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Studies on Lens-Regeneration in Anuran Amphibia

Preliminary Observation and Experiments

by

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With 12 figures in the text

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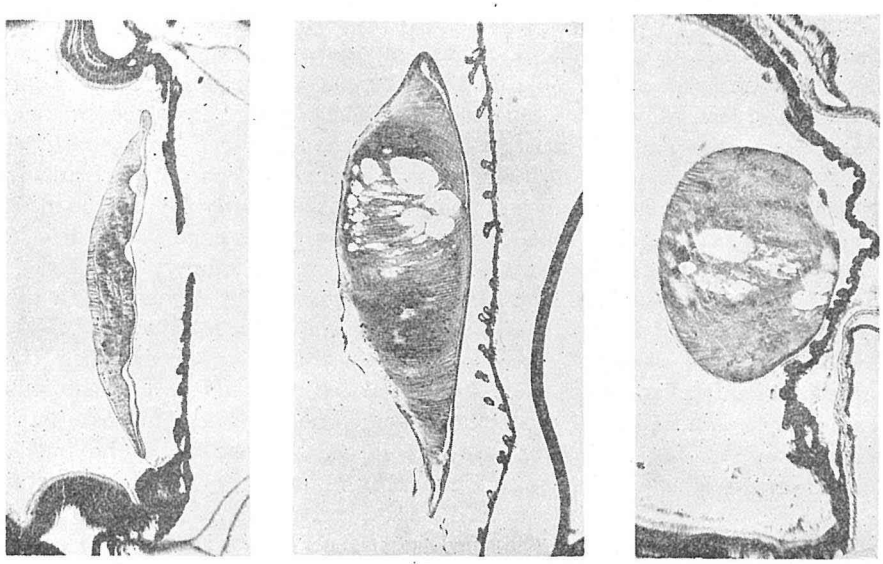
The so-called "lens-potency" of the iris of anuran Amphibia becomes extinct early during larval life (cf. MANGOLD 1931 p. 314). If, therefore, the lens is removed from the eye of frogs after metamorphosis, it is not replaced and the eye remains without lens. Practically however, a frog's eye thus operated on is found to have a lens again after a few weeks. The new lens had to have a different source of origin outside the iris, since this structure in frogs as has just been mentioned no longer possesses potency for lens production. If, on the other hand, the operation was imperfect, the lens may have been derived from the remnant of the old one, which is quite a plausible consequence of regeneration even in case of the lens. But this commonest fact, so far as I am aware, has not particularly been investigated among Anurans, being perhaps shadowed behind the most surprising process of lens-regeneration from the upper edge of the iris in Urodeles. In this fact lies the reason for the present study.

Normal sequence of regeneration. Lenses invariably regenerate in the fully formed eye of *Rana japonica, Rana nigromaculata, Rana rugosa, Hyla arborea* and *Bufo vulgaris*. According to WACHS (1914) who studied the same problem on larval Urodeles such as *Triton* and *Salamandra*, the lens fragment remaining in the eye-cavity is sometimes resorbed but sometimes regenerated. To me however, the lens in this case seems to be but a regulation product of a lens-fragment and I am quite sceptical as to whether it would further develop into a definitive organ. In a Japanese Urodele, *Triturus pyrrhogaster* a damaged lens is never retained in the adult as well as in the larva; it disappears sooner or later to be replaced by the one that comes from the edge of the iris. Moreover, the lens of this Urodele is easily removed without the rest. In the Anurans on the contrary—in those species above named at least—total extirpation is rather difficult and in the majority of cases after the operation, the epithelium and the

cuticular capsule, empty and collapsed, are still retained. These remnants of the lens are observed in sections as a double layer of thin lamellae, with cellular elements interposed between them also usually arranged in two layers. The lamellae consist of less easily stainable (homogenous) substances and at the wound, where the lens was extirpated, edges sometimes curl up like a clock spring.

In the next stage the wound is healed and the cellular layers expand more or less laterally through cellular division—the mitotic figures are particularly abundant at the folded margins—and the epithelial remnant is converted into an exceedingly flattened vesicle. This is often so much flattened that an interspace is hardly visible between the two layers of cells. But that the structure represents the lens-primordium from which a new lens starts its development, cannot be doubted, as the further development is carried out exactly as in the embryonic fashion as is the case also in the regeneration of lens from the upper edge of the urodelan iris: after being hollowed out slightly or even without such a preliminary change of form the lens-primordium becomes solid through the gradual elongation of the cells of its inner side facing the retina. The process goes on more vigorously in the middle of the layer and weakens gradually on both sides, while the outer side of the vesicle remains without change as a thin epithelial layer over the distal surface of the growing lens. Fig. 1 represents the state of regeneration at about 10 days after the



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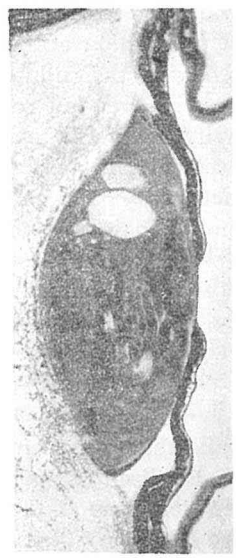
Figs. 1-3. Three successive stages of lens-metamorphosis of the lens-epithelium in *Rana nigromaculata,* 10, 16 and 26 days respectively after removal of the lens in late spring. Attention should be paid to the structure of new lenses which are highly vacuolated. Photograph ×28.

operation, and fig. 2 that about a week later. The lens 10 days later still, namely 26 days from the beginning, is shown in fig. 3.

A glance at these figures will at once show that the regenerated lenses are highly vacuolated particularly in median parts of the fibrous nucleus. Such is, however, by no means peculiar to those here presented as examples for illustration of the experiment, but is a character quite common to all so far as observed. Another characteristic point of regeneration of the anuran lenses is that being exceedingly flattened and greatly elongated the primordia, centres of fibrous differentiation easily form at more than one point and often result in bi-, tri-, or even pluri-nucleic lenses (see fig. 5 for example). Frequently they are separated into so many independent lenses connected only by a narrow bridge of cellular or non-cellular element. Fibrous differentiation may take place in a fragment of epithelium without first building up a vesicular structure. It makes no difference in this process whether the fragment is confined within the cuticular capsule or is extruded into the eye-cavity.



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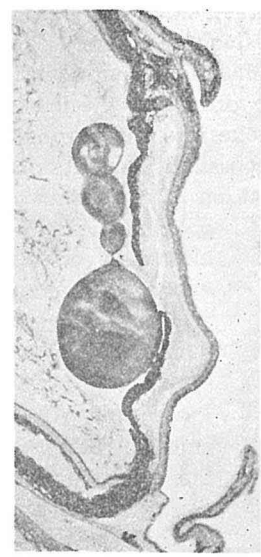

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Figs. 4-5. Two different types of regenerated lenses with respect to the number of fibrous nuclei in *Rana japonica*. Specimens are 23 days old; operation was done in late summer. Photograph ×28.

In the adult *Triturus pyrrhogaster* out of 12 cases of iris pieces tranplanted into the eye of the same species, OGAWA (1921) obtained no instance of lens metamorphosis even after 33 days. But this negative result was subsequently replaced by a positive one through the systematic investigation of NAKAMURA (1935, 1936) in the same Urodele; he showed that metamorphosis of the transplanted pieces of iris into lens or regeneration of the lens from the upper edge of the iris, takes more than 20 days even in the most favourable time in summer (25-30°) and the process is paralyzed in low temperature under 15-20°C. The lens development of the epithelium in frogs in late autumn, when the regeneration at the iris of the Urodele had almost stopped, took place even in less than 20 days. From this simple comparison a greater potency of lens formation seems to be preserved in the lens-epithelium of Anurans than in the iris of Urodeles, but it should not be forgotten that the proof of potency depends much on the experimental conditions. (In

cold winter season lens metamorphosis does not take place even in the anuran lens-epithelium.)

Determination of self-differentiation. The fact of regeneration from the remnant of an incompletely removed lens was mentioned according to WACHS (1914, p. 403) by RANDOLPH (as early as 1900) in a rabbit before WACHS (1914), and TÖRÖ (1932) described it also in larval Urodeles, but the process of regeneration was first adequately studied by IKEDA (1932, 1934 a) on two Japanese lizards, *Lacerta serpa* and *Lacerta vivipara.* According to this author "the lens-fibres are formed always from the cells of the posterior wall of the epithelial remnant and never from portions of the anterior wall, i. e. they differentiate in the proliferation zone of the normal growth of the lens." Therefore, the axis of regenerated lenses falls in that of the eye with the fibrous nucleus always on the retinal side. The fact in the case of Anurans is exactly the same. But IKEDA doubted whether this determination of the lens-axis in regeneration is actually due to an inductive effect of the retina. To investigate this question, he drew out the lens from the eye and re-implanted the epithelial part alone into the lensless cavity. By this experiment he demonstrated the possibility of fibrous formation in varied positions with regard to the position of the retina, sometimes "ganz umgekehrt wie bei normal Verhalten" and almost on the corneal side. From these experiments IKEDA (1934 a, p. 35) finally arrived at the conclusion that the place where the lens regenerates is not determined by the surrounding structures —especially by the vitreous humour and the retina—but the factors rest in the epithelium itself, localized in particular in the proliferation zone of the normal growth of the lens. To verify the conclusion he (1934 b) next transplanted the lens-epithelium of an Urodele, *Hynobius lichenatus* (=*unnangso*) into the brain cavities, and was thus able to certify self-differentiation of the lens-epithelium in abnormal positions.

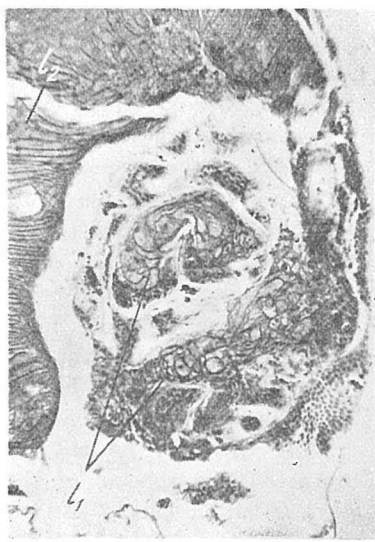
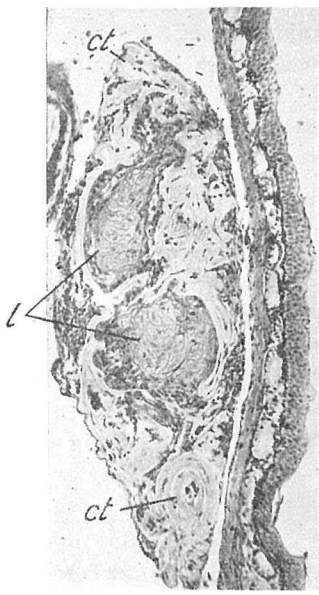


Fig. 6. Fibrous differentiation of the lens-epithelium transpalnted into the lens-primordium of the homoioplastic host (*Rana nigromaculata*); l_1—fibres developed from the graft, l_2—the host fibres. Specimen was fixed 20 days after operation in early summer. Photograph ×75.

Though less distinct than in *Hynobius*, self-differentiation of the lens-epithelium into fibres can be demonstrated also in frogs. Fig. 6 is the result of transplantation of the lens-epithelium into the cuticular capsule from which the lens has been removed. Two distinct masses of modified cells (l_1) are clearly shown beside the strongly deve-

loped lens-fibres of the host (l_2). This example can by no means be considered above criticism, since the graft is still confined within the sphere of retinal influence, namely in the eye-cavity. In the next example however, in which the same transplantation is done into the lymph cavity outside the eye, fibrous metamorphosis of the graft is also distinct as in the preceding case (fig. 7). That the lens-epithelium can self-differentiate is almost



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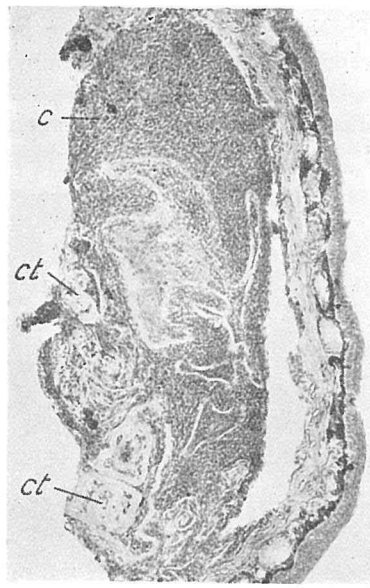

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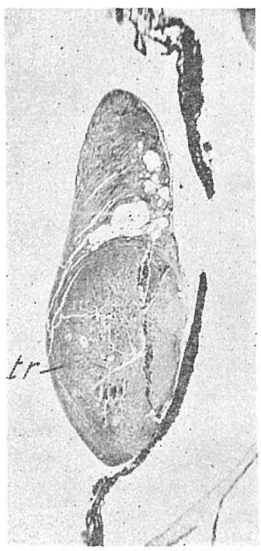
Figs. 7–8. Results of transplantation of the same lens-epithelium as before into a brachial lymph cavity. In the specimen of fig. 7 fibrous metamorphosis takes place in the graft, while in that of fig. 8 the graft dissociates into isolated cells which augment to a great number; *c*—dissociated cells, *ct*—rest of cuticular capsule, *l*—lentoid cells. Specimens were fixed about 20 days after operation. Photograph ×75.

beyond dispute. Only the process takes place unmistakably in a less degree than in the urodelan *Hynobius lichenatus*. Moreover the transplants into the lymph cavities, if conditions are favourable for them, easily dissociate into cells which augment to a great number as in the culture in vitro (fig. 8). The fibrous metamorphosis occurs only when the graft is contained in the cuticular capsule and the cells keep the original arrangement of the epithelium. Another interesting point of the anuran lens-epithelium which differs from that of the lizards is that the fact of self-differentiation cannot be manifested independently in the presence of the retina. So long as the inductive influence of the latter is operative, development of the lens or more precisely the differentiation of the lens-fibres is always under control of this power.

Check of regeneration. Wachs (1914), basing upon Spemann's idea that retina stimulates the iris to develop into lens while presence of the latter inhibits the process of regeneration, established in Urodeles a hypothesis of secretory equilibrium between the two agents. Investigation was made as to whether the secretory mechanism of regeneration-check holds true here in the case of the anuran lens-regeneration from the lens-epithelium. It was variously tested, by transplanting a half or quarter of a lens or sometimes an entire one, of the same or different species into the epithelial vesicle formed soon after the removal of lens. But in no case, whether homoioplastic or heteroplastic, was the graft found to act as more than a mechanical obstacle; the development of new lenses was otherwise quite normal (see figs. 9-10). The only peculiarity to be noticed was that the homoioplastic graft is incorporated into the new structure without visible signs of degeneration (fig. 9), while in heteroplastic combinations a clear space of some width invariably intervenes between the new and the old structures and the graft is often observed in degeneration (fig. 10). So that if anything can be said concerning chemical hindrance to the regeneration, it is to declare compatibility of the lens substance between different species, whereas the lens is reported as chemically uniform beyond difference of species (cf. Mangold 1931, p. 212).



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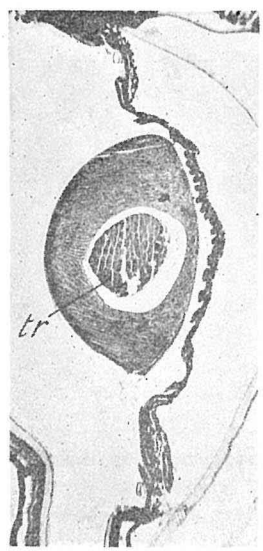

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Figs. 9-10. Lenses developed from lens-epithelia having respectively homoio- and heteroplastic lens-insertions. In the specimen of fig. 9 one half of the lens of the same species (*Rana nigromaculata*) and in that of fig. 10 an entire lens of *Triturus pyrrhogaster* were introduced; *tr*—graft. Specimens were fixed 20 days after operation in early summer. Photograph ×28.

On the other hand, the mechanical hindrance is most evident in the lens development, being the process taking place in a limited space of the vesiculiform lens-primordium within the cuticular capsule. Fig. 11 is the result of an experiment in which the lens regeneration is greatly checked though still not completely stopped by the presence of a paraffin sphericle inside the lens-vesicle. The same kind of regeneration-check is frequently brought about by an infiltra-

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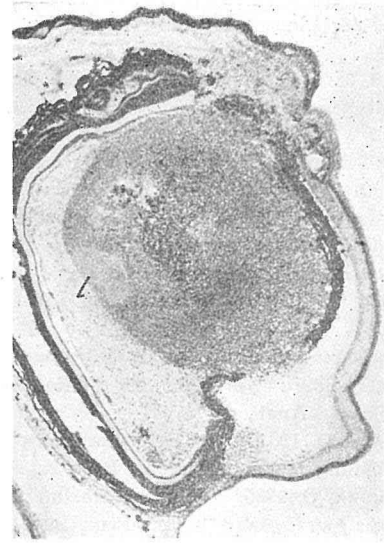

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Figs. 11–12. Two cases where lens development is greatly checked by the presence of foreign bodies—in fig. 11 by a paraffin sphericle and in fig. 12 through an infiltration of blood into the lens-primordium; *l*—lens fibres partly produced. Specimens were fixed 20 days (fig. 11) and 15 days (fig. 12) after operation. Photograph ×28.

tion of blood directly after the extirpation of the lens (fig. 12). And in such cases only fibrous differentiation may take place against the directive influence of the retina even on the corneal side.

Conclusion. Thus the lens-epithelium of anuran Amphibia is shown to possess highly regenerative potencies. It is, however, determined to a certain extent and can, when isolated, differentiate into fibres according to its own property. The development in the eye-cavity is ruled by the retinal influence, but is at the same time easily subdued by mechanical hindrance and fibres can be formed even in an abnormal position irrespective of the position of the retina. The so-called secretory equilibrium between the lens and the primodium—the lens-epithelium in this place—does not hold true in the present case.

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