CHAPTER 2

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

It is now well established that lipids serve as one of the main sources of energy for metabolism (Rossiter and Strickland, 1960; George and Berger, 1966). Schmidt (1966a) studied the distribution of the Sudanophilic lipids in the regenerating limb of the adult newt, *Diemictylus viridescens* and suggested that lipids may be playing a significant role in the biochemical events taking place during regeneration. With this supposition he further studied the distribution of non-acidic, acidic and phospholipids in the normal and regenerating limb of the newt, *D. viridescens* and concluded that lipids participate in the metabolism of the blastemal cells (Schmidt, 1966b). Woodland (1920) based on his studies on the histology of the normal and regenerating tail of the house lizard, *Hemidactylus flaviviridis*, called attention to the deposition of large amounts of lipids in the subcutaneous and submuscular adipose tissue regions. The present study was undertaken with the view to characterize these lipids and study their distribution pattern in the normal and regenerating tail of the house lizard, *H. flaviviridis* so as to throw some light on their metabolic significance during the process of regeneration.

MATERIALS AND METHODS

The normal and regenerating tails with atleast one or two segments of the normal tail stump were cut and fixed in 10% formol calcium (Baker, 1944) for 24 hours at room temperature. After processing for the various lipid tests as mentioned below the tissues were washed in distilled water and sectioned at 8 μ in a cryostat maintained at -20°C. For the demonstration of various types of lipids, the sections were stained with Sudan Black B, Fettrot 7B, Oil Red O, Nile Blue Sulphate and Acid Haematein.

Formalin fixed sections were stained with Sudan Black B, Fettrot 7B and Oil Red O. The staining for Sudan Black B and Fettrot 7B was carried out as described by Schmidt (1966a) and that for Oil Red O according to Lillie (1944) as cited by Pearse (1960). Control sections were treated with a mixture of chloroform-methanol (1:1 v/v) at 55°C for 24 hours to extract the lipids.

Tissues fixed in 10% formol calcium and chromated in 3% potassium dichromate were used for the Nile Blue Sulphate test. The preparation of the stain and staining procedure adopted was that of Cain (1947) as described by Pearse (1960).

Acid Haematein test for phospholipids was carried out according to Baker (1946) as cited by Pearse (1960). Weak Bouin's fixed, pyridine extracted tissues were used as controls.

OBSERVATIONS

NORMAL TAIL:

The cells of the beta and alpha layers of the old and new generations, stratum intermedium and stratum germinativum showed a diffuse cytoplasmic staining with Sudan Black B. However, small lipid globules of different sizes were seen to be located more towards the base of the cells of the germinativum layer and subjacent to the basement membrane all throughout (Figs. 1 & 2). The epidermal basement membrane stained negative with Sudan Black B. Apart from the stratum germinativum and lipid globules which were subjacent to the epidermal basement membrane, all other cellular layers of the epidermis stained negative for neutral lipid tests (Fettrot 7B, Oil Red O and the red oxazine reaction by Nile Blue Sulphate). The localization was similar to that of Sudan Black B. A diffuse cytoplasmic blue oxazine reaction (similar to Sudan Black B) was noted in all cellular layers of the epidermis whereas it was weak in the epidermal basement membrane. No phospholipids were noted in any of the epidermal layers.

The connective tissue fibres of the dermis and fascia stained negatively with Sudan Black B, Fettrot 7B and the red oxazine reaction of the Nile Blue Sulphate,

whereas a faint blue staining was noted with the blue oxazine reaction. Phospholipids were located in the blood cells, phagocytes and along the nerve innervations in the dermis.

The muscle tissue which gave a negative reaction for all other lipid tests showed a poor blue colour in the sarcoplasm of the fibres with Nile Blue Sulphate. Nevertheless, neutral lipid globules could be seen in between the fibres and interfascicular spaces. The subcutaneous and the submuscular adipose tissues stained intensely with Sudan Black B and for neutral lipids (Fig.3). The blue oxazine reaction and phospholipids were however negative.

The lipid globules of the vertebral marrow showed an intense staining reaction with Sudan Black B, Fettrot 7B, Oil Red O and the red oxazine reaction of Nile Blue Sulphate denoting the presence of neutral lipids (Fig.4). All other regions of the vertebrae stained negatively with Sudan Black B and other neutral lipid staining dyes, whereas, the vertebral body and the intervertebral cartilage revealed an intense blue oxazine reaction with Nile Blue Sulphate. No phospholipids were noted in any parts of the vertebra or vertebral column. The staining with Sudan Black B was greater in the white matter of the spinal cord than in the grey matter. Similar intensities of staining

were obtained by all the other lipid tests. An intense positive reaction for phospholipids was obtained which was higher in the white matter than the grey. The control sections also stained positively but the intensity of the reaction was relatively reduced.

REGENERATING TAIL:

Wound healing and Preblastemic phases:

The epithelium and the subapical cells showed diffuse cytoplasmic staining with Sudan Black B with lipid globules scattered in the subapical region (Figs.5 & 6). Similar localizations could be observed for all the other neutral lipid tests. The red oxazine reaction with Nile Blue Sulphate was positive only in the lipid globules of the subapical region. There was no detectable phospholipid staining in the epithelial and subapical cells. However, during the preblastemic phase, phagocytes with high phospholipids in their cytoplasm could be seen in the subapical region.

Blastemic phase:

The distribution of lipids in the stratified epithelium and mesenchyme cells was similar to that observed during the preblastemic phase (Fig. 7). There was a considerable increase in phospholipids in the cytoplasm of the phagocytes,

all over the blastema (Fig. 8).

Late blastemal, Differentiation and Growth phases:

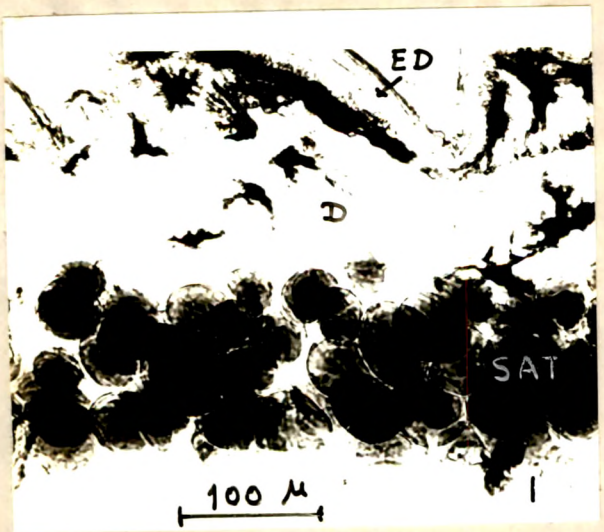
The differentiated mesenchyme cells which were at the distal end of the blastema gave a diffuse cytoplasmic staining with Sudan Black B and a negative reaction for neutral lipids. In the blastemal core, globules of neutral lipid were found to be scattered and a considerable reduction in the phospholipid loaded phagocytes was noticed. The differentiating epidermal layers viz. beta, alpha and germinativum layers showed diffused cytoplasmic Sudan Black B staining. Darkly stained lipid globules were found to be concentrated more towards the base of the cells of the stratum germinativum. The latter showed a similar staining pattern for neutral lipids also, whereas, the beta and alpha cells gave a negative reaction. The epidermal basement membrane was also negative to all lipid tests except for the blue oxazine reaction of Nile Blue Sulphate. In all the epidermal cell layers a diffuse blue oxazine reaction was obtained. Phospholipids were nil in the epidermal layers during differentiation.

The dermis when stained with Sudan Black B revealed a pale diffuse colour in the cytoplasm before the fibroblasts transform into the fibrocytes and connective tissue fibres. This pale staining was reduced further after

differentiation. All other tests for neutral lipids yielded a negative reaction and the blue oxazine reaction of the Nile Blue Sulphate was also very poor.

A diffuse reaction was obtained in the cytoplasm of the myoblasts and myocytes with Sudan Black B and a negative one for neutral lipids. The differentiated muscle fibres also stained negatively with Sudan Black B and for neutral lipids. Nile Blue Sulphate gave a poor blue oxazine reaction all throughout myogenesis and thereafter. Phospholipids were nil. Except for the high blue oxazine reaction for the cartilagenous neural canal, all other lipid tests remained negative throughout chondrogenesis.

The ependymal tube along which lipid globules were scattered, also showed a pale diffuse cytoplasmic staining with Sudan Black B and a negative reaction to all other neutral lipid dyes. During the early period of ependyma formation, no phospholipids were observed but during the late differentiation phase they were distributed throughout the length of the ependymal tube. The deposition of the subcutaneous and submuscular adipose tissues could be observed from the late differentiation and both adipose tissue regions remained negative to other lipid tests except those for neutral lipids and Sudan Black B (Fig. 9).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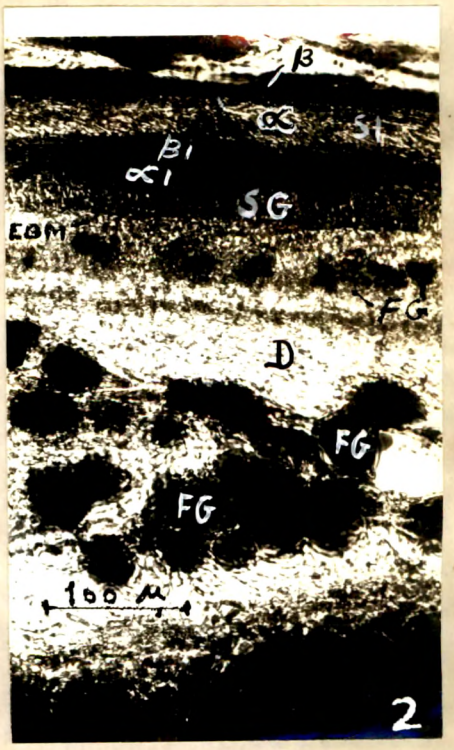
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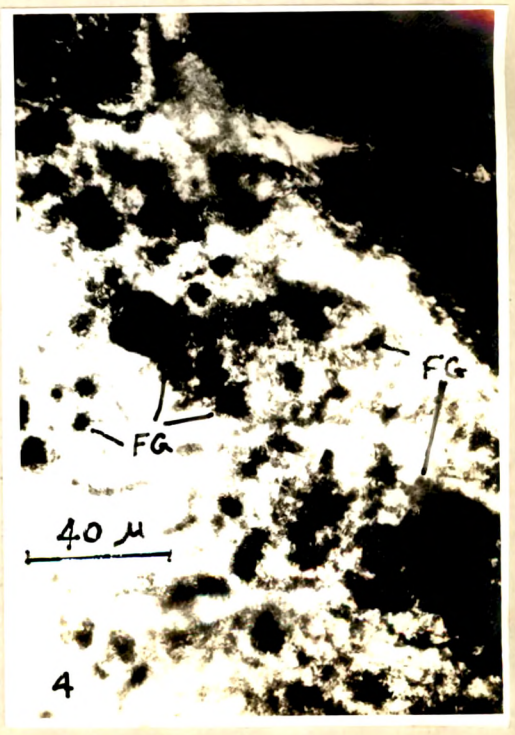
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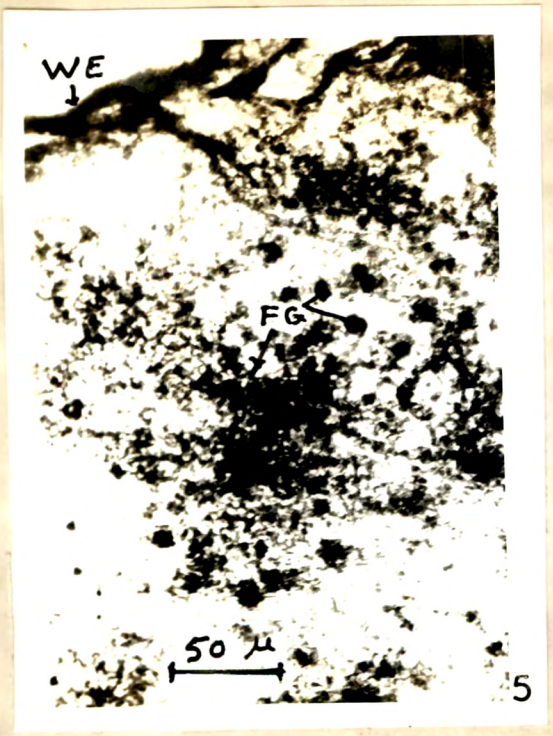

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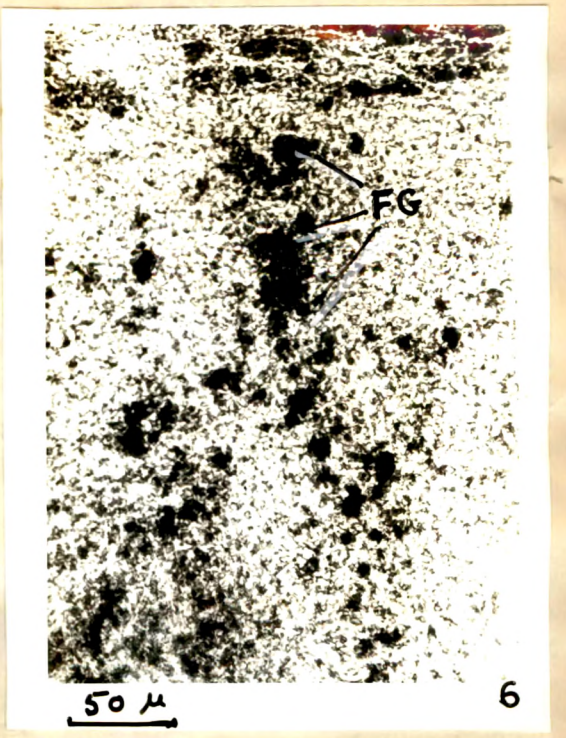

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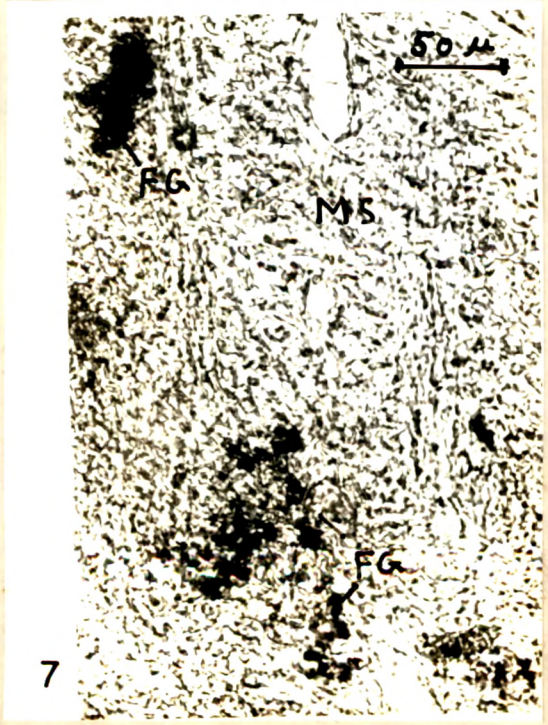

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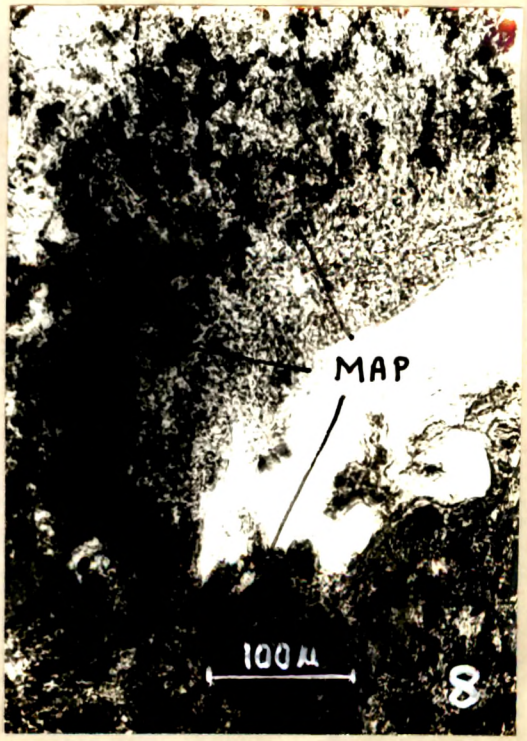

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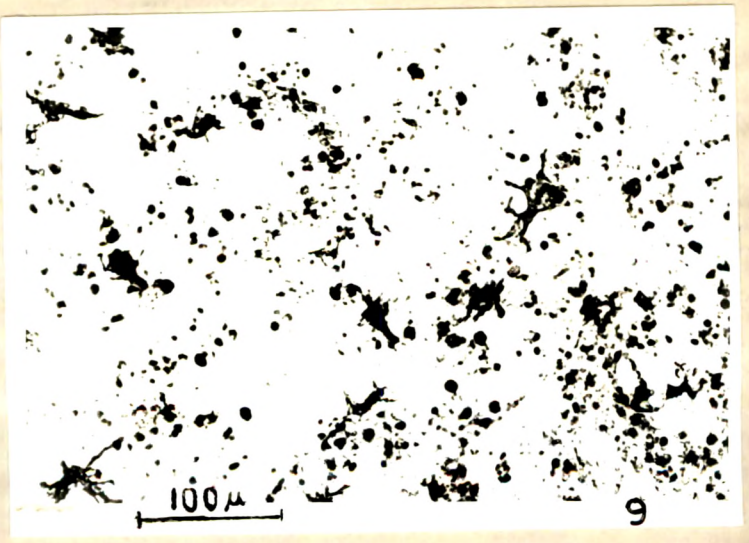
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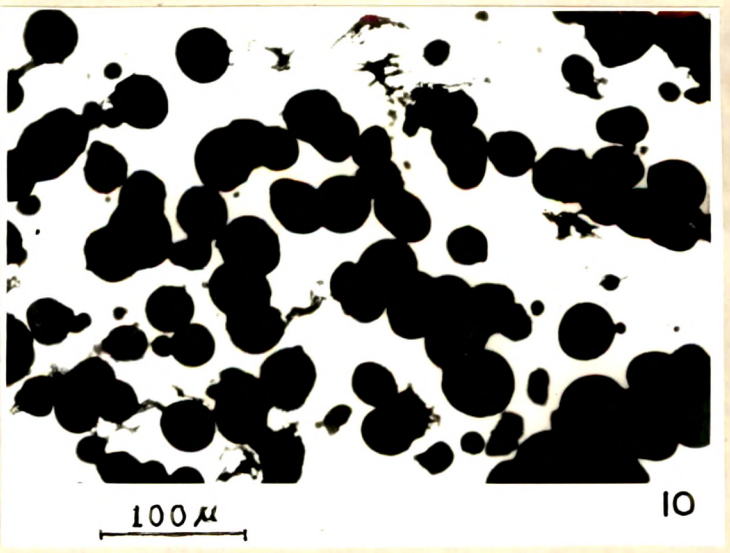
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EXPLANATIONS FOR FIGURES

Fig. 1. L.S. of the normal tail skin (dorsal side) showing the subcutaneous adipose tissue.

Fig. 2. L.S. of the normal tail skin (ventral side). Note the accumulation of fat in the subcutaneous adipose tissue and subjacent to the epidermal basement membrane.

Fig. 3. Submuscular adipose tissue loaded with fat.

Fig. 4. Vertebral marrow in the caudal vertebral body in the normal tail showing the lipid globules.

Fig. 5. The wound epithelium and the subapical region. Note the lipid globules dispersed in the subapical area over the wound surface.

Fig. 6. Subapical region of the regenerate (preblastemal phase), showing fat globules dispersed in the subapical tissue.

Fig. 7. Mesenchymal core of blastema showing fat globules.

Fig. 8. Mesenchymal core of blastema showing phospholipid in the macrophage cells.

Fig. 9. Initial stage of fat deposition in the submuscular adipose tissue in the regenerate.

Fig.10. Later stage of fat deposition in the submuscular adipose tissue in the regenerate.

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.

EBM - Epidermal basement membrane

ED - Epidermis

FG - Fat globules

MAP - Macrophage cells

MS - Mesenchyme cells

SAT - Subcutaneous adipose tissue

SG - Stratum germinativum

SI - Stratum intermedium

WE - Wound epithelium

The localization of lipids remained unchanged during the growth phase. A considerable increase in the subcutaneous and submuscular adipose tissue was observed during this phase (Fig. 10).

DISCUSSION

The observations made in the present study indicate that Sudan Black B stains lipids in general without any specificity, whereas Fettrot 7B, Oil Red O and red oxazine reaction of the Nile Blue Sulphate specifically stained the neutral lipids. The blue oxazine reaction of Nile Blue Sulphate on the other hand is known to denote the presence of acidic lipids. In the present investigation, all the tissues of the normal and regenerating tail stained in various intensities of blue colour with Nile Blue Sulphate. However, many of the tissues like connective tissue, muscle and cartilagenous neural canal did not stain with Sudan Black B, thereby indicating the absence of any type of lipids. Moreover, the blue oxazine staining was not strictly diffuse in the cytoplasm of all cells. Hence for the detection of acidic lipids a comparison was made with Sudan Black B staining. Wherever Sudan Black B gave a negative staining reaction and Nile Blue Sulphate a positive blue oxazine reaction, an absence of acidic lipids would be indicated. This staining reaction could therefore be attributed to the

presence of fatty acids or protein or to any other lipoprotein complex as suggested by Cain (1947a). The acid Haematein test stained phospholipids wherever present. The studies on the various lipids of the normal and regenerating tail revealed a diffuse localization in the cytoplasm and in the form of small to big globules.

In the epidermis, neutral lipids were seen to be diffused in the cytoplasm and as globules of varying sizes in the germinativum layer, whereas in the beta, alpha and intermedium layers it was absent. Nevertheless the diffused cytoplasmic staining obtained with Sudan Black B and blue oxazine reaction of Nile Blue Sulphate in all cellular layers of the epidermis possibly indicated the presence of acidic lipids in the above cells. The germinativum layer of the epidermis always appeared to be very active giving rise to new generations of beta and alpha cells and the stratum intermedium. A high level of metabolism could thus be expected within these cells with lipids serving as a source of energy for metabolic activities.

The various lipid tests showed that the subcutaneous and submuscular adipose tissues mainly comprised of neutral lipids, which could function as a reserve metabolite. It is interesting to note that some of the non-regenerating lizards like Calotes and Uromastix do not possess the above adipose tissue reserve.

Neutral lipids in the form of globules of varying sizes noted in the vertebral marrow, were diffused in the cytoplasm of the marrow cells. This tissue being actively involved in haemopoiesis, lipids may serve as an energy source for these cells.

The spinal cord showed the presence of neutral, acidic and phospholipids. An intense reaction obtained with the acid haematein test, was also noted in the controls but of a much reduced intensity. The presence of phospholipids in the myelin sheath of the nerve fibres have been reported (Baker, 1946; Cain, 1947b; Hori and Chang, 1963; Schmidt, 1966b), and cephalin, spingomyelin and lecithin could also be stained by the acid haematein test (Baker, 1946; Casselman, 1952). Comparing with the controls it is likely that the acid haematein reaction observed in the spinal cord may be due to the above mentioned components. The lipids present in the epithelium and the subapical cells during the wound healing and preblastemic phases and the striated epithelium and mesenchyme cells during the blastemic and late blastemic phases may serve as a source of energy for these cells. During these phases a high rate of cell division and proliferation was observed in the regenerates. The macrophages with high concentration of phospholipids in their cytoplasm were seen in abundance in the core of

the blastema but disappeared by the late blastemal phase. The role of these macrophages is not known, but it is likely that they may be disintegrating in the blastema giving out the lipids. A high concentration of unsaturated fatty acids has been reported in the blastemal cells of the regenerating tail of Xenopus laevis tadpole (Hess,1957). Recently,Schmidt (1966a,b) could also detect high lipid contents in the blastema of the regenerating limb of the newt D. viridescens. The results in the present study are in conformity with the above findings.

During the differentiation phase the localization of lipids in the epidermal layers and in the ependymal tube was similar to those in the late blastema. However, a considerable increase in neutral lipids in the subcutaneous and submuscular adipose tissue regions was observed. These adipose tissues were mainly laid out only during the late differentiation phase, suggesting that these regions serve as lipid stores.

The epidermal basement membrane, the connective tissue fibres of the dermis, fascia, muscle and the bony regions of the vertebrae were positive to the blue oxazine reaction of the Nile Blue Sulphate indicating the presence of acidic lipids. This was also found to be the case in the above tissues and the cartilagenous neural canal of regenerates. But the staining with Sudan Black B did not

show any lipid either diffused in the cytoplasm or as globules. Hence the blue oxazine reaction in the above tissues may be due to some components other than lipids.

The possibility of lipids participating in protein synthesis during regeneration has been suggested by Bodimer and Everett (1959) and Schmidt (1966b). In the regenerating tail of the house lizard, H. flaviviridis also, a similar function can be expected. Further it can be stated that lipids being one among the sources of energy for the cell maintenance and function, can actively participate in the metabolic activities of the tissues of the normal and regenerating tail of house lizard, H. flaviviridis.