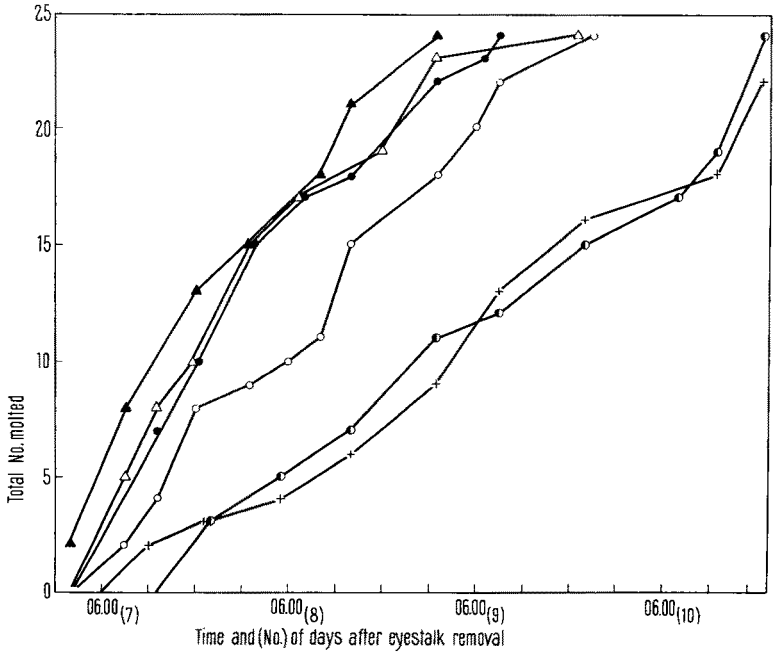

Fig. 2. Effect of time of dose after eyestalk ablation on rate of moulting. All experimental animals received crustecdysone in concentrations of 0.025 µg/g body weight. ◐ control, 0.02 ml van Harreveld's saline injected at 24 and 72 h; + control, 0.02 ml peanut oil at 24 and 72 h; o crustecdysone at 24 h; • at 48 h; △ at 72 h; ▲ at 24 and 72 h.

From the above experiments, it is evident that in the absence of eyestalks, crustecdysone in very small amount reduces the time taken to moult. However, the effect of crustecdysone is appreciable only after a period of 24 h or more following eyestalk removal. This time may be required for the blood titre of the MIH to drop and allow the animal to become responsive to the effect of crustecdysone. Also crustecdysone may initiate only some of the processes associated with moulting and it may be unable to exert its effect until other hormones have been synthesized by the Y-gland in response to the removal of the source of inhibiting hormone.

Résumé. La crustecdysone injectée dans l'écrevisse, *Procambarus sinulans* ne cause pas la mue chez l'animal intact mais l'accélère après ablation des pédoncules oculaires.

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23 October 1967.

Studies on Lens Regeneration in *Xenopus laevis*

Lens regeneration from the dorsal iris following lentectomy is a unique feature and is restricted to a number of species among the urodeles[1,2]. In the anurans, however, the 'lens potency' of the iris is lost during larval life[3].

The results of studies on regeneration from the dorsal iris in adults are not in agreement with each other, and if regeneration was found the conclusion was doubted[1,4].

Campbell[5] reported that in the adult *Xenopus laevis* lens regeneration from the dorsal iris following lentectomy occurs in 25% of the cases, and the time required for this is approximately 6 months. On the other hand, Overton and Freeman[6] and Freeman[7] showed that in the metamorphosed *X. laevis* lens regeneration appears to be absent, though it occurs from the inner cell layers of the cornea during the larval life. It was decided, therefore, to reinvestigate the problem of lens regeneration in adult *X. laevis*.

Methods. Sexually mature animals were purchased from a dealer in the Netherlands. Lenses were removed from the eyes under the dissecting binocular microscope while the animals were kept under anaesthesia with MS 222. From 5–188 days after the operation the eyes were extirpated for investigation. They were fixed in Bouin's solution and treated according to the usual histological procedures. Serial sections were cut to 10 µ thickness; they were stained with haematoxylin and eosin.

Results and discussion. As is shown in the Table the complete removal of the lens in the adult *X. laevis* was achieved successfully in a few cases only. In most of the cases some lens epithelium was retained within the collapsed capsule, as could be confirmed histologically

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Fixation after lentectomy (days)	Animals operated	Lenses removed	Incomplete removal	Complete removal
5	5	10	7	3
11	7	14	12	2
14	5	10	8	2
20	8	16	12	4
33	8	16	11	5
57	5	10	9	0
60	2	4	4	0
77	5	10	5	5
100	2	4	4	0
150	2	4	3	1
188	4	8	2	5

(Figure 1). In successive stages following the operation it was observed that a connection develops between the lens capsule and the inner cell layer of the cornea, which by this time has increased in thickness by several layers (Figures 2 and 3). The epithelial cells within the capsule start to divide and fill the cavity.

The lenses reconstituted from the epithelium are abnormal in histogenesis. They are bi-lobed and sometimes vacuoles are seen in the tissue, as described by OKADA[8] and STONE and SAPIR[4] in the regenerated anurans lens. Occasionally pigment cells can be observed, suggesting migration from the retina (Figure 4); these pigments can

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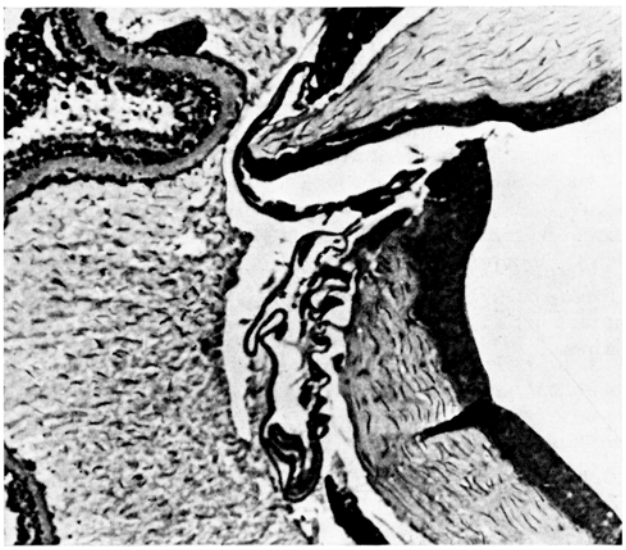

Fig. 1. 5 days after lens removal. The wound in the cornea is still visible. The collapsed lens capsule containing lens epithelium is observed. × 59.

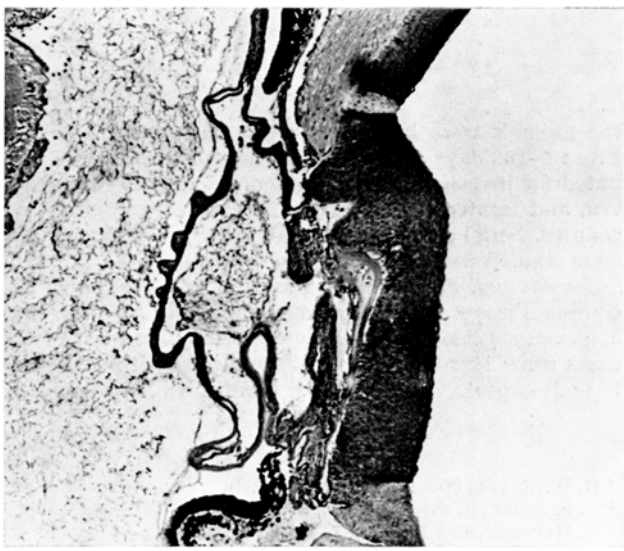

Fig. 2. 11 days after lens removal. The corneal wound is healed. The capsule starts to become enlarged. More epithelial cells are seen along the border of the capsule. × 59.

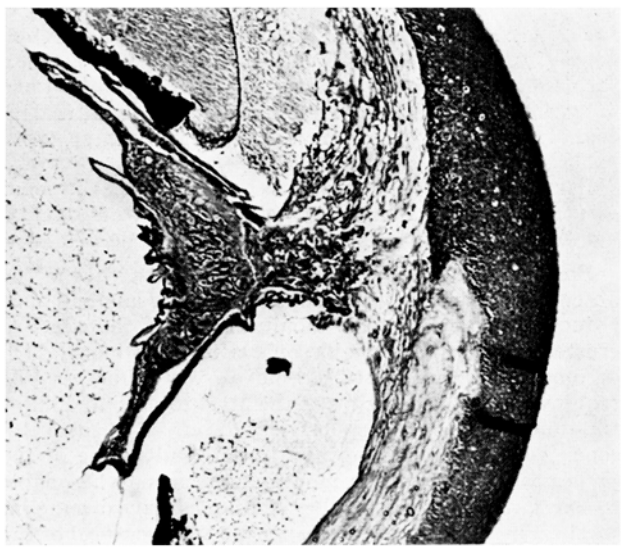

Fig. 3. 20 days after lens removal. The cavity of the lens capsule is filled with lens epithelial cells. A connection with the inner corneal cell layers is established. The inner layer of the cornea is increased in thickness. Pigment cells are also visible within the corneal cells. × 59.

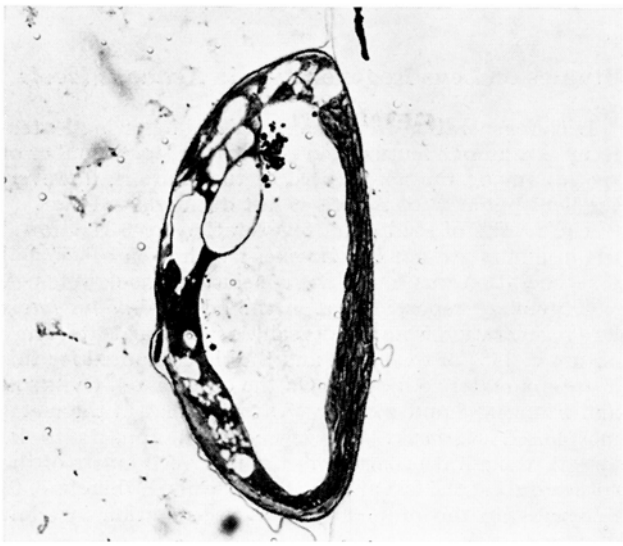

Fig. 4. 60 days after lens removal. The regenerated lens vacuoles contain pigment. Lens fibres are seen mostly at the periphery of the capsule. × 59.

also be found in the inner corneal layer (Figure 3). In most cases the lens retained a connection with the inner cell layer of the cornea by its capsule, which could be observed even 188 days after lentectomy (Figure 5). The reconstitution of the lens from the epithelium could be observed between 57 and 60 days and in some cases at an even earlier stage. In cases where the lens was removed completely, no evidence of lens regeneration from the dorsal margin of the iris could be found in this series of experiments, not even 6 months after the operation.

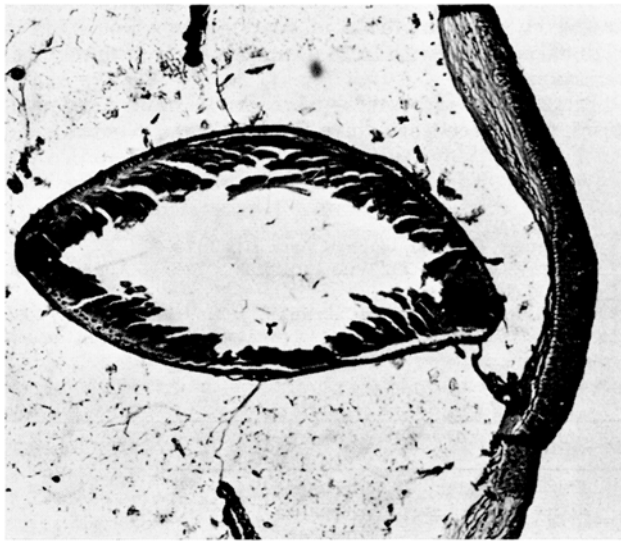

Fig. 5. 188 days after lens removal. The lens still retains connection with the inner corneal layer by the capsule. × 59.

The present results show that in *X. laevis*, as in other anurans according to reports so far, the complete removal of the lens is difficult. Where there was lens formation, it developed from the fragments of lens epithelium which were retained within the capsule, as has been suggested in the vacuoles (OKADA[9,10]).

Zusammenfassung. Es wurde die Regeneration der Augenlinse erwachsener *Xenopus laevis* nachuntersucht. Eine vollständige Entfernung der Linse gelang in wenigen Fällen. Die Neuentwicklung der Linse wurde nur von Linsenepithelresten, die nach der Lentektomie in der Kapsel zurückgeblieben waren, induziert.

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Department of Medical Anatomy and Embryology, State University of Utrecht (The Netherlands), 30 October 1967.

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PRO EXPERIMENTIS

A Sensitive Method for the Detection of Racemization in Peptide Synthesis

A few years ago we reported that diastereoisomeres of DL/DL dipeptides can be separated by paper[1] or thin layer chromatography[2]. This phenomenon allows one to evaluate the extent of racemization which occurs during introduction or removal of different protecting groups[3] used in peptide chemistry, or during the formation of the peptide bond[4].

In the latter case, phenylalanine or valine, *N*-protected by formyl or trifluoroacetyl residues were coupled with tertiary esters of phenylalanine or valine with the help of methods to be examined. The amount of the diastereoisomere D–L was estimated in free dipeptides.

Unfortunately such amino protecting groups (chosen due to the lack of other more appriopriate ones) are rather 'unnatural' and therefore inadequate. They can considerably diminish racemization and sometimes they can even protect the acylating acid against racemization[5].

Their removal by alkali or acid leads to partial hydrolysis of the peptide bond, and when its diastereoisomeres have the same Rf as the component amino-acids, such dipeptides cannot be used as test for the detection of racemization.

A perfect means for the protection of the amino group of the acylating amino acid would be incontestably acylamino acids or acyl peptides themselves. Unfortunately up to now only dipeptides could be resolved chromatographically into their diastereoisomeres.

To solve this problem we suggest: (a) to protect the amino group of the acylating amino acid with suitably *N*-protected amino acids or peptides, (b) to couple the peptide with tertiary butyl esters of L-amino acids with the help of the method to be examined, (c) to degrade the free peptide with the EDMAN method, and (d) to estimate the amount of diastereoisomere D–L in the dipeptide obtained.

After the synthesis of the peptide, the unreacted substrates must be removed from the reaction mixture by repeatedly washing out (under chromatographic control) in order to avoid the appearance of additional spots which

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