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

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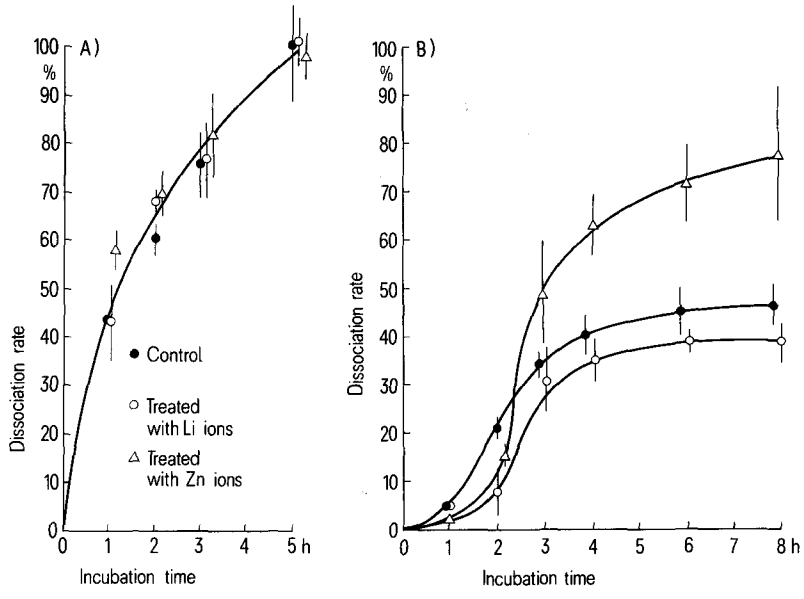
Summary. Cellular adhesion in sea urchin blastulae, normal and vegetalized by treatment with lithium ions, strengthened as development proceeded. This tendency was arrested in the embryos animalized by treatment with zinc ions.

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



Increase in the dissociation ratio of the blastulae of the sea urchin *Anthocidaris crassipina*. The embryos, cultured for 14 h (A) and 22 h (B) at 20 °C, were suspended in the mixture of 1 M glycine - 2 mM EDTA and Ca, Mg-free sea water (1:1): control (●), Li-treated (○), and Zn-treated (△). Each plot shows the average for triplicate counts. Vertical bar indicates SEM Student's t-test was applied to the difference between each treated group and the control at 8 h of incubation. For the Zn-treated group, $p < 0.01$; for the Li-treated group, $p < 0.02$.

number of these dissociated units increased gradually. Figures A and B show the increase in the dissociation ratio of 3 groups of the blastulae at 14 h and 22 h after insemination, respectively. The rate of increase in the dissociation ratio of the normal blastulae at 22 h after insemination was remarkably lower than that of the blastulae at 14 h after insemination, indicating that cellular adhesion of the blastulae becomes tight as a whole as development proceeds. This tendency was also observed in the vegetalized blastulae. At 22 h after insemination, the rate of increase in the dissociation ratio of the vegetalized blastulae was a little lower than that in the normal blastulae (fig. B). This difference means that the cellular adhesion in the vegetalized blastulae was somewhat tighter than that in the normal blastulae, which could be ascribed to the proportion of the endodermal cells to ectodermal cells being higher in the vegetalized blastulae than in the normal ones. In the animalized blastulae, however, the tendency of strengthening cellular adhesion was not remarkable compared with the normal and vegetalized blastulae, suggesting that Zn ions caused significant inhibition of the strengthening cell adhesion which is a prerequisite for gastrulation, since both the normal and vegetalized blastulae could form archenteron but the animalized ones remained permanently in the blastula stage at the time which gastrulation was expected to occur.

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Contradictory differential staining results with Coomassie Brilliant Blue and silver carbonate on sister chromatids[1]

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Summary. Coomassie Brilliant Blue, and silver stain, are used in electrophoretic gels to identify polypeptides. The relative staining intensity has been taken as indicating a quantitative difference between proteins. However, when these 2 stains were applied to 2 identically pretreated chromosome preparations, contradictory staining results were obtained.

Whereas Coomassie Brilliant Blue is regularly used by biochemists to identify polypeptides in electrophoretic gels and to determine protein concentration in solutions[2-4], a silver stain has long been used in many histological and cytological studies. Recently, by using silver to stain polypeptides in electrophoretic gels, a 100-fold increase in sensitivity has been achieved over Coomassie Brilliant Blue, and the method has also been used for quantitative evaluation[5-10]. In this communication we report a result showing that Coomassie Brilliant Blue and silver carbonate can give entirely different staining results on the same biological material.

Chinese hamster ovary cells were grown in an incubator at 37 °C 5% CO_2, in McCoy's 5a medium supplemented with 15% fetal calf serum, 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 0.03% L-glutamine. 5-Bromodeoxyuridine (BrdUrd, Sigma, final concentration 50 µM) was added to the culture for 24 h (about 2 cycles). Colcemid (final concentration 2×10^{-7} M) was added during the final 3 h of incubation. Chromosomes were prepared by the air-