rabbit heart[15]. The lack of effect of a low concentration (17 mM) of ethanol in the present study is in agreement with that found by others[14,15]. The effect of propylene glycol on NA release has not been investigated to our knowledge.

It is concluded that ethanol is suited to aid the dissolution of e.g. corticosterone in PSS when the latter drug is used as an uptake-2 inhibitor in ^{3}H-NA release studies. Propylene glycol, on the other hand, is not suitable for this purpose, since it enhances the passive ^{3}H-efflux from vascular adrenergic neurones preloaded with ^{3}H-NA.

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Lens regeneration from cornea in tail ectopic eyes of Xenopus laevis tadpoles

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Summary. Evidence was found that cornea in tail ectopic eyes of Xenopus tadpoles has the capacity to regenerate a new lens.

It has long been known that the dorsal region of the iris of certain larval and adult urodeles has the capacity to regenerate a lens should the existing one be removed from the eye[2]. In Xenopus tadpoles, however, lens removal is followed by regeneration of a new lens from the inner layer of the outer cornea[3]. Lentoids have also been reported to form in cornea of Xenopus tadpoles cultured in vitro in the absence of other eye structures[4], but in contrast, cornea transplanted to the tail fin apparently does not form a lens[5]. In the latter studies[5], cornea transplanted to the anterior chamber of lentectomized eyes, or to the limb bud blastema of Xenopus tadpoles, sometimes did form a lens, implying that in the tail there is either inhibition or absence of stimulation. We were interested to know whether or not lens regeneration would take place from cornea arising from tail ectoderm in eyes formed in tadpole tails as a result of prior optic vesicle transplantation to the tail bud.

Optic vesicle transplants were carried out essentially after the method described in Billett and Wild[6]. Nieuwkoop and Faber stage 24/25 embryos were placed in sterile 10% Holtfreter's solution, pH 8.0, and the pigmented epithelium overlying and adjacent to the optic vesicle was completely removed using tungsten needles. The optic vesicle was then excised by means of a fine hair loop and vitally stained in 1:200,000 solution of Nile Blue Sulphate in 10% Holtfreter's solution; this was to make it visible in subsequent transplantation procedures. Stage 28/29 embryos (tail bud stages) were used as recipients of grafts. Following anaesthesia in a 1:3000 solution of MS222 in 10% Holtfreter's solution, a small incision was made in the tail bud with a tungsten needle and a tunnel excavated such that its roof consisted of tail bud ectoderm. Stained grafts were then inserted keeping the orientation the same as in the normal forming eye, i.e. presumptive retina apposed to ectoderm. After grafts had healed in, embryos were transferred to 250 ml finger bowls containing 10% Holtfreter's solution where they remained for 24 h. They were then transferred to aged tap water and reared to stage 49/50.

Direct examination of ectopic eyes under a dissecting microscope indicated that cornea, as well as a lens and eye cup, had developed in 22 out of 100 successful transplants. Examples of such eyes are shown in figures 1 and 2. Confirmation of the presence of cornea comprised of an inner and outer layer (as in the normal tadpole eye) was obtained by subsequent histological analysis in 2 selected cases (figure 3). Lentectomy was performed on the remaining 20 stage 49/50 anaesthetized tadpoles. A small semi-circular cut was made through the outer cornea along

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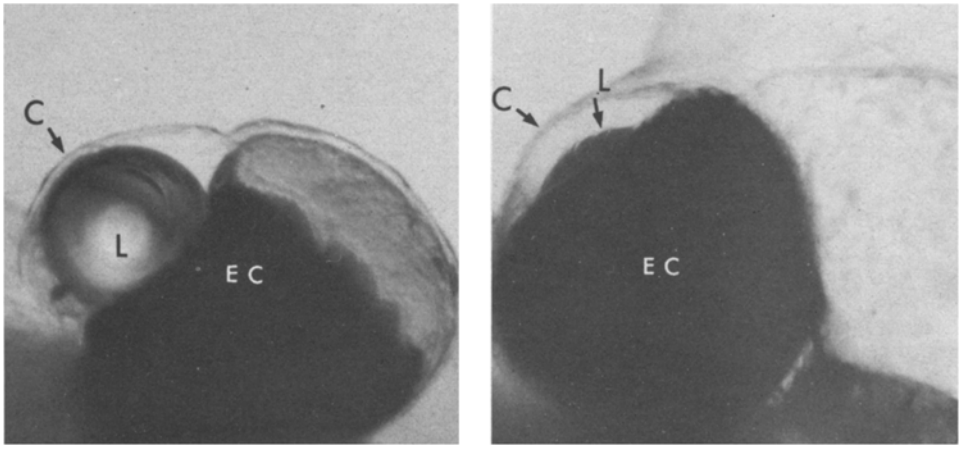

Fig. 1 and 2. Examples of ectopic eyes produced in tadpole tails showing cornea (C) and lens (L) differentiation. E. C., eye cup. ×60.

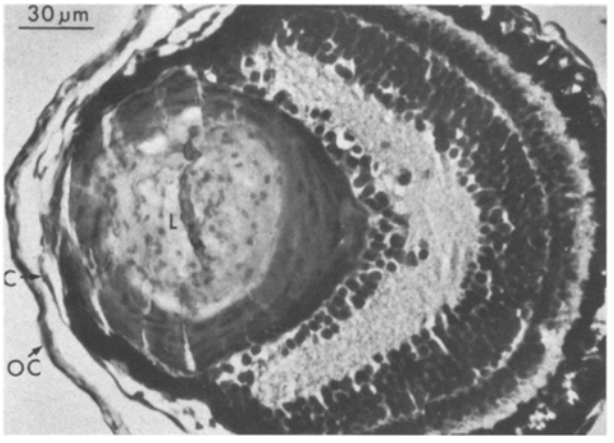

Fig. 3. T. S. of the ectopic eye shown in figure 1. Both inner (I.C.) and outer (O.C.) cornea can be seen to be present. L, lens. (Stained with haematoxylin and eosin.)

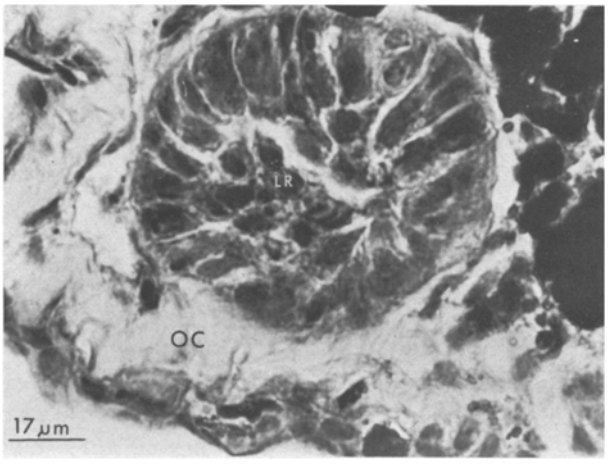

Fig. 4. T. S. of an ectopic eye 7 days after lentectomy showing an early stage 4 lens regenerate (L. R.) attached to the inner layer of the outer cornea (O.C.). (Stained with haematoxylin and eosin.)

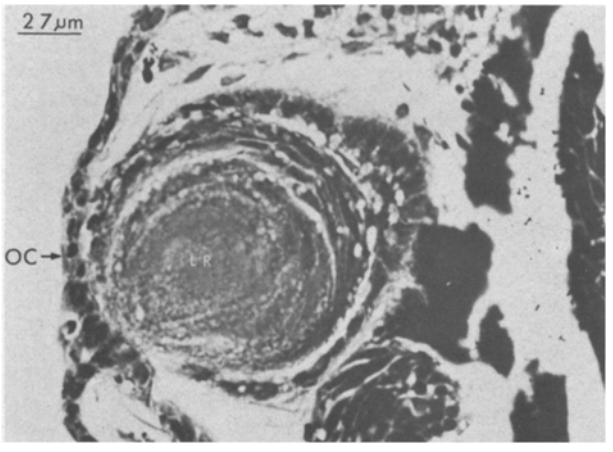

Fig. 5. T. S. of an ectopic eye 14 days after lentectomy showing a middle stage 5 lens regenerate (L. R.) still attached to the inner layer of the outer cornea (O.C.). (Stained with haematoxylin and eosin.)

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the ventral edge of the eye cup close to the corneal-epidermal junction, and the outer cornea was reflected dorsally. Pressure applied with forceps to the back of the eye then forced the lens through an incision, made in the inner cornea. Lentectomy was carried out in this way in both the ectopic eye and a normal eye of the same tadpole. In some cases, due to the eye being smaller in the tail and absence of the sclera making the eye more delicate, excessive damage occurred to the eye cup or the lens was incompletely removed; these animals were therefore excluded from the results. The operation was, however, judged to be successful in 11 tadpoles and these were subsequently sacrificed on days 3, 7, 10 and 14 after lentectomy.

One of the tadpoles sacrificed on day 7 was found to have regenerated an early stage 4 lens (staging according to Freeman[3]) in the ectopic eye (figure 4). The inner layer of the outer cornea appeared to be continuous with the developing lens (a condition similar to that found in the lentectomized eye of a normal tadpole) indicating that it was from here that the lens had originated. Enlargement of nucleus and nucleolus was also evident in the cells furthest from the cornea, this being the primary stage of lens fibre formation and characteristic of early stage 4 lens regeneration. In 5 animals sacrificed on day 14, middle stage 5 lens regenerates were found. Secondary fibre formation from the equatorial zone was evident and in the case shown in figure 5, the lens still remained attached to the inner layer of the outer cornea. The animals sacrificed on days 3 and 10 showed no evidence of lens regeneration.

These results show that lenses can regenerate from cornea of lentectomized tail ectopic eyes. In not regenerating a lens in every case, cornea in ectopic eyes is no different from that in normal tadpole eyes where in the present study 57% lens regeneration was found. Reyer[7] has reported that regeneration of a lens from iris tissue in tail ectopic eyes of Triturus tadpoles can occur after lentectomy. However, in Reyer's experiments optic vesicles with overlying presumptive lens ectoderm were transplanted and regeneration was from tissue of graft origin. Our results demonstrate that tissue which originates not from the graft, but from tail ectoderm, also has the capacity to regenerate a lens.

Lens and cornea in normal tadpole eyes are derived from head ectoderm which has not only come under the influence of the optic vesicle, but also of the archenteron wall. Non-neural tissue underlying the presumptive epidermis during neuralation has been shown by Jacobson[8] to also be of some significance in the induction of lens. Indeed, it has been suggested that the phenomenon of free-lens formation exhibited by certain Anurans including Xenopus[9], can be attributed to these non-neural tissues[10]. Evidence presented by Jacobson showed that the non-neural tissue effect on presumptive lens epidermis was not specific to head mesoderm; thus flank mesoderm could induce lens in presumptive lens epidermis. Clearly this effect is dependent on some property inherent in presumptive lens epidermis since neither head mesoderm nor flank mesoderm induced free lens formation in tail ectoderm[8]. The present study shows however, that optic vesicles, together with any mesoderm that may have inadvertantly been transferred, have the capacity not only to confer on 'unprimed' tail ectoderm the ability to form a lens, but to regenerate a lens as well. Furthermore, the presence of other eye structures can clearly offset any inhibitory influence(s) or lack of stimulation associated with tail tissues that have been ascribed to this region by corneal transplants[5].