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The Influence of Vitamin- A on Lens Regeneration in Indian Skipper Frog Rana Cynophlyctis

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Abstract

Regeneration is a development process in which the lost part of an organ is restored. This research paper includes the result of experiment made to study of lens regeration in froglet stage of Indian skipper frog RanaCynophlyctis treated with vitamin-A after lentectomy. The experiment was completed in a series and the animals of this series were divided into two groups after their lentectomy.

Group-A animals of this group were not treated with vitamin Aafter their lentectomy. It was found that no lens regeneration had occurred even in o single case.

Group-B : Animals of this group weregiven vitamin-A treatment after their lentectomy. It was found that lens regeneration has occurred in 90% cases, out of which 44.4% cases show-normal regeneration while in remaining 55.6% cases lentoid have been observed. Over all it was concluded that Vitamin-A induces the lens regeneration even in the froglet stage otherwise regeneration on ability have lost at this stage.

Keywords: Regeneration, froglet Indian skipper frog lens and lentectomy.

Introduction

The present study is based on investigations concerning the influence of vitamin A on lens regeneration in froglets of Indian skipper frog. Ranacynophlyctis.

It is well established that unlike urodels the ability of regeneration in the anurans is restricted to their larval period only. As the tadpoles grow and metamorphic changes are initiated the ability to regenerate gradually declines and in most of anurans it finally disappears before metamorphic climax. Regeneration is a developmental process in which the lost part of an organ is restored. It involves all those fundamental processes including cell proliferation, cell movements, morphogenesis, histogenensis and growth which occur during ontogenetic development in the embryonic stage. But lens regeneration differs from general regenerative process rather it provides clear example of "Metaplasia" during lens regeneration there is a transformation of one differentiated cellular types having a distinctive pattern of metabolic activities to another cellular types which is morphologically different from the original and which synthesized a different array of macromolecules. This type of regeneration is called "wolffian regeneration." Lens regeneration starts from the dorsal rim of the iris. During this process transformation of the iris into lens starts with depigmentation and is followed by multiplication of the cells, which become arranged into vesicle. Subsequently the cells in the posterior wall of the vesicle differentiated into lens fibers and finally a new lens is formed.

Vitamin A is known as to influence the developmental processes in a variety of ways and produces a number of teratogenic effects on embryos depending upon the stage when it is administered. Previous studies have shown that it also influences the processes involved in limb, tail and lens regeneration in anuran tadpole and newly born young mice. It is found that vitamin A accelerated lens regeneration in tadpoles and white albino mice of different developmental stages. It was found that the removed lens is restituted from dorsal iris cells in vivo. A factor controlling the differentiated state of the cells is found to be in the dorsal iris. Removal of this factor by somehow leads to the initiation of lens regeneration. In previous studies vitamin A was found to be very important chemical which removes the many restriction and enhanced the extent of dedifferentiation

of cells and thus accelerated limb regeneration even is adult frogs and lens regeneration in mature tadpoles.

The present investigations on the influence of vitamin A were motivated as mentioned earlier by recently finding by Swami (1993), Garg (1993), Jangir et al (1995,1997) and Ojha (2000); Shekhawat (1998,1999); According to these studies Vitamin A accelerates lens regeneration not only in amphibian tadpoles and chick embryos but also in newly born younger mice babies.

In view of the above present investigation were undertaken to study the effect of Vitamin A on lens regeneration in the froglets of Ranacynophlyctis. It was also decided to study the normal ontogenetic development of lens.

Aim of the Study

The significance of the above work is to provide evidence using dissociation and recombination techniques in vitro that treatment with Vitamin A results in the formation of lens even in the froglets stage whereas lens regeneration was not reported in the control or untreated animals.

Review of Literature

It is now intensively studied by several other workers that pigment cells in the dorsal iris dedifferentiate by changing their structure and function, lose their pigments and proliferate cells that differentiate in the new lens. (Okada, 1976, 77, 80, 83, 86; Mikami 1941, 1959, 1960, 1965; Hasegawa 1958; Stone, 1950 a, 50b, 1974; Yamada, 1967a and b; Dumont and Yamada 1972; Stone and Steintz, 1953; Reyer, 1954, 1962, 1970; Swami, 1993; Jangir et al 1995; Shekhawat, 1998; Jangir et al, 1997, 1999; Shekhawat, 1999; Swami Jangir 1999 and Ojha 2000.Jangir et at 2005 ;Henary and Tsonis 2010 ; Hamilton and Henary 2016 ; Kha et al 2018)

Hypothesis

Ourstudies isbased on the fact that vitamin A induces regeneration capacity in amphibians.

Material and Methods

For all experiments froglets of the frog Ranacyanophlyctis employed were of the same age at the time of operation. In all cases the right eye was operated i.e in all cases the operation wasperformed on the right eye under local anesthesia (xylocainc 2%) A longitudinal slit was made in the cornea of normal eye extending the middle of the pupillary space. The lens was extracted intact. The corneal mind then closed (fig-13).

The vitamin A preparation used was vitamin A palmitate (aqua sol 50000 I.U) the working solution of vitamin A was of 15 iu/ml. strength. 0.05 ml of this strength solution was injected intraperitoneally on alternate day after lentectomy.

The experiments were carried out at room temperature (34-36° C). The solution was prepared by dissolving a known quantity of this drug in a small amount of ethanol to make the stock solution and then diluting a certain quantity from the stock solution with appropriate amount of water.

The animals were preserved on day 3 day and day 25 after operations for histological study. The preservation of the animals atsimilar intervals was also done in control group for comparative study after keeping the animals in bouin' solution for 24-30 hrs and were transferred in 70% alcohol. For histological study the eyes were removed from the preserved animals and dehydrated in alcohol series cleaned in xylene and embedded in paraffin wax. The eyes were sectioned and stained with haemotoxylene and counter stained with eosin representative cases were photographed.

The experiment included in these 2 series were made on 4 group of froglets (A,B,C & D) experiments were performed on 120 animals most of the animal were kept alive for 25 days following operation but some of each group were sacrificed, fixed and their eyes sectioned. Beginning of the first day after operation is designated as day 1 while the day of operation was designated as day 0.

The plan of experiment was as follow:

Group A - Lentectomizedfroglets reared in water.

Gropu B - Lentactomized and vitamin A treated froglets.

Observations and Results

The observations are mainly based on histological study.The results are presented on the table 1. In the animals of group A lens regeneration has not occurred. fig-14 shows non-regenerating iris. The animals of this group were not treated with vitamin A after lentectomy, histologicaly there was no change in iris cell!)

In the animals of group B where vitamin A was injected intra peritoneally on alternate day after operation lens regeneration had occurred in 27 out of 30. Out of 27 regenerated lenses 12 were found quiet normal in shape and histological structures, whereas remaining 15 shows lentoid formation.

During lens regeneration lentectomy the two layers of pigmented epithelium of dorsal iris began to thicken (Fig.15). Figures 15 and 16 shows the thickening of iris layers and the nuclei of iris cells change their shape. Soon the cells become taller and the nuclei become more prominent. These figures also show that the pupillaiy margin become knob like.

Table Showing Lens Regeneration Percentage in Control and Vitamin-A treated animals

S. No.	Developmental phase of animals	Groups	No. of animals	No. of Regenerated Lens on Day 25 after operation	Percentage of Regeneration	Percentage of Non-regeneration
1	Froglet	**A** Control	30	Nil	Nil	100%
2	Froglet	**B** Vitamin A Treated	30	27 (12*+15**)	90 (44.4%+55.6**)	10%

*Normal shaped regenerated lenses ** Lentoids

This knob like structure continues until the free margin becomes swollen loop like structure. Scattered mitotic figures have also been observed. All these changes continue up to day 5 after operation in vitamin A treated animals. Then the cells start to dedifferentiate. They throw out their melanosomes (Fig. 16) and these melanosomes ingested macrophages that have entered from the wounded site. Dorsal iris cell continue to divide, forming dedifferentiate cells in the region of removed lens. The dedifferentiated iris cells now starts synthesizing the differentiate products of lens crystalline proteins. These proteins are almost similar as in intact normal lens development. Once a new lens has formed, the cells of dorsal iris cease mitosis.

The newly formed lens attached to the margin of dorsal iris. The lens is covered by cuboidal epithelium. The fiber growing area was usually more than one cell deep. The central cavity varies in size due to a plug of cells that pushed as a growth from the inner layer of iris into the neck of vesicle.

(Fig-17) shows the complete development and differentiated regenerated lens. The continuing differentiating inner segment of the fiber forming cells lying irregularly of the inner part of the lens vesicle. The distinguished feature was the formation of fibre hillock, the lumen of the vesicle was supposed to be filled by primary lens fibrenuclei, before the secondary lens fibers begin to form. Now the secondary lens fibers begin to differentiate and grow from the peripheral epithelium but did not entirely enclose the primary lens fibre nucleus. The further progress of growth and differentiation of the secondary lens fibres around the central nucleus was easy to follow. The lens at this time was becoming better defined spherical body. At this time the primary lens fibre nucleus was completelysurrounded by the rapidly growing secondary lens fibres. Lens was increasing much in size and the outer surface of the lens became completely surrounded by the developing capsule in 25 days old regenerates (Fig-18). The appearance of this membrane intervening between the lens body and the cells of the dorsal iris could be considered as the movement when the lens was detached. In the next stage of its development the lens had returned to its normal status, the nuclei of the primary lens fibres faded out. At this time lens was growing rapidly by apposition of secondary lens fibres.

Further the nuclei of the secondary lens fibres were progressively disappearing. It covered a long period in which the further growth of the lens carried towards final size. The final phase IV was the period of growth which continued about 25 days after operation or later. Mitosis continued in the lens epithelium. In the central lens fibres the nuclei eventually degenerated.

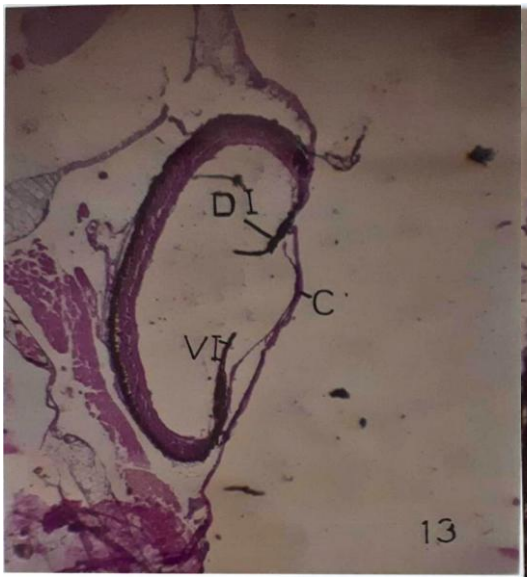
The representatives of complete lens regeneration in the animals of treated group B are shown in (Figs-19). All the regenerated lenses of 25 days old reached stage IV. The distinguished feature is reported in the arrangement of differentiated secondary fibers. Here all the fibers are arranged in transverse fashion (Fig 19) Moreover on day 25 particularly in the regenerated lenses of treated group B fiber forming cells became vacuolated. The bulk of lens now composed of layers upon layers of these fibre cells. Thus the fiber cells formation represents the final stage of lens cells differentiation. Finally the fiber cells lose their replicative activities and essentially enter the permanent stationary phase.

In some cases it is observed that vitamin A treated regenerates contain peculiar lens fibers at stationary phase. The lens fibers of the central zone became either wavy and /or vacuolated. Disintegration of individual cell, pycnosis of nuclei or cytoplasmic vacuolization arc also common features. However, in several cases the regenerated lenses are almost similar to that of the normal lens. Morphological too, the shape of regenerated and normal intact lenses was same. In some cases the size of normal intact lens of 25 days post-metamorphosed froglet is comparatively larger than that of regenerated lens however, the transparency of the both lenses normal and regenerated lenses was exactly similar. In most of the cases the eye with regenerated lens looks as normal as intact eye on day 25 after operation.

In 15 out of 27 regenerates only lentoid have been reported. The regenerated tissue was simply in the form of small nodulated form or lentoid. The microphotographs in (Figs. 20, 21 & 22) show the various degree of lentoid formation.

It has been observed that the origin of lentoid is almost similar to the lens formation i.e. they also originate from the dorsal iris in lentectomizedfroglets. In a few cases lentoid are lacking lens epithelium (Fig-20). This was characterized by desegregations of individual cells, pycnosis of nuclei and cytoplasmic vacuolization. The lens fiber area show a well advanced state of cellular differentiation as indicated by morphology and stain ability of nuclei as well as of the cytoplasm. In many cases the shape of lentoid is typically elliptical and elongated (Fig- 21, 22).

In some lentoid, the lens epithelial cells formed as aggregate of the lens fiber cells. The fiber cells show a stage of differentiation relatively advanced compared to the size of regenerates. Thus without consideration of size of lentoid the lens fiber area showed a well-advanced state of cellular differentiation cytoplasmic vacuolization and irregular arrangement of lens fibers was the common feature of all these lentioids.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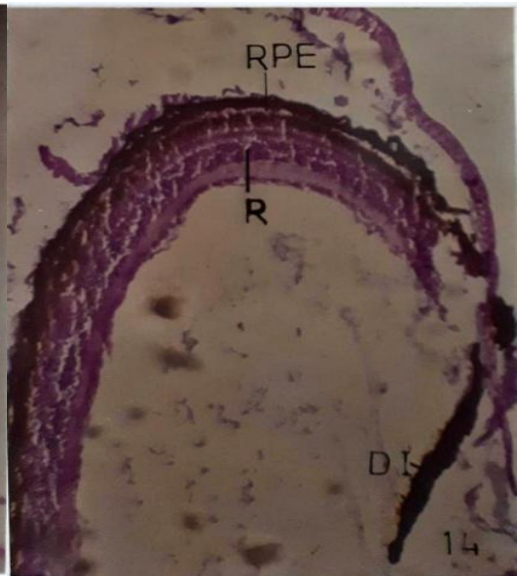
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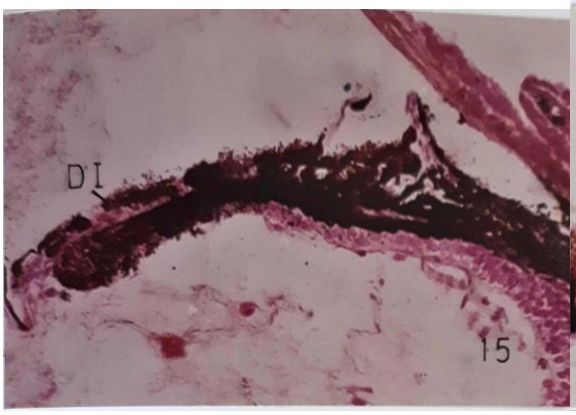
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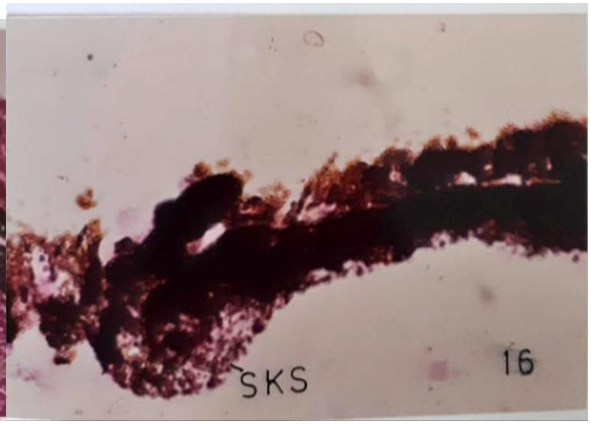
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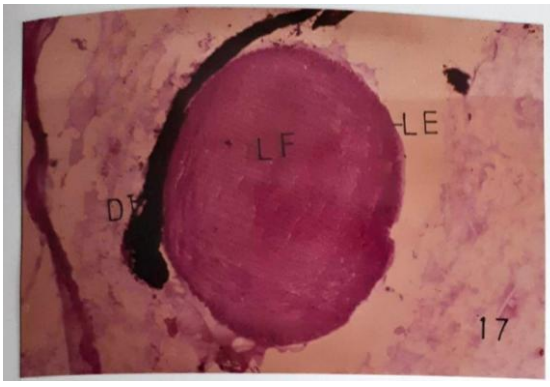
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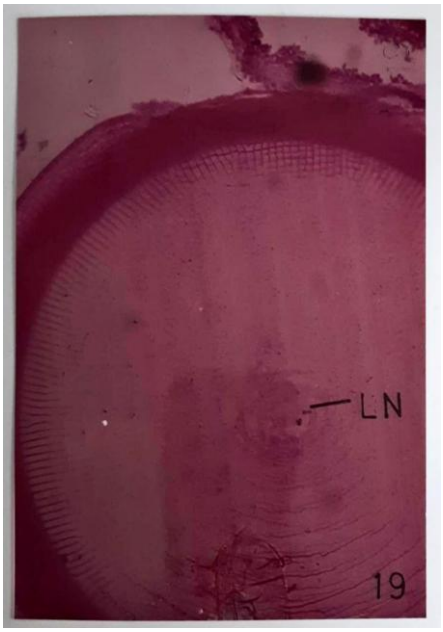

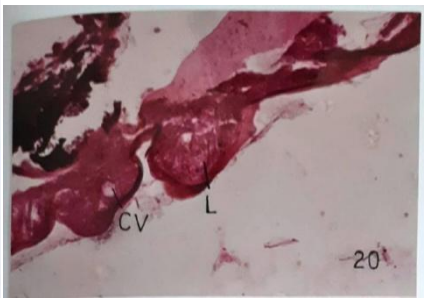

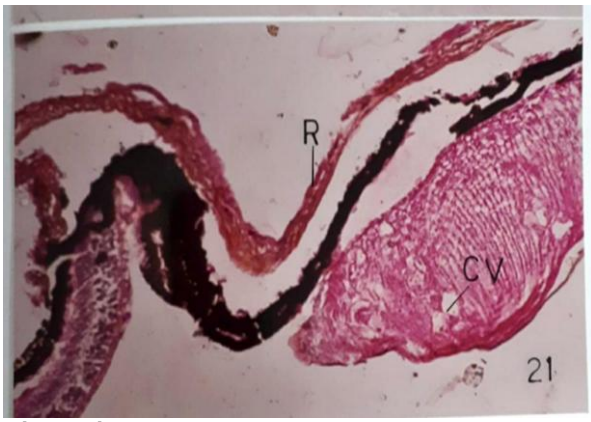

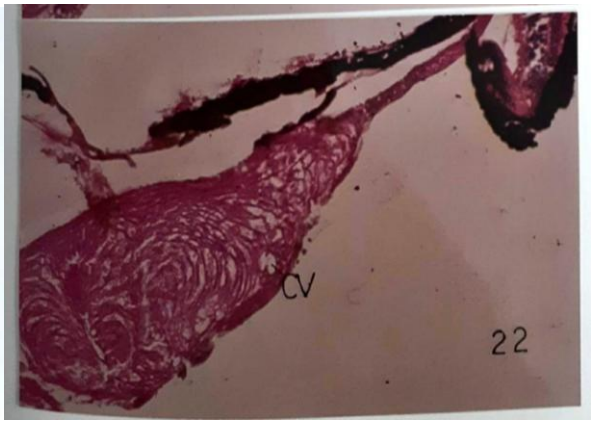

Discussion

From the present studies several conclusions of fundamental nature emerge which make it possible to advance certain plausible hypothesis. Recently De Longh and MC Avoy (1993) and Lovicu et al. (1997) studied the role of fibroblast growth factor in lens development. They concluded that FGF is involved in lens differentiation and growth through out life and distinct expression of FGF is associated with elongating primary fiber cells. They also suggested that from embryonic day 20 on wards lenses showed strongest expression of FGF on RNA in the transitional Zone, Where epithelial cells differentiate into fibers. Messenger RNA for FGF were localized in the ocular tissues near the lens and bordering the ocular median particularly the cornea, Ciliary body, iris and neural retina.

The above discussion regarding the morphogeneesis and differentiation of lens would also be of some use for the analysis of the mechanism of the lens or lentoids formation from the iris.

Present research work have demonstrated that vitamin A induced and accelerated the lens regeneration in the froglets otherwise they have lost this capacity. The froglets of control group did not show lens regeneration however the treatment with vitamin A had undoubtedly brought about the lens regeneration in 90 % cases.

The present results are supported by the observation made by Mitashov (1996). He observed that the removed lens was restituted from the iris cells in vivo. He also suggested that a factor controlling the differentiated state of the cells was found in the dorsal iris during lens regeneration, removal of this factor in the early stage of cell transformation leads to the initiation of lens regeneration.

Vitamin A was found to be very important chemical which removes the many restriction and accelerated limb regeneration even in the adult frogs (Niagi, Jan it & Sharma 1 9g9), Jangir (1980) suggested that vitamin A prevented those factor which promote differentiation and enhanced the extent of dedifferentiation of cells.Polezhaev (1972) has also suggested that if dedifferentiation is augmented by some means, regenerative ability can be induced or enhanced. In this view tissue destruction liberates certain biological active substances, possibly protein or of nucleic acid nature, which lead to tissue dedifferentiation and activate self proliferation ability finally resulting in tissue or organ regeneration. Niagi

et al (19) noted that retinoid treatment improved regeneration ability in the limbs of adult frogs in which it was lost and could revive it some extent similar results have been observed in the present study, the froglet have lost the regenerative capacity but vitamin A induced and brought about the lens regeneration.

It was observed by several workers that vitamin A enhances the DNA and RNA synthetic activity. Thus the data indicate that a change in Pattern of transcription of genetic information is involved in the mechanism of regeneration tissue transformation. So in the harmony with in the idea that enhancement of RNA Synthesis observed in the iris cells after lens removal is one of the essential steps of the tissue transformation. In addition to it, vitamin A was injected in lentectomizedfroglets which could enhance RNA synthesis activity in the present study. It might be the cause of induction of lens regeneration in froglets. This hypothesis is also supposed by the finding of (Jangir et al 1995; 1997 and 1999).

However regarding the mechanism how vitamin A affects the cells to increase the development potencies. Madan (1988) suggested that retinoids enter the cells either via unidentified surface receptor or by lipopilic intercalation through the membrane and then bind to cytoplasmic binding proteins (Chytill and Ong. 1984) like CRABP (cellular retinoic acid binding protein), the complex then transported to the nuclei where it ultimately alters the pattern of gene activity whatever, the mechanism adopted, vitamin A accelerated the regenerative potency in amphibian limbs as well as in the lens of froglet in the present study.

In the present study it has been shown that the proliferation zone of the lens regenerating system represents the pool of cells which are derived from the lens epithelium and gradually transform into fibre cells this zone histologically well defined and shows expression progressive differentiation of the some cell population observation suggests that all cell of the proliferating zone posses the competence for transforming into fiber cell. Accelerated morphogenesis and differentiation of Vitamin A treated regenerated lens have been observed in the present study.

Some general conclusion derived from the present results and was discussed. It was concluded that Vitamin A induces the lens regeneration even in the froglet stage otherwise regenerative ability have lost at this stage.

Lens regeneration occurs from the dorsal iris. From the present studies several conclusions of fundamental nature emerge which make it possible to advance certain plausible hypothesis.

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