DOI-10.53571/NJESR.2019.1.1.30-36

Sequential Events of Regeneration of lens in young Swiss albino mice under the influence of vitamin A

Dr Swati ojha

Shri Khushal Das University, Hanumangarh [Raj]

(Received 1January 2019/Revised 15 January 2019/Accepted 25 January 2019/Published 30January 2019)

Abstrect

The process of lens regeneration in vitamin A treated animal of all group was almost similar. During lens regeneration two layer of pigmented epithelium of dorsal iris begin to thicken ; soon the cell become taller and nuclei become more prominent; a knob like structure at the pupilary margin grows continue and then the cells starts to dedifferentiate forming a vesicular lense; dedifferentiated iris cells now start redifferentiation and crystalline fiber developed; once the new lens has formed the cells of dorsal iris stops mitosis and then primary and secondary lens fibers begins to form.

Keywords :- Regeneration, Vitamin A , Differentiation , lens

Introduction

Regeneration is development process in which lost part of an organ is restored. It involve all thos e fundamental process including cell proliferation ,cell movement ,morphogenesis, histogenesis and growth ,which occur during ontogenetic development in the embryonic stage. But lens regeneration differ from general regeneration process rather it provide a clear example of " metaplasia" . During lens regeneration there is transformation of one one differentiate d cellular type having a distinct pattern of metabolic activities to anather cellular type which is morphological different from the original which synthesizes a different array of macromolecule.

The present investigation on influence of vitamin A on lens regeneration were motivated as mentioned earlier by recently findings by Swami (1993) , Jangir et al (1995), and Shekhawat (1999). Acording to these studies vitamin A accelerate lens regeneration not only in amphibian tadpole and chick embryo but also in newly born young mice babies .

Several worker were also confirmed that vitamin A excess not only adversely affect the embryo and larva development in a series manner . It also produce interesting effect on regeneration both limb and tail in amphibian tadpole .

In view of above report the present investigation is undertaken to study the effect of vitamin A on lens regeneration in 7^{th} day young as well as 15^{th} and 35^{th} day old mice.

Material and Methods

For the present investigation 7, 15, 35 day old Swiss albino mice were employed . 60 mice were used for this purpose.

The experiments were completed in two series:

1. Series I . The mice of this series were treated as control group and were not given any treat after their lensectomy
2. Series II. Following lencectomy the mice were treated with vitamin A at various interval . The mice of this series were divided into following groups.

A. Group A - 7 day old animal lensectomised and treated with vitamin A
B. Group B - 15 day old animal lesectomised and treated with vitamin A
C. Group C - 35 day old animal lensectomised and treated with vitamin A

The experiment were terminated on day 35 after operation . In all cases lens of right eye were removed .

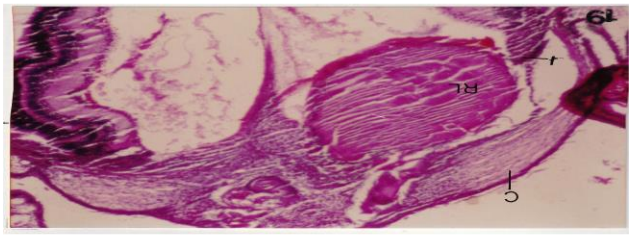
The vitamin A preparation was vitamin A palmitate .The working solution was of 30 IU /ml . strangth 0.05 ml. of this solution was injected intraperitonialy on alternate days after lensectomy.

The mices were preserved in day 1,2,3,5,7,15 and 35after operation for h istological study

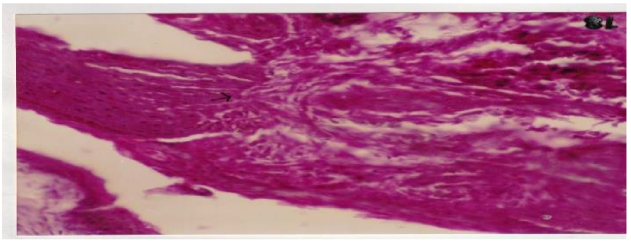
For histological studies the eyes were removed from the preserved animals and dehydrated in grades of alcohol cleaned in xylene and embeded in paraffin wax of 57 C. The eyes were sectioned and stained with haematoxylene counter stained with eosin .

Observations and results

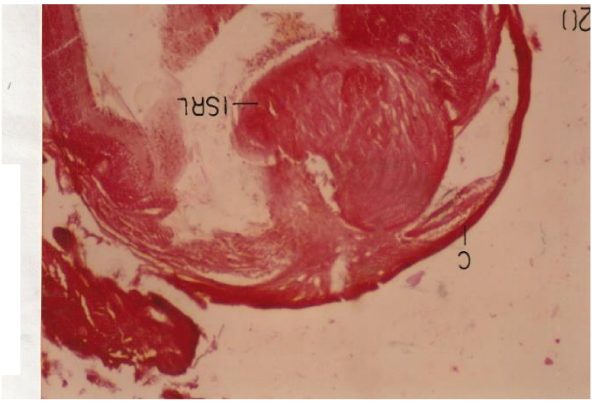
The experiment includes in the series were made on 3 groups of mice [A B C].After there lansectomy most of the mice were kept alive for 35 days following operation but some of each group were sacrificed; fixed an there eyes sectioned at various intervals for histological studies.



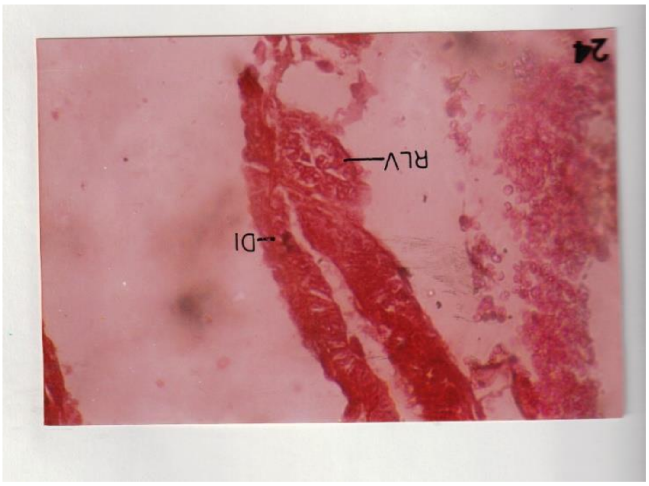
Figer 2 L.S through the eye showing regenerated lens attached with cornea at one side and with dorsal iris with other hand



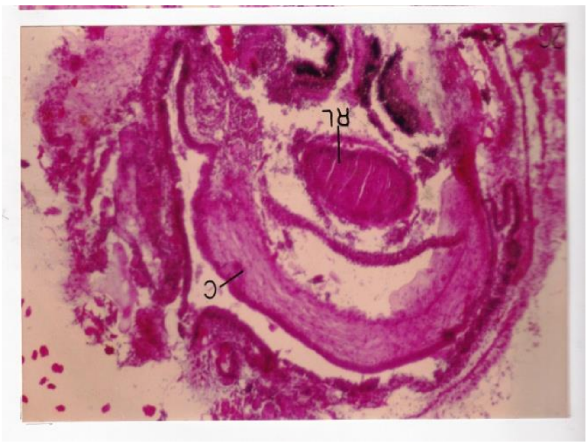
Figer 3 L.S through the eye showing cell migration through cornea to give rise in lense in control group



Figer 4 L.S through the eye showing irregular shaped regenerated lens of control group



Figer 5 L.S through the eye. Dorsal iris showing regenerated lens vesicle at the free end of dorsal iris



[Day 7 old mice]

Figer 6 Dorsoventral section through eye showing newly formed regenerated lens . primary lens fiber forming cells are visible

In the animal of group C [35 day old mice,] ,lens regeneration had not occured but in a few case lentoid developed.

In the animal of 7 day old control group A the lens regeneration had occur only in 3 out of 15 cases while the animals of vitamin A treated group of same age show 95 % regeneration .In the two out of 15 operated cases regeneration of lens initiated from the inner layer of cornea. Figer-1 shows the regenerated lens which had reached stage II by day 15 after operation . The regeneration appeared as epithelial vesicle associated with the inner layer of cornea . In one case lens regenerated from dorsal

iris and this lens too associated with iris on one hand and corneal epithelium on the other hand [Fig.--2].This lens so formed is irregular in shape and reached in stage II.

Graph: 1 Swiss albino mice showing lens regeneration percentage in 7 ,15 and 35 day old mice of control and vitamin A treated mice.

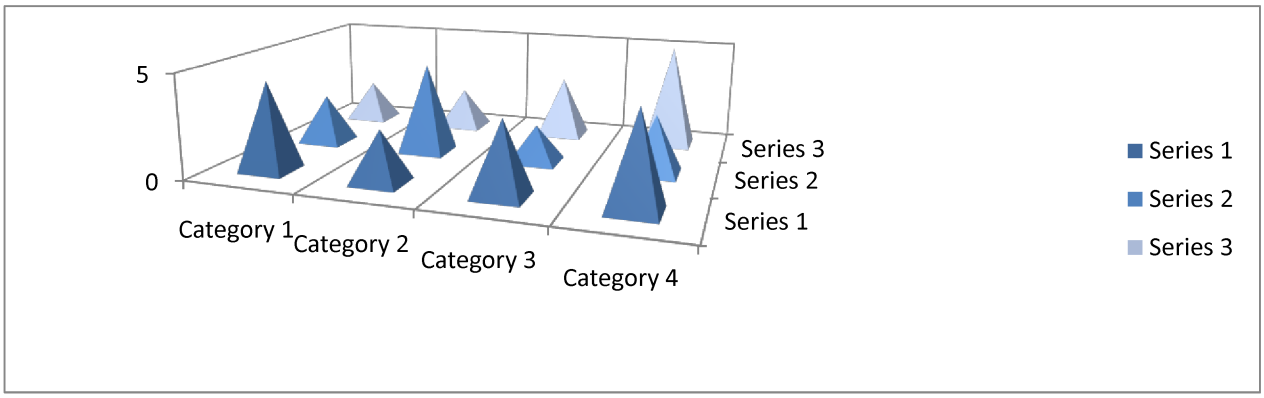


Table 1 Swiss albino mice showing lens regeneration percentage in 7 ,15 and 35 day old mice of control and vitamin A treated mice.

Sr. No.	Age of mice	Group	Number of animal used	No. Regenerated lens on day 35 after operation	Percentage of Regeneration
1	7 Day old	A [control] Vitamin A treated	15 20	3 19	20% 95%
2	15 day's	B[control Vit. A treated]	10 20	Nil 14	00% 70%
3	35 Day's	C[Control Vit A treated]	10 20	Nil 3	00% 15%

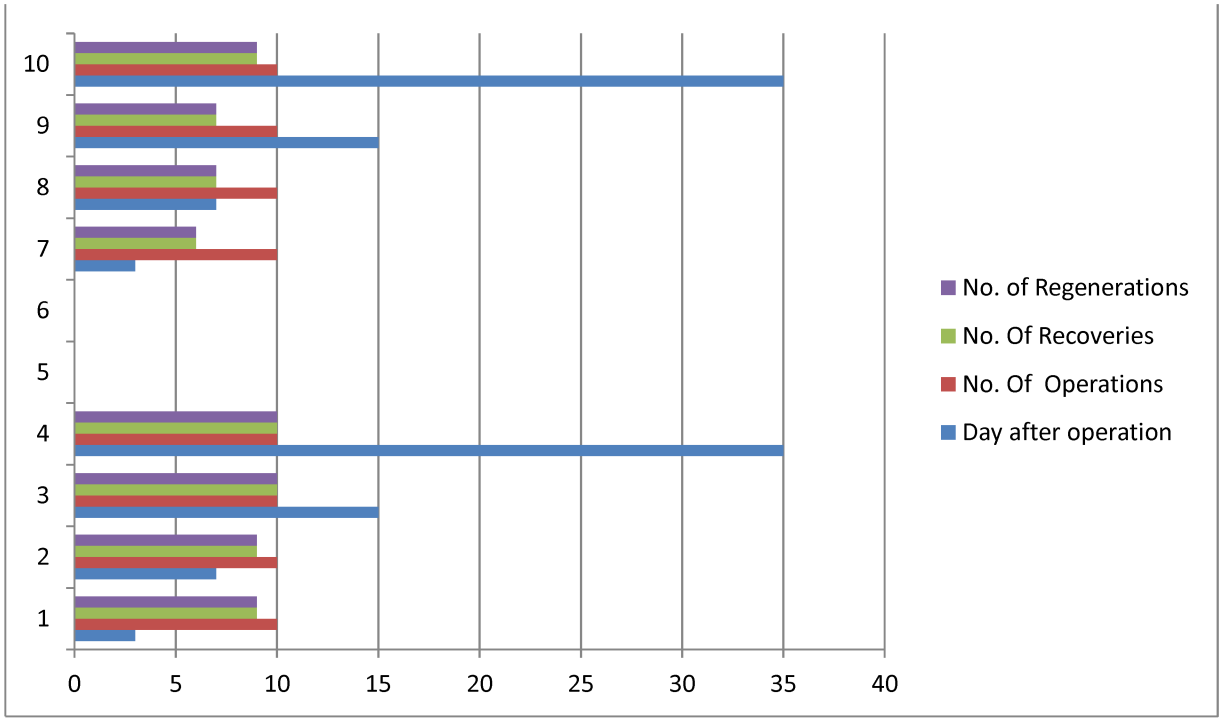
Table 2 : Swiss albino mice showing the stages of lens regeneration in vitamin A treated animals.

S. No		Day after operation	No. Of Operations	No. Of Recoveries	No. of Regenerations	Stages of regenerations
1 2 3 4	A [7 day old]	3 7 15 35	10 10 10 10	9 9 10 10	9 9 10 10	I and II stage III III and IV IV

5 6 7 9	B [35 day old]	3 7 15 35	10 10 10 10	6 7 7 9	6 7 7 9	I II III III#

 # Arrested stage III

Graph II Showing lens regeneration in vitamin A treated animals.



In the animal of 7 day old control group irregular shaped lens have been reported. In most of the animal of control group A, B and C no regeneration was observed.

Vitamin A influence lens regeneration not only in the animal of 7 day old but also of 15 and 35 day old group However the percentage of lens regeneration declined as the animal ages. It was 95% in 7 day old and 70 % in 15 day old animal. 35 day old animal do not show any regeneration but small and irregular shaped lentoid have been reported. [Table 1 and 2 graph 1]

The following breaf sequence of events occuring during lens regeneration is divided into 12 stages that are grouped in four major periods.

A. **Latent Period -Excision of lens is followed by Latent Period of a few days .T his period is a time of considerable changes . The border of the dorsal region of iris thicken and a cleft arises between inner and outer lamellae; amoeboidal cells moves from a stroma on the surface of epithelium and into a cleft . this movement is followed by a period increase in the rate of incorporation of labeled uridene and into ribosomal RNA [Reese et al,1969]**

B. Initial period- The begning of this period is signaled by the first appearance of depigmented cells at the dorsal papillary margin . The pigment is engulfed and carried away by invading amoeboidal cells. As more cells lose their pigments they forms a hollow epithelial vesicle . At the end of initial period the cells of inner wall of epithelium elongate and protrude into the leumen , forming few irregularly arranged lens fibers . some of these have left the cell cycle that is stop dividing . At this time lens specific protein the crystalline appeared which are otherwise not present in the iris.

C. Lens Fiber Differentiation - now the lens fiber differentiation begins. First formed primary lens fiber now push to the front of the vesicle forming a nucleus behind the lens epithelium . The later than proleferates the secondary lens fiber from the equatorial zone where they meet to the primary lens fiber nucleus. At about 20 days after operation the nucleus of primary lens fiber is symmetrically enclosed by secondary lens fiber .

D. Period of Growth – this period continues until about the 30 day after the operation . In the center lens fibers the nuclei eventually degenerated and DNA synthesis also gradually decreases.

Discussion

Results of the present study shows that vitamin A initially enhance the lens regeneration in 7 day old mice but also induce regeneration capacity in 15 and 35 day old adult mice too,otherwise lens regeneration capacity had been lost in 15 to 35 day old mice. However it seems that the does of vitamin A used 30 IU/ml was not upto mark that is why in the 35 day old mice only lentoid would have been developed.

Swami [1992] and Garg [1993] have also reported accelerated lens regeneration in amphibian and chick embryo under the influence of vitamin A.

It is quite possible that vitamin A might have affected the cell surface or intrcellular space stabilizing factor of dorsal iris epithelial cells and induce the cell in trensdifferentiation .Eguchi {19] reported in his cell culture study that PECs differentiation from fully grown human eye readly transdifferentiated into lens phenotype.

The results of the present study confirm the previous report of beneficial influence of vitamin A on lens regeneration in young 7 day old mice but also induce regeneration of lens in 15 and 35 day old adult mice too. Even in the series II group C in which lens regeneration had not occurred in control group ; vitamin A induce lens regeneration in 15% cases . However all these regenerat es were irregular shaped lenses or lentoids

The lentoid operated as various shaped mounds like structure of different sizes. All lentoids are containing larger cells with intracellular space.

Lentoid have been reported only in older treated mice.

Most of the lentoids are elliptical in shape each well differentiated into lens fibers but with vacuolated cytoplasm . Not only the shape, but the number of lentoids also very from 1 to 3. This may be due to vitamin A enhanced dedifferentiation activity.

References:

Eguchi,G and Wantanabe, k 1973 Elicitation of lens formation from the ventral iris epithelium of the newt by a carcinogen ,N-methyl-N –Nitro-N nitrosoguadimine.

Jangir O.P; Singh D.S.; Swami K.K 1995 A study of lens regeneration in the tadpole of Rana Cynophlyctis under the influence of vitamin A. XI National Symposium Developmental Biology pp30

Jangir O.P, Singh D, : Garg and Goswami M K 1995 A study on regeneration ability of lens in post netal mice under the influence of vitamin A ; XI National Symposium Developmental Biology. Rohtak pp31

Jangir O.P., Shekhawat D.S, Garg Shushma, and Goswami M 1995 A study of lense regeneration in the tadpole of Rana cynophylictis under the influence of vitamin A ; IX National Symposium Developmental Biology. Rohtak pp31

Ojha Swati 2000 Experimental studies on molecular espects of ontogenesis and regeneration of lens in young mice. Ph D Thesis, M.D.S. University, Ajmer

Shekhawat D S , Jangir O.P, Ojha Swati; Sharma Renu, and ojha swati 1997 Histological study of lens regeneration in young mice under the influence of Vitamin A; International symposium on Development Growth and differentiation ; Mahabaleshwar.

Shekhawat Digvijay Singh , Jangir O.P, Aacharya; , Suthar P; 1999 Effect of vitamin A excess on lens regeneration in newly born mice. J. Zool. Socity of India . Dessertation submitted for

Swami K. 1993 . A study of ontogenetic development of eye and morphogenetic changes during lens regeneration in the tadpoles of Rana cynophlyctis under the influence of vitamin A.