the objective (Nikon UVF 10 objective, covered with Saran Wrap for water immersion use) and the preference for penetrating the cells at a 45° angle to the plane of the slice. In the inverted configuration (Nikon UVF-20 objective) the electrode was left in the cells and we monitored electrical activity during optical measurements. Generally, optical resolution was reduced in the inverted microscope as a result of light scattering in the tissue, because injected cells were not usually near the optical surface. However, in the few injected cells that were located near the bottom of the slice, optical resolution improved. With the exception of Fig. 3, A and B, all figures in this report were made from images acquired on the upright microscope. Both microscopes were fitted with cooled charge-coupled device (CCD) cameras (Photometrics, model 220 on the UEM, model 80 on the IM35) with computer-controlled data acquisition. The $[Ca^{2+}]_I$ was determined from images at 340- and 380-nm excitation by using the ratio method (*5*). Details of data acquisition, background subtraction, calibration, and other experimental considerations have been given elsewhere [J. A. Connor, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6179 (1986)]. Reported Ca^{2+} levels assumed a viscosity correction corresponding to a 30% reduction in the 340-nm/380-nm fluorescence ratio [_____, P. E. Hockberger, H. Y. Tseng, *J. Neurosci.* 7, 1384 (1987); M. Poenie, J. Alderton, R. Steinhardt, R. Y. Tsien, *Science* **233**, 886 (1986)]. CCD camera exposure times were 5 s for each fluorescent image used to produce the maps in Fig. 1, 0.9 s for the images in Fig. 2, and 250 ms for the images in Fig. 3. Frame pairs were separated by <0.5 s.

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23 June 1988; accepted 3 October 1988

Growth and Transparency in the Lens, an Epithelial Tissue, Stimulated by Pulses of PDGF

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The rat lens undergoes dramatic growth during early postnatal development. Lens weight increased by a factor of 23 in 26 days. Growth rate per day oscillated between 0 and 87 percent. A new culture system was designed to study the oscillations in growth during development. Lens growth and transparency in vitro required pulsatile delivery of platelet-derived growth factor (PDGF) in HL-1 serum-free medium. Continuous delivery of HL-1 medium with PDGF or pulsatile delivery of HL-1 medium without PDGF resulted in lens opacity and no growth. These results provide direct evidence that PDGF stimulates an epithelial tissue and that oscillations in growth occur during normal development of the rat lens.

THE RAT LENS IS AN IDEAL MODEL for studies of normal growth and development of an epithelial tissue (*1*). In the embryo, an invagination of surface ectoderm results in the formation of a lens vesicle (*2*). The lens becomes a solid cellular tissue, without vessels and nerves, when the posterior epithelial cells elongate to fill the vesicle. The anterior epithelial cells migrate laterally, elongate, and differentiate into mature lens fibers, which constitute the body of the lens. An unusual feature of the lens is that the apical surfaces of the epithelial cells face inward and attach to lens fibers (*2*). The outside of the lens is covered by a thick basement membrane, the lens capsule, that influences the elastic, nutritional, and transport properties of the lens. All nutrients for normal growth and development come from the surrounding aqueous and vitreous fluids and pass through the lens capsule.

A high concentration of protein is required if the mammalian lens is to function as a transparent, refractile optical element (*3*). The lack of blood vessels and nerves in the lens is consistent with the general classification of an epithelial tissue and is necessary for transparency. Numerous gap junctions allow intercellular transport of the ions and small molecules required for lens growth (*2*).

External factors that may regulate lens growth include insulin-like growth factor, fibroblast growth factor, and epidermal growth factor, which are thought to be present in the surrounding aqueous and vitreous humors (*4–6*). These factors have been tested in lens epithelial cell culture and may act alone (*4, 6*) or synergistically (*5*). Growth factors may be responsible for major changes in transparency, hydration, wet and dry weights, protein content, gene expression, and cell shape that occur during the first 30 days of postnatal rat lens development (*2, 7, 8*). Despite many attempts at lens organ culture under conditions that would permit the identification and isolation of regulatory factors, an ideal culture medium for normal lens development has not yet been identified (*5, 9*). Cultured lenses typically lose transparency and have lower rates of mitosis and protein synthesis than are found in vivo (*9*). No culture systems are known to be suitable for studies of the development of lens transparency. Our goal was to develop an in vitro system for studies of external factors that regulate lens growth during the development of transparency.

A systematic evaluation of changes in wet and dry weights during normal develop-

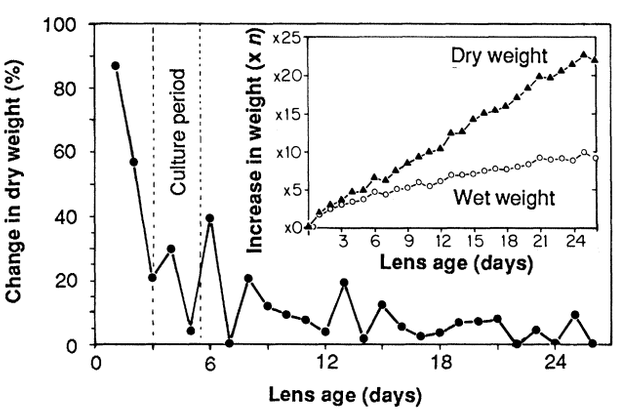

Fig. 1. In vivo lens development. Rates of increase in dry weight oscillated between 0 and 87% per day. Dry and wet weights increased by factors of 23 and 10, respectively, between birth and 26 days (inset). The *y*-axis in the inset indicates multiples of birthweight. Ten to 27 nonlittermate rats were killed, at each age. We avoided diurnal effects by conducting all experiments between 1 and 3 p.m. After enucleation, lenses were removed by posterior cuts through the retina. Wet and dry weights were measured on an electrical analytical balance (Mettler AE163). Lenses were dried at 80°C to constant weight. The time period selected for organ culture is shown by the vertical dashed lines. The percent change in dry weight was calculated by the formula $(DW_{x+1} - DW_x)/DW_x \times 100$ where DW_{x+1} is lens dry weight on day $x + 1$ and DW_x is lens dry weight on the previous day. The standard error is contained within the symbols in both plots. Instead of growing continuously (*21*), the lens showed large oscillations during neonatal development.

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ment provided the basis for the design of a lens culture system. Lens weight increases dramatically during in vivo development (Fig. 1, inset); lens dry weight increased from 0.3 to 6.97 mg, and wet weight increased from 2 to 20 mg. These in vivo values agreed with the general patterns of growth in the rat lens (*8*) and provided a detailed evaluation of growth rates per day for comparison with lens growth in culture.

Large oscillations in the rate of lens growth in vivo (Fig. 1) were observed. The oscillatory growth was consistent with reports that, following birth, lens epithelial cell proliferation and aqueous intraocular pressure display cyclic variations (*10*). The oscillations in Fig. 1 resemble oscillatory responses of glandular organs to cycling hormones (*11*). In these glandular tissues, oscillations in cellular responsiveness and growth are caused by cycling polypeptide hormones (*11, 12*); constant hormone levels inhibit the cellular responses by receptor downregulation (*13*). The in vivo data suggested that the design for a culture system must consider the oscillating growth of the lens. Aqueous humor from humans and rabbits contains platelet-derived growth factor (PDGF)–like activity (*14*) that may arise from nearby ciliary and retinal-pigmented epithelium (*15*). We seriously considered PDGF as the external growth factor for in vitro studies when we found that fetuin in HL-1 serum-free medium significantly improved lens culture conditions (*16*). Fetuin contains PDGF (*17*). It is possible that PDGF acts to stimulate oscillatory growth during lens development in vivo.

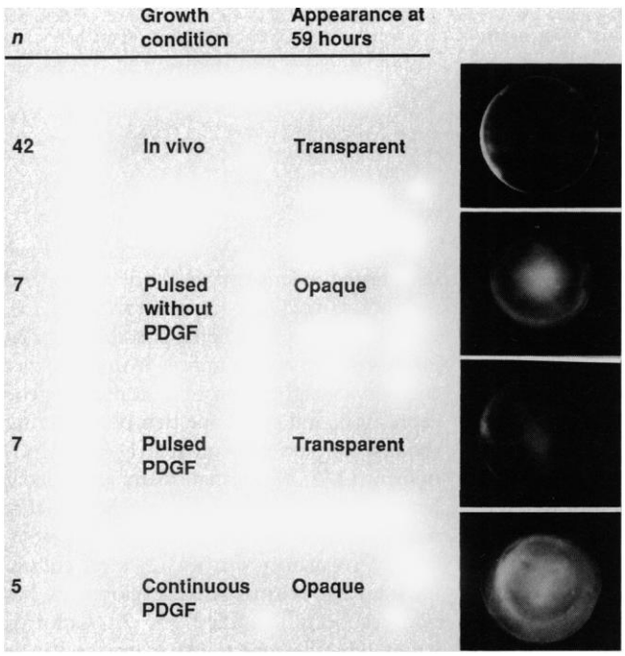

Fig. 2. Evaluation of PDGF effects on lens transparency in vitro. Lenses were cultured in an enriched HL-1 serum-free medium (*22*). Pulses of medium without and with PDGF were delivered to the lens through the perfusion chamber at a flow rate of 2.8 ml/hour for 1.25 hours every 4.75 hours in an atmosphere of 4% CO_2 and 96% air. Medium plus PDGF flowed continuously through the perfusion chamber at rates of 0.4, 0.5, 1.4, or 2.5 ml/hour. Column 1 lists the number of lenses observed under the growth conditions given in column 2. Column 3 indicates the appearance of the lens after 59 hours. Column 4 shows photographs of the lenses at 59 hours. Lenses receiving pulses of medium without PDGF remained transparent for 31 hours and became opaque by 59 hours. Continuous delivery of medium plus PDGF at all flow rates caused the lenses to become opaque by 20 to 30 hours. Pulsed delivery of PDGF produced lenses that remained transparent for the entire culture period.

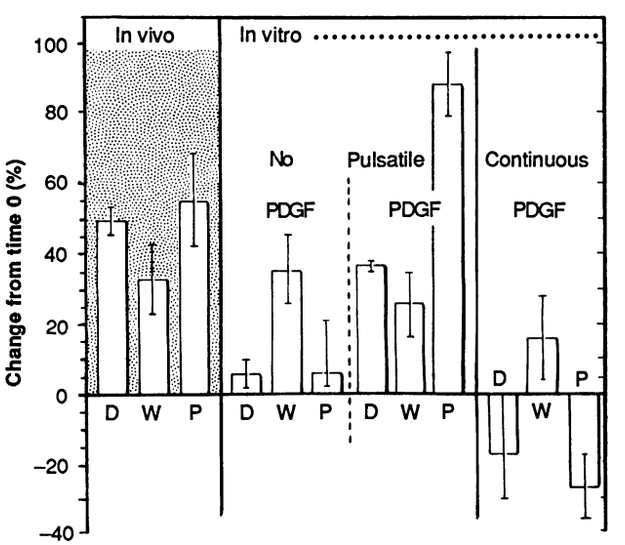

Fig. 3. Evaluation of PDGF effects on lens growth in vitro. Dry weight (D) and wet weight (W) and soluble protein concentration (P) were measured after lenses received pulsed delivery of medium without PDGF or with PDGF or with continuous delivery of PDGF. Three-day-old lenses were cultured under the same conditions as in Fig. 2 (*22*). The percent increase from time 0 to 59 hours in D, W, and P in vivo is compared to the percent increase in vitro. Standard error bars are shown. The difference between lens dry and wet weights in vivo and that with pulsatile delivery of PDGF is 12 ± 5% SEM and 7 ± 14% SEM, respectively, which is not statistically significant ($P < 0.005$). Pulsed delivery of medium plus PDGF stimulated increases in protein that were nearly normal. The percent increase in dry and wet weights were calculated by the formula given in the legend to Fig. 1. Increase in soluble protein was determined by $[(P_{59} - P_0)/P_0] \times 100$ where P_{59} is the concentration of soluble protein in a lens after 59 hours in culture and P_0 is the concentration of soluble protein in the uncultured contralateral lens at the start of the experiment. Protein concentration was determined by the Bradford assay (*23*) after lenses were lyophilized, homogenized, and resolubilized in 120 m*M* sulfate-phosphate buffer (*p*H 6.9); insoluble material was removed by ultracentrifugation.

Lenses from 3-day-old rats were selected for culturing because in vivo data indicated that major changes in growth, as defined by increased weight, occurred between day 3 and day 6. Lenses increased 74% in dry weight, 55% in wet weight, and 95% in soluble protein content during this 72-hour period. We compared the effect on transparency of pulsatile delivery with continuous delivery of PDGF during this period of very rapid growth. Lens transparency was found with pulses of PDGF; continuous delivery of PDGF produced opacity. Figure 2 presents a photographic comparison of lens transparency after 59 hours. In vivo, lenses were transparent at time 0 (postnatal day 3) and after 59 hours (postnatal day 5.5). Lenses cultured with pulses of HL-1 medium without PDGF became opaque by 59 hours, even though this medium contained insulin, a hormone that stimulates lens epithelial cell mitosis (*5*). In contrast, lenses cultured with pulses of HL-1 plus PDGF remained transparent. Lenses cultured with continuous delivery of HL-1 plus PDGF became irreversibly opaque within 20 to 30 hours.

Normal lens development was accompanied by significant increases in dry and wet weights and in soluble protein concentration. The effect of pulsatile delivery of PDGF on these parameters is shown in Fig. 3. In vivo, lenses increased 49% in dry weight and 33% in wet weight. Lenses receiving pulses of HL-1 without PDGF increased only 6% in dry weight and 36% in wet weight. In contrast, lenses receiving pulses of PDGF increased 37% and 26% in

dry and wet weight, respectively. Continuous delivery of PDGF decreased lens dry weight by 16.5% and increased wet weight by 16.5%. Transparency developed when dry weight increased faster than wet weight. A gain in wet weight without concomitant gain in dry weight has been frequently observed in cataractous lenses (*18*) and was consistent with the opacity observed in lenses receiving pulsative delivery of medium without PDGF or continuously exposed to PDGF (Fig. 2). These results demonstrate that pulsatile delivery of PDGF is necessary for lens growth and transparency in vitro.

The effect of pulsatile delivery of PDGF was even more dramatic on the soluble protein content of the lens (Fig. 3). In vivo, soluble protein concentration increased 56% after 59 hours. In vitro, an 88% increase in soluble protein resulted when lenses received pulses of PDGF. Lenses that received pulses of medium without PDGF increased only 6% in soluble protein, and lenses receiving continuous PDGF decreased 26% in soluble protein. The loss in soluble protein and the loss in dry weight in lenses receiving continuous PDGF is similar to what occurs in human cataractogenesis, in which proteins are degraded or released from damaged lens fibers (*19*). These results demonstrated the importance of pulsatile delivery as opposed to continuous delivery of nutrients to cultured lenses. Only lenses receiving pulsatile delivery of PDGF simultaneously increased in dry weight, wet weight, and soluble protein content and remained transparent after 59 hours in vitro (Figs. 2 and 3).

Our results suggest that the target cell populations and long-term biological effects of PDGF need reassessment. PDGF stimulates protein synthesis in many mesoderm-derived cells and in some glial cells (*20*). Our results show that PDGF stimulates the lens, an epithelial tissue of ectodermal origin, during development. The growth patterns and response of lens cells to circulating factors, such as PDGF, can now be evaluated under controlled conditions by means of our in vitro system. Our data suggest that PDGF or other hormones act as pulsatile regulators of lens growth and development. Both the source of PDGF and the receptors for PDGF need to be identified in vivo. Experiments are under way to characterize the activity of PDGF isoforms (AB, BB, and AA) required for normal lens growth. The lens is an excellent model for studying the factors that regulate oscillatory growth, differentiation, and gene expression.

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24. We thank E. Raines for valuable advice and support of this project and R. Blandau and L. Langley for advice and expertise in culture techniques. We also thank T. Stebbins for graphic design. Supported by grants EY-04542 and EY-07031 from the National Eye Institute and a grant from the Oculon Corporation.

21 June 1988; accepted 6 September 1988