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Experimentos sobre la regeneración del cristalino de *Diemyctylus*.

1. El autor describe con detalle la regeneración del cristalino en *Diemyctylus*.

2. Algunos de los experimentos se han llevado a cabo en la porción superior del iris. La porción inferior es incapaz de producir un cristalino, aun cuando se interrumpa la regeneración en la porción superior. Haciendo una incisión en forma de colgajo en la porción superior del iris, la regeneración que sigue tiene lugar de diferentes modos. Un trozo de iris transplantado en la retina puede provocar la regeneración del lente mejor que en la cámara del ojo.

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EXPERIMENTS ON THE REGENERATION OF THE LENS IN DIEMYCTYLUS

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FOUR FIGURES

INTRODUCTION

In embryonic development the lens is formed from the ectoderm covering the eye cup. If the larval or adult Urodele is experimentally deprived of its lens, regeneration takes place from the dorsal side of the iris, which has no relation with the normal embryonic development of the lens. This striking fact of heteromorphosis, which was first discovered by Colucci,¹ later led many investigators² to study this problem further from various points of view. Not only have descriptions of the regular regenerating process been given in detail, but also the factors that stimulate and determine the formation of the lens, the reason that the seat of regeneration is exclusively in the upper iris, and many other questions have been investigated.

In the present investigation the author has studied further the regular regeneration of the lens, and in relation to it has also made a number of experiments upon the dorsal part of the iris (upper iris).

MATERIAL AND METHODS

Adult specimens of *Diemyctylus* were used exclusively as material for the present study.

The method of operation is as follows: The whole body of the animal, except the head, is wrapped in cloth. A piece of cloth is put into the mouth in order to keep the eye protruded.

¹ See Emery ('97).

² Wolff (larva and adult of *Triton taeniatus*), Fischel (35-mm. long larva of *Salamandra maculosa*), Müller (3- to 6-cm. long larva of *Triton*), Wachs (larva of *Salamandra perspicillata*, *Triton taeniatus*, *cristatus*, *axolotl*), etc.

Under the binocular, an incision is made into the nasal part of the cornea with a small iridectomy scapel and then the cut is extended horizontally. Flowing off of the aqueous humor can be prevented until the cut is finished by slight pressure of the back of the scapel against the wound; in this way prolapse of the iris is avoided. Compression on the eye causes the lens to slip out easily through the wound. The right eye alone was operated upon. After the operation, the animals were put directly into the water. The wound heals quite easily and the eye recovers its original form.

For convenience of description, I shall suppose the eye axis to be in sagittal direction.

RESULTS OF EXPERIMENTS

Regular regeneration of the lens

As already known, the first changes that take place after extirpation of the lens are depigmentation of the epithelial cells of the dorsal part of the iris and thickening of the upper iris edge. Concerning the point where depigmentation first begins, however, existing statements are not exactly in agreement. According to Wolff and Müller, "die innere Lamelle der Iris beginnt ihr Pigment zu verlieren," while Fischel says "die erste Veränderung besteht darin, dass die Zellen ihres hinteren epithelialen Blattes pigmentärmer werden und die Depigmentation greift auch über den Pupillarrand hinaus eine Strecke weit auf das vordere Blatt über." Wachs mentions simply that "die Umschlagstelle des äusseren in das innere Blatt entpigmentiert sich;" my observation agrees with this latter statement. The first change, pointed out by Wolff and Müller, corresponds to a slightly later stage.

The first depigmentation occurs usually not in the whole upper iris edge, but just in its middle part, only now and then being displaced laterally or medially. Simultaneously with depigmentation, or rather prior to it, the iris edge undergoes thickening and presents a club shape if seen in sagittal section. The thickening is caused by the splitting open of the two epithelial layers of this part and also by the increase of their height. Mitosis cannot be seen in this stage. Several round cells, richly provided

with pigment granules, appear in the space produced by the splitting of the two layers. These round cells were considered by Wolff and Müller as leucocytes. Concerning the separation of the two epithelial layers of the iris, Wolff is inclined to assume that it is caused by pressure of the leucocytes which enter between them. On the contrary, Fischel states as follows: "Mit einer einfach mechanischen Erklärung kann man hier nicht auskommen . . . Die Zahl der Leukozyten ist eine zu variable und ihr Durchtritt durch die Iris erfolgt in zu unregelmässiger Weise und nicht an allen Stellen, um eine so gleichmässige, allseitige Abhebung bewirken zu können." It is not justified, however, to count the presence of the leucocytes as a cause of separation, because even in cases where no leucocyte is visible, separation of the two layers can be seen.

Thickening as well as depigmentation increases and extends from the middle part of the edge in various directions. The iris edge prevents a more distinct swelling. Especially cells of the inner layer increase in their height. Although the cells are arranged usually in a single layer in the beginning of regeneration, they may consist in this stage already of two layers. As a result of depigmentation, nuclei are revealed gradually in epithelial cells of both layers. While Wolff, Müller, and Fischel point out depigmentation in the inner layer, it takes place at first also in the outer layer in slight degree. The split between the two layers becomes more marked and extensive, and round pigment cells are to be seen in the cavity. As Fischel mentioned, depigmentation and thickening take place also in the lower iris edge in this stage and extend gradually toward other regions.

Still later, epithelial cells in the iris edge lose their pigment more and more, finally becoming entirely pigment free. The same process extends in the inner layer dorsally from the edge. Depigmentation in the outer layer, however, reaches only such degree that the nuclei become visible in the cell bodies. Sometimes the whole inner layer from the iris edge to the pars ciliaris may lose its pigment, but in other cases, on the contrary, total depigmentation is restricted to the iris edge and does not extend further, the rest of the iris containing more or less pigment all the time.

Between the pigment-free cells of the inner layer, occasionally a few round cells can be seen, which are larger than the iris epithelial cells and richly laden with pigment granules. It is difficult to decide whether they are leucocytes or modified epithelial cells, though the former assumption seems more likely. The upper iris, thus depigmented, elongates at the same time downward.

The upper iris edge becomes more enlarged by the increase of cell height as well as by the widening of the split, and thus forms a round vesicle. Mitosis is often seen here. The part which connects the enlarged iris edge with the original iris—the stalk—becomes gradually thinner and the split between the two layers here diminishes, so that the cavity of the iris edge does not communicate with the original split of the iris. Then the cells in the posterior pole of the vesicle grow higher and at the same time thinner in a radial direction. The adjoining cells also become successively high. In short, the cells in the posterior wall take up a concentric arrangement and protrude into the cavity of the vesicle. These are lens fibers. As the development proceeds, the cavity is narrowed by these lens fibers and finally disappears. Here a solid lens is formed, which is still connected by the stalk with the original iris. The cells constituting the stalk are flat or cuboidal and often small.

When the lens grows and attains a pretty advanced stage, pigmentation comes back gradually to the iris epithelium and the thickening also decreases. In this way the normal state is restored. Sometimes the pigmentation process in the iris epithelium is retarded, though the thickness returns to the norm or vice versa. The lens separates from the iris and remains in the pupillar region. The lens may either simply separate from it or, prior to separation, be connected with it by fibrous ligament. No consideration is given at present to the question as to how this ligament was formed. On the other hand, the lens may or may not become connected with the lower iris until it separates from the upper iris. This connection occurs directly or by fibrous ligament. The epithelium of the lens is sometimes pulled out where the lens is in connection with the iris, namely, about in the

equatorial zone. Owing to this projection, a cavity remains between the lens epithelium and its substance, though later both projection and cavity disappear. This connecting zone is variable in its position, deviating anteriorly or posteriorly in different grades from the equator; the position of the lens accordingly is also variable. According to Fischel, "die Art und Weise der Lösung dieser Verbindung lässt es zweifellos erscheinen, dass sie lediglich durch das Gewicht der wachsenden Linse erfolgt; hat dieses eine bestimmte Grösse erreicht, dann wird die Verbindung gelöst." But I think it is most unlikely that separation takes place by the growing weight of the lens, because the lens, after separation from the iris, still remains in contact with the iris in the pupillar region.

The lens capsule is formed at first on the anterior surface of the lens while the lens is in connection with the iris.

In normal embryonic development, pigment cells are found sometimes in the cavity of the lens vesicle; the same phenomenon is seen occasionally also in the regenerating lens. Besides the change in the iris epithelium, it is observed now and then that the gold pigment cells in the iris stroma extend to the surface of the lens.

Time of regeneration

Concerning the time required for the regeneration of the lens, Fischel ('00) states the following: "Ebensowenig glaube ich thermische Einflüsse eine Wirkung zusprechen zu können. Denn die Regeneration erfolgt im Sommer nicht rasher als im Winter, trotzdem der Temperaturunterschied des Wassers, in dem die Tiere gehalten werden, gewiss kein unbeträchtlicher ist." My results show, on the contrary, that the time required for regeneration is different according to the season and individual variety. For instance, thickening in the upper iris edge appears in five days after operation in summer, while it begins in nine days after operation in late autumn. Again, for the first appearance of the lens vesicle, it takes two weeks in summer and more than three weeks in autumn. Since the operated animals were all killed

before complete development of the lens, the exact time necessary for the whole process cannot be given here. In general, after the development of the lens attains a certain stage, further change is slow.

In order to confirm the possibility of regeneration in winter, ten animals were operated upon the middle of December, and the eyes were examined after forty days, but no indication of regeneration, such as depigmentation and thickening of the iris epithelium, could not be found at all. In this case the temperature of the aquarium, where the animals were kept, ranged from 7° to 4°C. Difference in duration of regeneration according to the season and absolute lack of regeneration in winter seem most likely to depend upon temperature, though food might play some part.

Individual difference in time of regeneration is fairly marked, even if the animals are kept under the same conditions; twenty specimens were studied three weeks after operation, and the results showed that the regenerated lenses are variable in size, the largest being several times larger than the smallest.

EXPERIMENTS ON THE IRIS

Lack of regeneration from the lower iris

In regeneration of the lens from the upper iris, depigmentation occurs also in the lower iris. Fischel considers this change as participation of the lower iris in the first stage of regeneration. But true regeneration never takes place from the lower iris. Fischel ('00, '03) demonstrated one such case and maintained his interpretation against Wolff's objection, but his case is not without doubt, because the small lentoid, which is in connection with the lower iris and also in contact with the larger regularly formed lens, might have arisen from the latter and only secondarily come into connection with the lower iris edge.

Now the question naturally arises whether regeneration may occur from the lower iris, if it is prevented from occurring from the upper iris. For this study, total synechia of the upper iris was brought about in the following way. The cornea was cut horizontally in the upper part. After removal of the lens through

this wound, the upper iris was also drawn out and kept in place. All animals, ten in number, were killed in from fifteen to twenty-two days after operation. In case of successful operation, the anterior surface as well as the edge of the upper iris fused with the cornea. In four cases synechia did not occur and the lens vesicle started to regenerate from the edge more or less normally. In the other five specimens no typical regeneration took place from the upper iris, which fused tightly by its entire surface with the cornea, though the iris edge itself was rather free in these cases. The reason for the absence of regeneration from the upper iris edge in spite of its relative freedom is hard to ascertain, but insufficient blood supply due to fusion might account for it.

In all these five cases the lower iris presented no indication of regeneration at all, except the usual slight changes. Thus it was established that the lower iris is incapable of producing a lens even when regeneration from the upper iris is prevented.

Regeneration after making a flap from the upper iris

It is not without interest to find out where regeneration will start when a flap is made from the upper iris. The iris is cut above the blood vessel which encircles the pupil, together with the cornea. After being stretched by pressure of the lens the iris is then cut at one end and the lens extracted. The flap is then pushed toward the middle of the pupil to prevent fusion. This operation was done upon forty-five specimens. In a number of cases regeneration occurred quite normally, probably owing to the healing of the flap with the original iris. In others the flap disappeared and the lens was formed from the shortened iris, while some cases presented abnormalities as results of operation. Description of the latter cases is given here.

A. Twelve days after operation. The stump of the flap fused with the lower iris edge, dividing the pupil into two parts. At the fused part the flap is thick and irregular in its cell arrangement and some of these cells show distinct depigmentation, but there is no sign of regular regeneration. Depigmentation is not seen at the lower iris. The upper iris is thick and the pigment-free part

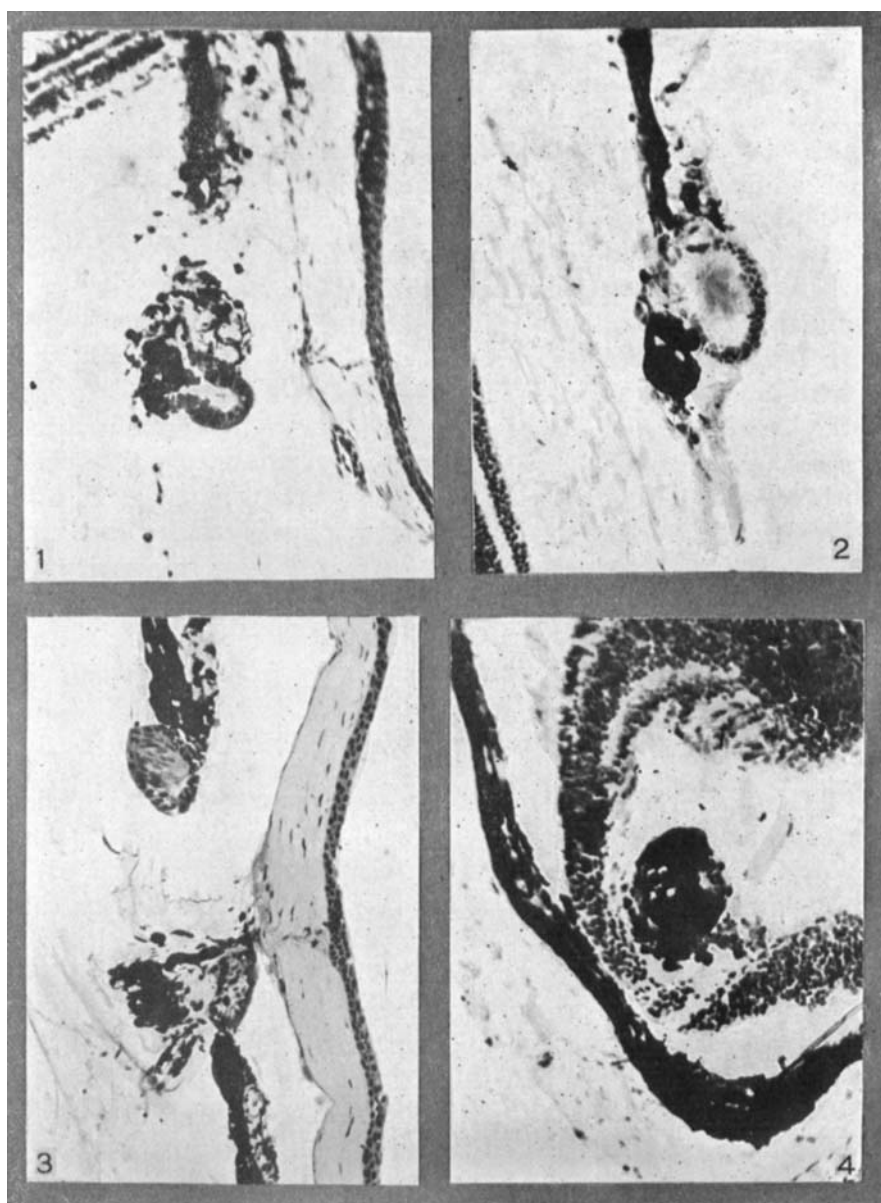


Fig. 1 Exp. C. Regeneration of the lens from an iris flap. The lens vesicle is formed anteriorly. No sign of regeneration at the shortened iris edge. $\times 80$.

Fig. 2 Exp. D. Regeneration of the lens from the cut edge of the iris. The lens vesicle is formed from the cut edge, while the iris flap remains unchanged. $\times 80$.

Fig. 3 Exp. A. Regeneration of the lens from a piece of the iris transplanted to the chamber. $\times 80$.

Fig. 4 Exp. C. Regeneration of the lens from a piece of the iris transplanted to the retina. $\times 80$.

of the inner layer is rather limited in its extent. Thus the pupil has in its upper margin the cut and the intact edge of the iris. The lens vesicle regenerated from the intact side and not from the cut edge.

B. Thirteen days after operation. In this case also the iris flap fused with the lower iris edge; but, in contrast to the former case, regeneration took place both from the cut iris edges as well as from the flap, the latter being converted totally into an incomplete lens vesicle.

C. Fourteen days after operation (fig. 1). The stump of the iris flap fused with the original upper iris, while the middle part of the flap is free from it. Almost the whole flap is changing into a lens vesicle, which turns toward the cornea. The cells enclosing the vesicle are columnar, pigment-free, and arranged partly in two layers. The cavity contains pigment cells and clusters. It can be easily concluded that the vesicle is the flap itself, because the iris ströma is attached to its anterior side. Although the cut edge of the original iris is quite free and two epithelial layers are fused together, no indication of true regeneration, such as thickening and depigmentation of the epithelium, can be seen, except that there is slight decrease of pigmentation.

D. Twenty-eight days after operation (fig. 2). In this case the flap remained unchanged and lens regeneration started from the cut edge of the iris. A microscopical study reveals a well developed lens vesicle between the original iris and the flap, which is closely attached to the vesicle and gives no depigmentation or other sign of regeneration. As the lens vesicle is pretty well developed, there is no connection with the cut edge of the iris, though in the inner layer distinct depigmentation is visible. This vesicle was formed undoubtedly from the upper iris.

As the results are different in the individual cases, no general conclusion can be drawn from this experiment.

Effect of turning up of the upper iris edge

When the lens regenerates from the iris edge, the lens fibers are formed always from the posterior wall of the lens vesicle. This led Wachs to assume that the direction of the lens fibers is determined by the presence of the retina. To examine how far this assumption is true, the iris edge was intentionally turned up, in such a way that the upper iris was cut at both sides and turned up in front of or behind the iris. Fifteen specimens were operated upon. In many cases, the bent iris did not remain in place, but came back to the original position, starting normal lens regeneration. Five cases disclosed some variations.

A. Thirteen days after operation. The iris has been only partially turned backwards, the lens vesicle accordingly was partially turned backward.

B. Sixteen days after operation. The lens vesicle regenerated from the iris edge posteriorly and slightly dorsally, without taking the normal downward direction, so that the lens fibers run as a whole from above downward postero-anteriorly.

C. Eighteen days after operation. The iris had been turned up anteriorly. No typical regeneration was obtained except slight depigmentation and grouping of cells at the edge.

D. Twenty-one days after operation. The iris was shortened. From the edge a relatively flat lens was formed. The lens fibers run as a whole slightly from above downward postero-anteriorly.

E. Twenty-three days after operation. Double lenses were formed. One is normal; the other quite abnormal and projects into the anterior chamber from the upper part of the former, the two lenses being partially continuous. A piece of the iris is attached behind the lens. The iris may have been severed by the operation in this case.

If the retina alone determines the direction of the lens fibers, the latter, whatever position the lens may take, must maintain as a whole a sagittal direction, because the retinal influence should remain the same. However, in the above cases the lens fibers

take sometimes a slightly downward direction. I am inclined to think that some factor influencing the direction of the lens fibers might also exist in the iris itself, because the direction of the axis of the lens seems to be vertical to the stalk of the lens, even in abnormal position.

Regeneration from transplanted pieces of iris

Fischel and Wachs studied cases where the lens regenerated from isolated or transplanted pieces of iris. In fourteen larvae Wachs cut the piece from the upper iris after extraction of the lens and left it in the posterior chamber. He found after some time that in six cases the pieces had slipped out, in one case it had remained in the corneal wound, in six cases they had fused with the iris, and in one case a lens was formed from the isolated piece, though incompletely. He transplanted also pieces of iris taken from other animals into the eyes of three specimens, but in all cases the pieces fused with the iris and then started to form lenses.

In fifteen specimens I made transplantations of upper iris pieces, taken from other individuals, into eye cavities. Examination after from thirteen to thirty-three days revealed that the pieces had remained intact in twelve cases; in nine cases they were in contact with the corneal wounds and in three cases they were apart from the cornea. The remaining three cases presented no trace of the transplanted pieces. None of these twelve cases gave pictures of regeneration, except that there was distinct depigmentation in one case.

Then I tried to transplant the upper iris pieces to the retina. Twelve specimens were operated upon and examined after from nineteen to twenty-six days. The results were as follows: In three cases the iris pieces were absent in nine cases the transplantation was successful. Among the nine successful cases, five showed regeneration (four in the retina and one in the anterior chamber), and four cases showed no regeneration.

Description of the regenerated cases follows:

A. Nineteen days after operation (fig. 3). What was unsuccessful in the former experiment, was achieved in this case. The transplanted iris piece is located in the anterior chamber near the lower iris edge, instead of being attached to the retina. The lens vesicle was formed anteriorly from the piece. The vesicle is elliptical. The cells are pigment-free, partly cuboidal, partly columnar, and arranged regularly in a simple layer. It is connected by a stalk with the iris piece. From the upper iris there is regular lens regeneration.

B. Twenty-two days after operation. The transplanted iris piece rests upon the lower part of the retina, which consists here of only two layers (inner reticular and outer nuclear). This is regenerated retina. The piece presents a round cell mass, amidst which there is a small vesicle, surrounded by a single layer of cuboidal pigment-free cells. Mitosis is seen in these cells. This structure must be considered as an early stage of a regenerating lens vesicle. It is somewhat striking that the vesicle was formed amidst the cell mass of the piece, while it usually starts from the periphery.

C. Twenty-two days after operation (fig. 4). The iris piece, resting on the retina, forms a round cell mass, measuring $17\ \mu$ in diameter. In the center a cavity is formed, which is partly filled with cells continuous with the wall. The wall consists of one or two layers of cuboidal or columnar cells. Some of them are pigment-free and show mitosis. This also should be considered as a lens vesicle.

D. Twenty-four days after operation. The iris piece, transplanted into the retina, consists of two parts, pigment-containing and pigment-free. In the latter there is a vesicle consisting of cuboidal or columnar pigment-free cells. The cavity of the vesicle is small. Although this is imperfect, it also can be considered as a lens vesicle.

From the above it is seen that when pieces of iris are transplanted to the retina, they regenerate far better than in the eye cavity. Of course in all these cases regeneration remains, as might be expected, incomplete and does not attain even the stage of formation of lens fibers, but the structures formed are

recognizable as lenses by their depigmentation, their cell arrangement and their general form.

The fact that regeneration takes place more readily after transplantation to the retina seems to be explicable in one of two ways: 1) The iris piece is better nourished in the retina than in the eye cavity or, 2) the retina has special ability to induce the regeneration from the iris. This agrees with the assumption made by Wachs. At present it cannot be decided which possibility is more probable.

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