Effect of vitamin A on lens regeneration in pigs

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Intraperitoneal injections of vitamin A (0.5 ml of 1500 IU/ml) to lentectomized pigs on alternate days up to 60th day after lentectomy induced lens regeneration in not only 10 days old young ones but also in 40 and 100 days old pigs. Lens regeneration did not occur even in a single case of control groups. In shape, size, transparency and histological features regenerated lenses were similar to normal intact lenses. The experimental model is the first to show that mitogenic and dedifferentiate activity of vitamin A can induce iris pigmented epithelial cells to trans-differentiate into new lens in pigs.

Keywords: Lens regeneration, Pigs, Vitamin A.

Lens regeneration provides a clear example of metaplasia or trans-differentiation process by which differentiated cells alter their identity to become some other distinct cell types. Lens regeneration is reported in amphibians19. Lens regeneration occurs from non-ocular tissue (dorsal iris) in urodele amphibians4,5. However, the capability of iris and retinal pigmented epithelial cells (PECs) to trans-differentiate into lens is reported to be not restricted to urodeles only but is widely conserved in vertebrates; and this phenomenon is said to be often observed in some fishes and avian embryos10. Although good lens regeneration from PECs in vivo has been seen in a few species, in cell culture PECs of almost all vertebrates can switch differentiation to acquire the characteristics of lens10,11.

The overall process of lens regeneration in newts has been reviewed by Eguchi and Kodama12. They found that after lentectomy in the adult newt the dorsal pupillary margin of the iris epithelium begins to change. Intercellular communication between the cells of the pigmented epithelium rapidly reduces as a result of inflammatory reactions. During the initial phase the condensed chromatin of the PEC nuclei becomes progressively dispersed, the nuclear volume increases and melanosome are discharged by the active participation of macrophages, which appear around the dorsal margin and often invade the interlaminal space of the iris pigmented epithelium. Later on, DNA replication followed by mitosis is initiated in depigmented PECs whose progeny form a vesicle corresponding to the lens vesicle. Immediately after the formation of lens vesicle lens fibers begin to differentiate at the posterior wall.

Recently, vitamin A was found to induce and accelerate lens regeneration not only in anuran amphibians but also in mammals like Swiss albino mice and rabbit13,14. Excess of this vitamin had been reported to destabilize cell membrane, stimulate synthesis and release of lysosomal enzymes into extracellular environment, causes dissolution of bone and cartilage due to degradation of the matrix by the released lysosomal hydrolases, liberating cells capable of mitosis15. Increased mitosis in certain vitamin A treated tissue explants in vitro was also observed by Fell and Rinaldini15. Maden16 suggested that retinoic acid (RA) is the bioactive metabolite of vitamin A, which acts on cells to establish or change the pattern of gene activity. Tini et al.17 studied the effects of retinoids on lens development and reported that RA a natural endogenous morphogenetic agent which acts as regulator of gene expression in the lens. Tsonis et al.18 studied the effect of retinoids on urodelian lens regeneration. They found that lens regeneration was dramatically affected by inhibition of the synthesis of retinoic acid.

Shekhawat et al.13 successfully induced lens regeneration in Swiss albino mice by treating them with vitamin A. On similar lines, the effect of vitamin A was also studied on corneal healing19 and lens regeneration in guinea pigs20. Vitamin A causes homeotic transformation of tail cells into limbs21,22.
These known properties of vitamin A motivated the present work to explore the influence of vitamin A on lens regeneration in pigs of different age groups. After getting success in induction of lens regeneration in mice, guinea pigs and rabbits, the phenomenon has now been studied in a higher mammal like pig, which is anatomically similar to human.

### Materials and Methods

The experiments were carried out on 10 pigs each of 10, 40 and 100 days old having 2, 3 and 5 kg. body weight respectively. Lentectomy was done under local anesthesia (2% xylocaine). A fine longitudinal slit was made in the cornea of the right eye under a stereoscopic binocular microscope. The complete intact lens was extracted through the incision. Out of these operated animals, 15 were treated with vitamin A and the remaining 15 were kept untreated as controls. Following the operation, 0.5 ml. of 1500 IU/ml vitamin A solution was injected intraperitoneally on alternate days up to the day of termination of experiment (60 days after operation). Sham injections were given to the animals of untreated group animals (it was done so to equalize trauma effect in animals of the both groups).

The vitamin preparation used was vitamin A palmitate. The solution was prepared by dissolving a known quantity of this drug in a small amount of ethanol to make a stock solution and then diluting it with appropriate amount of distilled water. Eyeballs were removed on days 7, 15 and 60 after operation for histological evaluation.

### Results

The results presented in Table 1 show that vitamin A can successfully induce lens regeneration in pigs. Lens regeneration was not found in untreated control group of any age only in one case a small lentoid like structure was reported. In vitamin A treated animals lens regeneration occurred in 80% individuals of different age groups (in 12 out of 15).

The morphological features like shape size and transparency of regenerated lenses were found similar to that of normal intact lenses. The right operated eye with regenerated lens gives normal appearance on the day of termination of experiment (Fig.1). This operated eye with regenerated lens was found normal in function. It was tested by its normal responses by putting black tape close to the intact left eye.

In the present study, vitamin A was found to induce lens regeneration in animals of all the three age groups (10, 40 and 100 days old) but the percentage of regeneration was found to decline with age. In the 10 days old pigs 100% lens regeneration was seen in operated eyes, whereas it was 80% and 60% in 40 days and 100 days old pigs respectively (Table 1).

Histological changes in regenerating lenses of vitamin A treated animals were almost similar as reported by Shekhawat et al. in Swiss albino mice and Sharma Manshi et al. in rabbit. Histological

### Table 1 — Effect of Vitamin A on lens regeneration in 10, 40 and 100 days old pigs

<table>
<thead>
<tr>
<th>Group</th>
<th>Age of animal (days)</th>
<th>No. of animals employed</th>
<th>Days of preservation after operation</th>
<th>No. of operated animals preserved</th>
<th>Lens regeneration occurred (Number)</th>
<th>Percentage of Lens regeneration</th>
</tr>
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<tbody>
<tr>
<td><strong>A</strong></td>
<td>10</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>10</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>20</td>
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<tr>
<td></td>
<td>100</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>6.6</td>
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<tr>
<td></td>
<td>15</td>
<td>5</td>
<td>7</td>
<td>1</td>
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<td></td>
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<td>60</td>
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A = Vitamin A treated; B = Control, Untreated
study revealed that during lens regeneration after lentectomy the pupillary margin of dorsal iris epithelium begins to change. Intercellular communication between the cells of pigmented epithelium rapidly reduces as a result of inflammatory reaction. The two layers of pigmented epithelium of dorsal iris begin to thicken and the nuclei of iris cells change their shape. The condensed chromatin of PEC nuclei becomes progressively dispersed. The nuclear volume increases, the melanosomes move toward the peripheral region of PECs at the dorsal margin of the iris. The PECs then discharge melanosomes, which appear around the dorsal margin. Mitosis is initiated in depigmented PECs (Fig. 2). The pupillary margin of

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**Fig. 1** — Normal looking right eye with regenerated lens of 60 days vitamin A treated animal. **Fig. 2** — Microphotograph of a section through dorsal iris of the eye of vitamin A treated animal (preserved on day 7) showing bilaminar layers and a slit like cleft between two layers (100 x). **Fig. 3** — Microphotograph of a section through the eye of vitamin A treated animal (preserved on day 7) showing development of lens vesicle from dorsal iris (100 x). **Fig. 4** — Microphotograph of a section through regenerating lens from dorsal iris of the eye in vitamin A treated animal (preserved on day 15). Primary lens fiber forming cells are visible (100 x). (DI = dorsal iris; CL = cleft; PLF = primary lens fibers; LV = lens vesicle; RL = regenerated lens; C = cornea; SLF = secondary lens fibers; AC = aqueous chamber; VH = viterous humor; ON = optic nerve; L = lentoid).
the iris became knob-like. The formation of this knob-like structure continued until the free margin became a swollen loop-like structure. All these changes continued up to day 7 after the operation in vitamin A treated animals. Then the cells started to dedifferentiate and they threw out more melanosomes. These melanosomes were ingested by macrophages that entered from the wounded site. Dorsal iris cells continued to divide forming a vesicle-like structure in the region of the removed lens (Fig.3). Now the vesicle differentiated into a new regenerated lens. Once the new lens formed, the cells of the dorsal iris ceased mitosis. The newly formed lens was surrounded by a lens epithelium whose cells were cuboidal and slightly taller than before. In addition, lens fiber formation was initiated in the inner surface of the vesicular lens. Cells began to elongate and entered the lumen of the vesicle. The lumen was filled by primary lens fiber nuclei before the secondary lens fibers began to form. Later on, the secondary lens fibers began to differentiate and grew around the central nucleus and the regenerated lens became a better-defined structure. In the next stage the lens got detached from the dorsal iris and returned to its normal status (Figs.4, 5). At last, the nuclei of the secondary lens fibers progressively disappeared. Fig.6 shows a photograph of a hand section of the operated eye of vitamin A treated animal with regenerated lens. It shows the placement of regenerated lens into normal position by day 60 after operation. The distinguishing feature of vitamin A treated regenerated lens was the arrangement of differentiated secondary fibers. In most of the cases the fibers were arranged in a transverse fashion.

In some of the regenerated lenses cytoplasmic vacuolization was also seen; otherwise the regenerated lenses were found similar not only in shape, size, transparency but also in histological features to normal intact lenses.

In untreated operated control animals, lens regeneration did not occur; only in one case a nodulated lentoid structure was found to develop.

Fig. 5 — Microphotograph of a section through the eye of vitamin A treated animal (preserved on day 60) showing regenerated lens with secondary lens fibers. The lens epithelium becomes thin and flat (50 x). Fig. 6 — Photograph of a hand section of the eye ball of 60 days old vitamin A treated animal. The section shows regenerated lens (RL) and other components of eye. Fig. 7 — Microphotograph of a section through dorsal iris of the eye of 10 days old untreated control animal (preserved on day 60) showing nodulated lentoid. (100 x) Abbreviations are same as in Figs 1-4.
(Fig. 7). Histological study revealed that the structure so developed was almost similar to normal lens but of smaller size.

**Discussion**

The main finding of this study is that vitamin A can induce and accelerate the lens regeneration even in 100 days old young pigs. In the previous studies vitamin A was found to induce lens regeneration not only in amphibian tadpoles but also in adult frogs, Swiss albino mice, guinea pigs and rabbits.

Lens regeneration from non-ocular tissue (dorsal iris) has been well documented in amphibians. However, recently it has been observed that the capability of iris and retinal pigmented epithelial cells (PECs) to transdifferentiate into lens is not restricted to urodele amphibians only but is widely conserved in vertebrates. Although good lens regeneration from PECs in vivo has been seen in a few species of fishes and amphibians but in cell culture study, PECs of almost all vertebrates can switch differentiation to acquire the characteristics of lens. Eguchi reported in his cell culture study that PECs dissociated from fully-grown human eyes readily trans-differentiated into lens phenotypes in the manner observed in chick embryo PECs.

It is well studied that lentectomy stimulates the iris epithelial cells of the newt's eye to undergo DNA synthesis and proliferate. Concomitantly with these processes, melanosomes disappear from the pigmented epithelial cells of iris and thus undergo dedifferentiation, some cells retreat from the cell cycle, elongate and proceed to synthesize lens specific proteins and transform into lens fibers.

Similar to earlier studies on lens regeneration in frogs, mice, guinea pigs and rabbits, vitamin A was found to induce lens regeneration in pigs too. However, the exact nature of vitamin A effect is still not known but observations revealed that dedifferentiation is pre-requisite for regeneration. One possibility of induction of lens regeneration by vitamin A may be due to its enhancing mitogenic activity and dedifferentiative process. Several workers on limb regeneration of anuran tadpoles also studied similar effects of vitamin A. Increase and prolonged mitotic activity and delay in re-differentiation were noted in the blastema of vitamin A treated cases. It is also expressed that retinoid treatment increases the potency of the blastema by causing more intense dedifferentiation of its cells and reactivating all the limb-forming genes. Increased acid phosphatase activity in the treated blastemas of frog is correlated with the process of dedifferentiation in regenerating limbs. Dedifferentiation of cells, a pre-requisite for regeneration, involves biosynthetic changes in the cells expressed in the production of new RNAs and proteins. These changes must be directed and controlled by certain genes reactivated under the influence of amputational injury and subsequent events.

Another pathway of vitamin A action might be through the influence on fibroblast growth factor (FGF). Fibroblast growth factor is found to control and induce the development and regeneration of lens. When the lens is removed, however, regeneration occurs by dedifferentiation of the pigment epithelial cells (PECs) of the dorsal iris. These cells proliferate and produce lens fibers and finally a normal polarized lens. It has been shown that FGF can initiate cell cycle events and cell division in the dorsal iris cells, which are the basis for lens regeneration. Some of the factors that have been studied in details are the FGFs and their receptors. During dedifferentiation of the PE from the dorsal iris, expression of FGF-1, FGF-2 and FGF-3 is prominent in the dedifferentiating cells and the subsequent regenerating lens vesicle and differentiating lens fibers. However, only FGF-1 product seems to be present specifically in the dorsal iris during dedifferentiation. Its role in regulating lens regeneration has also been strengthened in experiments where an inhibitor to FGFR-1 inhibited lens regeneration and lens fiber differentiation. In this sense, FGFR-1 can be regarded as the first known lens regeneration associated factor. Vitamin A might have enhanced the activity of FGFR-1 in the cells of dorsal iris and resulted induction of lens regeneration in the present animals otherwise these animals have lost this capacity during their evolutionary development. The present findings are also supported by Tsonis et al. They have also studied the accelerating role of retinoic acid in lens regeneration of urodele amphibians. In their study, a highly specific antagonist of retinoic acid (vitamin A) receptor (RAR) alpha was used in an attempt to study its function in lens regeneration. It was found that this antagonist inhibited lens regeneration and lens fiber differentiation.
Kumar and colleagues also reviewed similar type of observations in different vertebrates and suggested that the percentage of lens regeneration declined with the age of animal even in the experimental conditions. It was 100% in 10 days old young babies, 80% in 40 days old young pigs and 60% in 100 days old pigs. The declined trend of regeneration was also found in amphibian tadpoles, froglets, adult frogs and mice.

Thus, it may be concluded with the opinion that vitamin A enhances dedifferentiation of iris pigmented epithelial cells of pig and causes lens regeneration. Retinoids are thus a group of chemicals that can be employed for investigations of the molecular mechanisms responsible for homeotic transformation. Lens regeneration appears to be a suitable system for such investigation. The discovery opens the possibility that researchers might one day enhance the endogenous regenerative capacity of mammals by inducing cellular dedifferentiation in vivo.

Acknowledgement

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