The regeneration of the crystalline lens

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A brief account is given of renewed interest in lens regeneration in rabbits from the epithelial cells along the equator of the lens after removal of the crystalline body from the lens capsule. This interest is due to the claims by some recent investigators that better lens regeneration takes place in the presence of implanted cytolyzed fetal tissue within the lens capsule. However, overwhelming evidence has now accumulated to show that the presence of cytolyzing cells does not favor lens regeneration over that of normal controls. The evidence for lens regeneration after complete lentectomy is reviewed for all classes of vertebrates. After the original lens is removed from the eyes of several species of salamanders a new one regenerates from a budding process along the pupillary margin of the dorsal iris. Except for two species of fishes and one species of frog-tadpoles, lens regeneration following lentectomy has never been observed in any of the other vertebrates. Many experiments show that cells with the potential for lens regeneration in the newt eye are confined to the dorsal part of the eye and are scattered over the dorsal iris and even among the retinal pigment cells in the dorsal wall of the eye. Many experiments by the author on the eyes of adult newts are cited, dealing with the release of lens regeneration by a retinal factor and with the apparent inhibitor effect by living lens tissue.

The focus of attention upon lens regeneration is largely due to the fact that complete lentectomy in some members of one group of amphibians, namely, larval and adult urodeles, the salamanders, is followed by lens regeneration from the pupillary margin of the dorsal iris. This has been demonstrated many times. One then becomes curious to know whether or not the same phenomenon ever occurs in the eyes of other vertebrates.

Among the other groups of amphibians, the tailless anurans, there are a number of species in which the lens does not regenerate. In others it is not yet proved that a lens regenerates from the dorsal iris after complete lentectomy because of the possibility of incomplete lens removal in the material of those who claim it. This group should be investigated more thoroughly.

Sato found no lens regenerated from the iris of young Japanese frog larvae after lentectomy. However, his unusual finding was that only when iris epithelium or retinal pigment epithelium was implanted into a lentectomized eye did there appear in rare cases lens regenerates from the isolated epithelium. In his experiments the isolated epithelium most often gave rise to neural retina as in the case of implanted retinal pigment epithelium in the eyes of urodeles. Sato pointed out that the nutritional conditions of the lens and neural retina are normally quite different. Influenced by this fact he concluded that the nutritional condition prevailing in the pigmented epithelium may be one of the fac-

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tors which bring forth the capacity for either lens or neural retina formation.

Sato further suggests that the factors inhibiting lens regeneration in the tadpoles are probably the powerful proliferative capacity of the iris and natural tendency of the internal layer of the iris to form neural retina. These findings emphasize the importance of exploring further the conditions to be found in other anurans to uncover any hidden capacity for lens regeneration.

Sato also working on two species of Japanese fresh water fishes has offered the best evidence to date that lens regeneration takes place from the dorsal iris in this group of vertebrates after lentectomy.

There is no evidence of lens regeneration after complete removal of a well-developed lens in reptiles, birds, and mammals.

For a long time it has been known that the mammalian lens as well as the lens in other vertebrates is constantly supplied by new fibers from the epithelial cells along the equator of the lens capsule. Cocteau and d’Etoilles, Middlemore, Milliot found lens regeneration in rabbits following removal of the crystalline body from the lens capsule. Randolph in a fine series of experiments confirmed these findings.

Stewart and ‘Espenasse reported that two Russian investigators, Chanturishvili and Sicharulidze had concluded lens regeneration in rabbits was enhanced by implanting sterile cytolized fetal tissue into the lens capsule after removing the crystalline lens. Stewart and ‘Espenasse reported that they had repeated the Russians’ experiments on some rabbits. After a few weeks they examined by various techniques the living conditions of the regenerating lenses in the eyes with cytolized tissue and compared them with control eyes with simple extracapsular lens extraction. They concluded that, so far as the living conditions could reveal, the implants favored larger and better lens regenerates than in those of the controls. A histological study of regenerated rabbit lenses by Stewart revealed that larger lenses with more normal optic density regenerated if cytolized fetal tissue was implanted after extracapsular lens extraction.

Binder and associates and Agarwal and co-workers reported that implanted cytolized tissue did not induce larger lens regenerates in the rabbit. Agarwal, and associates, in a few experiments on monkeys, suggested that the implants might have induced larger lens regenerates. Pettit also studied lens regeneration and he suggested that the implants with prolonged irritation might have induced larger lens regenerates in rabbits.

My impression of the results of all of these investigators is that the larger lens regenerates depend upon the condition of the lens capsule. If it collapses, little or no lens tissue regenerates. If it remains open larger lenses are regenerated. Perhaps in many cases the introduction of cytolized tissue, which nearly always later degenerates, helps keep the capsule open and hence in counting the number of cases in an experimental group there may appear larger regenerates in those which have the implants.

In studies of embryonic lens development the presence of a cytolized cell or two may have occasionally been cited. I believe they have no significance with respect to lens formation. A few cytolizing cells can often be seen in various parts of developing embryos.

Now to get back to the interesting problems presented by the phenomenon of lens regeneration from the dorsal iris of certain salamander eyes first revealed in adult Triturus newts by Colucci in 1891. Since I have worked extensively with the American adult newt, Triturus viridescens, I shall deal largely with the results from several hundred of my own experiments over many years. A review of the contributions of other investigators not mentioned in the text will be found in the literature cited, especially in the fine review by Reyer.
Figs. 1 to 8A. These include drawings made from dissected anterior halves of adult *Triturus v. viridescens* eyes to show the inner surface of the black pigmented iris and its relation to the normal and regenerating lens. A photomicrograph of the same specimen in each case shows the dorsal iris and the lens regenerating from it. Days after lentectomy the stages (Sato stages) of lens regeneration are indicated in selected specimens to show the progress of development. They show the early thickening and depigmentation of the dorsal iris (Figs. 2 and 2A), the early vesicle formation (Figs. 3 and 3A), the lens vesicle filled with primary lens fibers (Figs. 4 and 4A), the early formation of secondary lens fibers (Figs. 5 and 5A), the rapid increase of secondary lens fibers (Figs. 6 and 6A), the detachment of the lens regenerate from the dorsal iris (Figs. 7 and 7A), and the rapidly maturing lens which will eventually reach the size of the original lens which was removed. (Figs. 1 and 1A from Figs. 3 and 3A; Figs. 2 and 2A from Figs. 4 and 4A; Figs. 3 and 3A from Figs. 5 and 5A; Figs. 4 and 4A from Figs. 10 and 10A; Figs. 5 and 5A from Figs. 16 and 16A; Figs. 6 and 6A from Figs. 18 and 18A; Figs. 7 and 7A from Figs. 19 and 19A; and Figs. 8 and 8A from Figs. 22 and 22A, Stone and Steinitz: J. Exper. Zool. 124: pp. 462 and 463, 1953.)
Although the rate of lens regeneration from the dorsal iris may vary in some individuals,21 Figs. 1 to 8A illustrate in a few selected specimens what takes place in the iris after the original lens is excised.

The dorsal iris which was quite thin (Fig. 1A) begins to be depigmented and to thicken along the mid-dorsal margin of the pupil within 10 days (Figs. 2 and 2A). Cell proliferation takes place and a vesicle develops during the second week (Figs. 3 and 3A). The vesicle soon becomes filled with primary lens fibers (Figs. 4 and 4A). Then the lens enlarges with the acquisition of secondary lens fibers (Figs. 5 to 6A). Within 4 weeks the lens regenerate becomes detached from its source of origin (Figs. 7 and 7A). During the second month the lens increases rapidly in size by the growth of many secondary lens fibers (Figs. 8 and 8A). The dorsal iris takes on the normal appearance again. This growth continues for several months until the lens regenerate attains the size of the original one.

The cells from which the lens regenerates appear to lie only in the dorsal region of the eye where they are widely scattered. The ventral half of the iris possesses no lens-forming cells.22 In the iris itself potential lens-forming cells are not confined to the pupillary border. This can be shown in the following experiments.

An incision is made in the cornea so that a corneal flap can be elevated to expose the dorsal iris. Slits are then made in the latter in various regions and into them small pieces of thin Pliofilm membranes are inserted to make permanent accessory pupillary openings. The corneal flap is then replaced and allowed to heal. Later the lens is removed and then a lens regenerates not only from the margin of the original pupil but in the accessory one as well. This is shown in 2 cases (Figs. 9 and 10). The histological appearance of the two lenses is shown in Fig. 11. The space between the two was occupied by the Pliofilm.

In Fig. 12 the accessory opening was more dorsal in position and as long as the original lens was present no lens regenerated in the new opening. Fig. 13 shows that, in the same eye, 32 days after lentectomy, a small lens had regenerated from the margin of the iris in the accessory pupil. A large lens regenerate lies in the normal pupil as well. The photomicrograph of the same eye in Fig. 14 shows the two lenses and the space occupied by the Pliofilm between them.

In Figs. 15, 16, and 17 a similar sequence of events is shown in another eye in which the accessory opening is near the border of the retina. After lentectomy a small lens formed from the border of the iris in the accessory opening below the Pliofilm membrane (Fig. 16). It is shown in Fig. 17 as a degenerating lentoid below the space occupied by the implanted Pliofilm. A large normal lens has regenerated in the primary pupil.

If permanent openings are made by inserting small pieces of Pliofilm into the dorsal retinal wall of the eye, small lenses will develop from retinal pigment cells at the periphery of the openings.23 If pieces of retinal pigment epithelium are isolated from the dorsal wall of the eyes of the adult newt and implanted into lentectomized eyes, they will give rise not only to neural retina tissue but also to small lenses.24 If all of the dorsal iris is removed, including some of the peripheral retinal wall, the dorsal iris regenerates from the retinal pigment cells. The regenerating iris gives rise to a lens regenerate as soon as the original lens is removed.24 This is not surprising, for the regenerated iris was derived from retinal pigment cells which were shown in the experiment mentioned above to possess cells with a capacity to form lenses.

The mid-central third of the dorsal iris is the most potent region for lens formation. If it is excised, rotated 180 degrees, even inside out (Fig. 18, A), a lens will develop in a lentectomized eye (Fig. 18, B) from that portion of the iris now at the
Figs. 9 to 17. Schematic drawings of the living left (Fig. 9) and right (Fig. 10) eyes 19 days after original lens was removed and 27 days after implantation of Pliofilm in the dorsal iris. Lens regenerates are about the same size in the primary and secondary pupils. (×35.)

Fig. 11 is a photomicrograph of eye similar to those in Figs. 9 and 10 showing the upper lens regenerate in the secondary pupil and the lower lens regenerate in the primary pupil. The space between the lenses locates the position of the Pliofilm. (×55.)

Figs. 12, 13, and 14 concern the same right eye showing the more dorsally placed secondary pupil 17 days after implantation of Pliofilm (Fig. 12) with original lens present and in Fig. 13 the small lens regenerate in the accessory pupil 32 days after the original lens was removed. (×35.)

The photomicrograph in Fig. 14 shows the primary and secondary pupillary spaces with their lens regenerates and the space occupied by the Pliofilm. (×55.)

Figs. 15, 16, and 17 concern the same left eye showing in Fig. 15 the more dorsally placed accessory pupil 17 days after the Pliofilm was implanted and in Fig. 16 the two lens regenerates 32 days after the original lens was removed. (×35.)

Fig. 17 shows the histological appearance of the small cataractous lentoid above the normal-appearing lens regenerate in the primary pupil. (×55.) (Plate 1 from Stone: J. Exp. Zool. 127: 488, 1954.)
Fig. 18. Schematic drawings showing the mid-dorsal segment of the dorsal iris excised and rotated 180 degrees and reimplemented inside out (A). Original lens was removed later (B). A lens regenerated eventually from the reversed pupillary margin (C and D) with lens fiber pole normally oriented toward the neural retina. Occasionally a lens regenerated from potential lens-forming host iris on either side of the graft (D). (Fig. 4 from Stone: Anat. Rec. 120: 611, 1954.)

pupillary margin (Fig. 18, C). In some instances a lens regenerate will also be released on either side of the graft (Fig. 18, D). Two separated potential lens-forming regions can also act independently if a piece of ventral non-lens forming iris is substituted for mid-dorsal iris.

Wounds such as several slits made in the dorsal iris do not initiate lens regeneration. It follows after a complete removal of the original lens or a fully regenerated one.

When the lens is removed from an eye, the loosened lens sometimes falls out of sight into the ventral vitreous chamber. After some delay a lens will regenerate from the dorsal iris and the dislocated lens will be in various stages of cataract formation. The cataractous lenses may be losing any inhibitory effect they may have possessed.

Eguchi noticed in the Japanese adult newts that, when the ventrally displaced lenses in the nnirillary space became cataractous, they inhibited lens regeneration until the regenerating lens was 25 to 28 days old and detached from the dorsal iris. It then possessed inhibitory activity.
younger smaller regenerating lens and found inhibition of lens regeneration. He concluded that a certain volume of implanted lens tissue must be attained to prevent lens regeneration and that it was not necessarily the age and quality of the implanted regenerating lens which was involved.

Since the presence of a large lens, even one from another species which does not regenerate lenses, is associated with inhibition of lens regeneration, I concluded that something might be given off by the lens into the aqueous humor in which the dorsal iris was bathing. This appeared to be substantiated by beginning on the second day and injecting daily for a long time into lentectomized eyes aqueous humor from eyes containing a normal lens. The best eyes carried for many weeks failed to regenerate a lens while daily injections of saline solution failed to inhibit lens regeneration.

Takano and co-workers repeated this experiment using the Japanese newt Triturus pyrrohogaster beginning at the second and seventh days after lentectomy. These eyes were followed for 20 to 35 days after operation in a few cases. There were also injections of various extracts not concerned with these comparisons. They often found, as I did, that the iris and retina were injured by this method. They reported lens regeneration as in controls in their best cases and concluded that aqueous humor from normal eyes did not inhibit lens regeneration by their methods.

One has to be quite skilled in this technique to control all phases of it, particularly in preventing leakage of the injected fluid. It is essential that an amount of aqueous humor be removed from the eye before an equal amount from the normal eye is injected. Otherwise the increased intraocular pressure will be accompanied by leakage of the injected material and invalidate the experiment.

An easier and safer approach to this problem was devised. A right eye was excised and a broad round opening cut in its nasal half. It was then implanted into a wound in the head so that the opening fused with a similar opening made in the temporal half of the host's right eye. Both eye units were lentectomized. A number of cases showed perfectly fused double eye units with a large common oval pupillary space in which both dorsal irises and lenses were bathed by the same aqueous humor. After several weeks, two large lens regenerates were seen and one was removed. Animals killed 42 days later showed that a lens had regenerated from the lentectomized unit in the presence of the other lens.

However, the fused eye units contained almost double the amount of the neural retina of the normal eye. It is known that a retinal factor initiates lens regeneration, and it is possible that the effect of a stronger retinal factor may have overcome any inhibiting capacity that the remaining lens unit might have possessed.

To clarify this, in a number of adult newts the ventral half of the right eye was cut away and the dorsal half of a donor eye grafted to it. The lenses of both halves were removed. Many of these half dorsal units fused perfectly and in several weeks gave the appearance of a single normal eye. Two large lenses regenerated, one from each dorsal iris. After many weeks one large lens was removed. Those eyes not injured by the second operation showed no lens regeneration from the lentectomized half in the presence of the remaining lens. Is it possible that the inhibiting influence involving the lens was emanating from something present in the aqueous humor and potent enough to overcome in some way the retinal factor?

The results of an earlier experiment also bear upon the relation of lens and aqueous humor when lens regeneration is inhibited. One can insert a round thin disc of Phlofilm through a corneal slit isolating the intact lens below it from the dorsal iris above (Fig. 19, A). The disc sinks into the retina medially and fits tightly against the cornea in front. This seals off the
stretched dorsal iris above in a chamber by itself so that the aqueous humor above is separated from that around the intact lens below. A lens regenerated from the isolated dorsal iris (Fig. 19, B). Possibly this is a result of the iris being removed from an inhibitory effect of something in the aqueous humor emanating from the intact lens.

The role of the retina in lens regeneration can be established as follows: When an eye has been lentectomized and the neural retina has also been removed, lens regeneration is retarded until the retina is regenerating from the retinal pigment cells.21

Lens regeneration is inhibited if the iris is isolated for a long time by the implanting of a plastic membrane between the iris and the neural retina (Fig. 20, A) in a lentectomized eye. If there is a small amount of neural retina on the iris side of the chamber (Fig. 20, B) a small lens regenerates. This seems to indicate that the size of the lens regenerate may be determined by the amount of neural retina to which the iris is exposed.

If the implanted membrane is extruded and a small opening remains permanent at the ora serrata, a small lens develops from the ciliary margin of the iris and another from the retinal pigment cells. When there is failure to isolate the iris and retina, a lens regenerates at the normal rate from the dorsal iris (Fig. 20, C and D).

When an implanted graft in a lentectomized eye partly encloses the pupillary margin of the dorsal iris and shields it from the neural retina no lens regenerates.24 The effect is the same as in those experiments where the iris was isolated.

The importance of the presence of the neural retina during the release of lens regeneration can be demonstrated in another experiment.25 If the entire neural retina and retina pigment cells in a lentectomized eye are excised, no neural retina can regenerate (Fig. 21) because of the lack of the retinal pigment epithelium. The eye becomes small but the surviving iris does not regenerate a lens. If, later, the dorsal iris (Fig. 21, B) or the entire iris (Fig. 21, C) is transplanted to a lentectomized eye in the environment of a normal neural retina, lens regeneration takes place from the graft as well as from the host iris.

Takano and associates23 mentioned that when retinal extracts were injected into lentectomized eyes the lens regenerates seemed to surpass the controls slightly both in size and in differentiation.

There is also evidence that the neural retina influences the formation of the mediolateral axis of the lens. The fiber-forming pole of the regenerating lens was always directed toward the retina even though the mid-dorsal iris was rotated 180 degrees inside out (Figs. 4 and 13, D).26

In the experiment mentioned above where there was a small segment of retina in the isolated chamber on the iris side of the implanted plastic membrane, the lens-fiber pole of the small regenerate usually pointed directly toward the island of retinal tissue. This is an area of research which should be explored extensively and correlated with histochemical techniques.

Takata25 working with adult newts and Ogawa37 with larval newts observed that RNase-sensitive basophilic substances and alkaline phosphatase increase markedly during rapid cell proliferation in the lens regenerate. These substances decrease as differentiation of the lens fibers takes place and prior to the completion of regeneration.
Fig. 20. Schematic drawings showing in central figure position of implanted Pliofilm membrane to isolate iris from retina in a lentectomized eye. A, no lens regenerates if iris is completely isolated. B, small lens regenerates if a small portion of neural retina remains on the iris side of the membrane. C, if the implanted membrane is ejected leaving an opening between iris and retina then, D, a lens regenerates into the primary pupil from the dorsal iris. Also a lens develops from the iris and retina on either side of the opening. (Fig. 1 from Stone: J. Exper. Zool. 139: 74, 1958.)

Yamada and Takata\cite{38} report that RNA synthesis is maintained on a high level in cells directly involved in lens regeneration until the cells are transformed into lens fibers. A part of the RNA synthesized in the nuclei is transferred to the cytoplasm during the formation of the lens vesicle. This continues to the time of the lens fiber formation when lens-specific antigens are detectable.

Just before this takes place these cells cease to synthesize DNA. However in the
cells of the lens epithelium DNA synthesis activity is high throughout lens regeneration, even though it was not evident in those cells which had differentiated into lens fibers.

Autoradiographic studies of protein synthesis with labeled precursors are being used by Takata and Albright along with immunological methods in the study of lens regeneration in the newts. They find that lens-specific antigens are not present during early lens formation but they do appear when the lens fibers differentiate. They conclude that at least some compo-
ponents of each of the major groups of lens protein, alpha, beta, and gamma crystallins, make their appearance together in the youngest primary lens fibers. This seems to be a critical stage in the progress of lens regeneration. It may be possible that by expanding these techniques in further studies some light may be shed on the mechanisms involved in the inhibition and release of lens regeneration.

A brief report by Karasaki has added some new knowledge about the ultrastructure of the regenerating lens through the electron microscope. Electron microscopic studies on the regenerating lens revealed that four days after lentectomy the mediodorsal iris cells show an increase in the number of primary nucleoli. The nuclei then enlarge and become spherical. Prominent nucleoli appear which have a granular cortex surrounded by a fibrous core. At this time the pigment granules of the depigmenting iris cells are extruded into the intercellular spaces where they are taken up by leukocytes. The cytoplasmic reticulum gradually disintegrates. Then the nucleoli become less prominent and the ribosomes increase in number in the cytoplasm. The cytoplasm of the lens fibers then acquires fibrous elements of low density corresponding to that of normal lens fibers.

Conclusion

Lens regeneration from the dorsal iris after the original lens is removed is probably limited to a few species among the lower vertebrates. This phenomenon is now known to occur in certain species of salamanders and apparently in two species of fishes, but in no other vertebrates.

Although lens regeneration from the pupillary margin of the dorsal iris has not yet been found in any anuran so far examined, there is evidence in the tadpole stage of at least one of the anurans that the capacity for lens formation is apparently hidden in the pigment epithelium of the iris and retina. It has been revealed that these pigment cells have a dual capacity to form either retinal tissue or occasionally lenses when transplanted into a lentectomized eye. It may be possible that in this case different nutritional conditions in the environment or in the graft itself direct the type of tissue which emerges from these pigment cells.

Since in anurans so far examined lens regeneration does not take place from the dorsal iris as it does in certain newts, it remains to be shown by further experiments whether or not these findings are widespread among other species of anurans. In fact, the same tests should be applied to early stages in the growth of the eyes of other vertebrates in which a lens does not regenerate from the pupillary margin of the dorsal iris after the original lens is removed.

In the experiments which are reviewed here on the eyes of the newt there were many which showed that the potential lens-forming cells exist in various regions of the dorsal half of the iris and also in the retinal pigment cells of the dorsal retinal wall. It is important to note that in all other cases cited where potential lens-forming cells are present they are also located in the dorsal half of the eye. From earlier experiments of Sato with rotated embryonic eyes of the newt it is apparent that the lens-forming area is polarized in the dorsal half of the eye in an early optic vesicle stage under the influence of the ventral fetal fissure. Previous to this, the entoderm of the archenteron and the prechordal mesoderm can already induce lens formation in the neighboring surface ectoderm. Then when the optic vesicle contacts the surface ectoderm it carries on its part in the induction and differentiation of the lens. It would be interesting to know through further experiments whether the inductive influence is centered in the dorsal part of the optic vesicle at the time when its future lens-forming area is polarized.

However, the existence of an embryonic lens in the newt eye in the first place has no role or influence in the development of
the mechanism which incorporates the capacity for lens formation later in the dorsal iris. Reyer\textsuperscript{44} has shown this conclusively in his experiments on newt embryos where, in the absence of the embryonic lens, a lens regenerates from the dorsal iris as soon as the latter is formed.

In a previous part of this text many experiments on newt eyes were cited to show that some retinal factor under favorable conditions induces lens regeneration from the dorsal iris. It may be a chemical factor but the mechanism involved is unknown.

The role the presence of a living lens plays in inhibiting lens regeneration is still not clear. It is not certain whether under normal conditions the lens shields the dorsal iris, whether it absorbs the retinal factor, or whether the lens gives off some inhibiting substance in the aqueous humor to neutralize the retinal influence. Further experiments must be devised to find the answers to these questions.

REFERENCES


34. Stone, L. S.: Unpublished data.


