Acid treatment and incubation in 2 × SSC with or without $Ba(OH)_2$ (*i* and *j* in the table) have produced some limited band formations. Without $Ba(OH)_2$ treatment (*i* in the table), faint bands have been observed (figure, *d*). In contrast, with the retention of $Ba(OH)_2$ treatment (*j* in the table) dark bands have been obtained (figure, *e*). However, such treatment conditions do not reveal all the bands that have been observed through the method *h*. The use of pectinase and cellulase with few drops of HCl as pretreatment before $Ba(OH)_2$ treatment (*k* in the table), has resulted into appearence of bands in the centromeric and terminal regions of the chromosomes (figure, *f*). Band formations in the interstitial regions of the chromosomes have been extremely poor. Use of the treatment conditions indicated as *h* in the table, has been extended for band formations to somatic chromosomes of Allium sativum and Zea mays. Chromosomes of these plant materials have also exhibited similar banding characteristics.

It is known that the acid-fixed, air-dried chromosomal preparations are left with some histones[9,11]. Treatment with $Ba(OH)_2$ followed with incubation in 2 × SSC at 60°C is considered relatively weak in comparison with NaOH treatment[6] for causing loss of protein or DNA. The treatment conditions containing in method *h* of the table, might have affected superficial DNA-protein associations sufficiently to alter the Giemsa staining reaction, but not significantly to alter the DNA content or packing, as might be expected from vigorous treatments with NaOH, trypsin, urea and $KMnO_4$. It appears that a significant factor which apparently determines band formation is connected with acid treatment. At least, that acid treatment plays a vital role has been documented by Brown et al.[13] in case of G-banding in mammalian chromosomes. Both the methodologies adopted by Schweizer[17] and Gill and Kimber[18] with regard to banding in plant chromosomes contained acid treatment in some or other form. It is known that HCl treatment causes loss of histone from the chromatin material completely[12,19] or differentially for some fractions of histone[9,20]. The characteristic disappearence of G-bands after introduction of HCl treatment in our preparations and the intensity differences observed in the C-bands with and without HCl treatment indicate that histones perhaps play a role in Giemsa band formation.

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Hypophysial hormones and a G_1 block in the lens epithelium of the adult frog (Rana pipiens)

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Summary. Mechanical injury in itself may be responsible for the transition of lens epithelial cells from the G_0 to the G_1 compartment of the cell cycle. This traverse which does not depend on DNA-dependent hypophysial hormones may be in part reversible.

A seasonal variation in mitosis in the anuran lens epithelium has been observed[1,2]. It was suggested that hormones may be influencing this mitotic fluctuation[3]. Hypophysectomy abolishes this seasonal variation in mitosis[4]. Even though injury-induced hyperplasia is characteristic of the lens of the intact frog, mitosis and DNA synthesis fail to occur in response to physical insult in the hypophysectomized frog. Incorporation of ^3H actinomycin D was found to increase in nuclei of lens epithelial cells from hypophysectomized-injured frogs as compared to hypophysectomized-non-injured animals. However, the highest incorporation was found in intact-injured animals. DNA synthesis occurred first near the central mechanical injury in the lens epithelium of hypophysectomized frogs given replacement therapy (hypophysial hormones); but DNA synthesis occurred first in the outer germinative zone in the lens epithelium of similar hypophysectomized frogs given replacement therapy but not given mechanical injuries. It was suggested that lens epithelial cells may proceed through part of the cell cycle without hypophysial hormones[5]. The present report documents further evidence suggesting that mechanical injury by itself may stimulate cells to proceed from G_0 to G_1 and that this transition may be partly reversible.

Materials and methods. Rana pipiens were obtained from Lake Champlain Frog Company, Alburg, Vermont or Ward's Natural Science Establishment, Inc., Rochester, New York. All frogs were maintained in 2 inches of water at 24 ± 2°C. Hypophysectomy was accomplished following a procedure of Hogben[6]. Only the adenohypophysis was removed. Sham operations were performed by drilling through the parasphenoid bone just anterior to the gland. In both cases cerebrospinal fluid was lost. 3 weeks were allotted before experiments were performed to allow levels of hormones to decrease. Mechanical injury was accomplished using a 0-gauge insect pin according to a previously mentioned method[7]. Frogs were anesthesized using Tricaine methanesulfonate (Ayerst Laboratories). The pin was inserted through the cornea and pierced the capsule, epithelium and cortex of the central anterior region of the lens. Lenses were dissected free from the globe and placed in ^3H-thymidine (s.a. 6.4 from New England Nucleur) at a concentration of 5 μCi/ml. The bathing solution consisted of the radioactive label added to Earle's salt solution (Grand Island Biological Company) adjusted to 280 mOsm. Incubation was

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for 1 h with subsequent fixation in Carnoy's. Whole-mounts were prepared following Howard's[8] method as modified by Rothstein[9]. Kodak NTB-3 emulsion was used for autoradiography with an exposure time of 3 weeks. All staining was done with Harris hematoxylin. Replacement therapy was accomplished by injecting i.p. 0.5 ml of a pituitary homogenate consisting of one adult adenohypophysis in 1 ml physiological saline.

Results. DNA synthesis in lens epithelium from intact animals normally occurs in the germinative zone only (figure 1, group 1, 126 and 132 h). Epithelial cells from intact frogs which have been pin-injured normally elicit DNA synthesis 42 h post-injury throughout the entire lens epithelium (group 1, 138 h). Hypophysectomized organisms had the right lens pin-injured at 0 h while at 96 h the left lens was pin-injured with replacement therapy given at this later time (group 2). If the cells in the right lens had progressed through part of the G_1 compartment of the cell cycle, they might be expected to synthesize DNA before those cells in the left lens epithelium. Results show that DNA synthesis begins in the right lens at 132 h (36 h after initiation of replacement therapy), whereas DNA synthesis in the left lens does not begin until 186 h (90 h after initiation of replacement therapy). Before this DNA synthesis (in both right and left lenses) there is a central migration of epithelial cells toward the injury site. However, pre-injury cell density is not restablished before the onset of this central DNA synthesis.

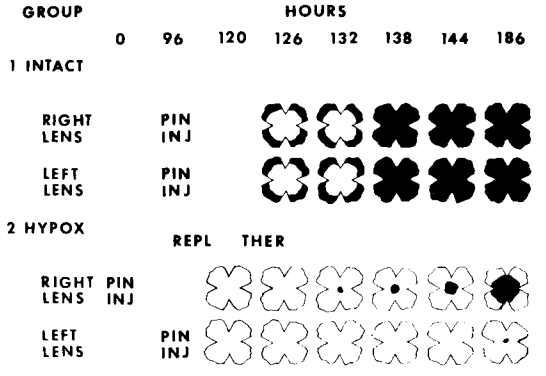

Fig. 1. Effects of replacement therapy on a long term and short term injury of lenses from intact and hypophysectomized (HYPOX) frogs. The star shaped figures represent lens epithelial whole-mounts. The shaded areas represent areas with DNA synthesis. 6 animals were used for each data point. See text for further discussion.

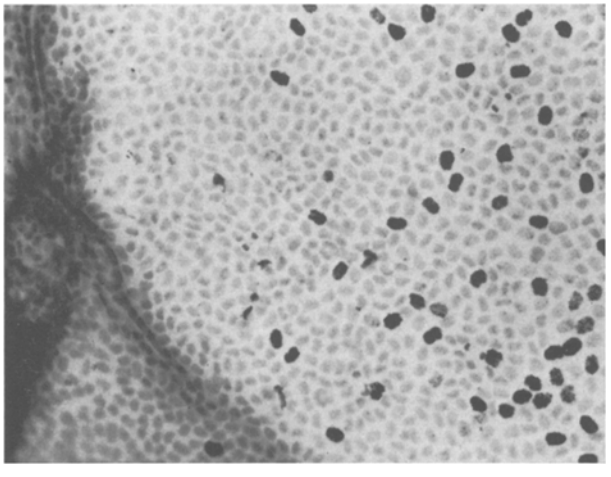

Fig. 2. DNA synthesis in a lens epithelium from a hypophysectomized frog given replacement therapy commencing 9 days after pin injury. Note the increase in cell density at the wound site (left) with the absence of DNA synthesis in this area. Incorporation and mitotic figures are located distally from the wound area. Hematoxylin 80 X.

Previous studies had shown that the injury area in lenses of hypophysectomized frogs eventually filled in with an abnormally high cell density 2 weeks after pin injury. This occurred at the expense of the middle area between the central injury and the outer germinative zone. This middle area had the lowest cell density at 2 weeks after injury in contrast to the injury locus which had the lowest cell density immediately following the injury. If cells near the pin injury remained in the advanced G_1 state then DNA synthesis should first occur here. If the cells had gone from the advanced G_1 state back to the earlier G_0 or early G_1 condition, then DNA synthesis might not initially occur immediately proximal to the injury focus. Hypophysectomized frogs were pin-injured with replacement therapy beginning 9 days later. The results are illustrated in figure 2. DNA synthesis occurred first neither in the germinative zone nor at the injured area. Instead DNA synthesis occurred in the cell sparsest region between the injury and germinative zone.

Discussion. Present evidence suggests that mechanical injury in itself (without the aid of hormones) may cause cells to traverse from G_0 to G_1 in the hyperplastic response in the lens epithelium of the adult frog. Further experiments suggest that this transition may be in part reversible. This premise does not indicate that hypophysial hormones are not affecting G_0 lens epithelial cells. DNA synthesis occurs at 186 h in figure 1, group 2, left lens; but DNA synthesis occurs at 138 h in figure 1, group 1, right and left lenses. In both cases pin injury was accomplished at 96 h and either exogenous or endogenous hypophysial hormones were present at this time. This difference of 38 h may suggest that there is a lag time so that inactive hypophysial hormones may be converted to active forms. The fact that an intracammeral injection of hypophysial hormones does not elicit DNA synthesis when used as replacement therapy in figure 1, group 2 lends support to this idea. However, lens epithelial cells from hypophysectomized frogs may be qualitatively different than lens epithelial cells from intact frogs and this may account for part of this 38-h-delay. Ultrastructural studies are now underway to answer this and other questions concerning the role of hypophysial hormones and wound hyperplasia in the lens epithelium of the adult frog.

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