CHAPTER 3

HISTOCHEMICAL LOCALIZATION OF LIPASE AND ESTERASE IN THE NORMAL AND REGENERATING TAIL OF THE HOUSE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Lipids serve as a source of energy for the cell maintenance and functions (Fredrickson and Gordon, 1958; Rossiter and Strickland,1960). It has been shown that lipids are component parts of most of the tissues in the normal and regenerating appendages of amphibians (Hess, 1959; Schmidt, 1966a,b). The presence of lipids in the normal as well as regenerating tissues of the lizard *Hemidactylus flaviviridis* (Chapter, 2), suggests the utilization of lipids as an energy source. Since the activities of lipase and esterase would be indicative of their hydrolysis which is a primary step before their utilization, a histochemical study of lipase and esterases of the various tissues of normal and regenerating tail of the house lizard, *Hemidactylus flaviviridis* was undertaken.

MATERIALS AND METHODS

The normal tail and the regenerates with atleast one or two segments of the original tail stump were cut and fixed in cold (4°C) 6% neutral formalin for 12 hours. After fixation, the tissues were washed thoroughly with

distilled water and sectioned at 10 to 15 μ on a freezing microtome. The sections were incubated at 37°C for 15 hours for the demonstration of lipase and esterase by the "Tween" method of Gomori (Pearse, 1954). The incubation media was prepared according to George and Scaria (1958). The rest of the procedure was the same as described by Pearse (1960). 'Tween 85' was used as the substrate for the demonstration of lipase and Tween 80, 60 and 40 were employed as substrates for esterases, of which, Tween 60 was found to give the maximum reaction. Gomori (1953) observed that Tween 80 was attacked only by true lipase. In the present study a more specific substrate, Tween 85 was employed to demonstrate true lipase activity as described by George and Ambadkar (1963). Therefore, the present observations were made from the sections treated in the medium where Tween 85 served as the substrate for lipase and Tween 60 for the esterase. The sections incubated in buffered media, devoid of the substrate and sections incubated after keeping them in distilled water at 80°C for five minutes, served as controls.

OBSERVATIONS

NORMAL TAIL:

The old and new generations of the beta and alpha cells in the epidermis were negative to lipase and

esterase activities, while the cells of the stratum intermedium and germinativum layers showed a low lipase but high esterase activity (Fig.1). The epidermal basement membrane did not possess lipase activity but esterase was observed in the subjacent region of the epidermal basement membrane. No lipase activity was noted in the dermis and the subcutaneous adipose tissue. However, the peripheral cytoplasm of the fat cells in the subcutaneous adipose tissue contained fairly high esterase activity (Fig.2).

The muscle fibres did not show any lipase or esterase activities. Like the subcutaneous adipose tissue, the submuscular adipose tissue had no lipase but only esterase activity. All cellular elements of the vertebrae were negative to lipase and esterase, but the fat cells and the marrow cells present in the vertebral marrow and the spine did show perceptible lipase and esterase activities. The spinal cord of the tail responded noticeably for lipase and esterase activities. Both these enzymes were more active in the cells of the grey matter than those of the white. The blood vessels and the blood cells were poor in lipase and esterase.

REGENERATING TAIL:

Wound healing and Preblastemic phases:

The epithelium during the wound healing and the preblastemic phases showed low lipase and high esterase

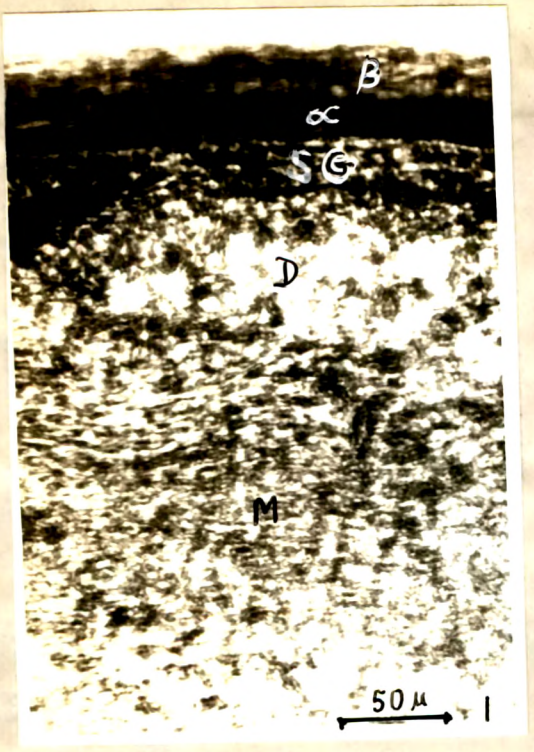
activities which was similar to that noticed in the epidermis of the skin over the stump (Fig.3). The blood cells, macrophages and lymphocytes which accumulated beneath the subapical region during the above phases also showed poor lipase but high esterase activity.

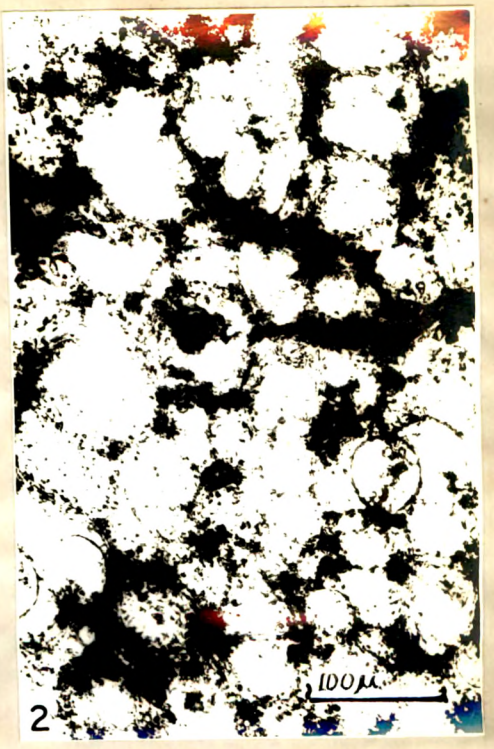
Blastemal and Late blastemal phases:

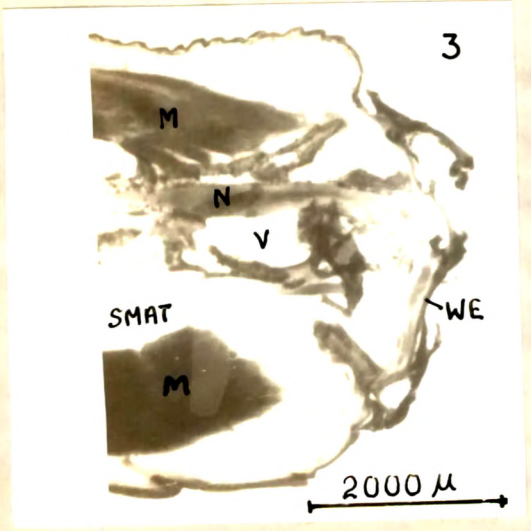
The lipase and esterase levels of the stratified epithelium of the blastema remained the same as in the wound healing and preblastemic phases. The mesenchyme cells forming the core of the blastema showed fairly high lipase and esterase contents (Figs. 4 & 5).

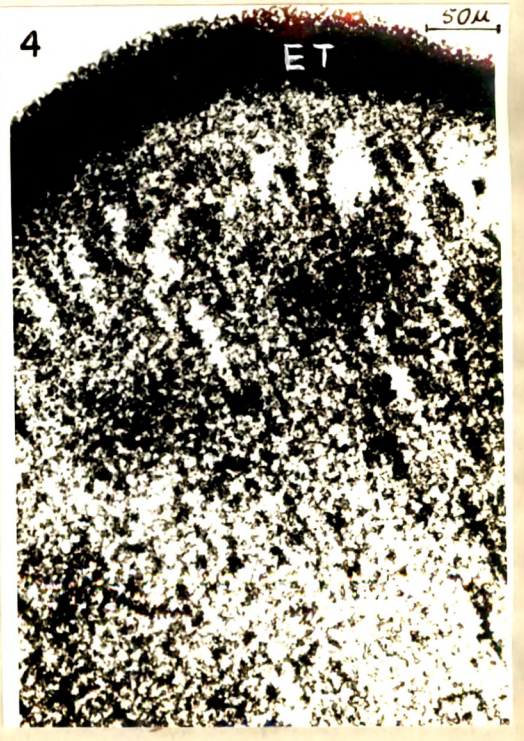
Differentiation and Growth phases:

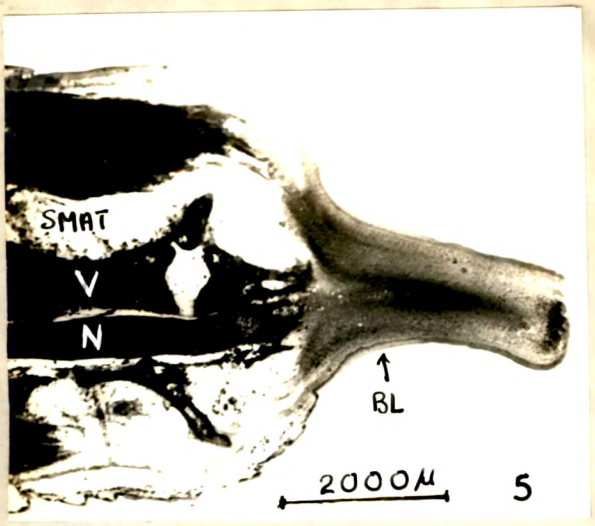
During the differentiation phase the epidermis was poor in lipase but had high esterase activity in the cells of the stratum germinativum and the enzymes were absent from the alpha and beta cells (Fig.6). The early fibrocytes in the dermis possessed a low but noticeable lipase activity while the esterase activity was quite pronounced. During further growth the lipase activity gradually decreased and finally disappeared from fully differentiated and matured fibrocytes. During this phase, the esterase activity remained quite high (Fig.7). During different stages of myogenesis, viz. myoblasts, myocytes and the muscle fibres, lipase and esterase activities were poor. The subcutaneous and


β
∝
SG
D
M
50 μ
1

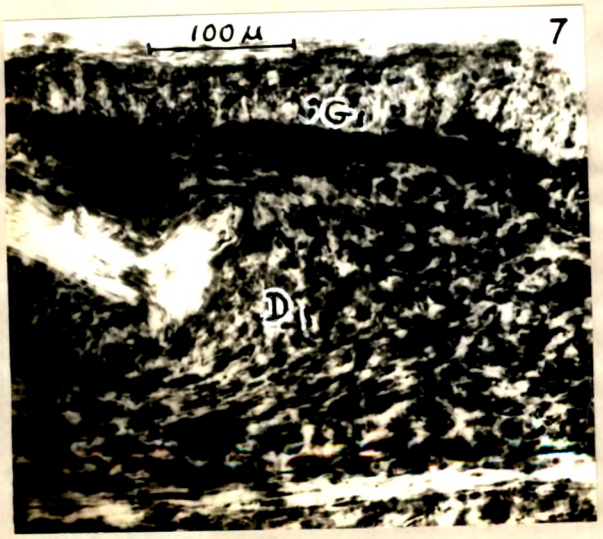

100μ
2


3
M
N
V
SMAT
WE
M
2000 μ


50μ
4
ET


SMAT
V
N
BL
2000M
5


100M
ED
SG1
D
6


100M
7
SG1
D1


8
C
EP
C
100M

EXPLANATIONS FOR FIGURES

Fig. 1. L.S. of the normal tail skin (ventral side) showing esterase activity in the stratum germinativum and epidermal basement membrane.

Fig. 2. Subcutaneous adipose tissue showing esterase activity.

Fig. 3. L.S. of the regenerate (wound healing stage) showing esterase activity in the wound epithelium and subapical cells.

Fig. 4. Epithelium and mesenchyme cells of the blastema showing esterase activity.

Fig. 5. L.S. of the regenerate (late blastemal phase) showing esterase activity in the blastema.

Fig. 6. L.S. of the skin in the regenerate (differentiation phase) showing the esterase activity in the differentiating stratum germinativum.

Fig. 7. L.S. of the skin in the regenerate (late differentiation phase) showing esterase activity in the fibrocytes of the dermis and stratum germinativum of the epidermis.

Fig. 8. L.S. of the ependyma showing low but uniform esterase activity.

ABBREVIATIONS

α - Alpha cells (old generation)

β - Beta cells (old generation)

BL - Blastema

C - Chondrocytes

D - Dermis

D_1 - Dermis in the regenerate

ED - Epidermis

EP - Ependyma

M - Muscle

N - Nerve cord (spinal cord)

SG - Stratum germinativum

SG_1 - Stratum germinativum in the regenerate skin

SMAT - Submuscular adipose tissue

V - Vertebral column

WE - Wound epithelium

submuscular adipose tissues showed high esterase activity. The chondroblasts differentiating from the mesenchyme cells showed neither lipase nor esterase activities.

The glial cells and the cells of the ependyma right from the beginning of their differentiation till a well defined ependymal tube was formed, revealed a low but uniform lipase and esterase levels, which were lesser than in the spinal cord of the normal tail (Fig.8). Once the differentiation of the tissues took place, the growth commenced. There was no change in the intensity of the lipase or esterase activities in the growing cells of the different tissues of the regenerating tail of the lizard, except for those mentioned earlier viz. fibrocytes where lipase was totally nil during the growth phase.

DISCUSSION

It has been shown that considerable amount of lipids are present in the adipose tissues and other tissues of the normal and regenerating tail of the house lizard, H. flaviviridis (Woodland, 1920 and Chapter 2). The presence of lipase and esterase indicates the utilization of lipids as one of the sources of energy for the cell metabolism and regenerative activities. The observations on the localization of lipase and esterase in the tissues of the normal and those of the regenerating lizard tail, showed esterase

as small particulate precipitates in the cytoplasm of most of the tissues but the lipase was localized only in few tissues.

In the epidermis fairly high esterase activity was observed in all the epidermal cell layers except for the outer most beta and alpha cells. A low lipase activity was restricted to the cells of stratum intermedium and and germinativum. Esterase granules were found aggregated subjacent to the epidermal basement membrane. From the study of lipids in the tissues of the tail, it is evident that the lipids were in high concentration in the cells of stratum germinativum and intermedium. The high activity of the enzymes in these cells may help for utilization of the lipids for metabolic processes including division of the cells of stratum germinativum which provides new cells periodically when ecdysis takes place. The fat cells of the subcutaneous and submuscular adipose tissues contained intense esterase activity but the lipase activity was nil. The occurrence of a true lipase in the adipose tissue of pigeon and certain other vertebrates had been demonstrated using both histochemical and biochemical methods (George and Eapen, 1958a,b; 1959a,b,c). In the present study, the subcutaneous and submuscular adipose tissues were shown to be negative to lipase but revealed intense esterase activity. This suggests that in the adipose tissue of the tail only short chain fatty acids are present and utilized.

High concentrations of lipase has been shown by quantitative estimations in the flight muscles of insects such as dragon flies, butterflies and moths (George et al., 1958; George and Bhakthan, 1960a,b), in the red muscle of fish (George, 1962; George and Bokdawala, 1964), pigeon pectoralis muscle (George et al., 1958). The muscle fibres in the tail of the house lizard, H. flaviviridis, did not show any lipase or esterase. Also the lipids were absent from the tail muscle fibres (Chapter 2). However, a high concentration of glycogen was noted in the muscle fibres (Chapter 5). The tail being an active organ in the lizards, a greater level of glycogen as the chief metabolite is quite understandable.

The marrow tissue of the caudal vertebra showed lipase and esterase activities. The fat present in the marrow cells provides energy for their metabolism and function, i.e. haemopoiesis. Thus, the lipolytic enzymes in these cells confirm the belief that fat is one of the sources of energy for the purpose. In the spinal cord the lipase and esterase activities were seen more in the grey matter than the white matter. This observation indicate, a difference in the metabolism of the two morphologically different parts of the spinal cord.

An increase in the esterase activity in the wound epithelium during the wound healing phase was noted in the guinea pig (Raekallio and Levonen, 1963), in the granulosa

tissue of the guinea pig (Shishkin, 1959) and in the granulosa of the rat skin wounds (Argyris, 1956). A considerable esterase activity in the subapical tissues during the wound healing, preblastemic and blastemic phases were observed in the lizard, H. flaviviridis, where lipids seem to provide energy for the active epithelial and subapical cells. It has been shown that the lipids were in fairly high concentration in the mesenchyme cells of the blastema (Chapter 2), whereas the glycogen content of these cells was very poor (Chapter 5). It is observed from the present study that the lipase and esterase activities in these cells was higher and the phosphorylase activity was almost nil (Chapter 5). These observations suggest that early blastemal cells use mainly lipids as the source of energy and not carbohydrates.

During the differentiation phase esterase activity could be observed in the cells of the dermis, fat cells of the subcutaneous and submuscular adipose tissues. This again is correlated with high concentration of lipids in the same tissues indicating fat metabolism. The lipase which was noticed in the early fibroblasts,reduced as these cells differentiated and matured into fibrocytes, which indicates that in the early fibroblasts long chain fatty acids are being utilized which later are replaced by short chain fatty acids.

The various stages of myogenesis viz. myoblasts, myocytes and myofibres showed poor but perceptible lipase and esterase activities. The findings that very poor low concentrations of lipids were noted in these cells and that glycogen appeared at a much later period, suggested that during the early period of myogenesis the cells are adapted for lipid metabolism and later a shift towards the glycogen metabolism can be expected. The ependyma showed relatively poor lipase and esterase activity compared to that noticed in the normal spinal cord. Since the ependyma does not resemble the normal spinal cord morphologically, it is reasonable to assume that it does not attain the functional state of the normal spinal cord and that the poor activity of these enzymes may be due to the low metabolic activity of this tissue.

From these findings it is likely that during the early phases of regeneration viz. preblastemic, blastemic and early differentiation, the regenerate tissues may be adapted for an aerobic metabolism involving the use of lipids as the fuel for energy.