CHAPTER 5

HISTOCHEMICAL LOCALIZATION OF GLYCOGEN AND PHOSPHORYLASE IN THE NORMAL AND REGENERATING TAIL OF THE HOUSE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

The energy problem in the regenerating appendages of amphibians has been investigated by Needham (1952), Hess (1959) and Schmidt (1960, 1966a,b). Considerable work on the glycogen and the glycolytic enzyme phosphorylase in the adult and embryonic tissues of various vertebrates has been done by Mancini (1948), Montagna (1949), Oconnor (1957), Bergman (1960), Grillo (1961), Engel (1961), Falin (1961) and Cosmos (1966). However, the role of glycogen and phosphorylase in the regenerating appendages of vertebrates has not been thoroughly investigated. The possibility that an endogenous energy supply might exist in the various cells and could be utilized by the normal and regenerating tissues led to the present histochemical study of glycogen and phosphorylase in the normal and the regenerating tail of the house lizard, *Hemidactylus flaviviridis*.

MATERIALS AND METHODS

The normal and the regenerated tails with at least one or two segments of the original tail stump were cut and immediately blotted to remove blood and tissue fluids.

The amputated tails were then kept on a microtome chuck in a cryostat maintained at -20°C. They were sectioned at 8 μ and then processed for the histochemical demonstration of glycogen and phosphorylase.

For glycogen, sections were mounted on slides without any adhesive and fixed in alcoholic picro-formol for two hours at -20°C. After fixation the sections were washed thoroughly with absolute alcohol till all the picric acid was completely removed and then stained for glycogen using the PAS techniques (Pearse,1960). Slides treated with salivary amylase or diastase served as controls.

The localization of phosphorylase was carried out according to the method of Eranko and Palkama (1962), using glucose-1-phosphate as the substrate. The sections were directly transferred to the incubation medium at room temperature for 20 minutes. The incubated sections were brought down to water through 40% alcohol and treated with Gram's iodine solution for one to three minutes. They were mounted in a mixture of Gram's iodine and glycerine (5:1) and immediately examined under the microscope. Unincubated sections treated with Gram's iodine and mounted in glycerine, served as control.

OBSERVATIONS

NORMAL TAIL:

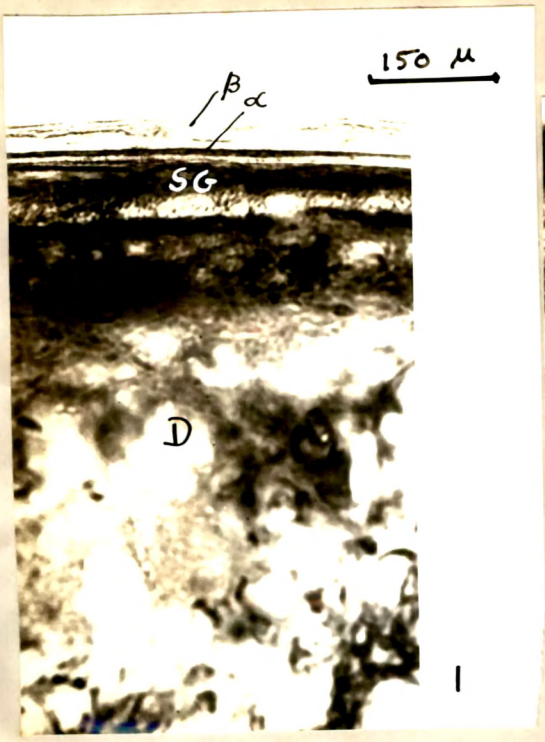
In the epidermis the beta and alpha cell layers of the old generation did not show either glycogen or phosphorylase, whereas, in these cell types of the new generation a low glycogen and phosphorylase activity was observed. A moderate concentration of glycogen and phosphorylase was noted in the cells of stratum intermedium and stratum germinativum in the epidermis (Figs. 1 & 2). The connective tissue fibres and fibrocytes of the dermis, the epidermal basement membrane and fascia connecting the dermis with muscles did not show either glycogen or phosphorylase.

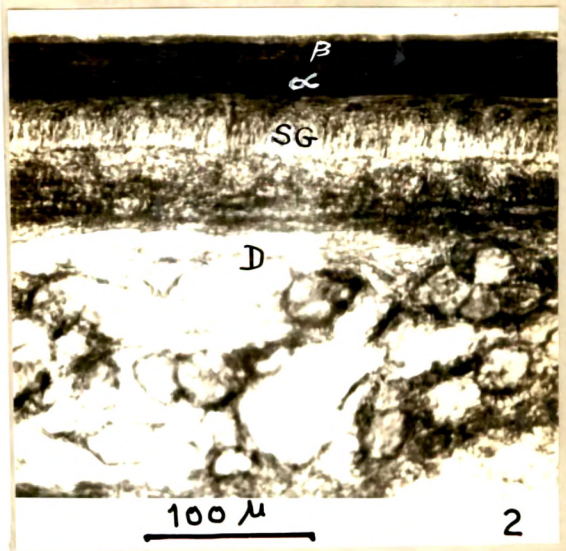
The muscle fibres contained a varied concentration of glycogen, wherein it was more in the fibres present at the periphery than those in the interior of fasciculi (Fig.3). The phosphorylase activity was proportional to the glycogen content in the muscle fibres (Fig.4) and was localized in the sarcoplasm and the mitochondria. The cells of the subcutaneous and submuscular adipose tissues as well as the cellular elements of the vertebral column did not possess glycogen or phosphorylase. The grey matter of the spinal cord showed high concentration of glycogen and phosphorylase while they were negligible in the white matter.

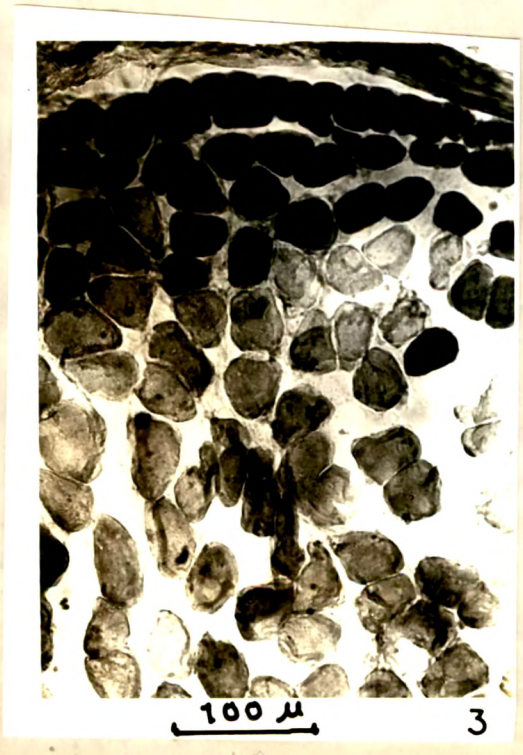
REGENERATING TAIL:

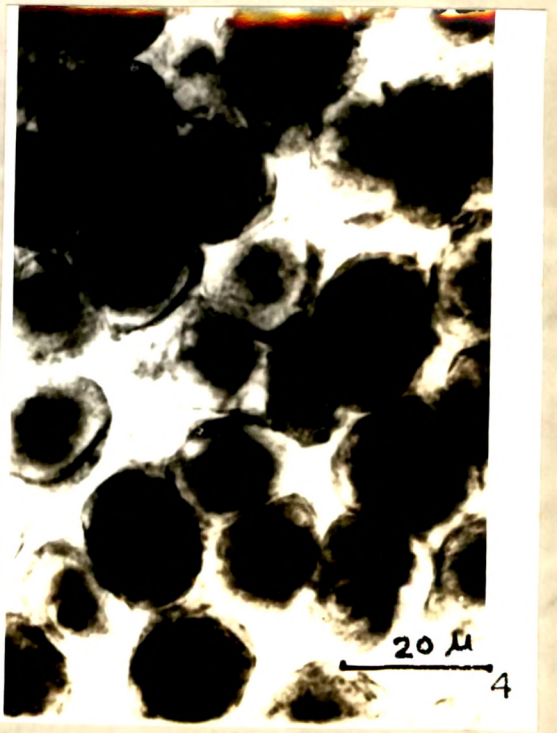
The epithelial cells during the wound healing and the preblastemic phases showed poor glycogen and phosphorylase activity (Fig.5). The subapical cells beneath wound epithelium were negative to glycogen and phosphorylase. During the blastemic and late blastemic phases when the epidermal region at the base of the regenerate started stratifying, low glycogen and phosphorylase activity was noted in the stratified epithelium. However, this metabolite and phosphorylase activity was more in the basal stratum germinativum compared to the distal stratified epithelium (Fig. 6). The mesenchyme cells of blastema and late blastema did not possess glycogen or phosphorylase.

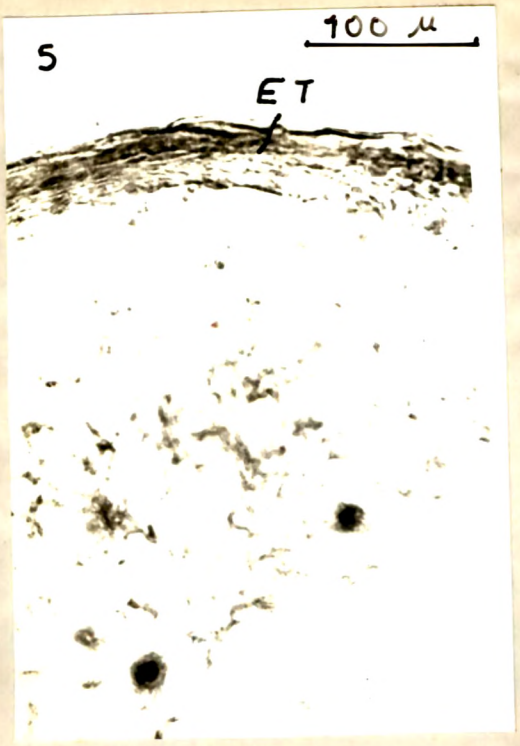
During differentiation and thereafter, the differentiated cell layers of the epidermis had low glycogen and phosphorylase in the beta and alpha cells, while a relatively high concentration was noted in the cells of stratum germinativum (Fig.7). Like the normal tail tissues, epidermal basement membrane, dermis, fascia, subcutaneous and submuscular adipose tissues and cartilageous neural canal in regenerating tail during differentiation and later did not contain any glycogen or phosphorylase. During different stages of myogenesis fairly high amounts of glycogen ~~was~~ were noted in the mononuclear myoblasts, myocytes and myofibres but the phosphorylase activity appeared only when the myofibres were just differentiated

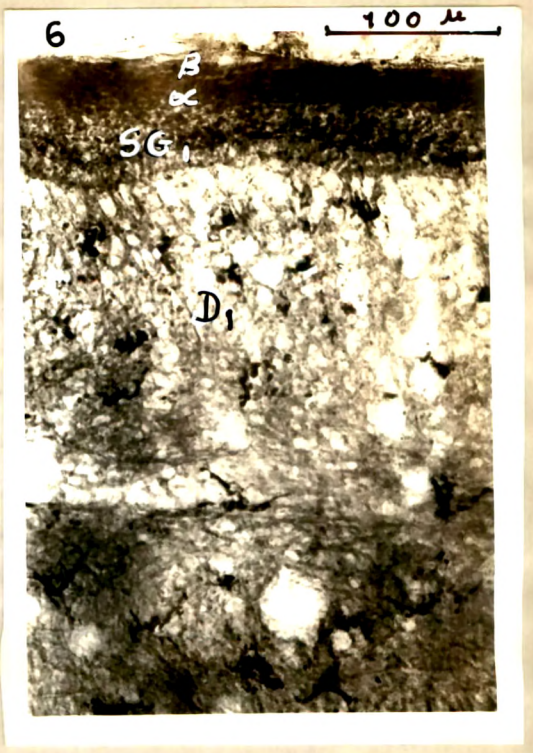

150 µ
β
α
SG
D
1

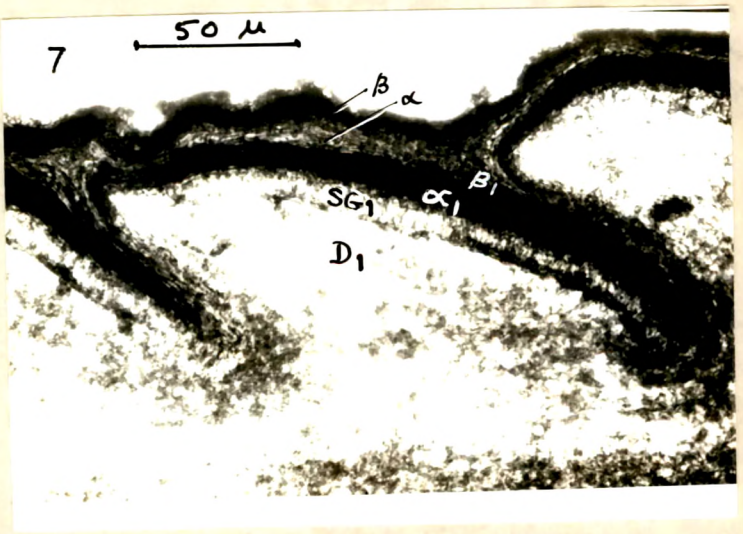

β
α
SG
D
100 µ
2


100 µ
3


20 µ
4


5
100 μ
ET


6
100 μ
β
α
SG1
D1


7
50 μ
β
α
SG1
α1
β1
D1

EXPLANATIONS FOR FIGURES

Fig. 1. L.S. of normal tail skin (ventral side) showing localization of glycogen in the stratum germinativum of the epidermis.

Fig. 2. L.S. of normal tail skin (ventral side) showing phosphorylase activity in the stratum germinativum.

Fig. 3. T.S. of a fasciculus of the caudal muscle showing glycogen. Note the high concentration of glycogen in the peripheral fibres of the fasciculus.

Fig. 4. T.S. of caudal muscle fibres showing phosphorylase activity.

Fig. 5. Part of epithelium (preblastemic phase) showing poor glycogen concentration.

Fig. 6. L.S. of the regenerate (early differentiation phase) showing glycogen in the stratum germinativum.

Fig. 7. Phosphorylase activity in the stratum germinativum during the scale formation in the regenerate skin.

ABBREVIATIONS

α - Alpha cells (old generation)

α_1 - Alpha cells (new generation)

β - Beta cells (old generation)

β_1 - Beta cells (new generation)

D - Dermis

D_1 - Dermis in the regenerate

ET - Epithelium

SG - Stratum germinativum

SG_1 - Stratum germinativum in the regenerate

(Fig.7). The enzyme activity increased thereafter and reached more or less the same level of intensity as seen in the muscle fibres of the normal adult tail.

The ependyma which first appeared at the blastemal stage showed very poor concentration of glycogen and phosphorylase and the intensity remained the same even in the fully regenerated tails.

DISCUSSION

In the normal tail skin of H. flaviviridis low glycogen and phosphorylase activity was noted in the beta and alpha cells of the new generation whereas in the cells of stratum intermedium and stratum germinativum the concentration of glycogen and phosphorylase activity was relatively high. The presence of glycogen in the epidermal cells of the newt, Diemictylus viridescens had been reported by Schmidt (1962) where he had observed an increase in the glycogen content in the cells towards the outer layers of the epidermis. It was reported that in the skin epithelium of man and other animals the glycogen was present (Bradfield, 1951; Kasabyan, 1956; Berlin 1958,1959). From his observations Bradfield (1951) surmised that the epithelial cells must accumulate a large amount of glycogen to maintain a certain level of anaerobic glycolysis which represents a source of energy for synthetic processes in the regenerating

epithelium where synthesis of the protein, keratin, is most important. Montagna and Ellis (1958) reported the histochemical localization of phosphorylase in the skin of man and other animals and observed that the enzyme localization corresponded with the distribution of glycogen. From their studies they suggested the ability of the epithelial cells to synthesis, glycogen which may be expected to be utilized for obtaining energy for cellular functions like keratin synthesis, sweat production and cell proliferation. In the epidermis of H. flaviviridis the observations showed that highly active cells of the stratum germinativum and intermedium possessed fairly high amounts of glycogen and phosphorylase. The cells of the stratum germinativum were in a stage of active cell division in order to produce new generations of beta and alpha cells and those of the stratum intermedium, before the older generation of these cells were cast off. The glycogen present in these cells provided energy for the cell proliferation and the metabolic activities. It was also noticed that some glycogen and phosphorylase activity was also found in the beta and alpha cells of the new generation. Here, perhaps the glycogen is the energy source for the synthesis of keratin which finally keratinizes the newly formed cells when the older generation of the highly keratinized beta and alpha cells are sloughed off. The cells of the stratum intermedium must be having glycogen

for their metabolic activity till they disintegrate and moult off the outer keratinized layers.

An identical localization of glycogen and phosphorylase activity was found in the tail muscle fibres of the house lizard. The muscle fibres present in the periphery of the fasciculi contained more glycogen and phosphorylase activity than those present in the interior. Similarly, Le Blond (1950) reported the presence of glycogen in the striated muscle of rat; Engel (1961) in the cultured avian muscle; George and Naik (1960) in the breast muscle of pigeon and Bergman (1960) in frog striated muscle.

George and Naik (1958) studied the localization of glycogen in the breast muscle of pigeon and reported the presence of two types of muscle fibres: one loaded with large amounts of glycogen (broad fibres) and the other with greater quantities of fat (narrow fibres). They suggested that the source of energy for the metabolism of these two fibre types was different and that they were physiologically distinct. Similar observations were made by George and Nene (1965) and George and Susheela (1966) in the flight muscle of some birds and the diaphragm of bat respectively. Schmidt (1962) observed large amount of glycogen in the normal and regenerating muscle of the newt, D. viridescens. The muscle fibres of the lizard

tail are chiefly characterised by glycogen utilization. George and Naik (1958) suggested that glycogen loaded broad fibres in the pigeon breast muscle were of the quick contracting type compared to the fat loaded narrow ones. The glycogen containing muscle fibres from the tail of house lizard are also quick contracting which would indirectly help in causing violent and quick contractions of some of the muscle segments that may lead to autotomy.

Soon after amputation (about 2 hours), it was observed that the depletion of glycogen from muscle fibres adjacent to the cut surface took place. It was also noticed that as glycogen was being depleted from the muscle fibres, the phosphorylase activity in the corresponding areas was reducing. However, the intact muscle fibres close to the injured ones showed a higher intensity for glycogen and phosphorylase than the dedifferentiating fibres. These observations are supported by the study of glycogen in the regenerating limb of the newt, D. viridescens by Schmidt (1962). Increase in the lactic acid content in the tissues (chiefly muscles at the cut surface) during the preblastemic phase of limb regeneration in axolotl was reported by Okuneff (1933). Dickens (1951) found a rise in the lactic acid levels during anaerobic glycolysis. From the present observations it could be suggested that

anaerobic glycolysis must be taking place in the tissues at the time when depletion of glycogen occurs. Schmidt (1960) also arrived at a similar conclusion based on the study of glycogen in regenerating limb of D. viridescens. The depletion of glycogen from the cut end of muscle fibres suggests the utilization of the metabolite for the process. However, the gradual rise in glycogen and phosphorylase contents in the muscle fibres adjacent to the cut surface of the tail indicates the role of phosphorylase in the synthesis of glycogen, and the increase in glycogen leading to the establishment of the normal metabolism of the muscle fibres. The newly formed mesenchyme cells were devoid of glycogen and phosphorylase. But later, glycogen was noticed first in mononuclear myoblasts and then it gradually increased as the differentiation of myoblasts to myofibres progressed. The phosphorylase activity was noticed only when the myocytes transformed into myofibres. Phosphorylase was detected in the cardiac muscle of chick, about a day after the beginning of glycogen formation (Grillo, 1961). Similarly, glycogen appeared as early as the 4th day of development of the domestic fowl but phosphorylase was observed only on the 17th or 18th day of development (Cosmos, 1966). These observations and the present study on the regenerating muscle of the house lizard tail suggest that during the early phases of myogenesis, the myogenic cells were not adapted for an anaerobic

metabolism. So it is likely, as suggested by Schmid et al. (1959), and Robbins et al. (1959) that during the early period in the absence of phosphorylase, the glycogen is being synthesized in the presence of a different enzyme, Uridine diphosphate-glucose-glycogen synthetase.

Unlike in the normal nerve cord, the glycogen content and the phosphorylase activity in the ependyma ~~was~~ were very low. As the ependyma in the regenerating tail of the lizard does not function as an active nerve tissue, its activity must be very low (as cited by Brachet, 1950) since the tissues at the cut end of the lizard tail stump loses its glycogen. It is suggested that the metabolite is used for dedifferentiation processes and the formation of the mesenchyme cells.

During the early phases of tail regeneration the glycogen and phosphorylase were absent from the mesenchyme of the regenerates. Similar observations on the absence of glycogen in the blastema of Planarians and Asellus were reported by Needham (1952).

As the differentiation of various tissues viz. epidermis, muscle and ependyma progressed, the glycogen as well as phosphorylase appeared which suggests that the tissues mentioned above acquire glycogen for their cell metabolism only when they reach a certain stage of morphological and functional maturity.