Histological and Ultrastructural Study on the Effect of Vitamin A on the Regenerating Tail Fin of the Teleost Fish, *Oreochromis niloticus*

Atta, K.I.[1]; Abdel-Karim, A.E[2] and Elsheikh, E.H.[1]

[1]Department of Zoology, Faculty of Science, Zagazig University, Egypt.
[2]Department of Zoology Faculty of Science, Alexandria University, Egypt.
karimaatta29@yahoo.com

ABSTRACT: The present work was performed to show how vitamin A affects the regeneration of the tail fin of *Oreochromis niloticus*, investigated by light and electron microscopy. In this study at the second day postamputation the apical cap is well established at the regenerating tail fins treated with 8.I.U./mL of VA compared to the regenerating tail fins treated with 2 and 4 I.U./ml of VA. In the third day the blastema was well established. At the fifth day in the regenerating tail fins treated with 2I.U./ml of VA the bones become first visible at their distal tips, but in the others treated with 4 and 8 I.U./mL of VA the bones (lepidotrichia) are formed in the form of two parallel rows of lepidotrichia. In the seventh day, the segmentation started in the proximal region of the regenerating lepidotrichia and in the 15th day the fin ray is well established and restored the original form in the regenerating tail fins which treated with 8.I.U./mL of VA. By the 45th day postamputation the fin grows and reaches to the original length. It is also noticed that in the ultrastructural studies, the effect of vitamin A on the regenerating tail fins treated with 8 I.U./mL of VA , the cell density of blastema cells increases indicating an increase in their mitotic activity. Their number is much higher than that observed in those fins treated with 2 & 4 I.U./mL of VA. In the tail fins treated with 8 I.U./mL of VA, the formation of fiber connections between the lepidotrichia forming cells are much more pronounced than that observed in 2 &4 I.U./mL of VA treated cases.

[Atta, K.I.; Abdel-Karim, A.E and Elsheikh, E.H. **Histological and Ultrastructural Study on the Effect of Vitamin A on the Regenerating Tail Fin of the Teleost Fish, *Oreochromis niloticus*.** *Life Sci. J.* 2013; 10(2):1492-1508]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 203

Key words: regeneration, fin, teleost, vitamin A.

1.Introduction

Stages of caudal fin regeneration have been described (**Becerra *et al*.,1983; Géraudie and Singer, 1992; Johnson and Weston, 1995; Poss *et al*., 2000 and Abdel-Karim *et al*., 2003**). The wound is closed by a thin layer of epithelium within 24 hours of amputation. The blastema, a mass of mesenchymal cells thought to be pluripotent, is formed within 4 to 5 days postamputation. The blastema cells are formed through the process of dedifferentiation (**Abdel-Karim *et al*., 2003**). The onset of the next phase, regenerative outgrowth, is marked by deposition of new bone. It has been postulated that distal blastemal cells proliferate, while proximal blastemal cells differentiate into missing structures. Although, it has been assumed that blastemal cell proliferation is critical to regeneration, blastemal cell cycle properties during regenerative outgrowth or blastema formation have not been characterized. The cells of the fin blastema seem to maintain a memory of their position of origin, since only the missing part regrows, that the positional memory of the blastema can be respecified by treatment with the retinoid vitamin **A** (**VA**) and its derivatives (**Bryant and Gardiner, 1992**).

Recently, it was discovered and confirmed that retinoids may alter frequently with a predictable manner the positional information of the blastema cells in regenerating amphibian limbs(**Bryant and Gardiner, 1992; Michael *et al*., 1994$_{a\&b}$**) and in developing chick limbs (**Johnson and Scadding, 1991; Rowe *et al*., 1991 & Muneoka *et al*., 1992**). Actually, **Niazi and Saxena (1978),** were the pioneers in this respect since they described that vitamin A induces proximo-distal (PD) duplication in the regenerating limb of *Bufo andersonii* tadpoles.

Vitamin **A** is a low molecular weight, lipophilic molecule that acts on the nucleus to induce gene transcription. In amphibians and mammals, it induces the regeneration of several tissues and organs; it induces the "super-regeneration" of organs that can already regenerate such as the urodele amphibian limb by respecifying positional information in the limb. In organs that cannot normally regenerate such as the adult mammalian lung, vitamin A induces the complete regeneration of alveoli that have been destroyed by various noxious treatments. In all these cases, **VA** is required for the development of the organ (**Maden and Hind, 2003 & 2004; Bockelmann *et al*., 2010&Tu and Johnson, 2011).**

The present work deals with the study on the effect of vitamin A on histological and ultrastructural aspects of the regenerating tail fin of *Oreochromis niloticus*.

2.Material & Methods

The present investigation was performed on the freshwater teleost *Oreochromis niloticus*. 200 Specimens of the fish were collected from El-Abbasa fish farm, near Zagazig city. The body length ranged from 4.6 cm to 10.3cm and the weight from 3.8gm to 19.3gm. The fishes were placed into aerated aquaria under conditions of room temperature about 27°c±3°c. The photoperiod was 12 hours of light per day. Prior to fin amputation, fishes were anaesthetized with MS222(ethylaminobenzoate methane sulfonate) dissolved in tap water (1mg/L). Tail fins were amputated with microscissors at proximal level, removing 70% of the fin.

Preparation VA palmitate

Fishes were divided randomly into two groups. The first group (Control) was exposed to a water medium containing 1ml/L (ethyl alcohol).The second group was exposed to three different concentrations of **VA** palmitate. A rearing medium containing **VA** palmitate at the definitive concentration has been previously prepared as follows: an ampule of **VA** palmitate containing 1ml of vitamin A (equivalent to 300.000I.U.) was dissolved in 1ml of a absolute ethyl alcohol in a test tube. After shaking well to ensure complete dissolving, the content was transferred to a dark bottle and completed to 500ml with tap water. With great care the bottle was enveloped in a sheet of aluminium foil paper and kept in a refrigerator to be used at the time of operation.

Three different concentrations of the **VA** were prepared for the present study using tap water; 2 I.U./ml. ,4 I.U./ml. and 8 I.U./ml. Immediately after amputation of the tail fins, the operated fishes were transferred to water medium for recovery (about 5minutes), then gently to the desired concentrations of **VA** where they were kept there for three days. The medium was renewed the concentrations in the second and third day in order to maintain proper concentrations of the vitamin. For each experimental type, after treatment with the vitamin, the fishes were allowed to continue their development in normal tap water.

Histological Studies

In ***Oreochromis niloticus,*** the tail fins were amputated through the proximal level to investigate the histological structure of the control fins as well as the histological changes of the treated fins during the regeneration process. Both fixation and decalcification of the entire fins were achieved by fixating individual samples in aqueous Bouins fluid for 24h. After fixation, the samples were decalcified in solution of 2% acid alcohol (nitric acid +70% alcohol), then dehydrated and infiltrated with paraplast wax. Serial longitudinal sections of 5-7 µm thick were prepared, and stained with Haematoxylin and Eosin, as shown by **Drury & Wallington (1980)**.

Ultrastructural Studies

Transmission Electron Microscope

The regenerating fin used in this study were removed on the blastema stage and early redifferentiation stage. The regenerated parts were removed from their stumps, and were fixed by immersion in 2.5% glutraldehyde in 0.1M cacodylate buffer at Ph 7.2 for 2 hours, **(Gupta and Berridge, 1966),** washed in cacdoylate buffer for 30 minutes, post fixed in 1% osmium tetroxide with the same buffer at 4c° for one hour and washed again. The specimens were then dehydrated in ascending ethanol series and finally embedded in Araldite **(Luft, 1961)**. Tissue blocks were sectioned using Jeol and Reichert ultramicrotome and glass knives. Thick plastic sections were obtained, stained with 1% toluidine blue in 1% borax **(Trumpt *et al.,* 1961)** and were examined by a light microscope to define the desired area for ultrastructual investigation. Ultrathin sections were mounted on uncoated copper grids and double stained with aqueous urinyl acetate **(Stempack & Ward, 1964)** for 10 minutes and lead citrate **(Reynolds, 1963)** for 2 minutes. Grids were examined in a transmission electron microscope (**JEOL JEM-100 CX)**. Photomicrographs of sections in the different regions of regenerated fins were prepared to demonstrate the ultrastructure of the blastema cells as well as the early redifferentiating cells in the regenerating fins.

3. Results

I- Histological Studies:

Histological Structure of the Normal (Unamputated) Tail Fin:

The tail fin consists of the epidermis, dermis, connective tissue and the skeletal elements (lepidotrichia). The lepidotrichia extend like fingers from the base of the fin nearly to its margin (Fig.1). The lepidotrichia are made up of pairs of segmented hemirays facing each other and occupy a subepidermal position. Bone of both hemirays is subdivided longitudinally into segments separated by non-ossified “joints”. Most of the lepidotrichia form a few forks or dichotomies along the proximo-distal axis of the fin. The space between the two hemirays was filled with connective tissue, nerves and blood vessels. Each ray end distally with a row of rigid but unmineralized elastoidin fibrils named actinotrichia.

Effect of Vitamin A on the Histogenesis of the Regenerating Tail Fins:

The regeneration process has been divided histologically into five different stages: wound healing stage, dedifferentiation stage, blastema formation stage, early redifferentiation stage and late redifferentiation and growth stage. However, the

process of regeneration is continuous one. There are no discrete limits between those five stages.

2nd day postamputation:

Control fishes: As shown inFig.2a ,the wound surface is covered with an epidermal layer.

Treated fishes with VA: The wound surface is first closed by a thin epithelial layer. This layer becomes thickened to form an apical epidermal cap, this cap is well established at the regenerating tail fins treated with 8.I.U./mL of VA (Fig.2_d)than in the regenerating tail fins treated with the other two concentration of VA (Fig.$2_{b\&c}$).

3rd day postamputation:

Control fishes: It was noticed that , the wound epidermis is composed of two or three layers of epidermal cells which form an apical epidermal cap(Fig3_a).

Treated fishes with VA: Beneath the epidermal cap, a blastema is formed; which originated from the mesodermal cells. This blastema is composed of a mass of cells or a population of undifferentiated cells. It was obviously noted that, the cell density of blastema in the regenerating tail fins treated with 8.I.U./ml of **VA** is greater than that in the other two lower concentration of **VA** (Fig.$3_{b,c\&d}$).

5th day postamputation :

Control fishes: Dedifferentiation of distally located stump cells continued and gave rise to mesenchyme cells, which accumulated just under the overlying epidermis(Fig.4_a). These dedifferentiated cells had begun cellular proliferation to form the blastema.

Treated fishes with VA: At the beginning, fibroblast like- cells; in particular scleroblasts cells and the connective tissue, entered the space beneath the wound epithelium. Scleroblasts of both hemirays cover the amputation surface. Many of scleroblasts are closely attached to the ray stumps forming a scleroblast cap over the amputation bony lepidotrichia. When the ray formation started, the blastema is structured in a scleroblast cell mass and proliferative activity is observed in the connective tissue and frequently in the scleroblast ray coat. The formation of bones become first visible at the distal tip of the regenerating tail fins as shown in the regenerating tail fins treated with 2 I.U./mL of **VA(Fig.4_b)**. Where as in the regenerating tail fins treated with 4 and 8 I.U./mL of **VA** the bones (lepidotrichia) are formed in the form of two parallel rows of lepidotrichia (Fig.$4_{c\&d}$).

7th day postamputation:

Control fishes: The more well differentiated cells in the proximal region of the blastema are arranged along strip just beneath the epidermis, in close association with the basement membrane. These cells will form the scleroblasts. These scleroblasts form a continuous cellular layer and secrete the extracellular matrix. By that time ,the regenerating bone begins to appear as shown in fig. 5_a.

Treated fishes withVA: In the regenerating tail fins treated with 2 I.U./mL of **VA**, the bone is formed by two parallel rays (lepidotrichia) at the distal tip of the fins and separated from the stump lepidotrichia(Fig.5_b). In the regenerating tail fins treated with 4 I.U./mL of **VA**, the bone is formed from several pairs of parallel hemirays or lepidotrichia, which are separated from each other and also separated from the stump lepidotrichia(Fig.5_c). While in the regenerating tail fins treated with 8 I.U./mL of **VA**, in addition to ray formation, irregular bone deposition above stump rays (stump lepidotrichia) is occurred. These irregular bone are deposits are linked the stump rays to the reformed hemirays (Fig.5_d). It was observed that, the segmentation is started in the proximal region of the regenerating lepidotrichia (closer to the stump).

10th day postamputation:

Control fishes: The formation of the hemirays become first visible at the distal tip of the regenerating fins(Fig.6_a).

Treated fishes with VA: In the regenerating tail fins treated with 2 I.U./mL of **VA**, each parallel pair of lepidotrichia is connected to each other and also to the stump lepidotrichia. At the same time, that parallel pair of bone forming few segmentation(Fig.6_b). It was noticed that, in the regenerating tail fins treated with 4 I.U./mL of **VA**, the lepidotrichia are connected and extended from the stump to the distal tip of the regenerating fins(Fig.6_c).

In the regenerating tail fins treated with 8 I.U./mL of **VA**, the growth (elongation) of the regenerating fins continue by addition of new lepidotrichial segments to the end of the fins (Fig.6_d).

15th day postamputation:

Control fishes: It was found that, the lepidotrichia are developed and have begun to show segmentation(Fig.7_a).

Treated cases with VA: Later, the growing lepidotrichia became separated from the overlying by the invasion of the scleroblasts which moved in around the side of hemisegment. At this stage, the regenerating hemisegment is completely surrounded by a continuous layer of scleroblasts. The more laterally situated scleroblasts, located between the epidermis and the regenerating lepidotrichium, now displayed a reversed polarity and secreted extracellular matrix into the regenerating hemisegment. Thus, these scleroblasts are responsible for the thickening of the hemisegment by a positional growth and the newly synthesized, lateral lepidotrichial layers. The medial and the oldest part

became separated from the lateral, as shown in regenerating tail fins treated with 2 I.U./mL and 4 I.U./mL of **VA(Fig.7 $_{b\&c}$)**. In the regenerating tail fins treated with 8 I.U./mL of **VA**, the lepidotrichia become segmented and increased in the thickness. The fin ray is well established and restored the original form (Fig.7_d).

30th day postamputation:

It was observed that, a longitudinal section of a 30th day old regenerate illustrates the entire morphogenetic pattern of fin regeneration: the distal region resembles the process occurring during the first few days following amputation, while the older portions of the regenerated ray, closer to the stump, are restored nearly to their original form. There is no difference between the control cases and the **VA** treated cases because the fin ray is well established and restored the original form (Fig.$8_{a,b,c\&d}$).

45th day postamputation:

At this time interval growth was a continuous process in the teleost regenerating fin. The fin grows and reaches to the original length. This occurred by addition of ray segments to the end of the fin, rather than by increase in length of established ray segments as shown in fig.$9_{a,b,c\&d}$.

II- Ultrastructural Studies :

Ultrastructural Studies on the Effect of Vitamin A on the Regenerating Tail Fins :

(A) Blastema Cells :

The present study showed that, in the control tail fins at third day postamputation, the blastema cells revealed a heterogenous cell population Within each of these blastema cells (undifferentiated mesenchyme cells), a nucleus with nucleoli , mitochondria and rough endoplasmic reticulum (RER) are recognized, as shown in fig. 10_a.

By the third day postamputation, the blastema cells in the regenerating tail fins treated with 2 I.U./mL of VA, showed the same cell density as in the control blastema, the nucleus with nucleoli and the cytoplasm contains rough endoplasmic reticulumand mitochondria (Fig10_b).

By the same time, it was observed, in the tail fins treated with 4I.U./mL of **VA**, that the blastema cells showed an increase in their number than that found in the control and that treated with 2I.U./mL of **VA** (Fig. 10_c). Within each of these blastema cells, a nucleus, increase in number of mitochondria and rough endoplasmic reticulum, this indicates the increase of the synthetic activity as shown in fig. 10_c.

In the tail fins treated with 8 I.U./mL of **VA**, the cell density of blastema cells increases indicating an increase in their mitotic activity. Their number was much higher than that observed in those treated with 2 I.U./mL and 4 I.U./mL of **VA** (Fig.10_d).

The blastema cells, displayed great number of mitochondria and the rough endoplasmic reticulum, consisting of long parallel cisternae, with higher number of ribosomes indicate the increase of the synthetic activity of these cells as shown in fig. $10_{d\&e}$.

(B) Regenerating Lepidotrichia :

The bony fin rays (lepidotrichia) consists of two parallel, bowed strips of bone disposed bilaterally beneath the epidermis and partially enclosing the soft dermal components of the ray. The bony plates of a lepidotrichium resemble a pair of parentheses, ( ), each member of which can be conveniently called a hemiray. Bone of both hemirays is subdivided longitudinally into segments separated by nonossified "joints".

It was noticed that, there are more well differentiated cells of the blastema cells form scleroblasts (Lepidotrichia forming cells). These scleroblasts formed a continuous cellular layer and secreted the extracellular matrix. The osteoblasts of regenerating lepidotrichia come from preexisting scleroblasts or from multipotential connective tissue cells, which transform into osteoblasts (scleroblasts transform into osteoblasts). The osteoid matrix of a lepidotrichia had begun to calcify. The matrix is formed between the epidermis and underlying osteoblasts. Collagen fibrils are distinctly formed in the matrix. The extensive mineralization are formed in the matrix of the lepidotrichia and collagen fibrils. The mineralizing fibrils fuse into large masses so that the growing mineralizing sites are composed of multiple collagen fibrils.

In the control tail fins at fifth day postamputation i.e, during the early redifferentiation stage, the fibers connection between the lepidotrichia forming cells begin to appear. This is the first sign of Lepidotrichia formation as shown in fig. 11_a.

By the same time, in the tail fins treated with 2I.U./mL of **VA**, the blastema cells are differentiated into Lepidotrichia forming cells (LFCs) and connected to each other by bundle of fibers (Fig. 11_b).

By that time, in the tail fins treated with 4 I.U./mL of **VA**, formation of fibers connections are much more pronounced (well developed) than that observed in 2 I.U./mL of **VA** treated cases.The nucleus of the lepidotrichia forming cells (LFCs) is well preserved (Fig. $11_{c\&d}$). The presence of phagocytecell and degeneration of the nucleus were also observed as shown in fig $11_{c\&e}$.

It was observed that, in the tail fins treated with 8 I.U./mL of **VA**, at fifth day postamputation, fibers connections, between cells were formed (Fig.11_f) and these cells well developed and differentiated. The junction between lepidotrichia forming cells is

desmosomes type (spot desmosomes) and collagen fibers are recognized (Fig11$_{d\ \&g}$).

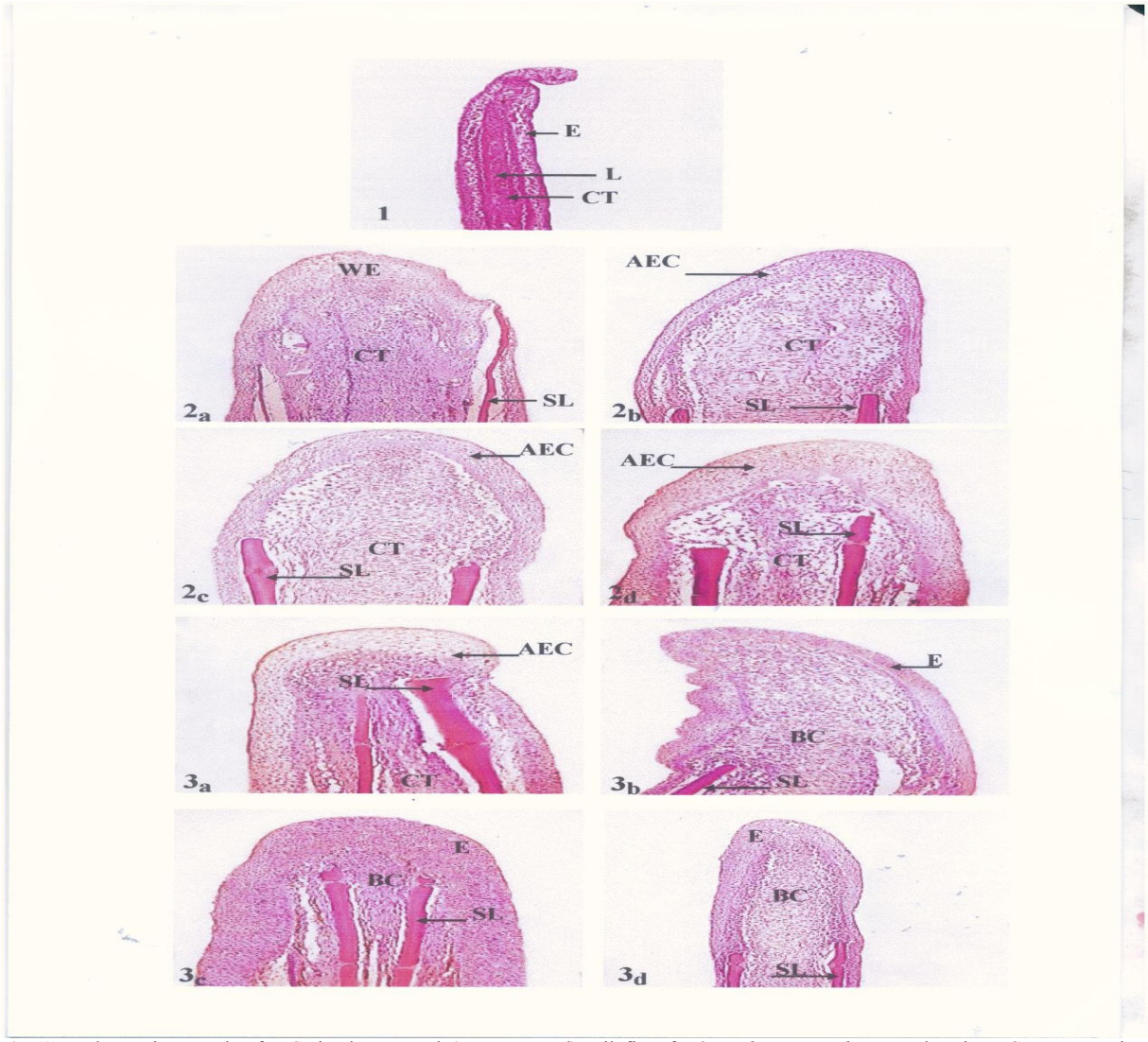


Fig.(1): photomicrograph of L.S. in the normal (unamputated) tail fin of *Oreochromis niloticus,* showing CT, connective tissue; E, epidermis ; L, lepidotrichia. (H &E) X: 200.

Fig. (2): photomicrograph of L.S. in the regenerating control and treated tail fin of *Oreochromis niloticus,* second day postamputation.

2a: Control fish, showing the wound is covered by a thin epidermal layer. WE, wound epidermis; CT, connective tissue; SL, stump lepidotrichia. (H&E)X: 300. Treated fishes, showing the wound epidermis increase in thickness and form the apical epidermal cap (AEC);CT, connective tissue; SL, stump lepidotrichia.

2b: Treated fish with 2 I.U. /mL of **VA**. (H&E) X: 300.

2c: Treated fish with 4 I.U. /mL of **VA**. (H&E) X: 300.

2d: Treated fish with 8 I.U. /mL of **VA**. (H&E) X: 300.

Fig. (3):photomicrograph of L.S. in the regenerating control and treated tail fin of *Oreochromis niloticus,* third day postamputation.

3a: Control fish, showing the apical epidermal cap (AEC) ;CT, connective tissue; SL, stump lepidotrichia. (H&E)X:300 Treated fishes, showing the blastema cells (BC); CT, connective tissue; SL, stump lepidotrichia.

3b: Treated fish with 2 I.U. /mL of **VA**. (H&E) X: 300.

3c: Treated fish with 4 I.U. /mL of **VA**. (H&E) X: 300.

3d: Treated fish with 8 I.U. /mL of **VA**. (H&E) X: 300.

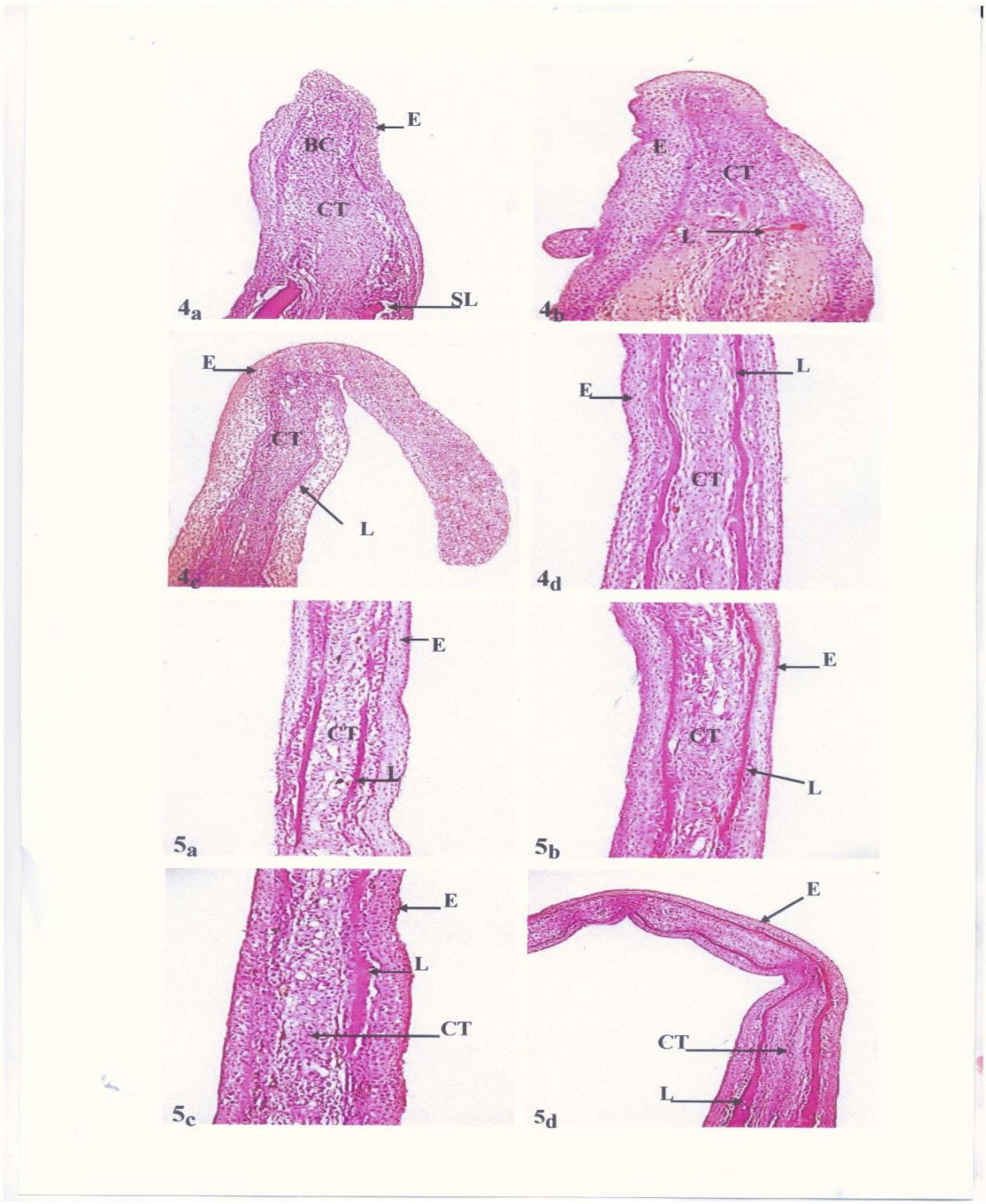


Fig. (4): photomicrograph of L.S. in the regenerating control and treated tail fin of *Oreochromis niloticus,* fifth day postamputation.
4a: Control fish, showing the blastema cells (BC); CT, connective tissue; SL, stump lepidotrichia. (H&E) X: 300. Treated fishes, showing the formation of bony lepidotrichia (L) ; E, epidermis; CT, connective tissue.
4b: Treated fish with 2 I.U. /mL of **VA**. (H&E) X: 300.
4c: Treated fish with 4 I.U. /mL of **VA**. (H&E) X: 300.
4d: Treated fish with 8 I.U. /mL of **VA**. (H&E) X: 300.
Fig. (5): photomicrograph of L.S. in the regenerating control and treate tail fin of *Oreochromis niloticus,* seventh day postamputation.
5a: Control fish, showing the formation of bone. L, lepidotrichia; E, epidermis; CT, connective tissue. (H&E) X: 300. Treated fishes, showing the formation of several pairs of parallel lepidotrichia (L) which are separated from each other; E, epidermis; CT, connective tissue.
5b: Treated fish with 2 I.U. /mL of **VA**. (H&E) X: 300.
5c: Treated fish with 4 I.U. /mL of **VA**. (H&E) X: 300.
5d: Treated fish with 8 I.U. /mL of **VA**. (H&E) X: 300.

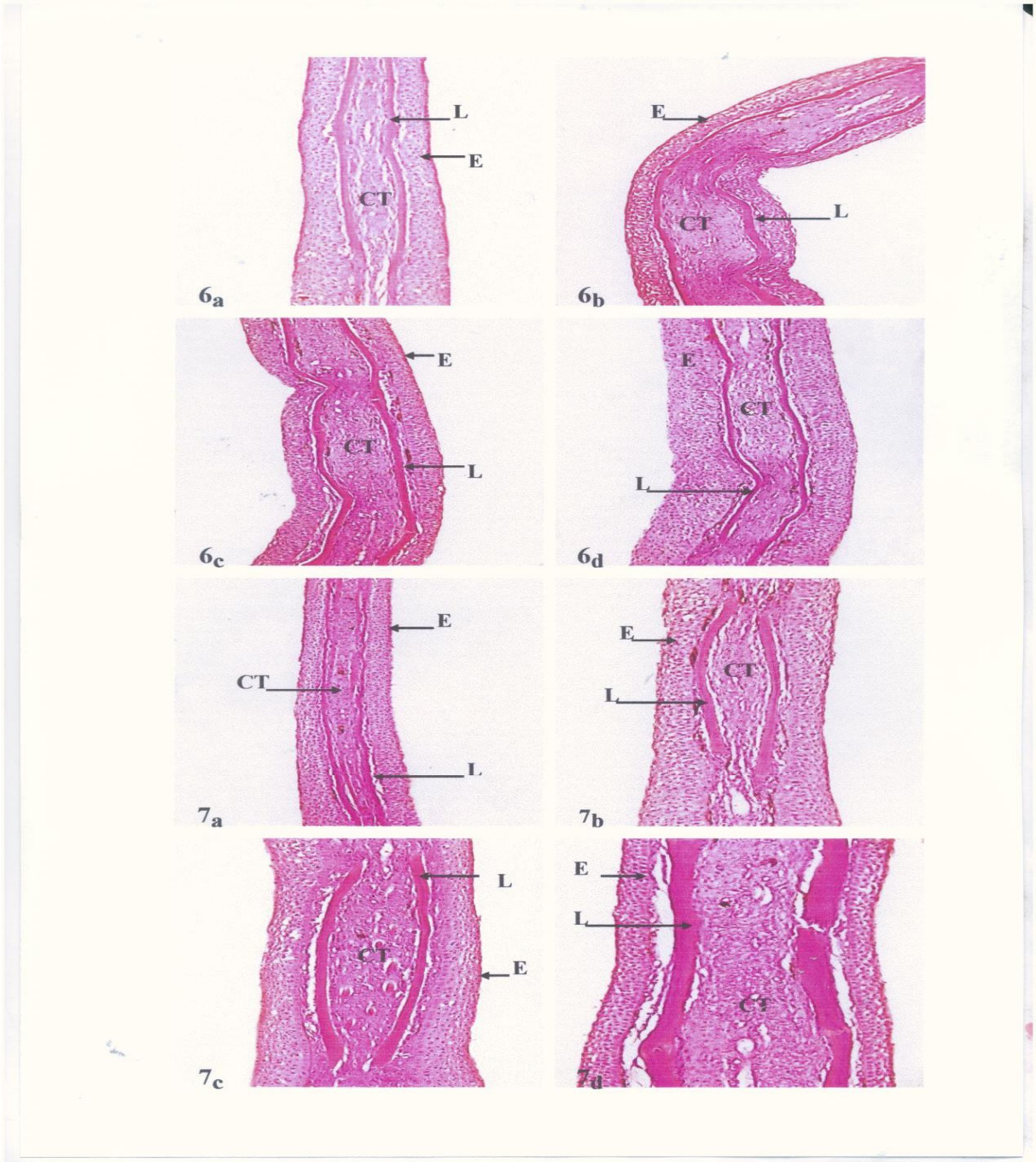


Fig. (6): photomicrograph of L.S. in the regenerating control and treated tail fin of *Oreochromis niloticus,* tenth day postamputation.
6a: Control fish, showing the formation of two pair of lepidotrichia (L); E, epidermis; CT, connective tissue. (H&E) X: 300.
Treated fishes, showing the lepidotrichia (L) become segmented and extended from the stump to the distal tip of the fin; E, epidermis ; CT, connective tissue.
6b: Treated fish with 2 I.U. /mL of **VA**. (H&E) X: 300.
6c: Treated fish with 4 I.U. /mL of **VA**. (H&E) X: 300.
6d: Treated fish with 8 I.U. /mL of **VA**. (H&E) X: 300.
Fig. (7): photomicrograph of L.S. in the regenerating control and treated tail fin of *Oreochromis niloticus,* fifteenth day postamputation.
7a: Control fish, showing the segmentation of lepidotrichia (L); E, epidermis; CT, connective tissue. (H&E) X: 300.
Treated fishes, showing the increase of thickness of lepidotrichia (L) and addition of new segment to it ;E,epidermis ; CT, connective tissue.
7b: Treated fish with 2 I.U. /mL of **VA**. (H&E) X: 300.
7c: Treated fish with 4 I.U. /mL of **VA**. (H&E) X: 300.
7d: Treated fish with 8 I.U. /mL of **VA**. (H&E) X: 400.

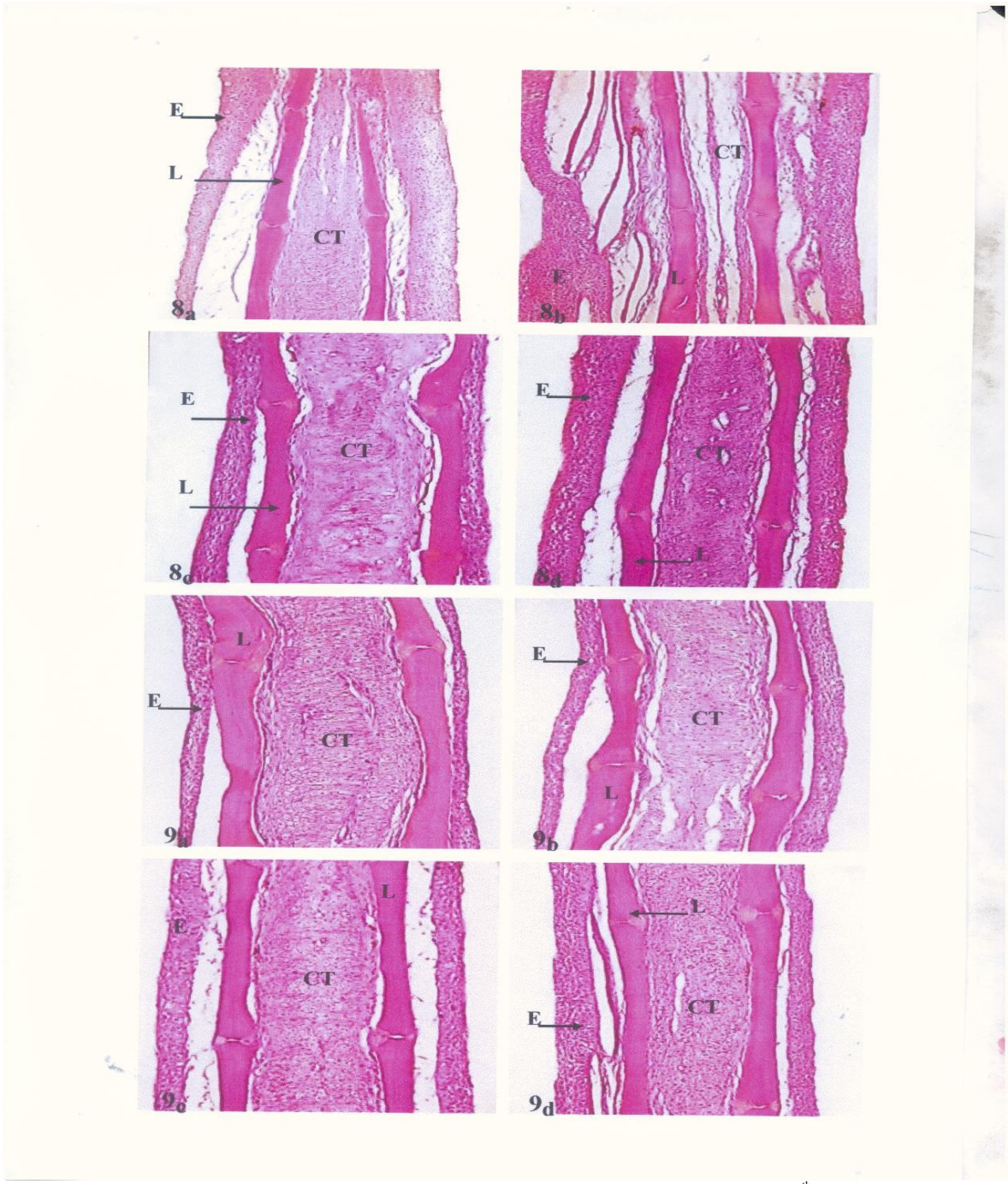
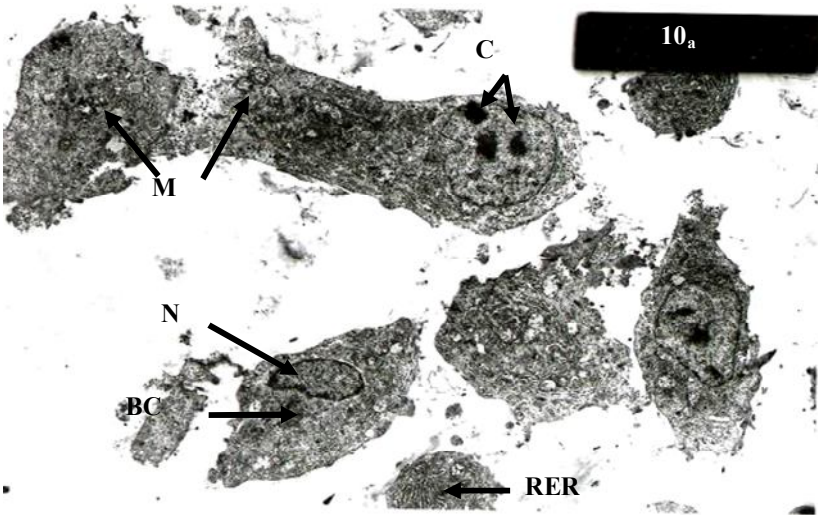
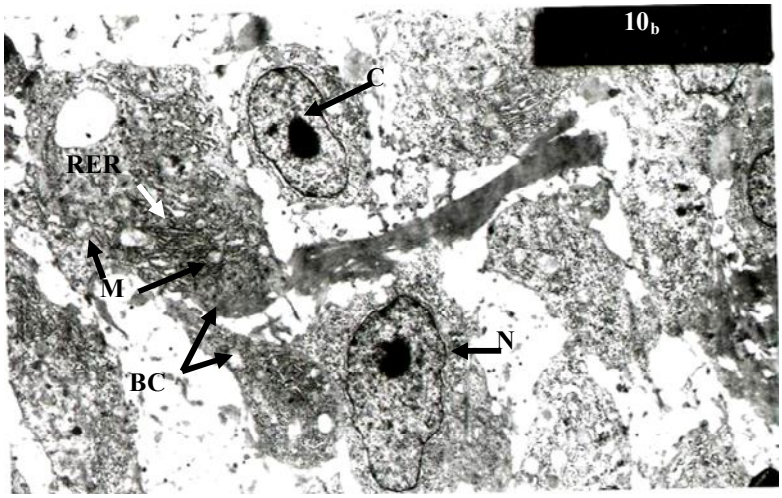
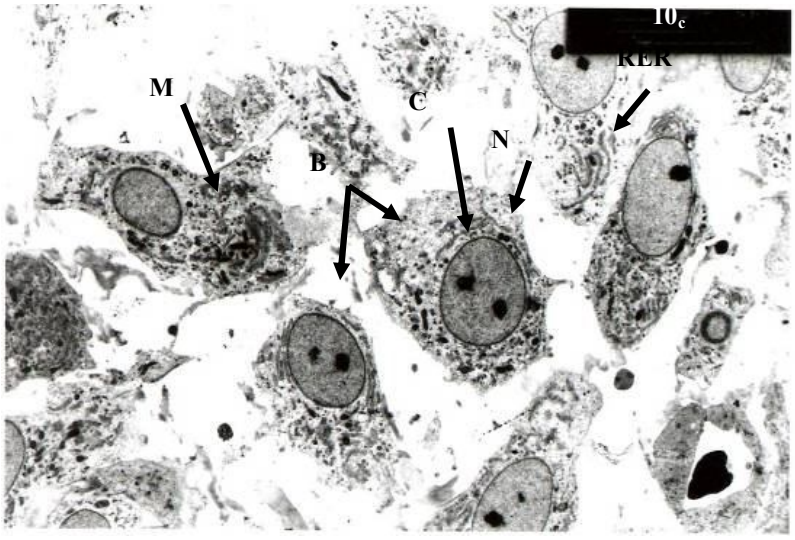


Fig. (8):photomicrograph of L.S. in the regenerating control and treated tail fin of *Oreochromis niloticus,* 30th day postamputation, showing a fully formed form of a regenerating fin ray (L) lepidotrichia; E, epidermis; CT, connective tissue.
8a: Control fish. (H&E) X: 400.
8b: Treated fish with 2 I.U. /mL of **VA**. (H&E) X: 400.
8c: Treated fish with 4 I.U. /mL of **VA**. (H&E) X: 400.
8d: Treated fish with 8 I.U. /mL of **VA**. (H&E) X: 400.
Fig. (9): photomicrograph of L.S. in the regenerating control and treated tail fin of *Oreochromis niloticus,* 45th day postamputation, showing that the fin reach to the original length and form, L, lepidotrichia; E, epidermis; CT, connective tissue.
9a: Control fish. (H&E) X: 400.
9b: Treated fish with 2 I.U. /mL of **VA**. (H&E) X: 400.
9c: Treated fish with 4 I.U. /mL of **VA**. (H&E) X: 400.
9d: Treated fish with 8 I.U. /mL of **VA**. (H&E) X: 400


10a
C
M
N
BC
RER


10b
C
RER
M
N
BC


10c
RER
M
C
N
B

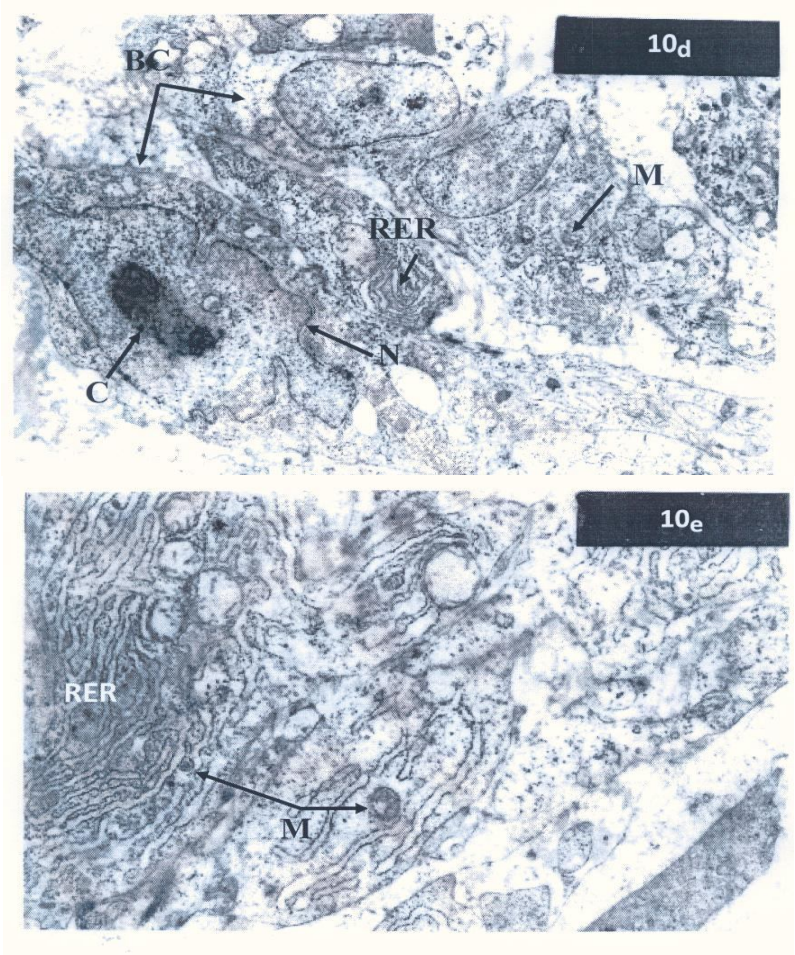
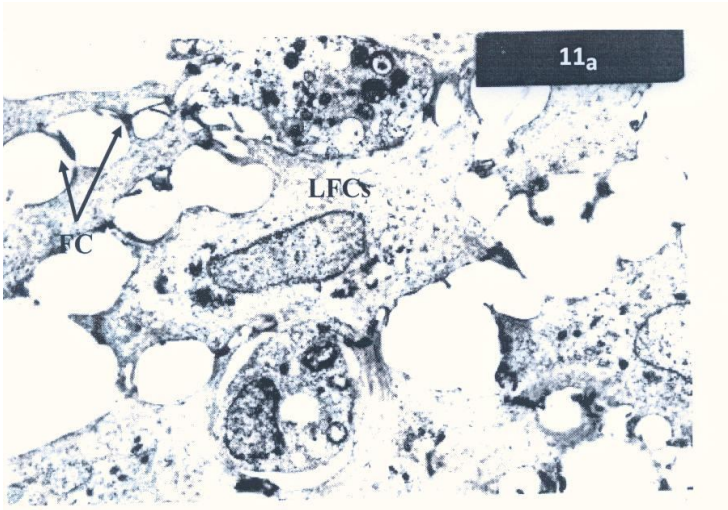
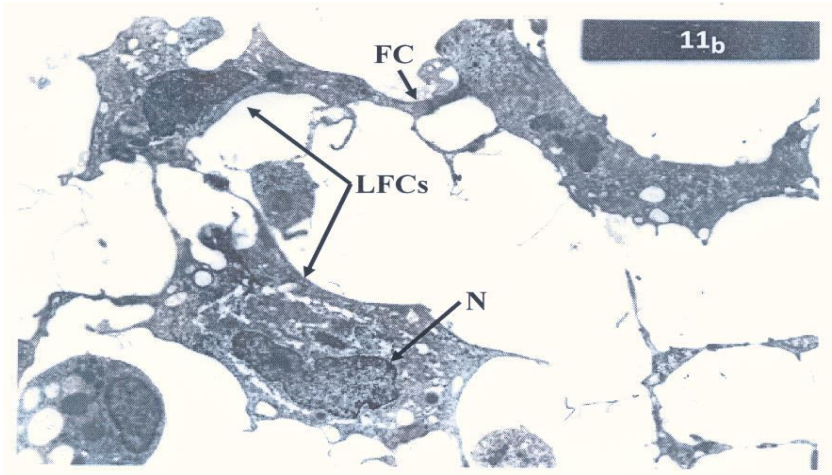
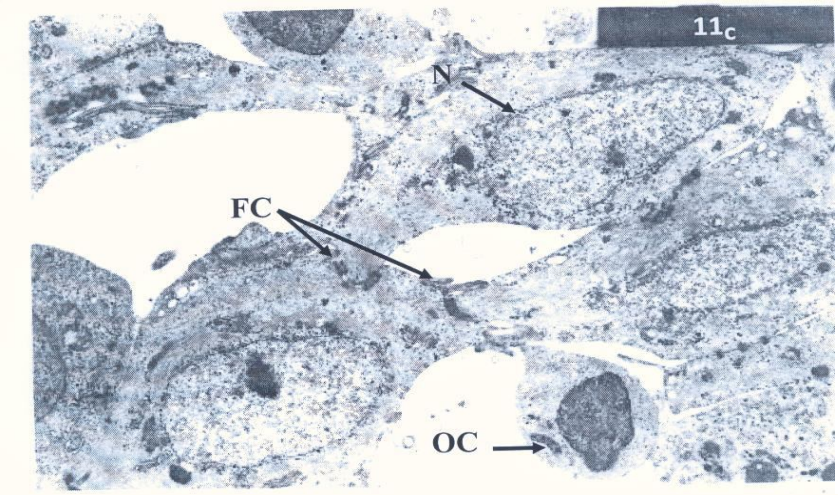
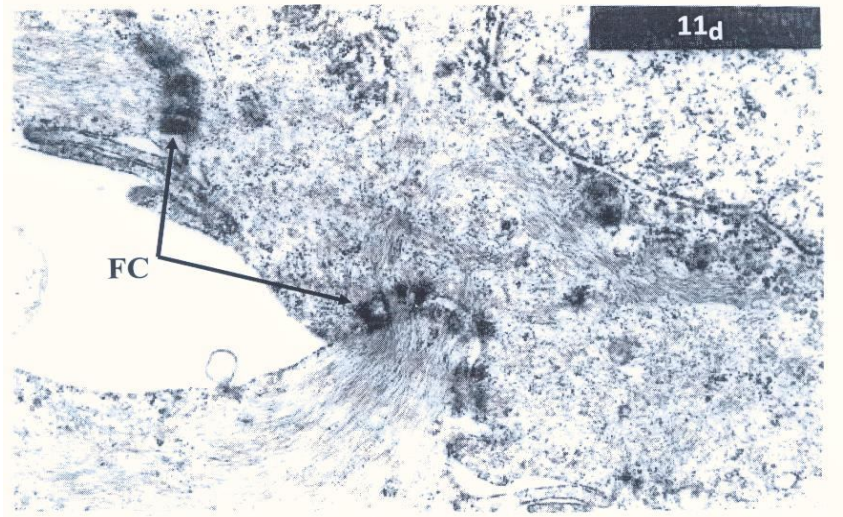


Fig.(10) : Electron micrograph of different cell types of blastema cells in the control and treated tail fins of *Oreochromis niloticus*, at the third day postamputation, showing the blastema cell(BC); N, nucleus; C, nucleolus/,m; M, mitochondria; RER, rough endoplasmic reticulum.
10a: Control fish. X: 2500 10b: Treated fish with 2 I.U. /mL of **VA**. X: 4000.
10c: Treated fish with 4 I.U. /mL of **VA**. X: 2000. 10d: Treated fish with 8 I.U. /mL of **VA**. X: 5000.
10e: Treated fish with 8 I.U. /mL of **VA**. X: 20000.




11b
FC
LFCs
N


11c
N
FC
OC


11d
FC

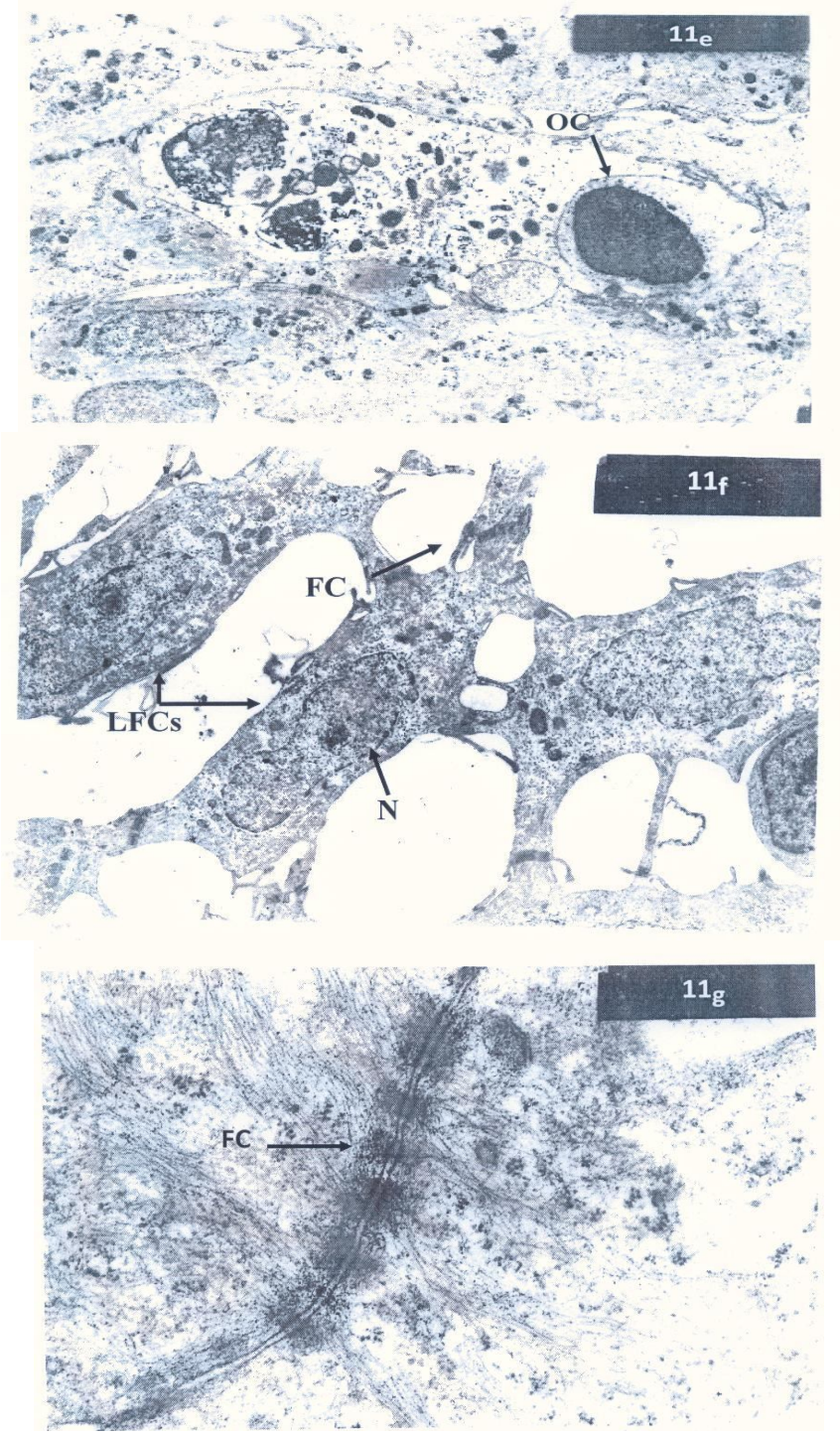


Fig. (11): Electron micrograph of lepidotrichia in the control and treated tail fins of *Oreochromis niloticus*, at the fifth day postamputation, showing the fibers connection (FC) between the lepidotrichia forming cells (LFCs) ; N, nucleus.
11a: Control fish. X: 4000. 11b: Treated fish with 2 I.U./mL of **VA**. X: 2500.
11c: Treated fish with 4 I.U./mL of **VA**,also showing OC, phagocyte cell. X: 7000. 11d: Treated fish with 4 I.U./mL of **VA**. X: 13000.
11e: Treated fish with 4 I.U./ mL of **VA**,also showing OC,phagocytecell. X: 5000. 11f: Treated fish with 8 I.U./mL of **VA**. X: 5000.
11g: Treated fish with 8 I.U./mL of **VA**. X: 30.000.

4.Discussion

The histological investigations of the present study have demonstrated that, the tail fin of *Oreochormis niloticus*is composed of segmented bony rays, or lepidotrichia. Each of them is consisting of a pair of concave facing hemirays that surround a mass of connective tissue including fibroblasts as well as nerves and blood vessels. These observations agreed with many other previous studies on teleosts fins (**Montes *et al*. ,1982 and Becerra *et al.*,1983)** showed that, each hemiray is a tile-like dermal bone also, called hemilepidotrichium is surrounded by a mononlayer of bone secreting cells, scleroblasts (lepidotrichia forming cells). Our observations demonstrated that lepidotrichia are connected by vascularized and innervated soft mesenchymal tissue, and are surrounded by a multilayered epidermis separated from the mesenchymal compartment by a typical basement membrane. Each of them is tapered distally by a group of actinotrichia (long rods of a collagen-like protein called elastoidin), then each of them presents a specific pattern of dichotomously branching points, also named forks or bifurcations. The whole structure is covered by non-scaled fish skin. This description is in accordance with many previous results on fin structure of teleosts (**Prenant, 1937**; **Montes *et al*., 1982; Becerra *et al.,* 1983; Santamaría and Becerra, 1991 & Géraudie and Singer, 1992*)***.**

As early as **1947, Blanc** metioned that, the fin skeleton of a teleost fish is formed by segmented rays (lepidotrichia), which together with the actinotrichia (fine rod bundles located at the apex of each ray) constitute the dermal skeleton. Similar to that observation, **Marí-Beffa *et al*. (1989)** insured that, the distal tips of the rays encase a tuft of actinotrichia (un mineralized fibrils of elastoidin).

Following removal, through the excision of a portion of the tail fin, it regenerated to restore its original length and size. The results of the present work have demonstrated that, the histological sequence of the different regeneration phases was found to be nearly similar to that described in many previous studies for other teleosts species such as **(Gerudie&Singer, 1992 and Abdel- karim *et al.*,2003).**

After two days, in control fishes , wound surface was firstly closed by a thin epithelial layer that was formed by migration of the neighboring epidermal cells of the stump. Also, others have reported, in their experiments on fin regeneration, that following the fin amputation, the cut surface of the stump is covered by a stratified wound epidermis during the first day (**Goss and Stagg, 1957 & Géraudie and Singer, 1992**). In treated fishes of the present work , the wound surface was first closed by a thin epithelial layer, this layer become thicker and forming an apical epidermal cap which is well established at the regenerating tail fins treated with 8 I.U./mL of **VA** compared to the regenerating tail fins treated with 2 I.U./mL of **VA** and 4 I.U./mL of **VA**.

The results of the present work have revealed that, after the third day, in control fishes there is a thickening of the epidermis by accumulation of additional layers. It may be suggested that, this increase in thickness is not only due to the migration of the stump cells but also due to the mitotic divisions in the apical epidermal cap which may perform an important role in the proliferation and differentiation of blastemal mesenchymal cells. It may also control the degree of cell differentiation. This finding matched with other previous studies.**Abdel-karim and Michael (1991)**have mentioned that, as a result of contribution of the dividing cells of the proximal epidermis in the formation of the AER (apical ectodermal ridge), more dividing cells being added proximo-distally. The increasing of mitotic index (MI) of the apical epidermis during AER formation leads to the suggestion that, some cells of the ridge come from local mitotic activity. This may revealed the important role played by the AER in the regeneration of tail fin. It may actively induce the mesenchymal accumulation, and as a result of the expanding and distal sliding of the whole covering epidermis, the mass of mesenchyme is formed. The AER may promote the proximo-distal regeneration. In contrast, **Becerra *et al. (*1983**) have shown that, this maturation process appeared to be dominated by migration events, not by proliferation. Also **Poleo *et al.* (2001)** stated that, in zebrafish, the apical epithelial cap is formed through cell migration but no evidence was found for proliferation in this tissue. Migration by epithelial cells and by cells of the intra-ray tissue contributes to the regeneration process.

Some investigators as **Santos-Ruiz *et al.*(2002**) have proved that fin regeneration in zebrafish involes activation of the cell cycle in the epidermal and the mesenchymal compartments, apparently in an independent way. This activation not only affects the neighborhood of the amputation plane but affects also long distance of the ray. Cells of mesenchymal origin, located far from the amputation plane, give rise to the blastema. This is located beneath a non proliferation apical epidermal cap, which is possibly receives an input of cells from the highly proliferating lateral epidermal surfaces. The epidermal elongation takes place through proliferation at these lateral surfaces. Our results also confirm the reports of **Poleo *et al.* (2001*)***on zebrafish, which have shown that, during fin regeneration a combination of cell migration and cell proliferation is responsible for the formation of the

epidermis and the mesenchymal compartements of the regenerate. It is thought that, a subset of cells in the basal layer of the wound epidermis stimulates alignment of blastema-derived scleroblasts, which deposit bone matrix to form new hemiray segments**(Sanatmaría *et al.*, 1996)**in Goldfish, *Carassius auratus*.

After third day, in treated fishes with **VA**, it was noticed that, beneath the epidermal cap, a blastema is formed from a mesodermal origin. The blastema is composed of a population of undifferentiated cells. It was found that, the cell density of blastema cells in the regenerating tail fins treated with 8 I.U./mL of **VA** is greater than in the other two concentrations of **VA** (2 I.U./mL and 4 I.U./mL). The present results are also in agreement with the observations of **Géraudie and Singer(1992)** which have a comparable caudal fin regeneration to newt limb regeneration, in particular with reference to the role that, retinoic acidand its receptors might play in the process. As in the newt limb, regeneration of the amputated fin is preceded by the formation of a wound epithelium, followed by the appearance of blastemal cells. In addition to a dense blastema that was formed at the amputated ends of each of the 18 lepidotrichia or bony rays, a less dense or soft mesenchyme was also formed that gives rise to the thin inter-ray tissue. Also, **Géraudie *et al.* (1994)** reported that, the regeneration of the pectoral fin in zebrafish, which was treated with retinoic acid, proceeds rather rapidly and a fully formed blastema is observed 3-4 days after amputation, and advanced regenerate is present after 2 weeks, and regeneration is apparently complete within 1 month.

On the other hand ,**White *et al.* (1994)**found that immediately exposure of the caudal fin of zebrafish to retinoic acid prevented regeneration over the course of the experiment. Regenerates harvested 24 hours following the addition ofretinoic acid, exogenous retinoic acid appears to be able to affect the organization of the blastema even after it has formed and regeneration has commenced. This would suggest that, the blastema remains sensitive to the effect of retinoi cacid throughout the course of regeneration.

In the present work, by the fifth day postamputation, in control tail fins, the blastema was well developed and begin their differentiation towards lepidotrichia forming cells (LFCs) in the proximal region, but the bone was still not formed. While in the treated fishes with different concentrations of **VA**, the well differentiated cells in the proximal region of the blastema were formed along a strip immediately beneath the epidermis. These cells which are in close association with the basement membrane, on either side of the fins will be called scleroblasts. The scleroblasts form a continuous cellular layer and secrete the extracellular matrix. Such a condition is the first sign of appearance of bone in the regenerating tail fins treated with 2 I.U./mL of **VA** while in the regenerating tail fins treated with 4 I.U./mL and 8 I.U./mL of **VA**, the formation of the hemirays (lepidotrichia) become visible at the distal tip of the fins. These observations are in accordance with the results in many observations as in *Fundulus heteroclitus* (**Blanc ,1949 ; Géraudie and Singer ,1979),** in teleost fish(**Géraudie, 1983and Becerra et al. ,1987),**in *Tilapia mossambica* **(Kemp and Park, 1970)**. They have suggested that after partial amputation of the fin, an apical blastema develops. From this blastema, cells differentiate and begin the synthesis of the lepidotrichial matrix (LM), which undergoes a sequential transformation from the initial deposition of each component to the final mineralization. This also was introduced by **Becerra *et al.* (1996)** in the teleost fins who suggested that the bone regeneration starts with differentiation of the blastemal cells in contact with the basement membrane into scleroblasts which synthesize and release the lepidotrichial matrix in the subepidermal space. Similarly **Santamaría *et al.* (1992)** after a detailed study on the tail fin regeneration in teleosts, found that each hemiray is formed by the synthetic activity of two different cell populations with a common origin. At first, the differentiating cells join the basement membrane and begin to release the lepidotrichial matrix components which polymerize in the subepidermal space. Then, some of these differentiated cells migrate laterally, occupying the space between the newly formed lepidotrichia and the basement membrane. Subsequently they begin to synthesize the hemiray from the opposite side. On the other hand, **Haga *et al.* (2002)** have reported that, characteristic deformities were induced by retinoic acid treatment of the Japanese flounder, *Paralichthys olivaceus* at 6-9 days post-hatching. Also, retinoic acid isomers induced deformities in the lower jaw, caudal fin and vertebrae. Caudal fin deformities included deformity of caudal bone complex and absence of the entire caudal fin. They have concluded that retinoic acid exerted toxic effects on the skeletal systems mainly through the RAR (Retinoic Acid Receptor) pathway.

The present investigations have demonstrated that in the seventh day postamputation, in the control tail fins, the scleroblasts of both hemirays cover the amputation surface, many of them are closely attached to the ray stumps forming a scleroblast cap over the amputation bony hemirays. When rays formation started, the blastema was structured in a scleroblast cell mass and proliferative activity was

observed in the connective tissue and frequently in the scleroblast ray coat. The formation of bone become first visible at the distal tip of the fins.These observations are in accordance with the results of many authors (**Blanc ,1949 Kemp and Park ,1970;Géraudie and Signer ,1979; Géraudie ,1983 and Bercerra *et al.* ,1987).** They have suggested that, after partial amputation of the fin, an apical blastema develops. From this blastema, cells differentiate and begin the synthesis of the lepidotrichial matrix (LM), which undergoes a sequential transformation from the initial deposition of each component to the final mineralization. This also was introduced by **Becerra *et al.* (1996)** in the teleost fins, who suggested that the bone regeneration starts with differentiation of the blastemal cells, in contact with the basement membrane into scleroblasts which synthesize and release the lepidotrichial matrix in the subepidermal space. It was noticed that, in the regenerating tail fins treated with 2 I.U./mL of **VA**, the first sign of bone formation leading to hemirays formation which becomes doubled along the fins. In the regenerating tail fins treated with 4 I.U./mL of **VA**, Two newly formed sisters hemirays appeared in addition to the first pair of hemirays, which are separated from each other and also, separated from the ray stump. In the regenerating tail fins treated with 8 I.U./mL of **VA**, more pairs of hemirays were added and irregular bone deposition above ray stump occurred. These irregular bone deposits linked the ray stump to the reformed hemirays. The formation of segmentation was started in the proximal region of the regenerating lepidotrichia (closer to the stump).

The present results have revealed that on the tenth day postamputation, in the control tail fins, the first pair of hemirays was formed and became doubled along the tail fins. By the same time it was noticed that in the regenerating tail fins treated with 2 I.U./mL of **VA**, each parallel pair of lepidotrichia was connected to each other and also, to the stump lepidotrichia. So few segmentations are formed in the proximal region closer to the stump. In the regenerating tail fins treated with 4 I.U./mL of **VA**, the lepidotrichia were segmented and extended from the stump to the distal tip of the regenerating fins. In the regenerating tail fins treated with 8 I.U./mL of **VA**, the growth and elongation of the regenerating fins continue by sequential addition of new segments at the distal end of each ray, when once formed they cannot be elongate. Therefore, growth of the fins occurs by increasing the number of segments, rather than by increasing the length of a fixed number of skeletal elements. In contrary, **Furukawa (1979)** who has stated, in his study on fin regeneration of *Oryzias latipes*, that more than five days after amputation further terminal newly formed ray segments were added concomitant with the elongation of the regenerating area.

In the present investigations, it was found that on the fifteenth day post amputation, in the regenerating control tail fins, the tail fins extended distally and more pairs of hemirays were added. The segmented lepidotrichia are developed and begin to branch and tufts of actinotrichia terminate each ray. Also **Santamaría and Becerra (1991),** in their studies of the tail fin regeneration in telelosts found that each ray is apically tapered with a double palisade of long rigid rods called actinotrichia, which may have morphogenetic activity. In the present work it was observed that in the regenerating tail fins treated with 2 I.U./ mL and 4 I.U./ mL of **VA**, the scleroblasts cells are responsible for the thickening of the hemisegment by a positional growth and the newly synthesized, lateral lepidotrichial layers. The medial and oldest part became separated from the lateral lepidotrichia. In the regenerating tail fins treated with 8 I.U./mL of **VA**, the lepidotrichial became segmented and increased in the thickness. The fin rays were well established and restored the original form. By this time, at different concentrations of **VA**, the lepidotrichia end distally with a row of rigid but unmineralized elastoidin fibrils, the actinotrichia. During the late redifferentiation and growth phase of the tail fins (by the thirtieth and forty –fifth day postamputation), the regenerating control and treated tail fins continued their growth by addition of segments to maintain their original length to reach approximately the same length after forty –five days.

The present results showed that, the regeneration rate of the regenerating tail fins treated with **VA** showed higher values than that of the control fins. This rate increases with the increase of **VA** concentration, from 2 I.U. / mL, 4 I.U. / mL to 8 I.U. / mL of **VA.**

In the present study, the ultrastructure of the blastema cells revealed that, in control tail fins at the third day postamputation, the blastema cells revealed a heterogenous cell population, within each of these blastema cells, a nucleus, mitochondria and rough endoplasmic reticulum are recognized. The density of the blastema cells was few. Also, in the regenerating tail fins treated with 2 I.U./mL of **VA**, the density of the blastema cells was the same as in control cases. While in the regenerating tail fins treated with 4 I.U./mL and 8 I.U./mL of **VA** the blastema cells showed higher density than that of the control and that treated with 2 I.U./mL of **VA**. The nucleus of those blastema cells displayed rough endoplasmic reticulum consists of long parallel cisternae. This indicates an increase of the synthetic activity of these cells.Similar results were obtained by **Kemp and**

Park (1970), in the tail fin of the *Tilapia mossambica*; they observed that, the abundance of the rough endoplasmic reticulum in blastema cells indicates that they are actively synthesizing protein.

Studying the ultrastructure of the bone (lepidotrichia), it was found that, in the control tail fins by the fifth day postamputation, few of fibers connection between lepidotrichia forming cells were formed. While in the regenerating tail fins treated with 2 I.U./mL of **VA**, the blastema cells differentiated into lepidotrichia forming cells and the bone (lepidotrichia) appear in the form of bundle of fibers, which are more than that in the control cases. In the regenerating tail fins treated with 4 I.U./mL of **VA**, several fibers connections were formed more pronounced than that in the control and treated tail fins with 2 I.U./mL of **VA**. In the regenerating tail fins treated with 8 I.U./mL of **VA**, the formation of fibers connections between cells was more than that found in the control and treated tail fins with 2 I.U./mL and 4 I.U./mL of **VA**. This indicated that, the bone of lepidotrichia were formed more pronounced in the tail fins treated with 4 I.U./mL and 8 I.U./mL of **VA**. Similar results were obtained by **Becerra *et al.* (1996)** in *Tilapia melanopleura, Cyprinus carpio* and *Carassius auratus*. They demonstrated that scleroblasts synthesize the extracellular matrix of the regenerating lepidotrichia. The deposition of collagen fibrils in the regenerating fins in order to form lepidotrichia.

Acknowledgement

The authors would like to express their deepest gratitude to Dr. A. E. El-Attar Prof. of Comparative Anatomy, Zoology Department, Faculty of Science, Zagazig Univ., for his interest and valuable suggestion.

References

Abdel–Karim, A.E. and Michael, M.I. (1991): Effect of the apical ectodermal rigde on mitotic index in hind limb bud mesenchyme in *Bufo regularis*. Qatar Univ. Sci., 11: 285 – 298.

Abdel-Karim,A.E.;Atta,K.I. and Elsheikh, E.H. (2003): Regeneration of the tail and pectoral fins of teleost: *Oreochromis niloticus*.J.Egypt. Ger. Soc. Zool., 42B: 39 -83.

Becerra, J,;Junqueira, L.C.U.; Bechara, I.J. and Montes, G.S. (1996): Regeneration of fin rays in teleosts: a histochemical, radioautographic and ultrastructural study. Archi.Histol.and Cytol., 59: 15-35.

Becerra, J.; Marí-Beffa, M. and Carmona, M.C. (1987): Cytological changes during the earlier stages of differentiation in tail fin regeneration of teleosts. Verh. Anat. Ges., 81: 689-690.

Becerra, J.; Montes, G. S.; Bexiga, S.R. and Junqueira, L. C. (1983): Structure of the tail fin in teleosts. Cell tissue Res., 230: 127-137.

Blanc, M. (1947): Structure histology des rayons de nageoires chez les poisons t**é**l**é**ostéens. Bull. Soc. Zool. Fr., 72: 17-22.

Blanc, M.(1949): Etude histologique de la r**é**g**é**neration des nageoires chez quelques poisson téléostéens. Arch. Anat.Microsc.Morp. Exp., 38: 52-64.

Bockelmann,PK.;Ochandio,BS.;Bechara,IJ.(2010) : Histological study of the carp tail fin (*Cyprinus carpio*, Linnaeus, 1758).Braz. J. Biol. Vol. 70 no.1Sao Carlos.

Bryant, S.V. and Gardiner, D. M. (1992): Retinoic acid, local cell-cell interactions, and pattern formation in vertebrate limbs. Dev. Biol., 152:1-25.

Drury, R.A. B. and Wallington, E. A. (1980): Carlton's Histological Technique. New York, Oxford.press.

Furukawa, A. H. (1979): Effect of radiation on fin regeneration of the fish *Oryzias latipes*, with special reference to dose- rate. J. Radiat. Res., 20: 215-223.

Géraudie, J. (1983): Morphogenesis of the dermal skeleton in the teleost fins in: Fallon JF. Caplan, AI (eds) limb development and regeneration, VoL. A. Alan R Liss. New York. PP. 289-297.

Géraudie, J. and Singer, M. (1979): Nerve-dependent macromolecular synthesis in the pectoral fin regenerate of fish *Fundulus sp*. J. Exp. Zool., 208: 281-286.

Géraudie, J. and Singer, M. (1992): The fish fin regenerate. Monographs in: Dev. Biol., 23: 62-72.

Géraudie, J.; Brulfert, A.; Monnot, M. J. and Ferretti, P. (1994): Teratogenic and morphogenetic effects of retinoic acid on the regenerating pectoral fin in zebrafish. J. Exp. Zool., 269:12-22.

Goss, R. J. and Stagg, M. W. (1957): Theregeneration of fins and fin rays in *Fundulus heteroclitus*. J. Exp. Zool., 136:487-508.

Gupta, B.L. and Berridge, M.J. (1966): Fine structural organization of the rectum in the blowfly *Calliphora erythrocephale* (Meig) with reference to connective tissue, trachea and neurosecretory innervation of the rectal papillae. J. Morph., 120: 23-82.

Haga, Y.; Suzuki, T. and Takeuchi, T. (2002): Retinoic acid isomers produce malformations in postembryonic development of the Japanese flounder, *Paralichthys olivaceus*. Zool. Sci., Oct; 19(10): 1105-1112.

Johnson, K.J.and Scadding, S.R. (1991): Effects of vitamin A and other retinoids on the

differentiation and morphogenesis of the integument and limbs of vertebrates. Can. J. Zool., 69: 263-273.

Johnson, S.L. and Weston, J.A. (1995): Temperature –sensitive mutations that cause stage specific defects in zebrafish fin regeneration. Genetics, 141: 1583-1595.

Kemp, N.E. and Park, J. H. (1970): Regeneration of lepidotrichia and actinotrichia in the tail fin of the teleost: *Tilapia mossambica*. Dev. Biol., 22: 321-342.

Luft, J. H. (1961): Improvements in epoxy resin embedding methods. J. Biothys. Biochem.Cyto., 9: 409-414.

Maden, M. and Hind, M. (2003): Retinoic acid a regeneration inducing molecule. Dev. Dyn., 226(2): 237-244.

Maden, M.and Hind, M. (2004): Retinoic acid in alveolar development maintenance and regeneration. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 29; 359(1445): 799-808.

Marí-Beffa, M.; Carmona, M. C. and Becerra, J. (1989): Elastoidin turnover during tail fin regeneration in teleosts. Anat. Embryol., 180: 465-470.

Michael, M.I.; Hassanien, A. M. M.; Aziz, F. K. and Ali, R.A. (1994a): Effect of electrical stimulation on regeneration of hind limbs in stages of the Egyptian toad, *Bufo regularis* Reuss after transection at the shank level. J. Egypt. Ger. Soc. Zool., 14 (B): 1-28.

Michael, M. I.; Hassanien, A. M. M.; Aziz, F. K. and Ali, R. A. (1994_b): Effect of electrical stimulation on regeneration of hind limbs in stages of the Egyptian toad, *Bufo regularis Reuss* after transection at the thigh level. J. Egypt. Ger. Soc. Zool., 14(B): 29-58.

Montes, G.S.; Becerra, J.; Toledo,O.M.S.; Gordilho, M.A. and Junqueira, L.C.U. (1982): Fine structure and histochemistry of the tail fin ray in teleosts. Histochemistry, 75: 363-376.

Muneoka, K.; Wanek, N.; Taylor G.; Hayamizu, T.F.; Trevino, C.; Shi, C.; Gardiner, D.M.; Andrson, R. and Bryant, S.V.(1992): Regeneration of chick limb bud is stimulated by the apical ectodermal ridge. Science, 182: 82-86.

Niazi, I.A. and Saxena, S. (1978): Abnormal hind limb regeneration in tadpoles of the toad, *Bufo andersonii* exposed to excess vitamin A. Folia Biol., 26: 3-8.

Poleo, G.; Brown, C. W.; Laforest, L. and Akimenko, M. A. (2001): Cell proliferation and movement during early fin regeneration in zebrafish. Dev. Dyn., 221(4): 380-390.

Poss, K.D.; Shen, J.; Nechiporuk, A.; McMahon, G.; Thisse, B.; Thisse, C. and Keating, M.T. (2000): Roles for Fgf signaling during zebrafish fin regeneration Dev. Biol., 222: 347-358.

Prenant, M.(1937): Sur la croissance des lépidotriches articulés chez les téléosteens. Bull. Soc. Zool. Fr., 62: 190-195.

Reynolds, E.S. (1963): The use of lead citrate at high PH as an electron opaque stain in electron microscopy. J. Cell. Biol., 17: 208-213.

Rowe.A.; Eager, N.S.C. and Brickell, P.M. (1991): A member of the RXR nuclear receptor family is expressed in neural crest-derived cells of the developing chick peripheral nervous system. Development, 11: 771-778.

Santamaría, J.A, and Becerra, J. (1991): Tail fin regeneration in teleosts: Cell-extracellular matrix interaction in blastemal differentiation J. Anat., 176: 9-21.

Santamaría, J.A.; Marí-Beffa, M. and Becerra, J. (1992): Interactions of the lepidotrichial matrix components during tail fin regeneration in teleosts. Differentiation, 49:143-150.

Santamaría, J.A.;Marí-Beffa, M.; Santos- Ruiz, L. and Becerra, J. (1996): Incorporation of bromodeoxyuridine in regenerating fin tissue of the Goldfish *Carassius auratus*. J. Exp. Zool., 275: 300-307.

Santos-Ruiz, L.; Santamaría, J. A.; Ruiz- Sanchez, J. and Becerra, J. (2002): Cell proliferation during blastema formation in the regenerating teleost fin. Dev. Dyn., 223(2): 262-72.

Stempack, K.G. and Ward, R. T. (1964): An improved staining method for electron microscopy. J. Cell. Biol., 22: 697-701.

Trumpt, B.F.; Smuckler, E. A. and Benditt, E. P. (1961): Method for staining epoxy sections for light microscopy. J. Ultrastruct. Res., 5.

Tu, S.; and Johnson , S.L. (2011): Fat restriction in the growing and regenerating zebrafish fin. Dev cell. 20(5): 725 -32.

White, J.A.; Beffa, M. B.; Jones, B. and Petkovich, M. (1994): A zebrafish retinoic acid receptor expressed in the regenerating caudal fin. Development, 120:1861-1872.