

Full Length Research Paper

***In vivo* study of lens regeneration in *Rana cyanophlyctis* under influence of vitamin A and ascorbic acid**

Sumer Singh^{1*} and O. P. Jangir²

¹Department of Biotechnology, Singhania University, Pacheri Bari, Jhunjhunu (Raj.)

²Developmental Biology Lab, Dungar College, Bikaner, Rajasthan

Accepted 18 June, 2013

After removal of lens from eye of *Rana cyanophlyctis*, the pigmented epithelial cells of dorsal iris started to proliferate and dedifferentiate and consequently regenerate lens. Vitamin A and ascorbic acid enhanced the percentage lens regeneration not only in young tadpoles but also in froglets. Lens regeneration ability declined with age of animals in both control as well as treated groups.

Key words: *Rana cyanophlyctis*, pigmented epithelial cells, vitamin A, ascorbic acid.

INTRODUCTION

Urodele amphibians are unique among vertebrates in their strong ability to regenerate lost part/ parts of body throughout their lives (Ferretti and Geraudie 1998; Wolpert et al., 1998) and in their striking resistance to tumor occurrence (Okamoto, 1987, 1988, 1997; Brockes, 1998). After lens removals through the pupil of the newt's eye, pigmented dorsal iris epithelial cells begin to dedifferentiate, proliferate and transdifferentiate into lens cells (Wolff, 1895; Kodama and Eguchi, 1995). In this Wolffian lens regeneration, it is noteworthy that only the dorsal iris can regenerate a new lens, while the ventral iris never shows such ability. Despite many morphological and biochemical studies on the process of lens regeneration, the difference between dorsal and ventral cells remains to be elucidated (Reyer, 1977; Yamada, 1977). In the present study, we proved that pigmented epithelial cells of dorsal iris have intrinsic ability to transdifferentiate into lens. Vitamin A and ascorbic acid was found to accelerate the percentage of lens formation from the dorsal iris in *in vivo* condition.

MATERIALS AND METHODS

For the present experiment, three different developmental stages: young tadpoles (3 toe stage), mature tadpoles (5 toe stage) and froglets of the frog, *Rana cyanophlyctis* were employed. For study of lens regeneration in young and mature tadpoles and froglets of the *R. cyanophlyctis* under the influence of vitamin A and ascorbic acid, animals of each group were divided as per plan of experiment (Table 1). Each age group contained 20 animals and thus the present experiment was performed on 180 animals. Each group contained controls (animals were not treated with vitamin A and ascorbic acid). In control group, animals particularly froglets, were given sham injection while in treated group, animals the respective chemicals (vitamin a and ascorbic acid) were injected on alternate day after operation upto the day of termination of the experiment.

RESULTS

The results are shown in Table 2. The experiment concerned the study of lens regeneration in young tadpoles, mature tadpoles and froglets of *R. cyanophlyctis* under the influence of vitamin A and

*Corresponding author. Email: sumer_raj_singh@yahoo.co.in

Table 1. Plan of experiment.

Group (age of animals at the time of operation)	Subgroup	Number of animals used	Day of preservation
G1-Young tadpoles (YT)	Control (YTG1C)	20	3
			7
			15
			40
	Vitamin A treated (YTG1VA) Ascorbic acid treated (YTG1AA)	20 20	-do- -do-
G2-Mature tadpoles (MT)	Control (MTG2C)	20	3
			7
			15
			40
	Vitamin A treated (MTG2VA) Ascorbic acid treated (MTG2AA)	20 20	-do- -do-
G3-Froglets (FT)	Control (FTG3C)	20	3
			7
			15
			40
	Vitamin A treated (FTG3VA) Ascorbic acid treated (FTG3AA)	20 20	-do- -do-

Table 2. Influence of vitamin A and ascorbic acid on lens regeneration in young, mature tadpoles and froglets of the frog *Rana cyanophlyctis*.

Group	Sub group	Number of animals used	Day of preservation	Number of animals preserved	Number of		Percentage (%) of regeneration
					Regenerates	Non-regenerates	
G ₁ -Young tadpoles (YT)	Control (YTG ₁ C)	20	3	4	-	4	60.00
			7	4	3	1	
			15	4	3	1	
			40	8	6	2	
	Vitamin A treated (YTG ₁ VA)	20	3	4	4	-	95.00
			7	4	4	-	
			15	4	4	-	
			40	8	7	1	
	Ascorbic acid treated (YTG ₁ AA)	20	3	4	3	1	85.00
			7	4	4	-	
			15	4	4	-	
			40	8	6	2	
G ₂ -Mature tadpoles (MT)	Control (MTG ₂ C)	20	3	4	1	3	55.00
			7	4	2	2	
			15	4	2	2	
			40	8	6	2	
	Vitamin A treated (MTG ₂ VA)	20	3	4	3	1	80.00
			7	4	3	1	
			15	4	4	-	
			40	8	6	2	
	Ascorbic acid treated (MTG ₂ AA)	20	3	4	3	1	75.00
			7	4	3	1	
			15	4	4	-	
			40	8	5	3	

Table 2. Contd.

G ₃ -Froglets (FT)	Control (FTG ₃ C)	20	3	4	-	4	
			7	4	1	3	
			15	4	1	3	20.00
			40	8	2	6	
	Vitamin A treated (FTG ₃ VA)	20	3	4	-	4	
			7	4	1	3	
			15	4	3	1	50.00
			40	8	6	2	
	Ascorbic acid treated (FTG ₃ AA)	20	3	4	1	3	
			7	4	2	2	
			15	4	2	2	45.00
			40	8	4	4	

ascorbic acid. After lentectomy, the operated animals were treated with vitamin A (30 IU/ml) and ascorbic acid (50 µg/ml) and preserved at various intervals of time (day 3, 7, 15, 40) for histological evaluation. Results were compared with that of untreated control group animals of the same age. Simultaneously, lens regenerative capability of different age group animals was also studied.

The results obtained in the present study show that lens regenerative power is present in the tadpoles of *R. cyanophlyctis*. However, it declined with the age of animal. It was 60% in young tadpoles; 55% in mature tadpoles and 20% in froglets of frog *R. cyanophlyctis*. In contrast to it, vitamin A and ascorbic acid were found to induce and accelerate lens regeneration in the animals of all ages. It was 95, 80 and 50% in young tadpoles, mature tadpoles and froglets, respectively whereas in ascorbic acid treated animals, the percentage of lens regeneration was 85, 75 and 45% in young tadpoles, mature tadpoles and froglets, respectively (Table 2). The declined trend of regeneration was also found in vitamin A and ascorbic acid treated animals with their age. The histological changes occurred in the lens regeneration of young tadpoles of sub group YTG₁C, YTG₁VA and YTG₁AA as follow: in the animals of this age group, lens regenerative events were found almost similar except their percentage. The events that occurred during lens regeneration are presented here through selective representative animals that were preserved at different time intervals. The representative cases were taken from vitamin A treated groups. In all cases (control as well as vitamin A and ascorbic acid treated animals), lens regeneration occurred from pigmented epithelium cells of dorsal iris. During lens regeneration, it was observed that after lentectomy, the two layers of pigmented epithelium of dorsal iris began to thicken and cleft arose between inner and outer lamella (Figures 1 and 2).

Figure 1 and 2 show the thickening of iris layers and cleft formation. The nuclei of iris cells change their shape on day 3 after operation. Soon the cells become

elongated and the nuclei become more prominent. Later on the pupillary margin becomes knob-like. This knob-like structure continued until the free margin became swollen loop-like structure by day 7 after operation (Figure 3). Scattered mitotic figures were also observed. All these changes continue up to day 7 after operation in vitamin A treated animals. Then the cells of dorsal iris started to dedifferentiate. They throw out their melanosomes and these melanosomes later on disappeared probably ingested by macrophages. Dorsal iris cells continue to divide forming dedifferentiated cells in the region of cleft (Figure 3). These dedifferentiated cells are considered as lens forming cells. The dedifferentiated iris cells by day 15 started synthesizing the differentiated products of lens crystalline proteins. These proteins were almost similar as in normal lens development. Once the new lens had been formed, the cells of dorsal iris ceased mitosis. Figures 4 and 5 show the newly formed vesicular cellular lens attached to the margin of dorsal iris. The cells in the wall of the vesicle are cuboidal and slightly taller but in addition to the fibre 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. Now onwards by day 15 after operation, lens fibre differentiation begins. Figures 6, 7 and 8 show the cellular regenerated lens still attached to the dorsal iris. The next phase of this development was shown by differentiation of lens fibres. On day 15 onwards, primary lens fibres started to appear (Figure 9). The primary lens fibres pushed to front of the vesicle forming a nucleus behind the lens epithelium.

Now the secondary lens fibres begin to differentiate but do not enclose entirely the primary lens fibre nucleus. At last the nuclei of the secondary lens fibres progressively disappeared. It covered a long period in which the further growth of the lens carried towards final size. The final phase was the period of growth which continued about 40 days after operation. The nuclei of lens fibres eventually degenerated (Figures 10 and 11). Figure 12 shows

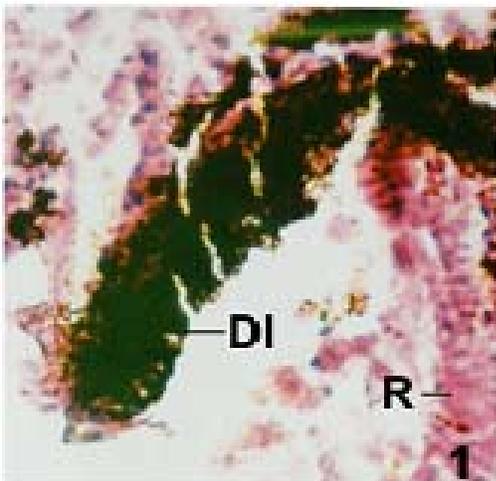


Figure 1. Microphotograph of a section passing through the lentectomized eye of vitamin A treated young tadpole of *Rana cyanophlyctis* showing bylaminal layers of iris (100X). DI, Dorsal iris; R, retina.

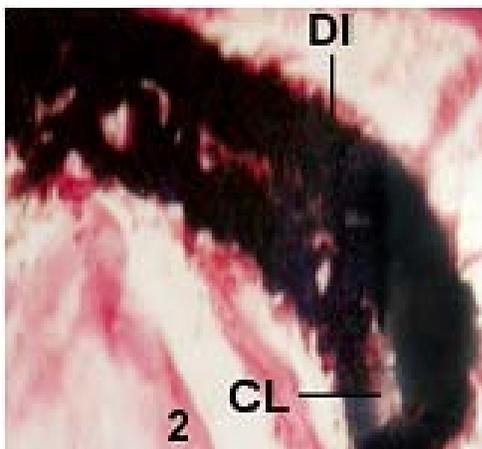


Figure 2. Microphotograph of a section passing through the lentectomized eye of 3 days vitamin A treated young tadpole showing cleft formation at the tip of dorsal iris (100X). DI, Dorsal Iris; CL, cleft.

concentrically arranged well differentiated secondary lens fibres in ascorbic acid treated animals. Similarly, well formed lens were also observed in untreated control group tadpoles (Figure 13). All these representative figures (Figures 11 to 13) clearly show well differentiated regenerated lenses in which secondary lens fibres are enclosing the primary lens fibre nucleus.

Similar types of changes have also been observed in regenerating cases of ascorbic acid treated animals and untreated control group tadpoles as well. In the control group, lens regeneration occurred from dorsal iris (Figure 13). The stages of lens fibre differentiation were found

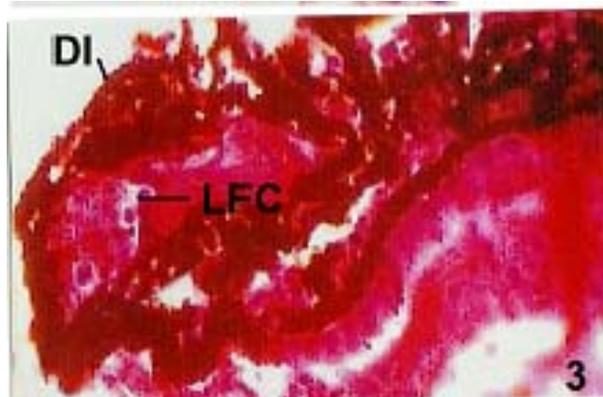


Figure 3. Microphotographs of sections passing through the lentectomized eye of 7 days vitamin A treated young tadpole of *Rana cyanophlyctis* showing didifferentiation of PECs and formation of lens forming cells into the cleft (100X). DI, Dorsal Iris; LFC, lens forming cells.



Figure 4. Microphotographs of sections passing through the lentectomized eye of 7 days vitamin A treated young tadpole of *Rana cyanophlyctis* showing differentiation of PECs and formation of lens forming cells into the cleft (100X). DI, Dorsal Iris.

exactly similar as reported in vitamin A and ascorbic acid treated animals. However, the percentage of lens regeneration was found low in controls. Lentoids have also been observed in tadpoles of all the groups particularly of mature tadpoles and froglets (Figure 14). The dorsal iris was found unchanged in non-regenerating cases as shown in Figure 15.

Lens regeneration in the animals of G₂ group (5 toe stage mature tadpoles)

The animals of this group were of mature tadpole stage. Similar to sub group YTG₁C, YTG₁VA and YTG₁AA, the



Figure 5. Microphotograph of a section passing through the lenctomized eye of 15 days vitamin A treated young tadpole of *Rana cyanophlyctis* showing formation of lens vesicle at the tip of dorsal iris (200X). LV, lens vesicle; DI, Dorsal Iris.

animals of this group were subjected to lentectomy in the right eye and were divided into three sub groups MTG₂C, MTG₂VA and MTG₂AA. The animals of MTG₂C sub group were not treated with any chemical and were considered as control group animals. Only sham injections were given after their lentectomy whereas the animals of sub group MTG₂VA and MTG₂AA were treated with vitamin A (30 IU/ml) and ascorbic acid (50 µg/ml) after their lentectomy.

In the animals of the control sub group, MTG₂C lens regeneration occurred in 11 out of 20. However, the percentage of lens regeneration was found high (80 and 75%) in vitamin A and ascorbic acid cases. The regenerated lenses were almost similar to that of young tadpole (YT) group animals as described earlier but in few cases regenerated lenses were found smaller and were of lentoid shape. Histological events of lens regeneration were found almost similar as described earlier viz. after lentectomy depigmentation of pigmented epithelium of dorsal iris, dedifferentiation of pigmented epithelial cells (PECs), proliferation, lens vesicle forma-

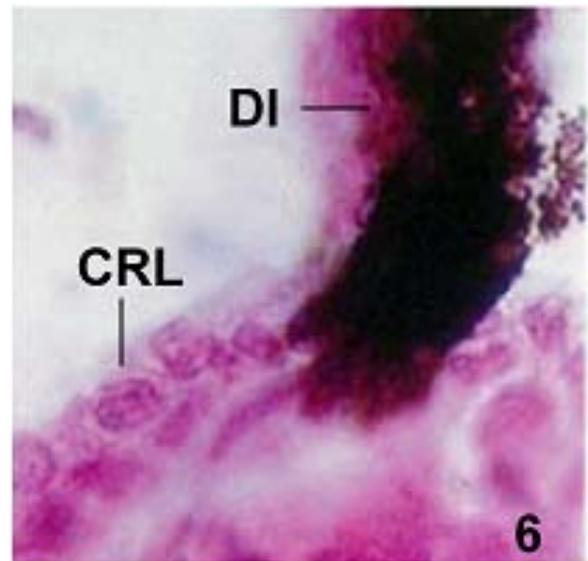


Figure 6. Microphotographs of sections passing through the lenctomized eye of 15 days vitamin A treated tadpole of *Rana cyanophlyctis* showing cellular regenerated lens (RL) attached with the tip of dorsal iris (200X). CRL, Cellular regenerated lens; DI, dorsal Iris.

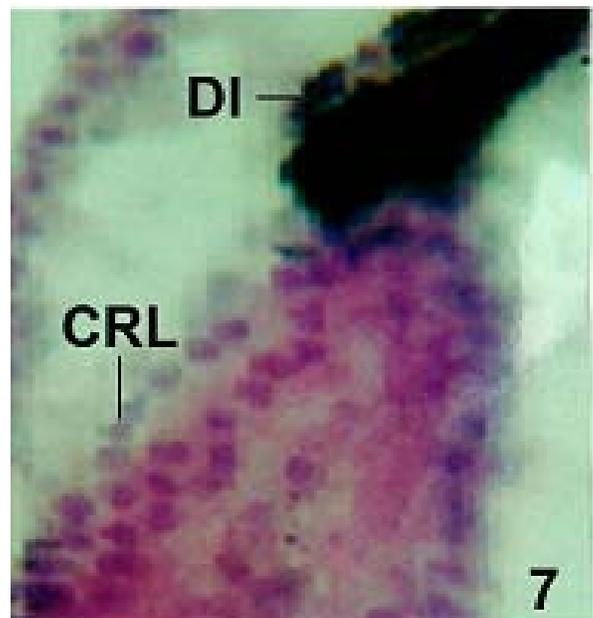


Figure 7. Microphotographs of sections passing through the lenctomized eye of 15 days vitamin A treated tadpole of *Rana cyanophlyctis* showing cellular regenerated lens (RL) attached with the tip of dorsal iris (200X). DI, Dorsal Iris; RL, regenerated lens.

tion, differentiation of primary lens fibres and secondary lens fibres. The regenerated lenses so formed started

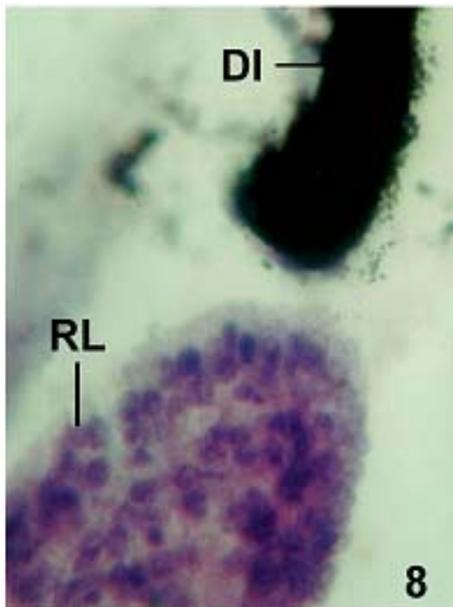


Figure 8. Microphotograph of a section passing through the lentec-tomized eye of 15 days vitamin A treated young tadpole showing further differentiation of regenerated lens (RL) (200X). DI, Dorsal Iris; RL, regenerated lens.

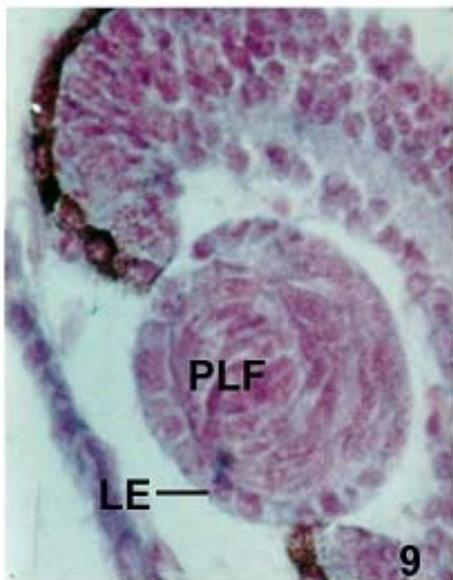


Figure 9. Microphotograph of a section passing through the lentec-tomized eye of 15 days vitamin A treated young tadpole showing regenerated lens with primary lens fiber (PLF) (200X). LE, Lens epithelium; PLF, primary lens fiber.

to grow and attend similar size and shape to normal lenses.

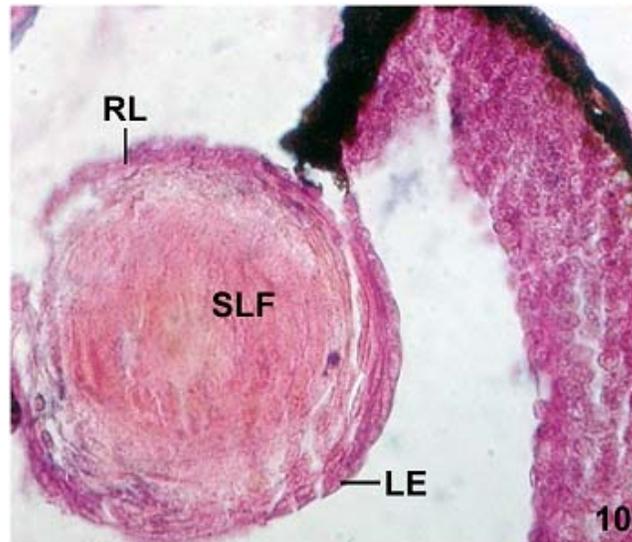


Figure 10. Microphotograph of a section passing through the lentec-tomized eye of 40 days vitamin A treated young tadpole showing regenerated lens (RL) with epithelium and secondary lens fibers (200X). LE, Lens epithelium; RL, regenerated lens; SLF, secondary lens fiber.

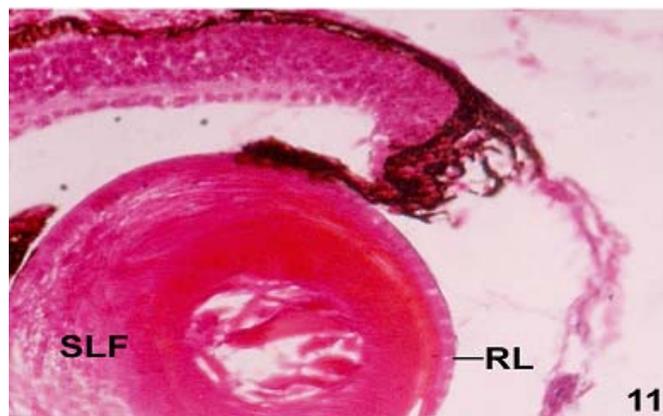


Figure 11. Microphotograph of a section passing through the lentic-tomized eye of 40 days vitamin A treated tadpole showing well differentiated regenerated lens (100X). RL, Regenerated lens; SLF, secondary lens fiber.

Lens regeneration in the animals of G₃ group (Froglet stage)

The animals employed were newly metamorphosed froglets. The method of lentic-tomy and mode of treatment was the same as employed for the animals of G₂ group. After lentic-tomy, operated animals were divided into three sub groups: FTG₃C, FTG₃VA and FTG₃AA controls, vitamin A and ascorbic acid treated animals, respectively. Normal lens regeneration was not reported in any animal of FTG₃C control group. However,

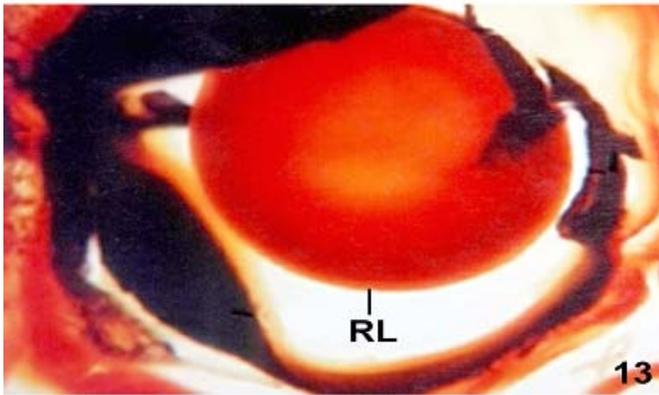


Figure 13. Photograph of a hand section of lentectomized eye of untreated tadpole (control) showing well differentiated and spherical regenerated lens (50X). RL, Regenerated lens.



Figure 14. Microphotograph of a section passing through the lentectomized eye of 40 days vitamin A treated tadpole showing lentoids (LD) formation (100X). LD, Lentoids.

in four out of 20 animals, lentoid formation was reported. In vitamin A (FTG₃VA.) and ascorbic acid (FTG₃AA) treated animals, lens regeneration occurred in 50 and 45% of animals. The regenerated lenses of these animals too, were found abnormal in shape. Majority of them were smaller in size and variable in shape. Histologically, it has been observed that the origin of lentoid is almost similar to lens formation, that is, they also originated from the dorsal iris in lentectomized iris. In a few cases, lentoids lacked lens epithelium. This was characterized by segregation of individual cells, pycnosis of nuclei and cytoplasmic vacuolization. The lens fibre area showed a well-advanced state of cellular differentiation. In many cases, the shape of lentoid is typically elliptical and elongated. In some lentoids, the lens epithelial cells formed as aggregation of the lens fibre cells. The lens fibre cells showed a stage of differentiation relatively advanced, in comparison to that of regenerated lens of



Figure 15. Microphotograph of a section passing through the lentectomized eye of 40 days vitamin A treated tadpole showing non-regenerating dorsal iris, the both layers of dorsal iris (bilaminar layer) remain closed (50X). NRDI, Non regenerate dorsal iris.

vitamin A treated and ascorbic acid treated animals.

DISCUSSION

The results of the present study showed that both vitamin A and ascorbic acid can induce and accelerate the transdifferentiation of retinal iris PECs into lens *in situ*. In these experimental conditions, vitamin A and ascorbic acid increased the percentage of lens regeneration in all the three developmental stages of the frog, *R. cyanophlyctis*. As shown in Table 2, the increased percentage of lens regeneration in vitamin A treated animals was 95% in young tadpoles, 80% in mature tadpoles and 50% in froglets. Similar trend was also found in the animals of ascorbic acid treated groups. It was 85% in young tadpoles, 75% in mature tadpoles and 45% in froglets. As shown in Table 2, the percentage of lens regeneration in control group animals was low. Thus, from the present results, it can be concluded that both vitamin A and ascorbic acid are good models for accelerating lens regeneration in the anuran amphibian (*R. cyanophlyctis*). The results also reveal that the regenerative ability in the present species of the frog employed (*R. cyanophlyctis*) declined as the animal ages.

Lens regeneration from non-ocular tissue (dorsal iris)

has been well documented in urodele amphibians (Reyer, 1977, 1954; Eguchi and Itoh, 1982; Eguchi, 1988). However, recently it has been observed that the capability of retinal iris PECs to transdifferentiate into lens is not restricted to urodeles amphibians only but is widely conserved in almost all vertebrates (Okada, 2000; Jangir et al., 2000, 2001, 2005; Acharya et al., 2003; Shekhawat et al., 2001). It is well studied that lentectomy stimulates the iris epithelial cells of the newt's eye to undergo DNA synthesis and proliferate (Eisenberg and Yamada, 1966; Yamada and Roesel, 1969; Reyner, 1971). Concomitantly, with these processes, melanosomes disappear from the pigmented epithelial cells of iris and thus undergo dedifferentiation. After completion of the phase of dedifferentiation, some cells retreat from the cell cycle, elongate and proceed to synthesize lens specific proteins and transform into lens fibres (Reyer, 1971; Tsonis et al., 2004). Similar types of regenerative steps of lens regeneration have been observed also in the present treated animals.

The present results confirm the previous report of beneficial influence of vitamin A on lens regeneration. Shekhawat et al. (2001) and Jangir et al. (2005) discovered that vitamin A could induce dedifferentiation in the dorsal iris cells to bring about regeneration of lens. Dedifferentiation is supposed to be prerequisite for regeneration. It is generally an accepted view that if dedifferentiation of an extraordinary degree could be brought, then the cells acquire new developmental potencies. Grigoryan and Mitashov (1991) also studied the effect of retinoic acid on regeneration of the crystalline lens in adult triton and found additional lens from the dorsal iris.

Workers from the same laboratory also observed that vitamin A induced and accelerated lens regeneration in amphibian tadpoles, froglets and chick embryos, swiss albino mice, guinea pigs, rabbits and pigs (Shekhawat et al., 2001; Swami, 1992; Swami and Jangir, 1997, 2000, 1995, 1997; Garg, 1993). The exact mechanism of Vitamin A action is still not well known. However, Chytil and Ong (1984) and Maden (1988) suggested that retinoids enter the cells either via some surface receptors or by lipophilic intercalation through the membranes and then bind to cytoplasmic retinal binding proteins (RABP). Chytil and Ong (1984) and Petkovich et al. (1987), also reported such binding proteins in most of the cells; one specific for retinoic acid, cellular retinoic acid binding protein (CRABP) and other specific for retinal, cellular retinal binding protein (CRBP). The complex is then transported to the nuclei where it ultimately alters the pattern of gene activity. Maden (1988) noticed that the level of CRABP are low in the unamputated axolotl limbs and have risen 3-4 fold in blastemal stage and then decrease again during redifferentiation. This striking correlation suggests that CRABP may indeed be playing a role in the respecification of positional information. Thus, it can be suggested that retinoids play an important role

in the transmission of their legends from the cell membrane to the nucleus where the pattern of gene activity may be altered. Whatever, the mechanism adopted, this can be equally applicable to the observations made by Jangir (1980), Jangir and Niazi (1978) and Niazi et al. (1985, 1989) in limb regeneration of amphibian tadpoles. Vitamin A excess is also found to cause dissolution of cartilage and bone matrix *in vivo* and *in vitro* leading to liberation of healthy, dividing and undifferentiated cells. It is believed to do so by reducing the stability of lysosomal membrane and liberation of hydrolytic enzymes from them (Fell and Thomas, 1960; Dingle et al., 1961; Dingle et al., 1963; Fell and Rinaldini, 1965; Roles, 1969). Polezhaev (1972) have also suggested that if tissue destruction and dedifferentiation is augmented by some means, regenerative ability can be induced or enhanced. In this view, tissue destruction liberates certain biologically active substances possibly protein or nuclei acid in nature, which lead to tissue differentiation and active self-proliferation finally resulting in tissue or organ regeneration.

Similar to present results Jangir et al. (2005), reported that vitamin A induces lens regeneration in mammals also. In support of vitamin A influence on lens regeneration, the report of Tsonis et al. (2000) is much relevant. They studied role of retinoic acid on urodele lens regeneration. They reported that when synthesis of retinoic acid was inhibited by disulfiram or when the function of the retinoid receptors was impaired by using an RAR antagonist, the process of lens regeneration was dramatically affected. In the majority of the cases, lens regeneration was inhibited and lens morphogenesis was disrupted.

Retinoids are found to activate DNA and RNA synthetic activity in several regenerative tissues. In contrast to enhancing DNA and RNA synthetic activity of vitamin A, Yamada and Roesel (1964) studied the effect of actinomycin-D on the lens regenerative system and found that injecting actinomycin-D can inhibit regenerative transformation of the iris cells into the lens. They suggested that the actinomycin-D primarily inhibits the synthesis of RNA which is dependent upon DNA as demonstrated in other system. It is in harmony with the idea that enhancement of RNA synthesis activity observed in iris cells after lens removal is one of the essential steps of the tissue transformation into lens. Thus, for the present observation, that is, enhancement of lens regeneration in vitamin A treated animals, it may be true that vitamin A injection in lentectomized mice could enhanced RNA synthetic activity and hence induction of lens regeneration. This hypothesis is also supported by previous finding of Jangir et al. (1995, 1997) and Shekhawat et al. (2001). The work of Harreno Saenz et al. (1994) on lens regeneration in adult newt also supports the present observations. They used antitumor drug 3-nitro benzothiazolo quinolinium chloride (NBQ) and cytotoxic drug doxorubicin for lens regene-

ration in adult newt. They found that NBQ accelerated cell proliferation while doxorubicin inhibits the same and thus NBQ was found to stimulate lens regeneration, independently of the time intervals and the stage of regeneration at which the drug was administered.

The present study and the results of previous studies too show that vitamin A accelerates the rate of mitosis after lensectomy and thus enhanced the dedifferentiation. Mc Devitt et al. (1982, 1990), and Yamada and Mc Devitt (1984) also reported that lensectomy in adult newt initiates the cell cycle and cell division in the dorsal iris cells which are main causative reason for the lens regeneration (Zalik and Scott, 1973, 1990). The pigmented epithelial cells from the dorsal iris dedifferentiate and subsequently transdifferentiate to form the regenerating lens. The restriction of this capability of the pigmented epithelial cells of the dorsal iris, together with the tissue's shorter cell cycle time (compared to ventral iris epithelium) after re-entry into the cell cycle, suggests that the capacity for lens regeneration may be related to mitogenic activity.

It is quite possible that vitamin A affected the cell surface or intercellular space stabilizing factors of dorsal iris epithelial cells and induced the cells for such transdifferentiation. Eguchi (1998) reported in his cell culture study that PECs dissociated from fully grown human eyes readily transdifferentiate into lens phenotype. In addition, Eguchi (1998) speculated that molecules detected in either cell surface or inter cellular space stabilizing the differentiated stage of PECs in the newt and that the loss of these molecules might be one of the key steps of lens regeneration from the iris epithelium.

Results of the present study are also being supported by the finding of Eguchi et al. (1974). In their finding, they observed that under certain conditions, dedifferentiation of extra ordinary cells enable the acquisition of new and higher developmental potencies. In their findings, they observed that under certain conditions, dedifferentiation of extraordinary cells enable them to acquire new and higher developmental potencies.

In the present study, ascorbic acid was found to accelerate dedifferentiation concomitantly increasing the percentage of lens regeneration in comparison to that of untreated control group animals. The exact mechanism of ascorbic acid on lens regeneration is not yet clear and the literature on this Ayurveda drug (with this respect) is very scanty. However, it is reported that a constituent of ascorbic acid have accelerating effect on wound healing (Hellman and Burns, 1958; Jagetia et al., 2003) and enhance proliferation and dedifferentiation of iris pigmented epithelial cells in culture medium. It is reported that ascorbic acid increases the formation of lentoids in culture medium (Kosaka et al., 1998). Good results have been seen in the treatment of diabetic cataract (Suryanarayana et al., 2004), watering of eyes, cough, asthma, bronchitis, peptic ulcer, cardiac disorders. It also improves the mitotic activity in the injured tissues. Thus

accelerating effect on lens regeneration in the developmental stages of the animal *R. cyanophlyctis* is a peculiar feature observed in the present study.

REFERENCES

- Acharya P, Shekhawat DVS, Ojha S, Sharma M, Jangir OP (2003). Effect of Vitamin A on lens regeneration in adult and old Swiss albino mice. National symposium on Dev. and Reproduction. Deptt. of Zoology, Karnataka University, Dharwad and Indian Society of Developmental Biologist. ISDB. ps-01
- Brockes JP (1998). Regeneration and cancer. *Biochem. Biophys. Acta.* 1377:1-11.
- Chytil F, Ong DE (1984). Cellular retinoid binding protein. In the retinoids. Ed. M.B. Sporn, A.B. Robert and D.S. Godman. New York, Academic Press. 2:89-123.
- Dingle JT, Leck JA, Fell HB (1961). Studies on the mode of action of excess of Vitamin A on the metabolism and composition of embryonic chick limb cartilage grown in organ culture. *J. Biochem.* 79:497-500.
- Dingle JT, Moore T, Shearman JM (1963). The influence of Vitamin A status on the proteolytic activity of lysosomes from the livers and kidneys of rats. *Proc. Nat Soc.* 22:183.
- Eguchi G (1988). Cellular and molecular background of wolffian lens regeneration. In "Regulatory Mechanisms in developmental process" (Eguchi, G. Okada, T.S., and Saxen, L., Eds.). Elsevier, Ireland. pp. 147-158.
- Eguchi G (1998). Transdifferentiation, Transdifferentiation in vertebrate cells in cell culture, pp. 242-258.
- Eguchi G, Abe SI, Watanabe K (1974). Differentiation of lens-like structures from newt iris epithelium cells *in vitro* USA. *Proc. Natl. Acad. Sci.* 71(12):5052-5056.
- Eguchi G, Itoh Y (1982). Regulation of the lens as a phenomenon if cellular transdifferentiation: Regularity of the differentiated state of the vertebrate pigment epithelial cell. *Trans. Ophthal. Soc. UK* 102:374-378.
- Eisenberg S, Yamada T (1966). A study of DNA synthesis during the transformation of the iris into lens in the lensectomized newt. *J. Exp. Zool.* 162:353-368.
- Fell HB, Rinaldini LM (1965). The effect of Vitamin A and C on cell and tissue in culture; in *Cells and Tissue Culture* (eds.) E.N. Willmer (Academic Press). Pp. 659-699.
- Fell HB, Thomas L (1960). Comparison of the effect of papain and Vitamin A on cartilage. The effect on organ culture of embryonic skeletal tissue. *J. Exp. Med.* 111:719-744.
- Ferretti P, Geraudie J (1998). *Cellular and Molecular Basis of Regeneration: From invertebrates to Humans.* John Wiley & Sons, Chichester.
- Garg S (1993). A study of ontogenetic development of eye and lens regeneration in brown leg horn chick under the influence of Vitamin A excess, - Dissertation (M.Phil.) M.D.S. University, Ajmer.
- Grigoryan EN, Mitashov VI (1991). The comparative characteristics of the effect of retinoic acid on the regeneration of the crystalline lens and the extremity in adult tritons. *Izv Akad. Nauk. SSSR (Biol.)*, Sep; 5:726-734.
- Harreno Saenz D, Ortiz JR, Baez A (1994). Effects of 3-NBQ and Doxorubicin on lens regeneration in adult newt a morphological study. *Differentiation* 55(3):169-74.
- Hellman L, Burns JJ (1958). Metabolism of L-ascorbic acid-1-L-14 in man. *J Biol Chem*, 230:923-930.
- Jagetia GC, Rajankant GK, Rao SK (2003). Evaluation of the effect of ascorbic acid treatment on wound healing in mice exposed to different doses of fractionated gamma radiation. *Radiat. Res.* 159(3):371-80.
- Jangir OP (1980). Experimental studies on the ontogenesis and regeneration of limbs in anuran *Bufo melanostictus* (Shneider). Ph.D. thesis submitted to University of Rajasthan, Jaipur.
- Jangir OP, Khachawa GS, Sharma R (2000). Study of fate of cytolysed iris implants into denucleated eye and lens regeneration in the tadpoles of *Rana cyanophlyctis*. A symposium of developmental regulation, Growth and differentiation. Feb 18-19, Pune. *Abst.* 25:17.

- Jangir OP, Modi D, Sharma M (2005). Effect of Vitamin A on lens regeneration in pigs. *Indian J. Exp. Biol.* 43:679-685.
- Jangir OP, Niazi IA (1978). Stage dependent effects of Vitamin A excess on limb during ontogenesis and regeneration in tadpoles of the toads, *Bufo melanostictus* (Schneider). *Indian J. Exp. Zool.* 16:438-445.
- Jangir OP, Shekhawat DVS, Acharya P, Swami KK, Suthar P (2001). Homoerotic regeneration of eye in amphibian tadpole and its enhancement by Vitamin A. *J. Biosci.* 26:577-581.
- Jangir OP, Singh DS, Swami KK (1995). A study of Lens regeneration in the tadpoles of *Rana Cynophlyctis* under the influence of Vitamin A. XI National symposium on Developmental Biology, Rohatak, pp. 30.
- Jangir OP, Singh DVS, Ojha S, Sharma R (1997). Histological study on lens regeneration in young mice under influence of Vitamin A, International Symposium on development, growth and differentiation. Mahabaleshwar 17-20
- Kodama R, Eguchi G (1995). From lens regeneration in the newt to *in vitro* transdifferentiation of vertebrate pigmented epithelial cells; *semin cell boil.* 6(3):143-149.
- Kosaka M, Kodama R, Eguchi G (1998). *In vitro* culture system for iris pigmented epithelial cells for molecular analysis of transdifferentiation. *Exp. Cell. Res.* 245:245-251.
- Maden M (1988). Vitamin A and its effect on pattern formation in the regenerating limb. *Proc. 6th M. Singer Symposium Ed. by Inouc et al.*, pp. 111-124.
- Mc Devitt DS, Brahma SK, Courtois Y, Jeanny JC (1990). Ontogeny and localization of Alpha A and Alpha B Crystalline during regeneration of the eye lens. *Exp. Eye Res;* 51(5):625-630.
- Mc Devitt DS, Brahma SK, Courtois Y, Jeanny JC (1982). Alpha, Beta and Gamma-crystalline in the regenerating lens of *Notophthalmus Viridescens*. *Exp. Eye Res;* 34(4):587-594.
- Niazi IA, Jangir OP, Alam S, Sharma KK, Ratnasarny C (1989). Vitamin A effect on limb regeneration: N studies on the tadpoles of anuran amphibian: In recent trends in regeneration research Ed. V. Mortis, S. Koussoulakos and H. Wallance (New York; Plenum Press): NATO-ASI Series, Series A:Life Sci. 172:355-370.
- Niazi IA, Pescitelli MJ, Stocum DL (1985). Stage dependent effects of retinoic acid on regenerating urodele limbs. *Roux's Arch. Biol.*; 194:355-363.
- Okada TS (2000). Lens studies continue to provide landmarks of embryology (developmental biology) Perspectives. *J. Bio. Sci.* 25(2):133-141.
- Okamoto M (1987). Induction of ocular tumor by nickel subsulfide in the Japanese common newt, *Cynops pyrrhogaster*. *Cancer Res.* 47:5213-5217.
- Okamoto M (1988). Inhibition of lens regeneration by nickel subsulfide in the Japanese common newt, *Cynops pyrrhogaster*. *Dev. Growth Differ.* 30:75-80.
- Okamoto M (1997). Simultaneous demonstration of lens regeneration from dorsal iris and tumour production from ventral iris in the same newt eye after carcinogen administration. *Differentiation* 61:285-292.
- Petkovich M, Brand NJ, Krust A, Chambon P (1987). A human retinoic acid receptor, which belongs to the family of nuclear receptors. *Nature* 33:440-450.
- Polezhaev LV (1972). Loss of restoration of regenerative capacity in tissue and organ of animals; Harvard, U.P. Cambridge, Mass.
- Reyer RW (1954). Regeneration of the lens in the amphibian eye. *Quarterly Review of Biology;* 29:1-46.
- Reyer RW (1971). DNA Synthesis and incorporation of labelled iris cells into the lens during lens regeneration in adult newts. *Dev. Biol.* 24:553-558.
- Reyer RW (1977). The amphibian eye, development and regeneration. In: *Handbook of Sensory Physiology VII/5: the Visual System in Vertebrates.* ed. F. Crescitelli (Springer, Berlin) pp. 309-390.
- Roles OA (1969). The influence of Vitamin A, C and E on lysosomes. *J. Lysosomes in Biology and Pathology.* Vol. V. Ed., Dagle JT, Fell HB (North Holland, Amsterdam). pp. 254-275.
- Shekhawat DVS, Jangir OP, Acharya P, Suthar P (2001). Lens regeneration in under the influence of Vitamin A. *J. Biosci.* (26):571-76.
- Suryanarayana P, Kumar PA, Saraswat M, Petrash JM, Reddy GB (2004). Inhibition of aldose reductase by tannoid principles of *emblica officinalis*; implication for the prevention of sugar cataract. *Mul. Vis.* 10:148-54.
- Swami KK (1992). A study of ontogenetic development of eye and morphogenetic changes during lens regeneration in the tadpoles of *Rana cyanophlyctis* under the influence of Vitamin A excess. Dissertation submitted for M.Phil. (2001). Dungar (Auto.) College, Bikaner, Univ. of Ajmer.
- Swami KK, Jangir OP (1997). Effect of Vitamin A on lens regeneration in the tadpoles of toad *Bufo melanostictus*. International Symposium on Development Regulation Growth and Differentiation. Dec. 17-20 Mahabaleshwar.
- Swami KK, Jangir OP (2000). A study of lens regeneration in the tadpoles of *Rana cyanophlyctis* under the influence of Vitamin A. A symposium of Development regulation. Feb 18-19, Pune.
- Tsonis PA, Del Rio Tsonis K (2004). Lens and retina regeneration: Transdifferentiation, stem cells and clinical application. *Exp. Eye Res.* 78:161-172.
- Tsonis PA, Trombly MT, Rowland T, Chandraratana RA, Del Rio-Tsonis K (2000). Role of retinoic acid in lens regeneration. *Dev. Dyn.* 219(4):588-593.
- Wolff G (1895). Entwicklungsphysiologische Studien. I Die regeneration der urodelenlinse; Wilhelm Roux Arch Entwickl-Mech Org. 1:380-390.
- Wolpert L, Beddington R, Brokes J, Jessell T, Lawrence P, Meyerwitz E (1998). Principles of Development. Current Biology Ltd, London.
- Yamada T (1977). Control Mechanisms in Cell-type Conversion in Newt Lens Regeneration (Monographs in Developmental Biology 13, ed. A. Wolsky). (S. Karger, Basel, Munchen, Pan's London, New York, Sydney).
- Yamada T, McDevitt DS (1984). Conversion of iris epithelial cells as a model of differentiation control. *Differentiation;* 27(1):1-12. Review.
- Yamada T, Roesel M (1964). Effect of actinomycin-D on lens regeneration system. *J. Embryol. Expt. Morphol.* 12:713-725.
- Yamada T, Roesel ME (1969). Activity of DNA replication in the iris epithelium by lens removal. *J. Exp. Zool.* 171:425-431.
- Zalik SE, Scott V (1990). *In vitro* development of the regenerating lens. *Dev. Biol;* 19(4):368-379
- Zalik SE, Scott V (1973). Sequential disappearance of cell surface components during dedifferentiation in lens regeneration. *Nat. New Biol.* 244(137):212-214.3