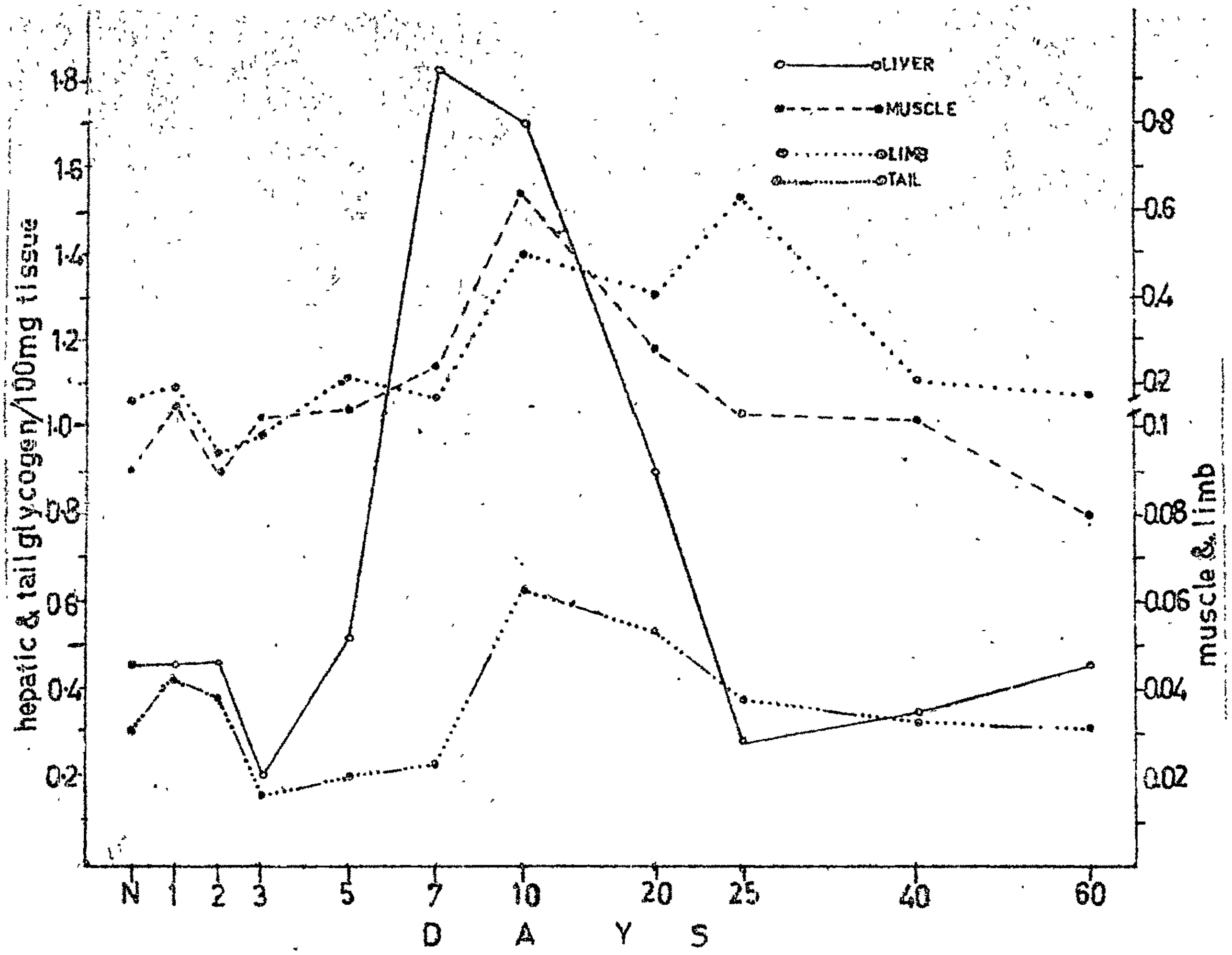

Fig. 2 : Graphic representation of the levels of glycogen in the liver, muscle, regenerate and limb during tail regeneration in the lizard, H. *flaviviridis*.

Table 3. Alterations in the levels of tissue glycogen content (mg/100 mg) post-limb amputation in *H. flaviviridis*.

Periods of regeneration in days	N	1	2	3	5	7	10	20	25	40	60
Liver	0.4556 ±0.003	3.446 ±0.347	3.5139 ±0.425	3.629 ±0.92	3.7336 ±0.174	2.9107 ±0.185 0.001*	2.870 ±0.521	1.572 ±0.007 0.0005*	3.070 ±0.0824	0.462 ±0.021	0.443 ±0.102
Muscle	0.0922 ±0.010	0.235 ±0.053 0.001*	0.114 ±0.0112	0.0961 ±0.007	0.0923 ±0.002	0.3214 ±0.124 0.0005*	0.1813 ±0.005	0.777 ±0.052	0.4003 ±0.017	0.1586 ±0.014	0.124 ±0.001
Tail	0.3066 ±0.03	0.1805 ±0.0715 0.0005*	0.204 ±0.004 0.001*	0.2741 ±0.013	0.1774 ±0.005	0.1932 ±0.053	0.94 ±0.001	0.955 ±0.014	1.229 ±0.024	0.714 ±0.0062	0.521 ±0.034
Limb	0.1607 ±0.0412	0.2491 ±0.018 0.0005*	0.07218 ±0.019	0.1624 ±0.015	0.1589 ±0.021	0.385 ±0.045	0.1216 ±0.010	0.408 ±0.016	0.586 ±0.013	0.433 ±0.019	0.328 ±0.008

± S. D.

* P value

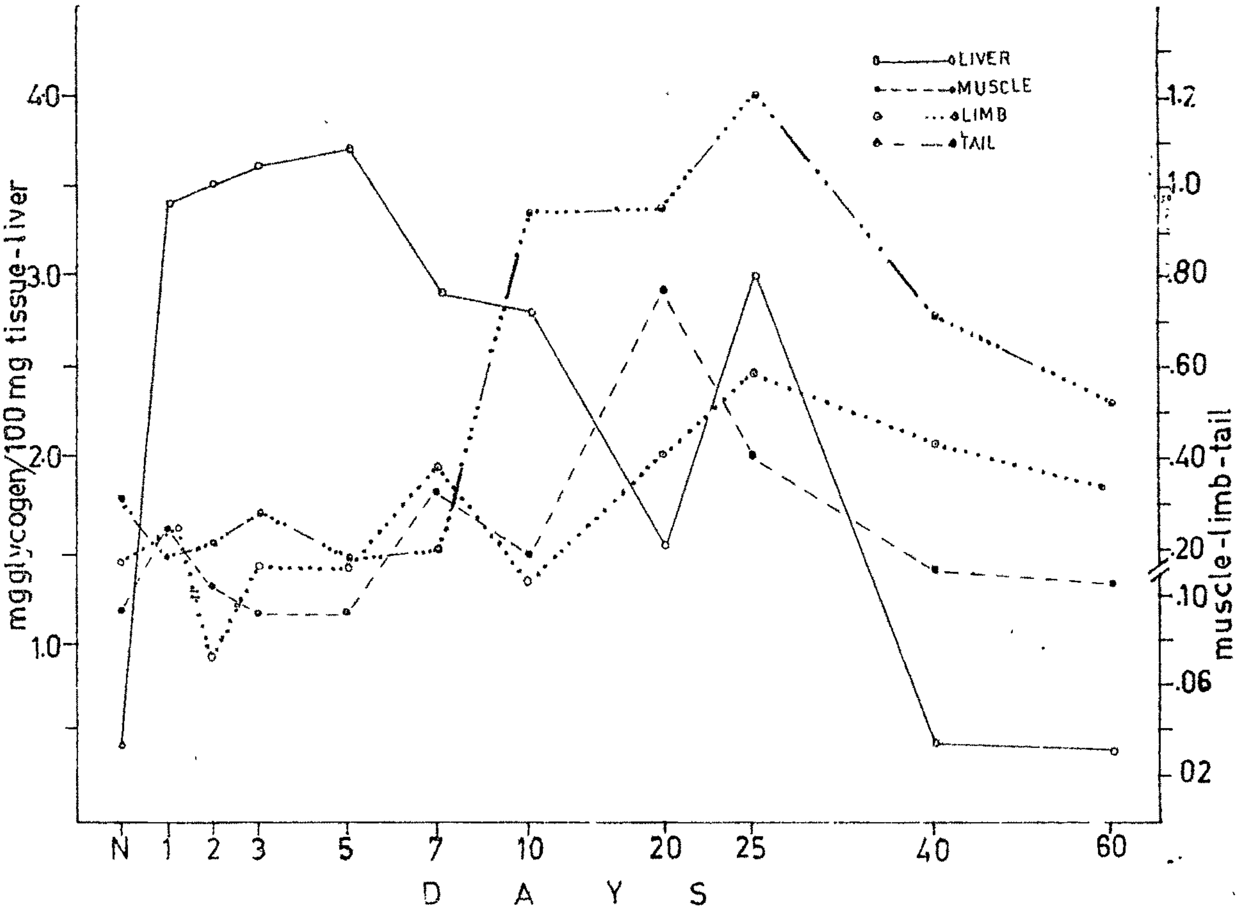

Fig. 3 : Graphic representation of the levels of tissue glycogen in the liver, muscle, tail and limb stump post-limb amputation in *H. flaviviridis*.

showed a sudden fall on the 2nd day and like wise in the above case two peaks were recorded one on 7th and the other on the 25th day.

DISCUSSION

Extensive studies carried out on carbohydrate metabolism in relation to tail regeneration in *M. carinata* have revealed depletion of caudal glycogen stores during wound healing and its build up and utilization during the post-blastemic and differentiation phases (Radhakrishnan and Shah, 1973), and significant participation of hepatic and muscle glycogen stores together with blood glucose as part of the collective systemic response (Shah *et al*., 1977, 1982). Present results obtained also show similar changes *in loco* and systemically, thus suggesting similarity in carbohydrate metabolism in response to regeneration in *Hemidactylus flaviviridis* as well. However, changes in blood glucose and muscle glycogen appear to be of a different pattern in *H. flaviviridis* as compared to those of *M. carinata*. Unlike in Mabuya which depicted a pronounced hyperglycemic condition during blastemic and early differentiation periods (Shah *et al*., 1977; 1982), and significant depletion of muscle glycogen during the first fortnight of regeneration (Shah *et al*., 1982). Hemidactylus, in the present study, depicted neither hyperglycemia nor muscle glycogen depletion at any stage of regeneration. In fact, muscle glycogen has shown an increase during the first fortnight. It is likely that these differences are mainly due to the different metabolic strategy adopted by these two lizards. In this respect, muscle glycogen may be considered to play a negligible role in the regenerative mechanics in Hemidactylus and may bear some correlation with the observed protein depletion from systemic sources (Chapter 2), a feature not shown by Mabuya. Whereas the lowered blood glucose levels during wound healing to blastema and during differentiation indicate the ready withdrawal of blood sugar for meeting the requirements of regeneration, the inability to attain a hyperglycemic level may be due to the insulin dependent carbohydrate metabolism characteristic of this lizard unlike Mabuya which is known to be glucagon oriented (Shah et al., 1977; 1982; Ramachandran et al., 1981).

A comparison of the events post-caudal autotomy and post-limb amputation indicate a similar pattern of changes in systemic glycogen stores (liver and muscle) and glycemic levels; however, the in loco response appears to be different, with limb amputation failing to show the adaptive alterations in glycogen content characteristic of tail autotomy. Apparently, both limb amputation (which fails to regenerate) and tail autotomy (which undergoes regeneration) elicit similar systemic carbohydrate responses, with local responses being of a dissimilar type. The in loco response in the case of limb is more or less comparable with the responses of the muscle and tail post-limb amputation and only with that of muscle post-caudal autotomy. Evidently, the limb response remains identical in either experimental condition in the form of increasing glycogen content (Fig. 2 and 3).

It may be concluded from the present study, that in an animal with a restricted regenerative potential, loss of any body extremity can elicit similar systemic response, but the capacity to regenerate solely depends on the ability of the local site to bring forth adaptive alterations and also to respond to the systemic factors.