The lens is derived from surface ectodermal cells which lie directly over the optic vesicle from which they are separated by the basement membranes and a thin interepithelial space. Early differentiation, following induction, involves forming a compact disk of these epithelial cells in which the intercellular spaces have disappeared and their height increased. This disk or placode invaginates to form a vesicle surrounded by the basement membrane mentioned before. This becomes converted into the capsule. The cells of the posterior wall of the vesicle elongate rapidly, at this time, fusing the vesicle and forming the primary fibers. Metamorphosis of the epithelial cells to fibers, both primary and secondary, consists of the formation of low density, diffuse clouds of minute fibrils. These are thought to be composed of the specific lens proteins, alpha, beta, and gamma crystallin. As these develop, the cytoplasmic organelles become reduced in number and segregated in the tips of the cells (fibers). Specific adult lens antigens are demonstrable as soon as morphological differentiation of the lens is observable. Experiments indicating an earlier appearance of these antigens are discussed. Induction of the lens is reviewed with emphasis on concepts which require "contact" between the optic vesicle and surface ectoderm, and the transfer of substances from the eye vesicle to the presumptive lens cells.

The purpose of this review is to describe the morphological aspects of lens differentiation particularly in its earlier stages, the time of appearance of specific lens proteins, the origin of the capsule, and the differentiation of lens cells into fibers. Particular attention will be paid to changes which occur during the process of induction.

The eye has been the object of an enormous amount of basic research in embryology. In fact, much of our understanding of the process of differentiation, generally, has been derived from experimental studies on ocular development, based largely on amphibian and bird embryos. Reviews of this subject which must be cited are those of Twitty, Coulombre, and Woerdeman. The results of these studies have not always been identical in the many species used, although one may have confidence that the differences which have been observed are variations in degree, due to temporal factors or environmental conditions, such as temperature. In general it may be said that a lens can be formed from any surface ectoderm if it should come in close relation to the optic vesicle at the correct stage of development, which is shortly before anatomical differentiation occurs. The optic vesicle is then said to have "induced" the formation of a lens from the overlying surface ectoderm. The lens is not only induced to form, but the direction of fiber growth, orientation of suture lines, and its size are governed by the optic cup with which it is associated. An excellent example
of this complex relationship has been published recently, and a general discussion of this subject by Coulombre will be found elsewhere in this Symposium. The means by which all this is brought about is still, however, a matter of investigation.

The process of induction should not be regarded as a single action, but rather as a series of separate modifying steps. Some aspects of the control of cell differentiation may involve inhibitory reactions rather than direct stimulation. For example, the lens-forming ability of cells peripheral to the early lens placode is apparently inhibited by the underlying tissue immediately after induction of lens formation in the cells lying directly over the optic vesicle. Possibly the formation of cornea and conjunctiva are thus insured, and the lens-forming area circumscribed. There is general agreement, particularly with respect to lens formation, that induction involves the passage of some substance(s) from the inducing optic vesicle cells to the surface ectoderm cells which forms the lens. Ribonucleic acid (RNA) or ribonucleoprotein (RNP) complexes have been suggested as likely inducers in lens as well as in other tissues. The importance of protein as well as RNA constituents of inducing tissue was emphasized by the fact that ribonuclease treatment only reduced but did not abolish its inductive ability. Other experiments which support the idea that induction depends on inducing substances are those in which non-living cells or their products have been shown to act as living cells, i.e., induce cellular differentiation. This concept, that induction involves transfer of materials, has been enlarged by Paul Weiss, who suggests a two-step reaction. The first step is highly specific by virtue of the shape of the reacting molecules (comparable to the “key-lock” specificity of antigen-antibody reactions), in which molecules of the surface of the inducing cells produce micro-openings in the membrane of the cells acted upon, thus permitting the second step of the transfer of substances from one cell to the other. Possibly, in some cases, molecules from the surface of the optic vesicle cells may become detached and diffuse to the surface ectoderm where they would act as suggested. In any event, these concepts emphasize the importance of the closeness, if not of actual contact, between the inductor and differentiating cells, which would affect the precision and ease of transfer of the inducing substances.

In understanding the development of the lens, one should visualize the original arrangement of presumptive lens and optic vesicle as groups of cells located in a single cell layer or sheet of ectoderm prior to neural tube formation. During this process the sheet of ectoderm folds so that the basal aspects of the lens and of the neural cells (that portion directed toward the underlying mesoderm) come to lie adjacent to one another. By the time this relationship occurs, the basal aspects of both epithelia rest on a basement membrane, and the apices of the lens cells are exposed to the amniotic cavity, in higher vertebrates, whereas the apical aspect of the neural cells faces the optic vesicle cavity, which is continuous with that of the ventricles of the brain. Therefore, the bases of lens and optic vesicle cells at this stage are in apposition to each other, separated only by a narrow space. Presumably the basement membrane is a product of the epithelial cells if it is formed as in the yolk sac.

At such a stage (26 to 27 days post fertilization in man) the surface ectoderm and the future lens cells are similar in appearance, form a cuboidal epithelium (Fig. 1), and are supplied with the usual cytoplasmic components, mitochondria, Golgi complex, endoplasmic reticulum, RNP particles, and glycogen. The future lens cells are firmly attached to each other by terminal bars located at the external surface. The cells also appear to be attached to each other at their bases, although no discrete attachment bodies are observed at that point. Between the cells are large lacunae, or intercellular vesicles
Fig. 1. Section through the optic vesicles of a 25 somite human embryo of 26 days' gestation. This embryo belongs to Horizon XII of Streeter. Note that neither the retina nor the lens has differentiated from the adjacent neural or surface ectodermal epithelium. Carnegie embryo 6697.

(Fig. 2). At this stage, and earlier, the surface and neural ectoderm are easily separated from each other. The first morphological evidences of lens formation and, therefore, of induction having taken place, consist of an increase in cell height, and a reduction in the lacunae with the resulting appearance of a more compact disk-shaped area of epithelial cells. The disk of surface ectoderm (lens) cells is then no longer easily separable from the underlying neural ectoderm (optic vesicle). The appearance of compactness is not the result of cellular proliferation, although the number of future lens cells increases, but is due to a change in cell orientation and closeness of cohesion, resulting in the formation of a discrete area known as the lens disk or placode14 (Fig. 3). It is the first morphologically identifiable lens.

This is also apparently the first stage in which characteristic adult lens antigens (proteins) appear. By application of sensitive modern immunological techniques, as many as 9 to 10 distinct antigens have been demonstrated in adult lenses.15 These techniques have, therefore, been extensively applied to problems posed by the developing lens. Interpretation of the results, however, is complicated by the many technical factors which may affect the results. Among the more obvious are the concentration and purity of the antigens and antisera employed. Studies of the effect of antibodies, contained in unfractionated sera, on living cells should take into consideration the possible effect of other substances in the sera which may affect the differentiating cells. The purity of the antigens used in preparing these sera is an obviously important factor in such studies. Even though objections may be raised in some cases, the advances made by application of immunological techniques to lens embryology are most impressive, and the future possibilities even more so. This subject has been reviewed recently in an exceedingly clear, critical, and concise manner by Zwaan in...
Fig. 2. An electron micrograph of a section through the future lens and tip of the optic vesicle of a stage 11, 13 somite chick embryo. "The cuboidal cells of the lens ectoderm are seen between the amniotic space (AF) in the upper left corner and the interepithelial space (I), which contains several interepithelial clouds (C) and which extends diagonally through the middle portion of the figure. The optic vesicle lies inferior to the interepithelial space. The lens ectoderm cells contain rounded nuclei (N), mitochondria (M), and occasional intracellular vacuoles (AD) and are widely separated in their middle regions by intercellular lacunae (L). Terminal bars (T) are seen at their apaxes. The cells of the optic vesicle in the lower portion of the electron micrograph are also widely separated by intercellular lacunae (L). These cells have rounded nuclei and numerous discontinuities of the plasma membranes (A)." (From Hunt, H. H.: Devel. Biol. 3: 175, 1961, Academic Press, Inc.)

his thesis, "Immunochemical analysis of the eye lens during development,"10 from the Laboratory for Anatomy and Embryology, University of Amsterdam. This Laboratory has continuously contributed to our knowledge of lens development for many years, and has pioneered in the application of immunological methods to these problems under the direction of Professor Woerdeman, with the aid of his associates Drs. ten Cate and van Doorenmaalen.

The results of numerous investigators, working mainly with chick embryos, may be summarized by stating that adult, organ-specific lens antigens can be detected reliably as early as the lens placode stage,17-20 or in the early lens cup.16 Therefore, the specific proteins (antigens) of the adult lens appear about the time of obvious morphological differentiation, and after induction has taken place. The earliest antigen(s) to be found in chick lenses were thought to belong to the alpha crystallin group by some investigators17 and to be a beta crystallin by others.16 (See article by Rabaey in this Symposium.) Alpha crystallin was identified by Zwaan after the lens vesicle was filled with primary fibers, and a gamma crystallin only after secondary fibers were well formed (fourteenth day of incubation). Takata and associates21 found, by immunofluorescence techniques, that the first demonstrable adult lens antigens in newt lenses were located in differentiating primary lens fibers. This is in agreement with Zwaan's observation just mentioned. Precise determination of the

Fig. 3. Section through the lens placode of a human embryo of 27 days' gestation. Horizon XIII. Carnegie embryo 8119.
The morphological stage at which each of the numerous adult lens antigens appears has not been made.

Data have been presented suggesting that adult lens antigens develop earlier than indicated above, and that they are present, not only in the lens, but also in the retina, the iris, and in the body as a whole. It has been postulated that as differentiation progresses the distribution of lens antigens becomes more and more restricted. The validity of these conclusions has been questioned. Zwaan was unable to demonstrate "lenslike substances" in the rump or eyeless head, skin, cornea, or brain tissue of chick embryos. Lenslike antigens were found, however, in the iris, retina, vitreous, and aqueous humor of the adult chicken. Possibly the lens antigens diffused, post mortem, into these tissues.

Indirect evidence of the early appearance of adult lens antigens has been sought by determining the effect of antisera to adult lenses on the developing lens, assuming that an effect on lens development would indicate an antibody-antigen reaction. Possibly the best-known experiments on the effect of antiadult lens sera on the developing lens is that of Guyer and Smith. They found that immunization of rabbits with lens substance resulted in cataracts in the offspring. Many attempts have been made to confirm this finding, most of which were unsuccessful.

Langman found that culturing an eye rudiment, prior to lens formation, in a medium containing antilens sera, resulted in destruction of the lens cells. Clarke and Fowler found that addition of lens sera to the medium in which an optic vesicle was cultured prevented the induction of a lens in the surface ectoderm in contact with the optic vesicle. Additional experiments are required to demonstrate that these effects were due solely to antibodies to specific adult lens proteins. The necessity of determining the specific nature of the antibody involved is emphasized by the report of Barber who produced cataracts in the young of mothers injected with emulsions of adjuvants and brain (not ocular) tissue.

Burke and Nace and Clarke have suggested that transitory lens antigens are present during development which are not identical with those of the adult lens. The existence of these has not been properly confirmed, and the experiments on which they are based have been questioned on technical grounds by Zwaan.

It has been shown that adult lenses of widely divergent species, e.g., fishes and mammals, contain some antigens in common, plus others which are more characteristic of their group. Bon and Vyasov, quoted from Zwaan, have considered the possibility that the development of lens proteins may recapitulate their phylogenetic development. It is suggested that embryonic mammalian lenses possibly may contain more antigens in common with those of primitive vertebrates than do the adult, and that some of these "primitive" antigens disappear as the lens matures. If this concept can be demonstrated, the transitory embryonic lens antigen would be established, and would acquire an evolutionary significance.

Returning to the earliest morphologically distinct lens rudiment, one notes that the lens placode and optic vesicles are separated by their respective basement membranes plus some material lying in the space between them. This space (Fig. 4) in the developing chick eye, was found to be only 0.08 μ in width, although a larger gap was reported by Hunt and Ferris and Bagnara. Due to their glycoprotein content, the basement membranes give a strong periodic acid-Schiff reaction. Future lens and optic vesicle cells have been studied carefully with both light and electron microscopes at this critical stage in the chick and mouse by several authors. A consensus of their results indicates that these cells are well supplied with cytoplasmic structures including, in particular, RNP particles which are presumed to cause the basophilia observed in light microscopy. The cytoplasm of the optic vesicle cells is
Fig. 4. Electron micrograph of a section through a developing chick eye, stage ca. 12 hours' incubation. The epithelium of the right side of the figure is the basal surface of the lens ectoderm (LE), and the epithelium to the left side is the apical surface of the optic vesicle (OV). These epithelia are separated by the interepithelial space (I). Vesicles (AC), free cytoplasmic RNP particles (Y), and rosettes (G) are seen in the cytoplasm of the optic vesicle. Agranular membranes (E) and vesicles (AC) are visible in the lens ectoderm cytoplasm. Separated from the respective plasma membranes (X) of the optic vesicle and lens ectoderm by a space approximately 400 Å are the respective basement membranes (B) of these two epithelia. Within the interepithelial space are found filaments (S), dense particles 100 to 150 Å in diameter (AB), and a single interepithelial cloud (C) beneath which is an artifactual break in the plasma membrane (A) of the lens ectoderm. (From Hunt, H. H.: Devel. Biol. 3: 175, 1961, Academic Press, Inc.)

Basophilic and presumably contains more RNP than do the presumptive lens cells in this early stage. Electron micrographs confirm that the optic vesicle cells contain more RNP particles than do the presumptive lens cells. As the placode forms, the basophilia of the apex of the optic vesicle cells decreases rapidly and that of the lens cells increases. This was confirmed by the electron microscope studies of Hunt, who showed that the relative concentration of free RNP particles in the lens cells increased greatly concomitantly with the formation of the compact placode and a decrease of this material in the retina. Some RNP particles were found between the two basement membranes, possibly in transit from the optic vesicle to the surface epithelium. The space contained, in addition to basement membranes, "clouds" of a basement membrane-like material sometimes associated with the RNP particles (Fig. 4). Weiss and Jackson noted an accumulation of vesicles in the optic vesicle cells, possibly suggesting the secretion of materials into this interspace. According to them, many minute fibrils were often arranged across this space, as if to assist in the transport of substances from the neural to the surface ectoderm. The observations of Ferris and Bagnara were compatible with those of Hunt and of Weiss and Jackson. Although the neural and future lens cells were often close to each other, no direct cellular contact was observed which, it would seem, might be involved in induction. The cells were always separated by their basement membranes and the space between them. It has seemed reasonable to many embryologists that "induction" might involve a transfer of inducing substances to undifferentiated cells. McKeehan tested this assumption by separating the surface ectoderm and optic vesicle in chick embryos by means of a piece of impermeable cellophane membrane which prevented induction or lens formation. This did not occur when a permeable agar membrane or a Millipore filter was used, nor was there evidence that cell processes bridged the gap of these pervious filters. A more direct attempt to determine whether lens induction involved the transfer of material from optic vesicle to surface ectoderm cells was made by Sirlin and Brahma. The cytoplasm of optic vesicle cells of amphibian embryos was labeled in vivo with D-3-phenylalanine-2,4-C and subsequently brought into contact, by transplantation, with unlabeled
smelser

Fig. 5. Section through the lens cup of a human embryo of 20 days' gestation. Horizon XIV. Carnegie embryo 7394.

Fig. 6. Section through a lens vesicle at the moment of separation from the surface ectoderm. A human embryo of 33 days' gestation. Horizon XVI. Carnegie embryo 6517. Note the primary lens fibers are beginning to form from the posterior wall of the lens vesicle.

surface ectoderm in which a lens was consequently induced. Radioautographs showed the induced lens cells to be radioactive, whereas the surrounding cells, which did not form a lens, were not. The isotope which they contained was necessarily derived from the labeled optic vesicles, and could represent inductive substances which had been transferred to them.

Byers and Porter have prepared electron micrographs of the developing chick eye at this stage, and discovered "microtubules" in early lens placode cells. These were very long (ca. 30 μ), straight, and slender, 230 Å in diameter, apparently quite rigid, and their tips appeared to be embedded in the terminal web, a cytoplasmic area adjacent to the cell surface characterized by a felt-like mass of fine fibers. Fine acidophilic fibers which possibly represent the microtubules were reported earlier in light microscopic studies. The rough-surfaced endoplasmic reticulum, mitochondria, and Golgi apparatus become more concentrated in the base of the cells at this stage. These microtubules will have disappeared when the conversion of lens cells to fibers occurs.

Following its establishment, the placode rapidly becomes indented, forming first a cup (Fig. 5), and then a vesicle, which finally pinches off from the overlying surface (now corneal epithelium (ca. 33 days post fertilization in man) (Fig. 6). Nothing is really known of the factors involved in this process, although McKeehan suggests that a local contraction of the terminal-bar network may play a role. At the point of detachment of the lens vesicle, some of the cells appear to disintegrate. At the moment of separation, the vesicle consists of a single layer of cells enclosed externally by their basement membrane, on the outer surface of which mesodermal cells and the capillaries of the tunica vasculosa lentis are found. These capillaries possess their own basement membrane, which is similar in structure to that of the lens vesicle. The lens basement membrane,
which has been visible in electron micrographs since before induction, increases in thickness until it is recognized as the elastic capsule of the lens which may be considered to be a hypertrophied and specialized basement membrane. The capsule increases in thickness by the addition of successive layers of material identical, or similar, to the original basement membrane. This process appears to be intermittent, since a multilayered structure results, as shown beautifully by Jakus, Cohen, and Brini and co-workers (Fig. 7). Whether this appositional growth results from layers added internally by the lens cells is not known. Its chemical composition is very similar to that of basement membranes. The lens capsule stains as do basement membranes (periodic acid-Schiff positive), except that its outer layers, which are continuous with the zonula fibers, are metachromatic. The similarity of lens capsule and basement membrane is impressively demonstrated by the fact that they contain identical, or very similar, antigens.

When the lens vesicle closes, the cells forming its posterior half have already started to lengthen rapidly (Fig. 6) continuing to do so until the lumen of the

Fig. 7. Electron micrograph of a section of the posterior aspect of the primary lens fibers and the adjacent lens capsule (on the left) of a 5 day chick embryo. External to the capsule is a condensation of the vitreous humor. The lens fibers are composed mainly of a low-density fibrous material. In addition, some vesicular profiles and slender mitochondria are seen. These are more numerous in the anterior and posterior ends of the fibers. (Araldite embedding.) (Courtesy of Dr. A. Brini.)
vesicle is filled (Fig. 8). These constitute the primary lens fibers. Their fine structure during this stage has been described. Minute fibrils begin to form in the cytoplasm of the cells, appearing as clouds of low density material which displace other intracellular structures, the mitochondria, for example, toward the ends of these elongated cells now called fibers. The differentiating cytoplasm contains, in addition to the fibrillar component, mitochondria, dense particles (presumably RNP), and considerable rough-surfaced endoplasmic reticulum (Figs. 7, 9, 10).

The secondary fibers are formed from the epithelial cells at the equator of the lens by this same process. However, since primary lens fibers fill the vesicle lumen at this stage, the secondary fibers, of necessity, extend around them, growing between them and the overlying capsule. According to Brini, the epithelial cells at this location contain relatively fewer mitochondria. Wanko and Gavin do not comment on their number, but remark that they are small, 1 μ or less, in length. Both laboratories noted much rough-surfaced endoplasmic reticulum in these cells, which is associated with protein synthesis. A finely fibrillar material appears in the cytoplasm as a low-density material, especially in regions of cytoplasmic prolongations. As these cells, or fibers, become older, the mitochondria and the rough-surfaced endoplasmic reticulum decrease in quantity but do not disappear. The fibrillar material finally fills the cell. Resnik, Wanko, and Gavin suggest that the fibrillar material is composed of the characteristic lens protein, i.e., alpha, beta, or gamma crystallin, a conclusion similar to that of Brini and Porte in their study of galactose cataracts.

Where the tips of the secondary lens fibers, growing from other regions of the lens, come in contact with their fellows, the lens sutures are formed. These are essentially planes arranged vertically to the surface of the lens, constituting an inter-
Fig. 9. Electron micrograph of a section of the anterior end of the primary lens fibers and the adjacent epithelium of a 5 day embryo chick lens. The junction between primary lens fibers (bottom) and anterior epithelium is shown. (Araldite embedding.) (Courtesy of Dr. A. Brini.)

Face where the tips of lens fibers come in contact with each other. Interdigitations plus a few desmosomes occur between the fibers which may serve to hold them more firmly together. With completion of the first layers of the secondary fibers, the basic anatomy of the lens has been attained. This occurs in the human embryo by about the seventh week post fertilization and, following this stage, the lens continues to grow by adding layers of secondary fibers to those already formed.

Figs. 1, 3, 5, 6, and 8 are photomicrographs of significant stages in lens development in the human. The sections are of embryos in the Carnegie Institute collection at Baltimore and were studied there. They represent stages, discussed in the text, which were the subject of experimental analysis in other species.

Figs. 2 and 4 are from the paper by H. H. Hunt, to whom we are indebted and whose permission to reproduce them is acknowledged with thanks.

Figs. 7, 9, and 10 are electron micrographs prepared by Dr. A. Brini of the University of Strasbourg, France. They are similar to figures illustrating his paper referred to in the text. The author is most grateful for Dr. Brini's permission to publish these figures.
Fig. 10. Electron micrograph of the nuclear zone of primary lens fibers of a 5 day chick embryo. This area is rich in dense particles and low-density fibrous material. Some mitochondria are seen. (Araldite embedding.) (Courtesy of Dr. A. Brini.)

Addendum

Since this manuscript was written, two very pertinent articles have become available to us. Matsumoto and Kawakami found during lens induction in Triturus pyrrohogaster numerous high density particles on the plasma membranes of the optic vesicle cells and the lens ectodermal cells where they were closely opposed. Many interdigitations of these membranes were observed and numerous small vesicles in this interacting zone. Their electron micrographs led them to deduce that these were micropinocytotic vesicles serving to transfer the inducing substance. Such vesicles were located around the Golgi apparatus and it is postulated that they arise as a consequence of the fragmentation of its cisternae.


REFERENCES

35. Cohen, A. I.: Electron microscopic observa-