CHAPTER - 11

HISTOENZYMOLOGY OF THE REGENERATING LACERTILIAN TAIL: 3. ATP: D-HEXOSE PHOSPHOTRANSFERASE AND ATP: D-GLUCOSE-6-PHOSPHOTRANSFERASE (HEXO AND GLUCOKINASES; E.C. 2.7.1.1. AND E.C. 2.7. 1.2.) ACTIVITY DURING REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA

Hexo and glucokinases, the two soluble enzymes catalyse the transfer of the terminal phosphate group of ATP to a D-hexose, with the liberation of one hydrogen ion. Glucokinase can only catalyse the phosphorylation of glucose, while, hexokinase can act on several different sugars (Walker, 1963; Walker and Rao, 1964). The phosphorylation of glucose is a necessary first step in both glycogenesis from glucose, as wellas the catabolic breakdown of glucose. Studies on the distribution of glycogen and phosphorylase (Radhakrishnan and Shah, 1973), and enzymes of both glycolytic pathway as well as TCA

cycle (Shah and Ramachandran, 1970, 1972, 1973, 1975, 1976) during tail regeneration in Mabuya carinata have suggested that the regenerate during the blastemic and differentiation phases might be dependent on blood glucose for their metabolic activities, and that gluconeogenesis and glycogenesis take place with the progression in differentiation. This aspect of carbohydrate metabolism could well be substantiated by an investigation of the presence and localization of the enzymes hexo and glucokinases during tail regeneration. Since the nonspecific hexokinase can phosphorylate any of the monosaccharides, an attempt to histochemically demonstrate the specific glucokinase would also lead to the demonstration of hexokinase and hence, the two enzymes cannot be histochemically demarcated. The present investigation undertaken principally to validate the intricacies of carbohydrate metabolism during regeneration thus could be construed to demonstrate the presence of both the enzymes in the regenerating system.

MATERIAL AND METHODS

The adult Mabuyas selected for the experiment

were maintained on a diet of insects in the laboratory. The autotomy of the tails was carried out as described in previous chapters. The normal and various stages of the regenerating tail after autotomy were blotted to remove blood and other tissue fluids and were immediately fixed on a chuck of a cryostat microtome maintained at -20°C. Longitudinal and trnasverse sections of 12-18 μ thickness were cut and incubated at room temperature for about 2 hours in the incubation medium prepared after Meijer (1967a) and described by pearse (1972). A few sections treated with water at 80°C before incubation and a few others incubated in a substrate blank medium served as the controls.

OBSERVATIONS

NORMAL TAIL

The highest concentration of these enzymes, in the normal tail was found to be localized in the nerve cord followed by the muscles and stratum germinativum respectively. Though the localization of the enzymes was noted to be chiefly sarcoplasmic,

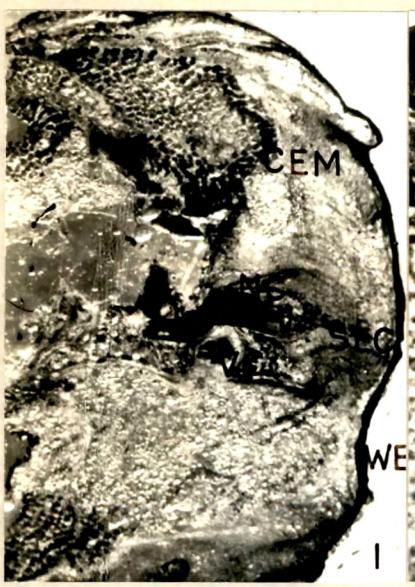
EXPLANATION TO FIGURES

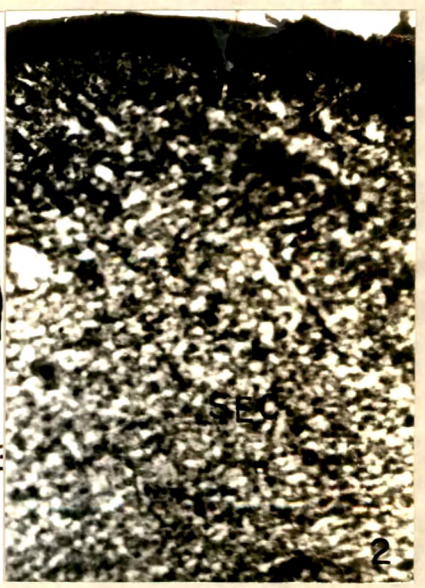
Fig. 1. Photomicrograph of L.S. of wound healing tail denoting hexokinase activity. CEM - Cut end of muscles; NC - Nerve cord; SEC - Subepithelial cells; VC - Vertebral column; WE - Wound epithelium.

Fig. 2. Wound epithelium (WE) with the underlying sub-epithelial cells (SEC) depicting high hexokinase activity. 50 X.

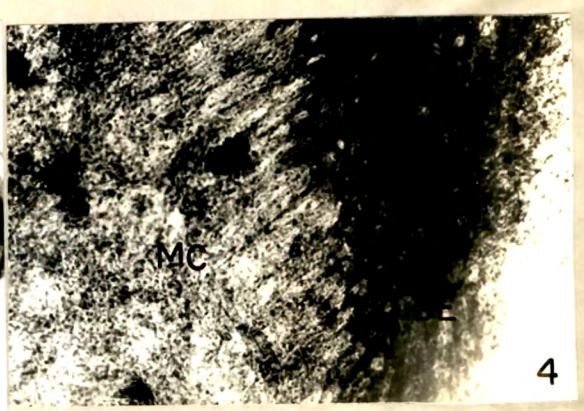
Fig. 3. L.S. of blastema showing hexokinase activity. BE - Blastemic epithelium; MC - Mesenchymal cells; NC - Nerve cord; VC - Vertebral column.

Fig. 4. Blastemic epithelium (BE) together with the mesenchymal cells (MC) showing enhanced enzyme activity. 125 X.


CEM
WE
1


SE
2


BE
MC
VC
3

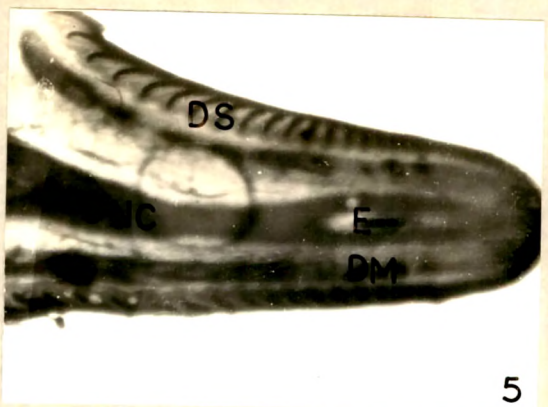

MC
4

EXPLANATION TO FIGURES

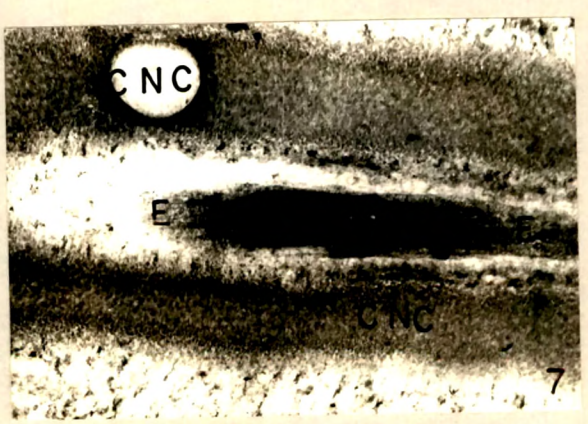
Fig. 5. L.S. of differentiating tail showing hexokinase activity. CNC - Cartilagenous neural canal; DM - Differentiating muscles; DS - Differentiating scales; E - Ependyma.

Fig. 6. Differentiating scales (DS) and differentiating muscles (DM) depicting hexokinase activity. 50 X.

Fig. 7. Cartilagenous neural canal (CNC) with ependyma (E) showing hexokinase activity. 50 X.


DS
E
DM
5


DS


CNC
E
CNC
7

some mitrochondrial localization was also evident, and, as in the previous studies, the muscle fibres could be distinguished into three types regarding their enzyme concentration and localization. The adipose tissue showed a mild response towards these enzymes and it was nil in all the other components of the tail.

WOUND HEALING PHASE

The wound epithelium in contrast to underlying subepithelial cells showed an appreciable hexo and glucokinase activity.

BLASTEMIC PHASE

Blastemic epithelium showed enhanced activity of both the enzymes as compared to the previous stage. The mesenchymal cells forming the bulk of the blastema also showed high reactivity of these enzymes.

DIFFERENTIATION PHASE

The stratified epithelial cells of the regenerate of the early differentiation phase showed a high enzyme concentration which appeared to diminish with the cellular differentiation of the skin and formation of the scales. Ultimately, with the complete

differentiation into epidermis and dermis, the stratum germinativum stood as the lone entity equipped with kinase activity. As myogenesis and chondrogenesis progressed, the differentiating myoblasts and chondroblasts showed increasing levels of kinase concentration finally attaining the highest level during the late differentiation phase. During early differentiation, the mononuclear myoblasts and myocytes displayed only cytoplasmic localization of the enzymes, whereas during late differentiation, the multinucleated myofibres also acquired the mitochondrial localization. The cells of the ependyma also showed high enzyme activity on the whole.

GROWTH PHASE AND FULLY REGENERATE TAIL

As in the case of other enzymes (Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975, 1976), hexo and glucokinase too started declining during this period, ultimately reaching in the fully regenerated tail the level of activity characteristic of the normal tail tissues.

DISCUSSION

Hexo and glucokinases are generally assumed to

aid in glucose uptake by the cells, and in this context, the intensity of these enzymes could be considered to provide an index to the uptake and incorporation of monosacharides into the cellular metabolic reactions. Viewed in this light, the moderate level of enzyme activity noticed in the muscles and skin of the normal tail, denotes the minimal dependence of these tissues on glucose and as such, these tissues are known to have a store of glycogen and lipids on which they could easily draw upon for their routine metabolic requirements. On the other hand, the high activity of these enzymes in the nerve cord is suggestive of the known fact that the nervous tissue is chiefly dependent on blood glucose for their respiratory activities.

The most significant feature regarding the distribution pattern of these enzymes during regeneration is the gradual but definite increase during the wound healing and blastemic phases ultimately attaining a peak level of activity during the differentiative process. Wound healing phase is known to be marked by a high rate of anaerobic

glycolysis (Rashakrishnan, 1972; Shah and Ramachandran 1970, 1972, 1976) and depletion of glycogen from the cut ends of the stump tissues (Radhakrishnan and Shah, 1973). In such a situation, the wound epithelium with its high hexo and glucokinase activity could be supposed to utilize the glucose supplied through blood.

A critical dependence on glucose by the regeneration blastema was inferred by Shah and Ramachandran, (1970, 1972, 1976) and Ramachandran, (1972) by taking into consideration the facts that, (1) the blastemic phase is marked by high activities of glycolytic enzymes such as LDH and Aldolase, (2) operation of HMP shunt pathway marked by the activity of G-6-PDH (Shah and Ramachandran, 1973), (3) synthesis of lipids as shown by the appearance of the same (Radhakrishnan, 1972) and (5) the absence of glycogen and phosphorylase (Radhakrishnaan and Shah, 1973). In this interesting biochemical environment, the sole source of cellular metabolite at this period appears to be the blood glucose, and the presently observed high hexo and glucokinase activity in the blastemal cells indicates the presence of an efficient mechanism for the maximal

uptake of glucose by these cells. The presence of a highly active hexo and glucokinase during differentiation also, is strongly suggestive of the fact that during this phase too, as during the blastemic phase, the cells of the regenerating system are increasingly dependent upon blood sugars. This would become rather apparent on an introspection and analysis of the information available from the previous studies. Such a critical evaluation reveals, that this phase is marked by anabolic reactions leading to the synthesis of many essential macromolecules and that in this molecular ecology of multifarious synthetic activities, the lipids that are elaborated during blastemic and early differentiation phases would tend to be rather inadequate; thus necessitating the presence of an efficient mechanism for the uptake of glucose from the blood. These facts are highlighted in the previous studies on tail regeneration in Mabuya mentioned earlier, and the currently demonstrated hexo and glucokinase activity conclusively helps in corroborating and strengthening the views elucidated therein. Further confirmation of the

dependence of the regenerating system on blood glucose is also available from the study on liver glycogen and blood glucose during tail regeneration in Mabuya carinata (Chapter - 3, Section - 1). It may be appropriate to recall here the contention of Banerjee and Ganguly (1962) that ascorbic acid has a stimulatory influence on hexokinase activity and hence the glucose uptake by cells. This contention finds its application in the regenerative mechanics, when the herein observed increased activity of hexo and glucokinase is coupled to the earlier report of a five fold increase in ascorbic acid content in the regenerate during differentiation (Ramachandran et al., 1975). Another interesting aspect is the revelation of the presence of two distinct types of enzymes ie., a specific glucokinase and a nonspecific hexokinase in the liver of mammals (Walker and Rao, 1964, 1963; Walker, 1963b; Spiro, 1958; Cahill et al., 1958; Long, 1952; Dipietro and Weinhouse, 1960). Extending further, Vinuela et al., (1963) based on their studies postulated that the unstable specific glucokinase is concerned with the phosphorylation of glucose leading to glycogen

synthesis and that the more stable nonspecific hexokinase is concerned with the phosphorylation of glucose leading to its catabolic breakdown. By extrapolating this suggestion on to the process of regeneration, it may be assumed, that a similar distinction exists and that both the enzymes may be operative during regeneration. If this be true, it could be construed that the nonspecific hexokinase would be active during the blastemic and early differentiation phases aiding in effective glucose catabolism, and that the specific glucokinase would be more active during the late differentiation phase leading to glycogen deposition in the differentiating tissues of the tail, which could well be correlated with the corresponding increase in glycogen in the tissues noted during this phase (Radhakrishnan and Shah, 1973).

The commencement of growth phase is marked by a declining activity of hexo and glucokinases which corresponds well with the attainment of a more or less normal pattern of metabolic and biochemical set up in the various differentiated elements of the regenerate and ultimately, in the fully regenerated tail, the hexo and glucokinase activity too settles down to the preautotomy moderate level as observed in the corresponding normal tail tissues.