ment of the transplant, and thereby regulate the rate of injection. Fourthly, the glass capillary used for making glass needle is of very fine caliber (0.8 mm outer and 0.6 mm inner diameters), and it causes least amount of physical damage to the host brain. And, it being of glass there is no danger of its inner wall rusting over time.

3. The use of the stereotaxic apparatus aids in transplantation of neural tissues in exactly defined structures of the host brain according to the stereotaxic coordinates. This in turn facilitates subsequent manipulations of the fully grown transplants, such as making electrolytic lesions in the transplants or injecting chemicals into them for the study of their connectivity, with a high degree of precision.

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2 G.D. Das, T.I.T.J. Life Sci. *4*, 93 (1974).
3 G.D. Das, B.H. Hallas and K.G. Das, Experientia *35*, 143 (1979).
4 B. Flerko' and J. Szentagothai, Acta endocr. *26*, 121 (1957).
5 P.G. Smelik, in: Neurohormones and neurohumors, p.277. Ed. J. Ariens Kappers. Springer, Wien-New York 1969.
6 U. Stenevi, A. Bjorklund and N.-A. Svendgaard, Brain Res. *114*, 1 (1976).
7 J.F.R. Konig and R.A. Klippel, The rat brain, a stereotaxic atlas. Williams and Wilkins, Baltimore 1963.
8 L.J. Pellegrino, A.S. Pellegrino and A.J. Cushman, A stereotaxic atlas of the rat brain. Plenum Press, New York 1979.

Lens regeneration from the dorsal iris in *Eurycea bislineata*, the two-lined salamander[1]

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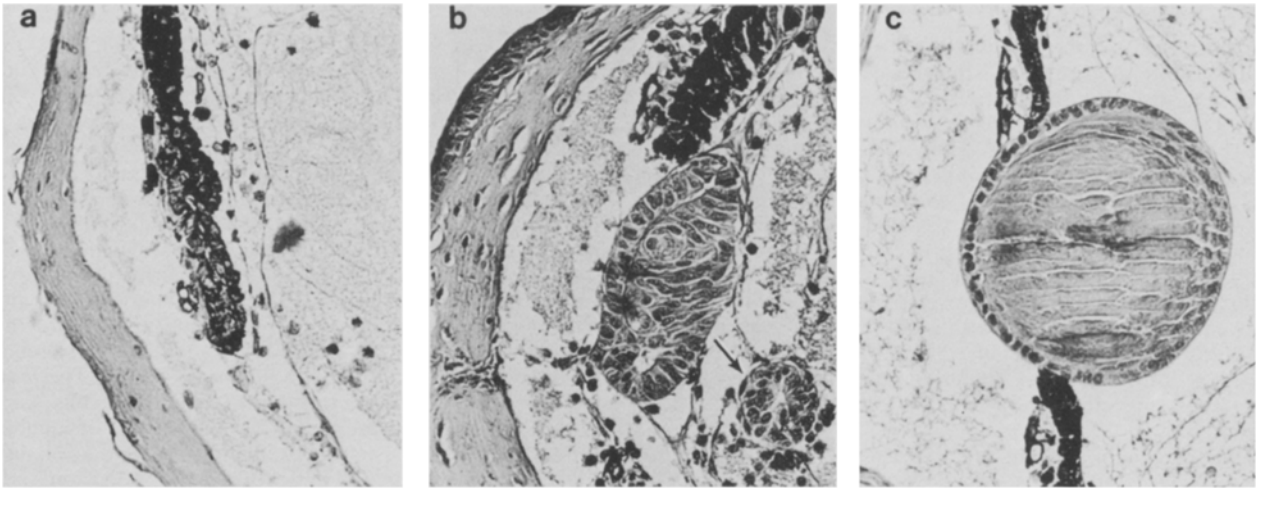
Summary. Following lens removal from the eye of adult *Eurycea bislineata*, the northern (USA) 2-lined salamander, it was found that this salamander has the capacity for lens regeneration. Its widespread distribution and high percentage of regenerative success suggests it as a suitable organism for the study of this differentiative phenomenon.

Eurycea bislineata, the northern 2-lined salamander, is a member of the family *Plethodontidae* (lungless salamanders) and native to the northeastern United States. Stone[3] reported that one species of the genus *Eurycea (E. lucifuga)* can regenerate the lens of the eye after lentectomy, while another in the same genus *(E. longicauda melanopleura)* cannot; both inhabit the twilight zone of caves. In order to determine the ability of *E. bislineata*, a much more common organism, to regenerate a lens (from the dorsal iris), histological examination, of eyes fixed at varying periods after lentectomy, was undertaken.

Adult salamanders were collected in Montgomery County, Pennsylvania, anesthetized using 1:2000 ethyl-m-aminobenzoate methane sulfonate (Tricaine, Sigma), and the lenses removed with the aid of a binocular dissecting microscope. A naso-temporal incision was first made in the cornea with a scapel. After the cut was completed, the open tips of a pair of watchmaker's forceps were pressed gently against the eye perpendicular to the incision. This pressure forced the lens out through the slit cornea. Only those animals whose lens was removed intact were utilized. The salamanders were then kept in a moist environment at 21 ± 1 °C until they were sacrificed at 2 weeks, 3 weeks, an 1 month post-lentectomy. The eyes were isolated and fixed in Bouin's fixative, then prepared for histological observation.

Morphological staging of lens regeneration as described by Yamada[4] was used to characterize the regenerated lenses seen in 10 µm serial sections. Briefly, these stages recognize initial depigmentation of cells along the margin of the



Representative lens regeneration stages of *E. bislineata* (hematoxylin and eosin; ×278): *a* stage III/IV - depigmentation of the dorsal iris epithelium and initiation of vesicle formation; *b* stage VIII, with 2° fibers beginning to form - the connection of the lens regenerate to the dorsal iris is not evident in this section (arrow indicates portion of tortuous neural retina); *c* stage XI - the fully-regenerated lens has detached from the dorsal iris and fills the pupillary space.

dorsal iris, followed by the formation of a lens vesicle, differentiation of primary and secondary lens fibers and detachment of the new lens from the iris[4-6]. Lens regeneration thus mimics in large part embryonic lens development as seen in other salamanders[7], the major difference being the prolonged attachment of the lens regenerate to the dorsal iris.

The 2-week specimens showed early stages (II to IV) of lens vesicle (i.e., with a cavity) formation (fig. a). By the 3rd week after lentectomy, lens regeneration was much more evident (fig. b) ranging from stage VI (1° fiber formation-to form the lens core) to stage VIII (2° fiber formation-to form the peripheral lens fibers). At 1 month a stage XI lens, with histogenesis complete (1° and 2° fibers with a monolayered anterior epithelium) and detached from the dorsal iris (fig. c), was found. The regenerated lens, albeit smaller at this time, is histologically identical to the original lens.

Of a total of 16 eyes examined, 14 showed evidence of lens regeneration. The 2 eyes that failed to regenerate a normal lens were in poor histological condition, showing evidence of hemorrhage and disorganization of the retina due to surgical trauma. The importance of the intact retina in lens regeneration in situ has been well-documented[4-6]. However, several other specimens with retina judged to be in fair or poor condition, i.e., folded or detached, did show evidence of lens regeneration. It should be noted that the stages of lens development noted in these damaged eyes were delayed, in comparison to those in eyes of good to excellent condition.

The capacity for lens regeneration from the iris is limited to some urodeles; in fact, a large number of salamanders have yet to be studied for their lens regeneration capability[3]. All species of *Triturus, Diemyctilus, Cynopus,* and *Notophthalmus* examined (at various times, reported as synonymous genera in the literature), are known to have this ability and *N. viridescens,* the spotted newt, is prototypical. In addition, species of different genera which can regenerate a lens include *Salamandra s. salamandra, Salamandra perspicillata, Pleurodeles waltlii, Typhlotriton speleus,* and *Eurycea lucifuga*[3]. The genus *Eurycea* is of particular interest because it demonstrates that closely-related species have different capacities for lens regeneration.

E. bislineata and *E. lucifuga* are capable of regenerating their lenses, while *E. longicauda melanopleura* is not. In future studies to elucidate the causal mechanism(s) of inhibition or release of the ability for lens regeneration, it may be helpful to determine how widespread this phenomenon is among salamanders, as well as anatomical differences, if any, that may exist in the eyes of different salamanders, to account for this. In addition, the discovery of the capacity for lens regeneration in a salamander so common and widely-distributed, may provide investigators with an organism to rival the utility of *N. viridescens* as the subject of choice for lens regeneration studies.

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2 To whom all correspondence should be addressed.
3 L.S. Stone, J. exp. Zool. *164,* 87 (1967).
4 T. Yamada, Curr. Top. devl Biol. *2,* 247 (1967).
5 R.W. Reyer, Q. Rev. Biol. *29,* 1 (1954).
6 T. Yamada, Monographs in Developmental Biology, vol. 13. Ed. A. Wolsky. Karger, Basel 1977.
7 D.S. McDevitt and S.K. Brahma, Devl Biol. *84,* 449 (1981).

Effects of zinc and lithium ions on the strengthening cell adhesion in sea urchin blastulae

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Summary. Cellular adhesion in sea urchin blastulae, normal and vegetalized by treatment with lithium ions, strengthened as development proceeded. This tendency was arrested in the embryos animalized by treatment with zinc ions.

It is known that the ectoderm of sea urchin plutei is easily dissociated into single cells but the archenteron is relatively hard to dissociate in Ca-free sea water[2], in 1 M glycine - 2 mM EDTA[3], or in 0.44 M sucrose - 1 mM EDTA - 10 mM Tris-HCl, pH 8.0[4]. Hence there is thought to be a difference in the tightness of cellular adhesion between the ectoderm and endoderm of the embryos. A quantitative approach, however, has not yet been attempted to determine the tightness of adhesion in embryos during differentiation into endoderm. In the present work we have modified the media for dissociation of sea urchin embryos[5,6] and examined the change of cellular adhesion of blastulae in 3 groups: untreated, vegetalized by Li ions[7], and animalized by Zn ions[8], in order to determine a difference in the tightness of cellular adhesion between the ectoderm and endoderm.

The sea urchin used in this experiment was *Anthocidaris crassispina.* Eggs and sperm were obtained by stimulating their ejection with a few ml of 0.5 M KCl pipetted into the body cavity, and the embryos to be treated with Li or Zn ions were transferred to sea water containing 16.5 mM LiCl or 1 mM $ZnCl_2$, respectively, and cultured for 8 h. The embryos were collected by centrifugation (150×g for 5 min) at 14 h (about 1 h after hatching) and 22 h (about 1 h before gastrulation) after insemination, then they were washed once with Ca-free sea water, and were suspended in the mixture of 1 M glycine - 2 mM EDTA and Ca, Mg-free sea water (1:1). The suspensions were incubated at 20 °C while being stirred gently with a glass blade. Aliquots, taken up at scheduled times, were transferred onto a hematocytometer plate and the total number of single cells and multicellular fragments dissociated from the embryo was counted. The dissociation ratio was determined as the percentage of the number of dissociated units against the total number which was obtained by completely dissociating the embryos in aliquots by extensive stirring. The total cell number was constant and the dissociated cells were moving actively with their cilia during the time of incubation in the mixture. The rate of increase in the dissociation ratio reflects the reduction in tightness of the adhesion.

When the blastulae were incubated in the mixture, cells having the shape of epidermal cells became spherical, outlines of the embryos started to break into multicellular fragments and single cells within 30 min, and the total