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The problem of lens regeneration in anuran amphibians at the postembrionic stage. VI. Lens removal experiments in Rana Graeca Tadpoles

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

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Embriologia e morfogenesi. — *The problem of lens regeneration in anuran amphibians at the postembrionic stage.* VI. *Lens removal experiments in* Rana Graeca *Tadpoles.* Nota di LUIGI BOSCO, SERGIO FILONI, CARLA CIONI e SERGIO BERNARDINI, presentata (*) dal Socio A. STEFANELLI.

ABSTRACT. — 50 larvae of *Rana graeca* at larval stage corresponding to 49-50 of *Xenopus laevis* were lensectomised.

In all cases examined the lens did not regenerate and there were no appreciable modifications in the cornea. In most cases, a detachment of epithelial laminae of the iris accompanied by a partial depigmentation was present.

KEY WORDS: Anura; Rigeneration; Lens.

RIASSUNTO. — *Il problema della rigenerazione della lente negli Anfibi Anuri negli stadi post-embrionali.* VI. *Esperienze di asportazione dello lente in larve di Rana graeca.* 50 larve di *Rana graeca* ad uno stadio di sviluppo corrispondente allo stadio 49-50 (sec. Nieuwkoop e Faber, 1956) di *Xenopus laevis*, sono state sottoposte a lentectomia. In tutti i casi esaminati la lente non rigenerava, e non sono state osservate modificazioni istologiche apprezzabili a carico della cornea esterna. In molti casi era possibile osservare il distacco delle lamine epiteliali iridee accompagnato da una parziale depigmentazione.

INTRODUCTION

Owing to intensive investigations on lens regenerating from iris in the Urodeles *Salamandridae*, cytologic and macromolecular events in the iris epithelial cells and tissue interactions involved during the regenerative process have been quite well established (Reyer, 1977; Yamada, 1977).

On the contrary the degree of knowledge on lens regeneration anura is limited. The data reveal that the adult anuran does not regenerate the lens, but the evidence collected by various Authors in larvae from different species is conflicting (Reyer, 1954; Scheib, 1965). Most of the Authors who have

(*) Nella seduta del 29 novembre 1986.

described cases of positive regeneration claim that it occurs through approaches of Wolffian regeneration.

Nevertheless, other Authors maintain that the positive cases cannot be interpreted as indicative of the capacity of the iris to regenerate a lens, but of incomplete lens removal and lens reorganization from lens fragments left *in situ* (Filoni *et al.*, 1976; 1977 *a*).

The only anuran tadpole in which lens regeneration has been fully demonstrated is *Xenopus laevis*, a species in which regeneration occurs at the expense of the corneal epithelium (Freeman, 1963).

The experimental analysis effected in this species has given a sufficiently clear picture of morphogenetic processes and tissue interaction during lens regeneration (Filoni *et al.*, 1979; 1980; 1983; Bosco *et al.*, 1979; 1981; 1985; Cioni *et al.*, 1982).

For some time, contemporaneously to casual analysis of lens regeneration in larval *Xenopus laevis*, we have been studying the lens forming capacity of various species of Anura using the same operating techinique for all species examined and guaranteeing total removal of lens. The species studied up to now are *Rana esculenta*, *Rana dalmatina*, *Discoglossus pictus*, *Hyla arborea*, *Bufo viridis* (Filoni *et al.*, 1976; 1977 *b*; Cioni *et al.*, 1979; Bosco *et al.*, 1983 *a*; 1983 *b*).

Material and methods

This research was carried out on larvae of *Rana graeca* lensectomized at stage corresponding to 49-50 of *Xenopus laevis* (according to Nieuwkoop and Faber, 1956), 50 operations were transferred to 10% Holtfreter solution and therafter gradually returned to dechlorinated tap water. Fifty operated larvae were fixed in Bouin's liquid in groups of 5, 3-5-7-10-15-20-25-30-45 days after the operation, 5 larvae were fixed immediately afterwards to serve as controls. After embedding in paraffin, the tadpoles were cut into 7 μm cross sections. The serial sections were stained either with hematoxylin-eosin or with Mallory-Azan's method.

Results

3 days after operation. The operated eye was slightly reduced in volume, and ematic elements were present in the vitreous chamber; the outer cornea was not thickened or it was so very slightly (Tav. I, fig. 1). The inner cornea was re-establishing its continuity. The entire edge of the iris manifested a partial depigmentation; at the level of the dorsal iris a thickening and detachment of the two epithelial laminae were observed (Tav. I, fig. 1). The ventral edge of the iris gave rise to vescicular expansions (Tav. I, fig. 1).

5 days after operation. The operated eye was still volumetrically reduced. The outer cornea did not show appreciable histological modifications. The dorsal edge of the iris was partially depigmented and there was a slight cleavage between the two epithelial laminae. The ventral edge of the iris showed a higher degree of reactivity than the dorsal edge, and it was possible to observe some depigmentation and thickening (Tav. I, fig. 2).

7 days after operation. The outer cornea was almost normal; the vitreous chamber, even if volumetrically reduced, had a greater volume than manifested on preceding days. The dorsal iris showed the same histological modifications observed previously. In all cases examined at the level of the ventral edge of the iris vescicular expansions slightly depigmented were observed (Tav. I, figs. 3-4).

10 days after operation. In one out of the 5 cases examined the operated eye was partially degenerated. In all other cases a thickening of the entire edge of the iris with a various degree of depigmentation was observed.

15 days after operation. The dorsal and ventral edges of the iris were partially depigmented, and the two epithelial laminae were detached in various degrees. It was also possible to observe a thickening of the choroid and of iris stroma (Tav. II, fig. 5).

20-25-30-45 days after operations. 20 days after operation the dorsal and ventral edges of the iris showed the same histological modifications observed on preceding days; vescicular enlargements which strongly reduced the pupillary hole up to this point obliterated it completely (Tav. II, fig. 6). These modifications were also evidenced in the following days and the histological picture remained unchanged (Tav. II, figs. 7-8).

DISCUSSION

This research revealed that larvae of *Rana graeca* show no lens-regenerating capacity at developmental stages corresponding to these at which *Xenopus laevis* tadpoles exhibit the highest lens regenerating capacity (Freeman, 1963; Campbell and Jones 1968; Waggoner 1973). Histological examination of the eye territories which in larval *Xenopus laevis* and *Triturus* have lens-forming competence (respectively outer cornea and dorsal iris) revealed that there were no appreciable structural changes in the cornea of operated eye; in many cases iris edge underwent a detachment between the two epithelial laminae; furthermore this cleavage was almost always accompanied by a partial depigmentation and thickening. These iris modifications correspond to the initial phases of Wolffian lens regeneration from dorsal iris, in larvae and adults of *Triturus* (Yamada 1977) however, in larvae of *Rana graeca* these structural changes in-

volve both dorsal and ventral iris and do not undergo further evolution until the end of the experiment. It is know that in *Triturus* these histological modifications of the dorsal iris are associated with nuclear activation of iris epithelial cells (IEC) as evidenced from progressive numerical and volumetrical increases of nucleoli with a preferential increase of granular component. These structural alterations of nuclei and nucleoli are immediately followed by the entrance of the IEC in active proliferation. While dividing they free themselves gradually from melanosomes (depigmentation). During multiplication and depigmentation phases, at the larval of pupillary edge of the two epithelial laminae detach each other and gradually form a lens vesicle *Yamada*, 1977.

On the basis of these data it is necessary to determine whether the histological modifications of the iris we observed in larvae of *Rana graeca*, as those previously observed in lensectomised larvae of *Bufo viridis* (Bosco *et al.*, 1983 *b*), really correspond to the Wolffian regeneration phases or if they depend on aspecific histological modifications due to lensectomy. This could be verified by studyng the ultrastructural modifications of nuclei and nucleoli of iris epithelial cells after lensectomy.

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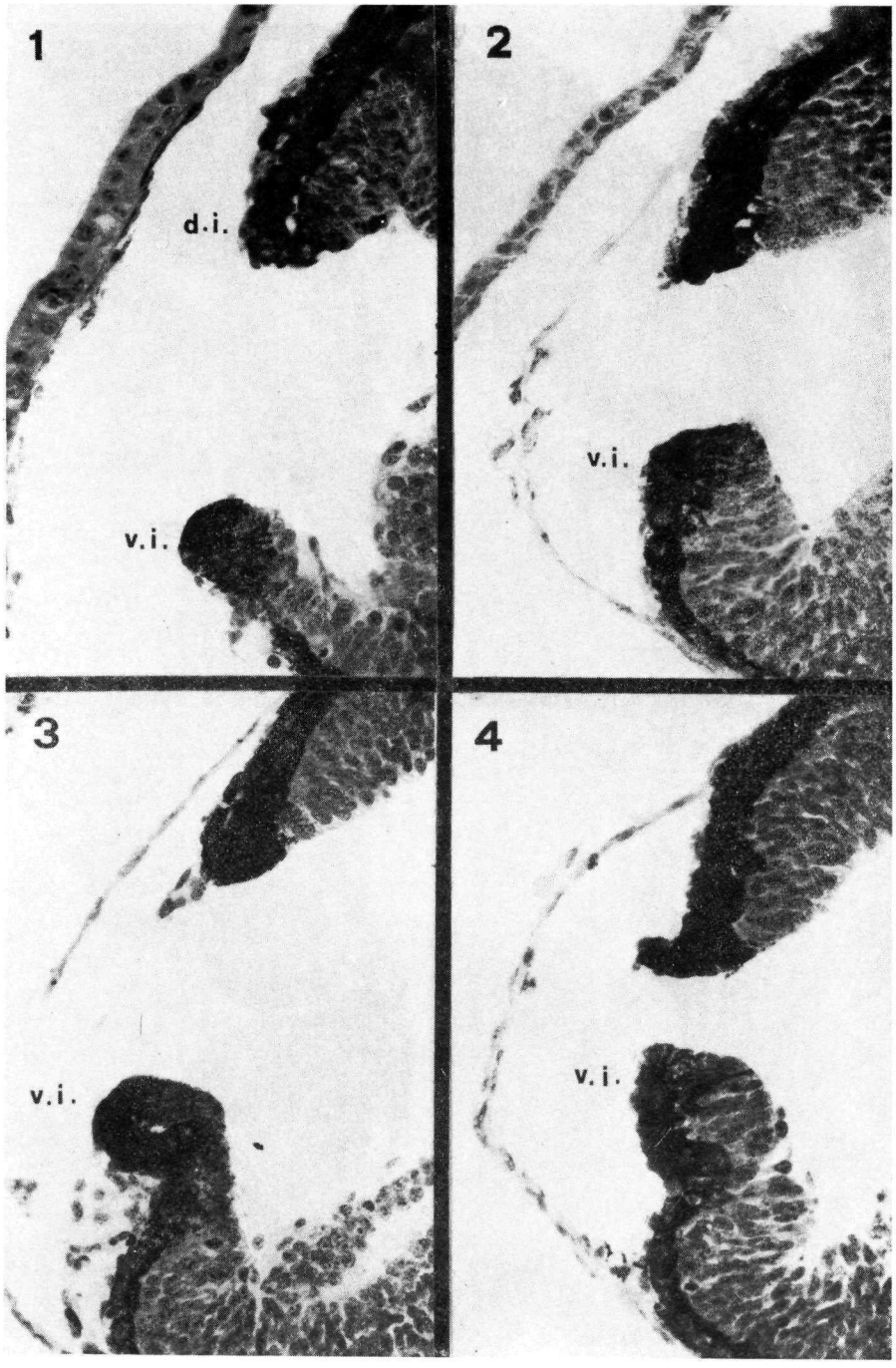

Fig. 1. – 3 days after operations: The dorsal iris (d.i.) shows a thickening and detachment of the two epithelial laminae; the ventral iris (v.i.) give rise to vescicular expansions (240 ×). Fig. 2. – 5 days after operation: At the level of the ventral iris (v.i.) it is possible to observe some depigmentation and thickening. (240 ×). Figg. 3-4. – 7 days after operation: The ventral iris (v.i.) gives rise to vescicular enlargements slightly depigmented. (240 ×).

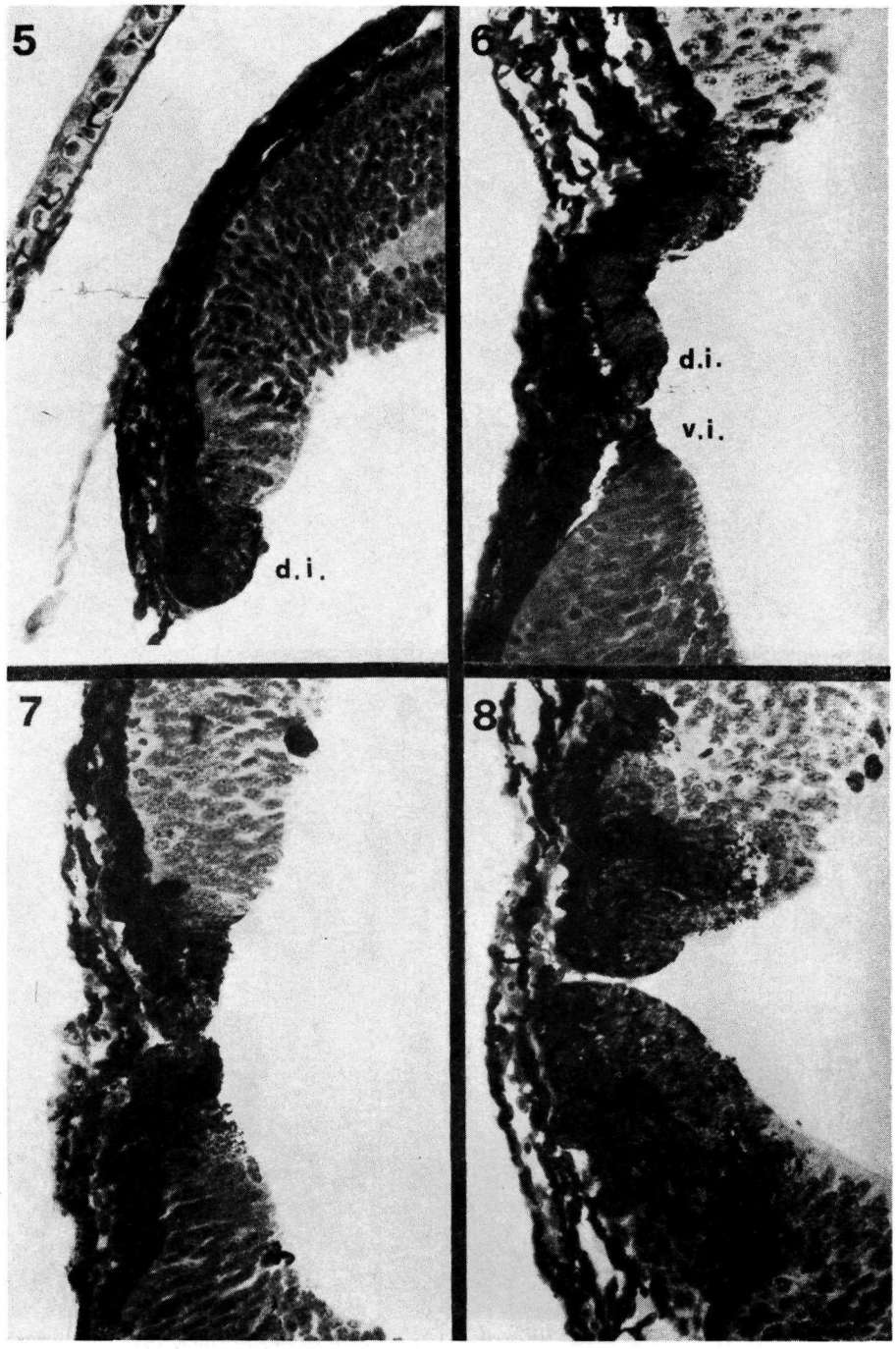

Fig. 5. – 15 days after operation: At the level of the dorsal iris (d.i.) it is possible to observe a thickening of the iris stroma. (240 ×). Fig. 6. – 20 days after operation: The pupillary hole is obliterated by vescicular enlargements of the dorsal (d.i.) and ventral (v.i.) iris. (240 ×). Figg. 7-8. – 30 and 45 days after operation: The modifications of the entire edge of the iris are the same observed 20 days after operation. (240 ×).