

CHAPTER 8

HISTOCHEMICAL LOCALIZATION OF NUCLEIC ACIDS IN THE
NORMAL AND REGENERATING TAIL OF THE HOUSE
LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

The changes in nucleic acids during the development of chick (Novikoff and Potter, 1948; Reddy et al., 1952; Marrian, 1956; Davidson et al., 1950; Gluck et al., 1964) and amphibia (Sze, 1953; Grant, 1958; Bieber et al., 1959; Lovtrup, 1959; Brown and Caston, 1962) have drawn much attention during recent years. The ribonucleic acid concentration in the regenerating Urodele limbs is reported to be higher than that noted in the normal ones (Ide-Rozas, 1936; Jacovleva, 1943; Roskin and Kharlova, 1943; Clement-Noel, 1944; Brachet, 1950 and Bodemer, 1962). These suggested an association of nucleic acids with increased metabolism, cell growth and their importance in the processes of protein synthesis. The present investigation was undertaken to study the localization and concentration of nucleic acids in the tissues of the normal and regenerating tail of the house lizard, Hemidactylus flaviviridis and their role in regeneration.

MATERIALS AND METHODS

The normal and regenerating 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 tails were then kept on a microtome chuck in a cryostat maintained at -20°C , and sectioned at 5μ . The sections were fixed in Carnoy's fluid or in 10% neutral formalin for two hours and processed for the histochemical demonstration of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) according to the Methyl Green-Pyronin Y method of Kurnick (1955) as described by Pearse (1960).

OBSERVATIONS

NORMAL TAIL:

The beta cells of the old generation of the epidermis did not show either of the nucleic acids. The rest of the cellular layers of the epidermis viz. alpha cells of the old generation, the cells of the stratum intermedium, beta and alpha cells of the new generation and the cells of stratum germinativum showed the presence of RNA in the cytoplasm and DNA in the nuclei (Fig.1). Since the nucleus occupied a large part of the cells of stratum germinativum the cytoplasm was comparatively less and it appeared that the RNA was relatively low. The cells of the beta and alpha layers of the new generation had considerably high content of RNA, whereas, that in the alpha cells of the old generation was slightly low and it was considerably less in those of stratum intermedium. The RNA reaction in the connective

tissue strands of the epidermal basement membrane was extremely low, but in the dermis the spindle shaped nuclei of the connective tissue fibrocytes showed DNA (Fig.1). In the dermal connective tissue fibres and cells, the epidermal basement membrane, the fascia and the adipose tissue, the concentration of RNA was very poor but the nuclei contained DNA.

The muscle tissue responded quite intensely for both the nucleic acids, RNA in the sarcoplasm and DNA in the nuclei (Fig.2). In the caudal vertebra, the nuclei of osteocytes, osteoclasts, osteoblasts, chondrocytes and the marrow cells showed fairly high DNA content whereas RNA was found to be quite low in the cytoplasm of the bone cells but high in the cells of the intervertebral cartilage and marrow cells (Fig.3).

Since the localization is nuclear, it was noted that DNA concentration was more in the grey matter than in the white. So also the RNA which was localized in the cell cytoplasm, was observed in greater concentration in the grey matter than in the white (Fig.4).

REGENERATING TAIL:

Wound healing phase:

The wound epithelium showed a concentration of RNA and DNA similar to that seen in the stratum germinativum

of the original tail stump. A uniformly distributed cytoplasmic RNA and nuclear DNA was observed in the subepithelial cells viz. lymphocytes, microglial cells, melanocytes, erythrocytes and macrophages (Figs.5 & 6).

Preblastemic phase:

The epithelium became stratified and a high concentration of RNA was noticed in all the cells of the epithelium. Accumulation of different types of cells as stated above were noted in the subapical region which showed uniform concentration of RNA in the cytoplasm and DNA in the nuclei.

Blastemic phase:

During this phase the cells of the stratified epithelium over the blastemal cone showed high concentration of RNA in the cytoplasm and DNA in the nuclei. The mesenchyme cells of the blastema showed high concentration of RNA and DNA (Figs. 7 & 8). The blood cells in the blastemal cone showed low RNA concentration similar to the one noticed in the blood cells under the wound epithelium.

Late blastemal phase:

During the late blastemal phase the epidermis over the regenerate was almost fully differentiated. All other

tissues had just started differentiating at the base of the regenerate, adjacent to the stump tissue. The epidermal cells appeared to possess a high RNA content which was similar to the concentration noticed earlier. However, the basal cells of ^{stratum} germinativum had comparatively more RNA than the cells of the same layer in the epidermis of the original tail skin. The differentiating mononuclear myoblasts, chondroblasts and the connective tissue fibrocytes showed a high concentration of RNA in their cytoplasm (Fig.9). The undifferentiated mesenchyme cells continued to show high RNA as well as DNA concentration.

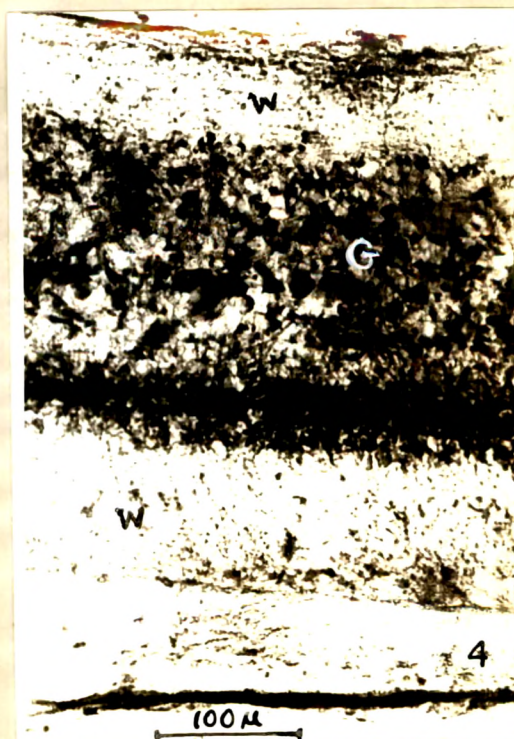
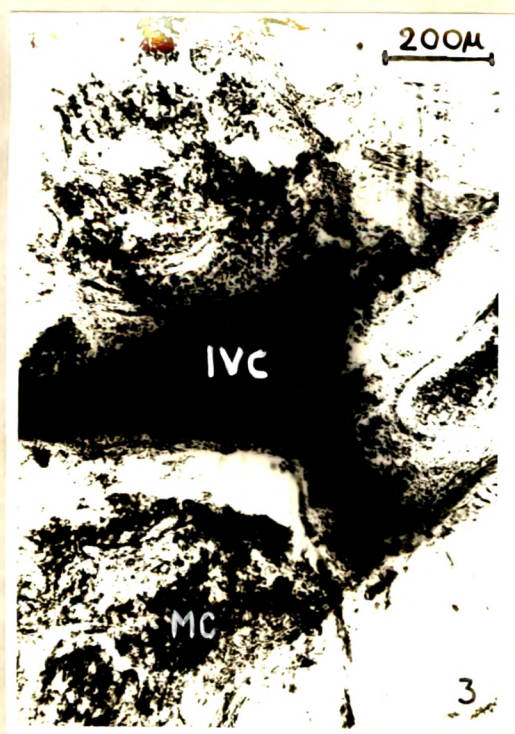
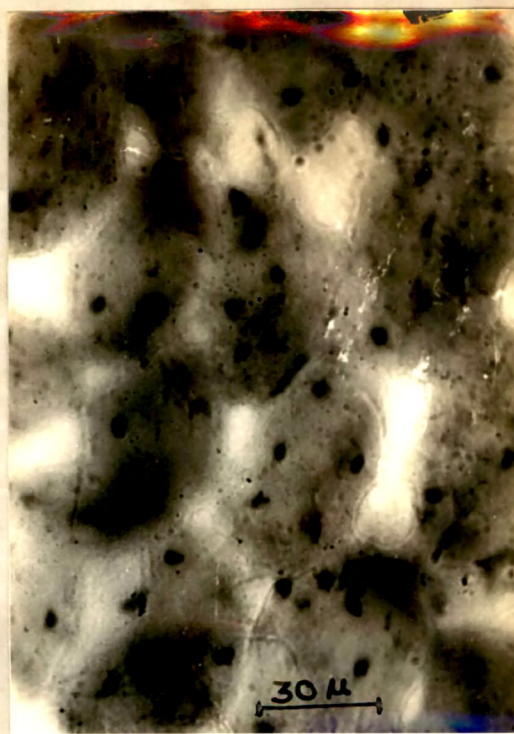
Differentiation phase:

The intensity of RNA and DNA in the epidermal cells during the differentiation phase was similar to that noticed in these cells at the late blastemal stage. However, the inward growth of the epidermis into the dermis, in order to begin the scale formation was noticed and at this phase RNA concentration in the cells of the stratum germinativum was very high (Figs. 10 & 11). Once this stage was over there was a decline in the concentration of RNA in the cells of stratum germinativum. The basement membrane, the connective tissue fibres of dermis and fascia which connect the dermis with muscle showed a low but perceptible RNA ^{Content} which remained ^{the} same even when the regenerate was fully grown (Fig.11). All the different stages during myogenesis, viz. the

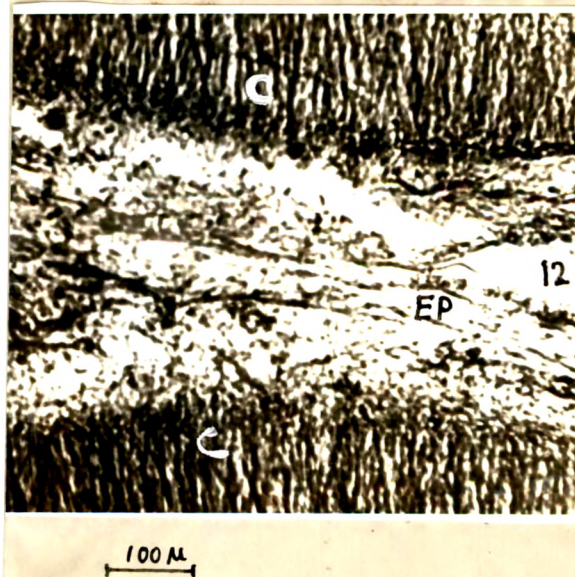
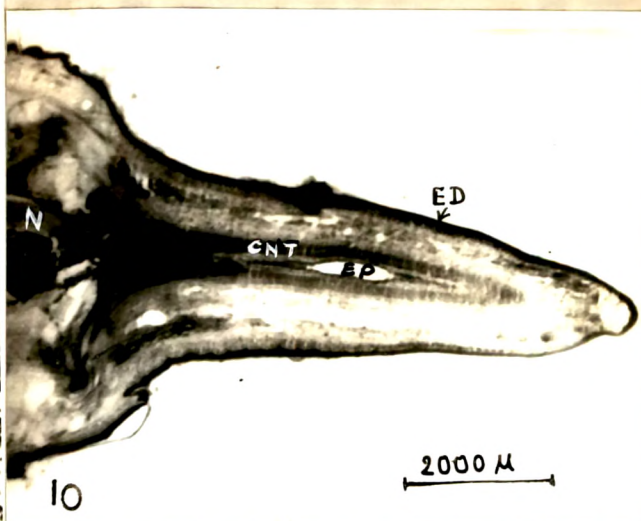
mononuclear myoblasts, myocytes and myofibres revealed a uniform cytoplasmic RNA and nuclear DNA distribution. In the submuscular adipose tissue, the fat cells and the connective tissue fibres showed both RNA and DNA. The cartilagenous neural canal also contained considerable RNA in the cytoplasm of the chondroblasts and chondrocytes (Fig.12). DNA was seen in the nuclei. The ependymal tube showed a uniformly high RNA response in the cytoplasm of the glial cells and along the fibres, whereas, DNA was observed in the nuclei (Fig.12).

Growth phase:

The general appearance of the epidermis of the regenerate became ^{the} same as that in the original tail skin. The RNA distribution and its concentration, which was quite high during the early phases of epidermis formation, reached the same level as found in that of the normal tail skin. During myogenesis also, there was an initial increase in the RNA but later when the growth phase started it decreased and ultimately reached the same level as noticed in the muscle fibres of the normal tail. The connective tissue fibres, fibroblasts of the dermis, fascia and adipose tissue showed no change in the RNA concentration. The cells of the cartilagenous neural canal were seen to possess a very high quantity of RNA even after the tail was fully grown.







EXPLANATIONS FOR FIGURES

- Fig. 1. L.S. of normal tail skin showing RNA in the cytoplasm of the cells of alpha layer, stratum germinativum of epidermis and along the connective tissue fibres of the dermis. DNA is seen localized in the nuclei of the cells of different layers of the skin.
- Fig. 2. T.S. of caudal muscle fibres showing RNA in the sarcoplasm and DNA in the nuclei.
- Fig. 3. L.S. of vertebral column at the intervertebral junction showing high concentration of RNA and DNA in the cells of the intervertebral cartilage and marrow cells.
- Fig. 4. L.S. of the spinal cord showing high concentration of RNA and DNA in the cells of grey matter.
- Fig. 5. L.S. of the regenerating tail (wound healing phase). Note RNA and DNA in wound epithelium and subapical cells.
- Fig. 6. Portion of Fig.5 enlarged showing RNA and DNA in the wound epithelium and subapical cells.
- Fig. 7. L.S. of the blastema showing RNA and DNA in the stratified epithelium and the mesenchyme cells.
- Fig. 8. Portion of Fig.7 enlarged showing RNA and DNA in the epithelium and mesenchyme cells.
- Fig. 9. L.S. of regenerate (early differentiation phase) showing RNA and DNA in the epidermis, myoblasts and fibroblasts of the dermis and submuscular connective tissue.
- Fig.10. L.S. of the regenerate (differentiation phase) showing RNA and DNA in different tissues, with its high concentration in the epidermis and chondrocytes.
- Fig.11. L.S. of the regenerate showing high RNA in the epidermal cells and the myocytes (note the early stage of scale formation).

Fig.12. L.S. of the regenerate passing through the ependyma and the cartilagenous neural canal. Note high concentration of RNA in the chondrocytes and moderate concentration of RNA in the ependyma.

ABBREVIATIONS

α	-	Alpha cells (old generation)
β	-	Beta cells (old generation)
C	-	Chondrocytes
CNT	-	Cartilagenous neural canal
D	-	Dermis
D ₁	-	Dermis in the regenerate
ED	-	Epidermis
EP	-	Ependyma
F	-	Fibroblasts
G	-	Grey matter
IVC	-	Intervertebral cartilage
M	-	Muscle
MB	-	Myoblasts
MC	-	Myocytes
MS	-	Mesenchyme cells
N	-	Nerve cord (spinal cord)
SAC	-	Subapical cells
SET	-	Subepithelial cells
SG	-	Stratum germinativum
V	-	Vertebral column
W	-	White matter
WE	-	Wound epithelium

DISCUSSION

There are two histological phases of the skin epidermis - the resting and the moulting phases. In the resting epidermal cells, the RNA concentration in the cells of the alpha layer and the stratum germinativum was considerably high while the cells of the beta layer contained no RNA. This is in conformity with the high protein synthesis by the alpha cells for keratinization and that by the cells of stratum germinativum for the growth which would enable active cell division prior to moulting. Once the cells of the stratum germinativum started dividing and gave rise to cells of the stratum intermedium and the new generation of beta and alpha cell layers, the RNA concentration in these cells decreased but that in the newly formed beta and alpha cells and in the stratum intermedium it was very high. The high concentration of RNA in the latter regions suggests that it must be for a higher rate of protein synthesis for growth and keratinization of beta and alpha cells and for growth and maintenance of the cells of the stratum intermedium. At this phase, these cells belonging to the old generation which were pushed above the stratum intermedium, showed low concentration of RNA in the alpha cells and ^{hi} nil in the beta cells. At the onset of moulting, the cells of stratum germinativum began disintegrating but prior to

this the RNA concentration in them was considerably reduced which indicated a regressive metabolism in these cells. Ultimately when they started disintegrating, they were devoid of RNA. Thus, the varied concentrations of RNA in the epidermis signifies the differences in the metabolic state of these cellular layers.

The concentration of nucleic acids in the muscles, bone cells, cartilage cells and the marrow cells was a little higher than that in the connective tissue fibres and the cells of the adipose tissue. Since there is a constant protein synthesis in the osteocytes and chondrocytes for the formation of the matrix, the RNA level was high in these cells. The high RNA content observed in the marrow cells denotes probably cell division and protein synthesis for the formation of new blood cells.

Bodemer (1962) reported no change in RNA concentration in the wound epithelium but as the differentiation of epidermis occurred, there was an increase in the level of the nucleic acid which however never exceeded the RNA level of the blastemal cells. In the regenerating tail of the house lizard, H. flaviviridis our observations showed that there was^{0.5} considerably high concentration of RNA in the wound epithelium which remained so even during the differentiation phase of the epidermis. The epithelial cells give rise to the stratified epithelium by a series

of cell divisions which later differentiated into the epidermis.

A high content of RNA in the blastema have been reported by several workers (Ide-Rozas, 1936; Jacovleva, 1943; Clement-Noel, 1944; Brachet, 1950). Similarly, in the blastemal cells of the regenerating lizard tail a high level of RNA concentration was observed. This high concentration of RNA can be correlated with higher mitotic activity and also higher rate of growth of the blastemal cells which requires ^ahigh protein synthesis.

Jacovleva (1943), Roskin and Kharlova (1944) and Laufer (1960) have showed an increase in the nucleoprotein content of the blastemal cells immediately prior to their differentiation. A further increase in the RNA was observed in the blastemal cells which showed the differentiation into mononuclear myoblasts, chondroblasts and ependymal cells. Of these the condensation of the chondroblasts was first to occur and there the first increase in RNA was noticed. Later the myoblasts acquire high RNA concentration. The localization was cytoplasmic and more near the nuclear border. As the differentiation progressed in some tissues there was a fairly high amount of RNA present in the myocytes and myofibres but when the myofibres started growing the RNA level decreased and

finally reached more or less the same level noted in the normal tail muscle fibres. As far as chondrogenesis was concerned the high level of RNA was regained in the chondroblasts and the chondrocytes of the cartilagenous neural canal. However, a little, but appreciable fall in the level of RNA was noticed in the chondrocytes compared to that in the chondroblasts. It may be noted that RNA is involved in protein synthesis to give rise to the collagen fibres of the matrix and for the proliferation of the chondrocytes.

The tail regeneration is a continuous process, though arbitrarily different morphological phases are recognised. It is quite logical to refer the RNA content in relation to the different histomorphological stages of the regenerating tail tissues. Thus it is suggested that the high concentration of RNA in the mesenchyme cells of the blastemal and differentiation phases of regenerating lizard tail is mainly due to the increase in their protein synthesising nature and high cell proliferation. The high RNA concentration during the differentiation phase is correlated with the growth of the differentiated cells.