CHAPTER 6

HISTOCHEMICAL STUDIES ON THE NUCLEIC ACIDS (DNA AND RNA) IN THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

Nucleic acids usually occur as nucleoproteins in conjugation with a basic protein such as histone or protamine. Being the fundamental genetic substance, DNA is capable of duplicating itself. It also serves as the template on which the RNA molecules are synthesized. The functions of nucleic acids in regeneration appear to be essentially the same as during ontogenesis (Brachet, 1947, 1950a, b).

The earliest cytochemical studies on nucleic acids in amphibian regeneration (Yakovleva, 1943a,b; Clement-Noél, 1944; Roskin and Karlova, 1944) were mainly of the RNA distribution. Litwiller (1939) had investigated nuclear DNA indirectly on the basis of its chromosomal association during the mitotic activities in regeneration. But the recent investigations at the molecular level with the aid of tritiated thymidine (Hay and Fischman, 1961; O'steen and Walker, 1961; Riddiford, 1960) have yielded clues of the existence of an association of nucleic acids

with increased cell metabolism, growth and protein synthesis.

Apart from the above mentioned reports none others are available regarding the distribution of DNA and RNA in the regenerating tissues, especially the reptilian ones. The only investigation on this line is that of Shah and Chakko (1972) who in their studies have followed the distribution pattern of DNA and RNA during regeneration in the tail of the house lizard, Hemidactylus flaviviridis. In the wake of the scarceness of literature and as a follow up of the work of Shah and Chakko (1972) it was thus deemed fit to investigate histochemically the distribution of DNA and RNA in another lizard, Mabuya carinata, also exhibiting excellent power of regeneration and belonging to the family Scincidae.

MATERIAL AND METHODS

The adult Mabuyas, maintained in the laboratory on a diet of insects were used as the experimental animals. The autotomy of the tail was induced as described in Chapter 1. The cut surfaces of the normal and regenerated tail pieces were blotted to remove

blood and tissue fluids and were fixed on a microtome chuck of a cryostat maintained at -20°C. Sections of 12-18 μ thickness were cut, mounted on a slide without any adhesive and fixed in Carnoy's fixative for two hours. After fixation, the sections were washed thoroughly in distilled water.

The histochemical demonstration of Ribonucleic and Deoxyribose nucleic acids (RNA and DNA) were carried out according to the Methyl green Pyronin-Y method of Kurnick (1955) as cited by Pearse (1960). The staining for DNA and RNA were carried out separately to have a clearcut localization of the nucleic acids. Identical sections treated with 4% Trichloroacetic acid (TCA) at 90°C for five minutes prior to staining served as suitable controls. Both the sample and control sections were dehydrated in alcohol and mounted in canada balsam.

OBSERVATIONS

NORMAL TAIL (Fig.1)

In the skin of the normal tail of the lizard the outer beta cell layers of the epidermis were negative for both DNA and RNA. However, the cells of the alpha

layer, stratum germinativum and fibrocytes of the dermis showed DNA and RNA in their nuclei and cytoplasm respectively. The muscles responded quite intensely for both the nucleic acids. The submuscular adipose tissue showed DNA and RNA in their peripherally situated nuclei and cytoplasm respectively. The caudal vertebral marrow cells and the osteocytes also showed fairly high concentrations of DNA and RNA. In the spinal cord, unlike the white matter, the grey matter showed a uniform distribution of both DNA as well as RNA.

REGENERATING TAIL

Wound healing phase: (Figs. 2 and 3)

The newly formed epithelium at the wound surface showed increased concentration of DNA and RNA in comparison to the adjacent stump epidermis. Uniformly distributed cytoplasmic RNA and nuclear DNA were observable in the subepithelial cells below the wound epithelium.

Preblastemic and blastemic phases: (Fig.4)

On the whole, the blastemic phase was found to depict ~~an~~ increased nucleic acid contents than the

EXPLANATIONS FOR FIGURES

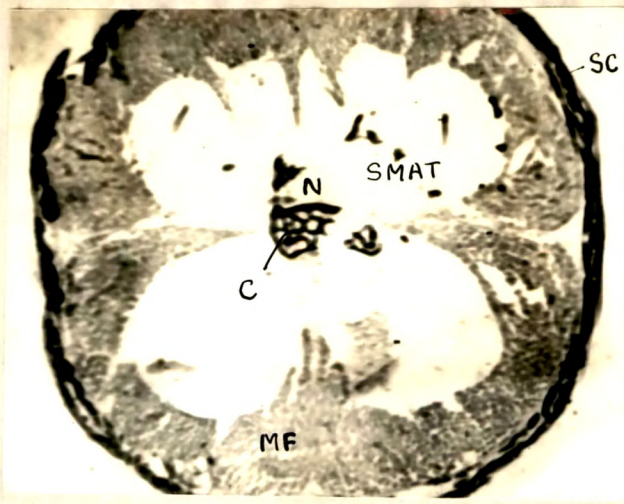
Fig. 1. Transverse section of the normal tail showing the RNA distribution. Note the RNA in the caudal muscles.

Fig. 2. Longitudinal section of the tail regenerate during the wound healing phase. Note the high RNA content in the wound epithelium.

Fig. 3. Magnified region of the wound epithelium revealing DNA in the wound epithelium and the subapical region.

ABBREVIATIONS

C	-	Centrum
CEM	-	Cut end of the muscle
CEV	-	Cut end of the vertebra
MF	-	Muscle fasciculus
SAR	-	Subapical region
SC	-	Scale
SCB	-	Scab
SMAT	-	Submuscular adipose tissue



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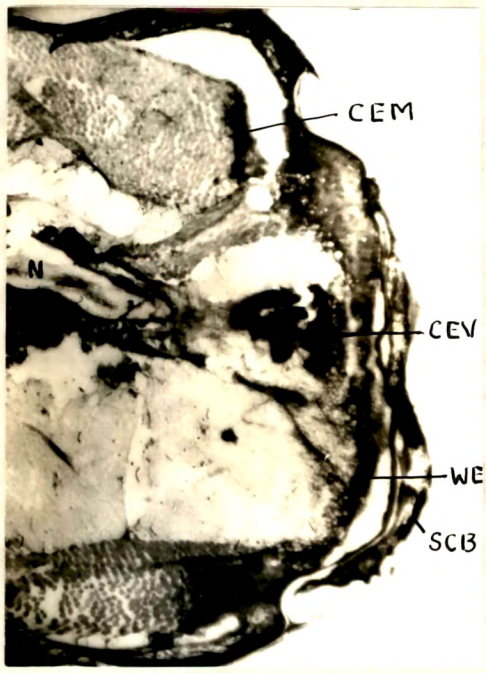

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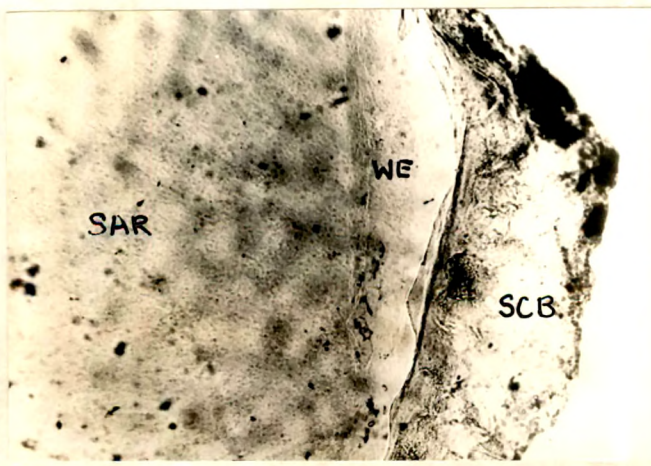

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EXPLANATIONS FOR FIGURES

Fig. 4. Photomicrograph of the tail regenerate at the blastemic phase revealing the RNA content in the mesenchymal cells and the blastemic epithelium.

Fig. 5. Longitudinal section of the tail regenerate during the early differentiation phase. Note the RNA content in the differentiating muscles, and the chondrocytes of the cartilagenous neural canal.

ABBREVIATIONS

BE - Blastemic epithelium

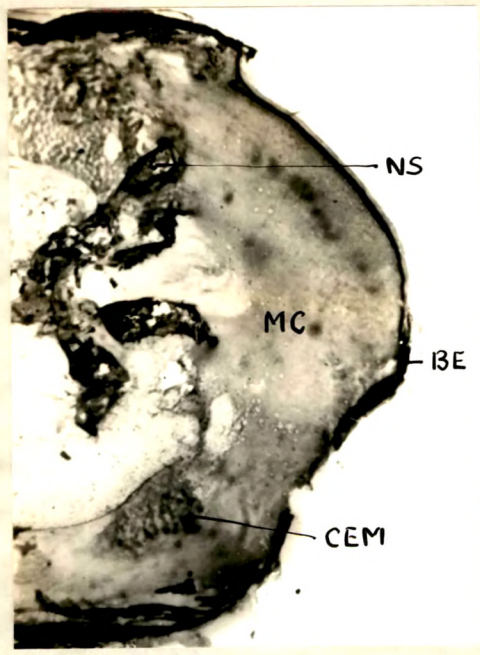
CEM - Cut end of the muscles

CHB - Chevron bone

CNC - Cartilagenous neural canal

MC - Mesenchymal cells

NS - Neural spine



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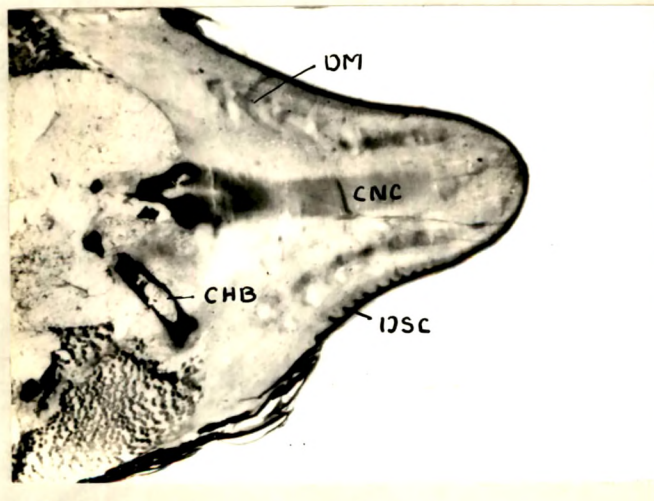

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EXPLANATIONS FOR FIGURES

Fig. 6. Photomicrograph of the longitudinal section of the tail regenerate during the differentiation phase revealing the RNA content in the various differentiating tissues. Note the high content in the cartilagenous neural canal, the differentiating scales and the differentiating muscles.

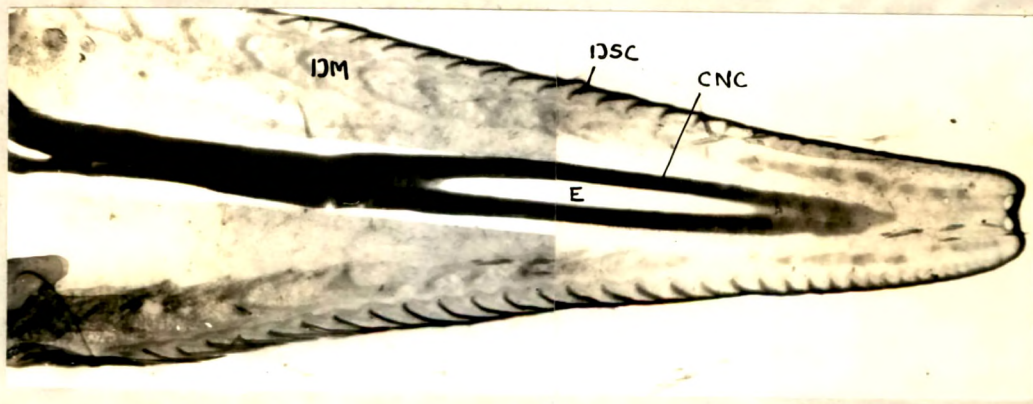
Fig. 7. Magnified region of the differentiating scales revealing the DNA content. Note the DNA content in the differentiating muscles and the dermal region.

ABBREVIATIONS

CNC - Cartilagenous neural canal

DM - Differentiating muscles

DSC - Differentiating scales



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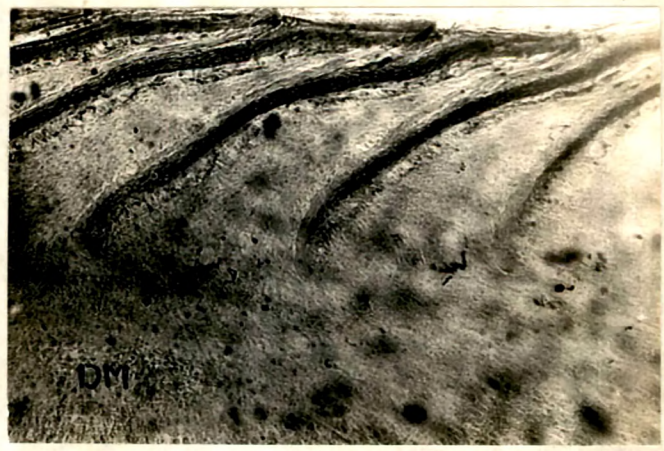

200 μ

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wound healing phase with both the stratified epithelium and the underlying mesenchymal cells responding with equal intensity.

Differentiation phase: (Figs. 4,5,6 and 7)

The intensity of RNA as well as DNA in the epidermal cells during the differentiation phase was almost similar to that which was noticed in the blastemic phase. During myogenesis, however, there was an increased concentration of both the nucleic acids, but on completion of myogenesis the myofibrils demonstrated a reduced content than the myoblasts and myocytes. The differentiating fibroblasts in the dermis region showed a high concentration of RNA. Whereas the differentiating submuscular adipose tissue displayed a uniform distribution of RNA and DNA; both the nucleic acids were found to be high in the chondrocytes of the cartilagenous neural canal with a very poor content in the ependyma.

Growth phase:

During the growth phase there was a slight decrease in the nucleic acid levels in almost all the growing tissues except in the chondrocytes of the cartilagenous

neural canal. As the growth progressed, a gradual decrease in the nucleic acid contents was noticed in all the tissues which finally reached a level in the fully regenerated tail identical to that of the normal one.

DISCUSSION

The presently reported DNA as well as RNA in the alpha cell layer and the stratum germinativum of the epidermis of Mabuya carinata is in agreement with the recent reports of Shah and Chakko (1972) in the house lizard, Hemidactylus flaviviridis. A correspondence between acid phosphatase activity and protein synthesis as been suggested by Vorbrodt (1958). Since the stratum germinativum layer is highly acid phosphatase active (Chapter 4; Schmidt, 1963a) and as the nucleic acids are also found to be localized in it, it could be safely assumed that they are of significance in the synthesis of protein as well as chromatin material required for active cell proliferation. The similar high concentration of the nucleic acids, specially RNA, in the outer alpha cells and concomitant presence of high acid phosphatase activity in these cells could be correlated with the

active keratin formation that leads to changing the alpha cells into beta ones, when the ecdysis takes place. The present suggestion gains validity by the reported presence of Glucose-6-phosphate dehydrogenase in the epidermis of Mabuya carinata (Ramachandran, 1972) and Hemidactylus flaviviridis (Magon, 1970).

The increased levels of nucleic acids observed herein in the wound epithelium might be correlated with the increased need of protein for the actively dividing epithelial cells which are simultaneously getting stratified. It could be noted in this connection that Brachet (1955, 1960) has suggested the existence of a close relationship between nucleic acids and protein synthesis. Further, Ramachandran (1972) and Magon (1970) have also suggested increased nucleotide metabolism during the wound healing phase based on their studies on Glucose-6-phosphate dehydrogenase (G6PDH) in the regenerating tail of Mabuya carinata and Hemidactylus flaviviridis respectively. Similar increase in the level of nucleic acids at the wound healing site have been reported by a number of workers (Clement-Noël, 1944; Roskin and Karlova, 1944; Tsanev, 1951; Washburn, 1954; and Shah and Chakko,1972).

During the blastemic phase of the tail regeneration, there was a marked increase in the DNA as well as RNA content. This becomes clear in the light of the reported concomitant increase in the G6PDH activity at this phase (Ramchandra[a,b]n, 1972; Magon, 1970; Schmidt and Weidman, 1964) as the HMP shunt pathway serves as the principal source of pentose sugars much needed for the synthesis of nucleosides, nucleotides and nucleic acids. A high content of RNA in the blastema has been reported by several workers (Ide-Rozas, 1937; Yakovleva, 1943a,b; Clement-Noel,1944; Brachet, 1930a; Shah and Chakko, 1972; and Schmidt, unpublished) in various regenerating systems. During this phase of active cellular proliferation the hydrolytic enzyme, acid phosphatase was also found to be very active in the regenerating tail of Mabuya carinata (Chapter 4).

A similar correlation between the increased activity of acid phosphatase and RNA was also reported by Schmidt (unpublished) in the forelimb of Diemictylus viridescens. A close relationship between the amount of RNA and acid phosphatase activity has been proposed

in the nerve cells for the release of phosphate ions for cell maintenance and function (Bodian and Mellors, 1944; Lavelle et al., 1954). Spiegelman and Kamen (1946) and Brachet (1950a) suggested that nucleoproteins may be used as a phosphate donor in protein synthesis. The present high concentration of RNA could be correlated with the high necessity of newly synthesized proteins for the mitotically active blastemal cells.

A peak concentration of DNA and RNA during differentiation is indicative of both a multiplication of genetic material as well as a high rate of protein turnover for the actively dividing and differentiating cells respectively. Similar suggestions regarding the high protein output during differentiation has been arrived at by Ramachandran (1972) and Magon (1970) by their observations of peak G6PDH activity in the tail of Mabuya carinata and Hemidactylus flaviviridis respectively. It is interesting to note here that the correlation observed in the present work between the parallel increase in nucleic acid content and alkaline phosphatase (Chapter 5) gains significance when viewed in the light of the suggestion that in the early developing nervous system, the alkaline phosphatase is

concerned with phosphate transfer in DNA metabolism (Rogers, 1960). Increased content of nucleic acids in the chondrocytes and dermal fibrocytes is quite probably related to the collagen synthesis for the matrix of the cartilagenous neural canal and the connective tissue at the dermis region.

With the completion of the hectic proliferative and differentiative phase the reduced metabolic activity of the various tissues is well exemplified by the decreasing content of nucleic acids, all throughout the growth phase. The gradual decline finally, with the attainment of physiological and morphological maturity of the fully regenerated tail got set the original level observed in the normal tail.

Thus it could be surmised that the high concentrations of RNA as well as DNA in the mesenchymal cells of the blastema and the differentiating tissues is mainly due to the high requirements of new protein molecules essential for the highly proliferating and differentiating cells during these phases of tail regeneration.