The present experiment has demonstrated that the synaptic terminal of the photoreceptor cell is severely damaged by exposure of the retina to bright visible light and that the occurrence of the pathologic changes in the synapse is simultaneous with that of the outer segment. The first changes in the synapses, the proliferation of paramitochondrial membranes in the rod spherule and the finely branched budding of the smooth endoplasmic reticulum in the cone pedicle, occur when the lamellar membranes of the tips of the outer segment show the earliest changes. Since other membranous micro-organelles in the photoreceptor cell are not affected during the earliest stage of light exposure, it suggests that the changes in the synapses are specific. Excess photic stimulation which has been transmitted from the outer segment may cause morphologic changes in the synapses. No appreciable changes are demonstrated in the postsynaptic processes.

Although the proliferating membranes in the rod spherule form lamellar sheaths directly outside of the mitochondria, these membranes originate from the smooth endoplasmic reticulum and contain no mitochondrial enzymes. Also, with continuation of light exposure, the proliferated membranes as well as the mitochondria undergo degeneration within a short period of time. The large mitochondria in the spherule may be susceptible to photic stimulation which is transmitted to this region. Further exposure causes alteration of synaptic ribbons and vesicles but these appear to be part of general atrophy and degeneration of the spherule.

Proliferation of the smooth endoplasmic reticulum in the cone pedicle occurs in the early stage of light exposure. However, similar budding of the smooth endoplasmic reticulum is frequently observed in various pathologic conditions of the retina. The appearance of these cisternae suggests new synaptic vesicle formation.

Proliferation of membranes in the synaptic organ by light exposure may be one of the efforts for maintaining the normal synaptic functions. However, the exact function of the proliferated membranes has not been explained in this study.

Presence of cone cells in the rodent retina has been reported by several authors and the structural characteristics of the cone synapse has been reported to be similar to those of other animals. This experiment has demonstrated not only the regular distribution of the cone synapses in the normal rat retina but the difference in light-induced cytologic changes from the rod cells. Also, it has been shown that the cone cell, including the synaptic terminal is less sensitive to light. This finding may be related to the fact that the cone cells often survive in various dystrophic conditions of the retina of animals and the human. However, this does not go along with the higher susceptibility of the cone cells in experimental light and threshold laser exposure to the monkey retina.

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Morphological changes preceding insulin-induced mitosis in cultured rabbit lenses were characterized. The fine structure of these lenses was compared with that of lenses exposed to cul-
ture conditions in which a mitotic response failed to materialize. Lenses were cultured in: (1) medium KEI-4, i.e., a completely defined medium, which retains the central epithelium in a nonproliferating state, (2) KEI-4 plus insulin, and (3) KEI-4 plus insulin, theophylline, and dibutyryl adenosine 3\':5\'-cyclic monophosphate (1-DBcAMP-T). The insulin-induced mitotic response was inhibited by dibutyryl cAMP and theophylline or papaverine or 8-bromoadenosine cAMP. At one hour, lenses cultured in KEI-4 alone or in KEI-4-1-DBcAMP-T showed a moderate increase in free ribosomes relative to zero hour controls. In contrast, lenses exposed to KEI-4-insulin exhibited a pronounced increase in the number of free ribosomes and had prominent nucleoli. An increase in the degree of folding of the cell membrane was detected in all lenses at three hours but was decidedly more prominent in lenses cultured in KEI-4-insulin. At seven hours, lenses exposed to KEI-4-insulin showed an increase in the size and apparent number of mitochondria. At all intervals, ribosomes were more numerous in lenses cultured in KEI-4-insulin. Lenses cultured in medium KEI-4 alone for 24 and 52 hours displayed a morphology ostensibly similar to that of zero hour controls. At 52 hours the epithelial cells of lenses exposed to insulin, DBcAMP and theophylline were multilayered and markedly elongated. The increase in ribosomes (one hour), changes in the cell membrane (three hours), and the heightened mitochondrial profile all precede the initiation of DNA synthesis and mitosis which commenced at 24 and 40 hours, respectively.

Although hormones are known to be mitogenic relative to both the amphibian and mammalian lens epithelium, information concerning the mechanism of hormone action at the cytological level is lacking. It would seem that the mechanism which maintains the central epithelium in the nonproliferating state or which underlies the release of the cells from this state might have a basis at the ultrastructural level. This study characterizes the effect of insulin on the fine structure of the cultured rabbit lens at various intervals prior to and accompanying the onset of cell division in the central epithelium. Since agents that purportedly elevate the intracellular level of cyclic AMP prevent the insulin-induced mitotic response, the effect of some of these compounds on lens fine structure is presented.

Materials and methods. New Zealand white rabbits 8 to 10 weeks old were killed by an air embolism. Eyes were enucleated and lenses isolated. Certain lenses were fixed immediately after sacrifice in a 1 per cent OsO4 as previously described. Others were cultured in medium KEI-4 or in medium KEI-4 containing insulin plus dibutyryl adenosine 3\':5\'-cyclic monophosphate, theophylline, papaverine or 8-bromoadenosine 3\':5\'-cyclic monophosphoric acid (sodium salt) at a final concentration of 10-3M (Sigma Chemical Company, St. Louis, Mo.). Medium KEI-4, developed by Kinsey and co-investigators, was prepared as previously described. The lenses were maintained at 33.8 ± 0.2°C. The cultured lenses were either fixed in a manner identical to zero hour controls or whole mounts of the entire epithelium were prepared and analyzed for mitosis. Some tissues were prefixed in 3.25 per cent glutaraldehyde and blocks were treated with a 1 per cent solution of alpha amylase (Sigma Chemical Company). Tissue from the central anterior polar region of the lens was embedded in Epon-Araldite and sections were stained with uranyl acetate and/or lead citrate as previously described.

Results. Lenses cultured in KEI-4 alone for 52 hours exhibited a pattern of mitosis comparable to that noted in vivo. The addition of insulin to medium KEI-4 engendered a pronounced mitotic response throughout the central epithelium which commenced at 40 hours and peaked at 52 hours. If papaverine or 8-bromoadenosine 3\':5\'-cyclic monophosphate was added to KEI-4 insulin, the mitotic response normally found at 52 hours failed to materialize. Information at the ultrastructural level was obtained on tissues fixed at 0, 1, 3, 7, 16, 24, and 52 hours.

Tissues fixed at 0, 1, and 3 hours. The fine structure of lenses fixed immediately after sacrifice was essentially similar to that previously described from this and other laboratories. The epithelial cells from lenses cultured for one hour in either KEI-4 alone or KEI-4-insulin or KEI-4-insulin containing dibutyryl adenosine 3\':5\'-cyclic monophosphate and theophylline (KEI-4-1-DBcAMP-T) exhibited an overall morphology similar to that observed in lenses fixed immediately after sacrifice. Lenses cultured in KEI-4 alone or in KEI-4-1-DBcAMP-T showed a moderate increase in the number of ribosomes relative to zero hour controls. In marked contrast, lenses cultured in KEI-4-insulin (Fig. 1) showed a pronounced increase in the number of free ribosomes and exhibited prominent nucleoli. Ribosomes were stained in the presence of uranyl alone; treatment with a-amylase did not affect their integrity. Thus, the plethora of cytoplasmic particles noted at this time cannot be attributed to monoparticulate glycogen.

The basal region of the epithelium of lenses cultured in KEI-4-insulin for three hours showed an increase in the degree of folding of the cell membranes. Such cytoarchitectural changes were evident but decidedly less pronounced in lenses cultured in KEI-4 alone or in KEI-4-1-DBcAMP-T. Free and membrane-bound ribosomes were increased relative to zero hour preparations, but
were decidedly more numerous in the lenses cultured in KEI-4-insulin. 

Tissue fixed at 7 and 16 hours. Lenses cultured in KEI-4-insulin for seven hours showed an increase in the size and apparent number of mitochondria (Fig. 2). Ribosomes, both free and membrane-bound, were clearly more numerous relative to one hour and three hour preparations. Even though the epithelium of lenses exposed to KEI-4-I-DBcAMP-T did not show the pronounced increase at seven hours, a moderate increment in the apparent number and size of mitochondria was noted at 16 hours. Although the ribosomal population was elevated in lenses cultured in KEI-
Fig. 2. Photomicrograph from a lens cultured for seven hours in KEI-4-insulin. Note the pronounced ribosomal population and the prominent mitochondria (m). x39,000.

4 compared to zero hour controls, it was not as pronounced as that observed in the insulin-containing media. Moreover, the ribosomal profile was not as pronounced in lenses cultured in KEI-4-I-DBcAMP-T as in lenses exposed to KEI-4-insulin. The epithelium of lenses exposed to KEI-4-I-DBcAMP-T exhibited a slight multilayering at 16 hours. In areas in which multilayering was observed, the cells were elongated in a plane parallel with that of the overlying lens capsule.

The increase in ribosomes (one hour), changes in surface membranes folding (three hours), and the heightened mitochondrial profile (seven hours) all precede the initiation of DNA synthesis and mitosis which commences at 24 and 40 hours, respectively.
Fig. 3. Photomicrograph from a lens cultured for 24 hours in KEI-4 alone. Except for a possible slight increase in mitochondria (m) there are no notable differences between this and zero hour preparations, X10,000.

Tissue fixed at 24 and 52 hours. Lenses cultured in medium KEI-4 alone for 24 (Fig. 3) or 52 hours exhibited a morphology similar to that of one hour preparations. However, the ribosomal population did not appear to be as high at 24 or 52 hours as it was at one hour. Not only do the central epithelial cells from lenses cultured in medium KEI-4 remain amitotic, but their fine structure is ostensibly similar to that noted in the normal noncultured lens. On the other hand, lenses cultured for 24 hours in KEI-4-I-DBcAMP-T exhibited a multilayered epithelium in some locations.

At 52 hours, the epithelium from lenses cultured in KEI-4-I-DBcAMP-T was multilayered and showed a more pronounced cellular elonga-
Fig. 4. Photomicrograph from a lens cultured for 52 hours in KEI-4-I-DBcAMP-T. The epithelium is multilayered and the cells are noticeably elongated. x14,000.

tion in the plane parallel to the overlying lens capsule (Fig. 4). Lenses exposed to KEI-4-insulin continued to show abundant free and membrane-bound ribosomes. Moreover, lenses exposed solely to insulin did not exhibit the pronounced cellular elongation which was noted in lenses cultured in the presence of the nucleotide, insulin, and theophylline. Lenses exposed to KEI-4-insulin for 52 hours exhibited some regions of slight multilayering although the majority of the epithelium was retained in the monolayered condition.

Discussion. The fine structural changes brought about by insulin are similar to those noted in previous studies wherein mitosis was triggered by the addition of serum to the culture medium. Thus, insulin not only substitutes for the serum components normally required for the mitotic activation in the cultured mammalian lens, but engenders a similar sequence of events at the ultrastructural level.

The ultrastructure of lenses cultured in an insulin-containing medium exhibited several changes that were not apparent in zero hour preparations or in lenses cultured solely in medium KEI-4. At one hour the epithelia of lenses cultured in KEI-4-insulin showed a pronounced increase in the number of free ribosomes and exhibited prominent nucleoli. The heightened ribosomal profile noted at one hour is in agreement with previous biochemical studies in which an increase in the level of uridine incorporation and a concomitant elevation of the binding of tritiated actinomycin-D were detected at one and three hours in lenses cultured in KEI-4-insulin.

Mitosis induced by mechanical insult to the lens in vivo or by exposure of the cultured lens to whole rabbit serum is also preceded by a heightened ribosomal profile. The ribosomal increase appears to be part of a continuum of cellular events, reflective of a common mechanism, that precedes the initiation of DNA synthesis and mitosis irrespective of the mode of stimulation. The fact that lenses cultured in medium KEI-4 alone or in KEI-4-I-DBcAMP-T did not show a mitotic activation, and at the same time exhibited fewer ribosomes than lenses exposed to KEI-4-insulin, suggests that a certain ribosomal threshold may be needed in order to permit cells in the G1 state to re-enter the cell cycle.

In other systems, the transition of cells from a quiescent to a dividing state is accompanied by an increase in the rate of ribosomal synthesis. The increase in ribosomes is thought to represent a physiological change which is an essential prerequisite for DNA synthesis. Insulin stimulates RNA and protein synthesis and influences the formation of ribosomes and their assembly into polysomes in a variety of cells. Moreover, in chick fibroblasts insulin brings about an increase in RNA polymerase activity which is associated with the formation of all major classes of RNA.

The basal region of the epithelium of lenses cultured in KEI-4 for three hours showed an increase in the degree of folding of the cell membrane. Insulin has been shown to engender changes in cell surface to other mammalian cells. Exposure of cultured fibroblasts to the hormone leads to the appearance of microvilli and to an aggregation of ribosomes. Although cell surface changes are clearly implicated in the control of cell division, a causal relationship between perturbations in the cell surface topography and those intracellular...
events that lead to the stimulation of mitosis, e.g., a derepression of the genome, remains to be elucidated.

In the present study the central lens epithelial cells cultured in KEI-4-insulin showed an increase in the size and number of mitochondria at seven hours of culture. In this respect, the central lens epithelial cells of the insulin-treated lens exhibited a morphology similar to that noted in cells residing in the germinative zone in the living animal. Increases in mitochondria have also been detected in the traumatized lens prior to the onset of cell division.

The epithelial cells of lenses exposed to insulin, dibutyryl cAMP, and theophylline were elongated in a plane parallel to the overlying lens capsule. Theophylline and cyclic AMP also engender a morphological change in cultured newt iris cells, which mimics that detected during Wolffian lens regeneration. Moreover, explanted chick lens epithelial cells become markedly elongated if exposed to insulin. Dibutyryl cAMP stimulates cell elongation in one-day-old rat lenses and simultaneously accelerates the appearance of lens-specific proteins.

Hormones are known to influence mitosis both in the mammalian and amphibian lens. Lenticular mitosis in larval amphibians can be initiated by thyroid hormones. In addition, thyroxin and growth hormone trigger a central mitotic activation in the epithelium of the adult frog lens. Studies from Rothstein's laboratory have shown that the lens epithelium of the hypophysectomized frog become amitotic; such lenses fail to undergo mitosis even if cell-to-cell relationships are disturbed by a mechanical needle injury. Injury related cell division can be reinstalled subsequent to the administration of somatotropin or whole pituitary extract. The effect, if any, of growth-promoting hormones on the mammalian lens in situ remains to be documented.

The isolation of insulin-like substances from rabbit serum and from the aqueous humor obtained from injured or inflamed eyes, which is known to contain a potent mitogen, is the focus of current studies. The presence of such factor(s) in the aqueous may be required for wound related mitogenesis and for the restoration of tissue transparency in the traumatized or cataractous lens.

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Key words: lens epithelium, ultrastructure, rabbit, mitosis, migration, organ culture, defined medium, papaverine, cyclic AMP, ribosomes, cell surface, mitochondria.

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Xerophthalmia. ANTOINETTE PIRIE.

Some characteristics of children with xerophthalmia are described and the difference in age between those with serious corneal xerophthalmia and those showing milder conjunctival xerophthalmia is noted. The various public health measures