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Changes in Cell and Organ Shape during Early Development of the Ocular Lens

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SYNOPSIS. It is currently fashionable to attribute changes in organ shape during development to the actions of microtubules and microfilaments on individual cells of the organ in question. In the case of the eye lens it has been proposed that cellular elongation under the influence of microtubules and/or apical contraction by microfilaments are responsible for the remodeling of the originally low cuboidal ectoderm into the tall and wedge-shaped presumptive lens cells. Invagination of the lens is thought to follow automatically. These ideas cannot account for certain observations on lens morphogenesis, such as the relatively fixed diameter of the organ rudiment during early development, which is incompatible with the supposed contraction of the rudiment.

We found that the area of contact between presumptive lens and optic cup does become fixed after a few hours of "induction." There is a remarkable correlation in time between this fixation, and the process of lens cell elongation and increase in lens cell density. We calculated that the latter two can, in fact, be accounted for by population pressure caused by continued cell division within the defined area of the lens rudiment. A mathematical model along these lines was developed, which explains lens invagination on the basis of cell number and size, extent of the area of contact between ectoderm and optic cup, and cell population doubling times.

We hypothesize that the prevention of lateral cell spreading within the lens territory, after the contact area becomes fixed, is a function of the build-up in extracellular materials in this area during the "induction period." Both lens rudiment and presumptive retina contribute to this extracellular matrix.

The continuous division of the embryo into cell populations with diverging developmental pathways is very often initiated by shifts in the topography of these cell groups relative to each other; the folding of epithelial cell sheets is one of the more common mechanisms by which these changes in position are accomplished. Formation of the vertebrate central nervous system, for instance, begins with a thickening of dorsal ectoderm, which then sinks below and separates from the remainder of the ectoderm to become the neural tube. Invagination of the placodes destined to become parts of sense organs, early development of the salivary glands and thyroid, and the formation of lung bud and pancreas rudiments from the wall of the intestinal tract are but a few, relatively well-studied examples of this widespread phenomenon.

It may be instructive to start out attempts to analyze the possible mechanisms underlying these morphogenetic movements with a list of some of the features that these systems have in common. *First,* inductive interactions with neighboring tissue(s) are important, if not essential un-

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der natural conditions, in bringing about the switching in developmental pathways. For example, neural plate formation and invagination are dependent on the underlying notochord and mesenchyme, and lens formation is brought about under the influence of the optic vesicle. *Secondly,* cell division continues, and perhaps even accelerates, during the morphogenetic changes. Evidence for the last possibility is weak, because the complete analysis of cell population kinetics that is necessary to reach definitive conclusions has been done only rarely. It is quite clear, however, that cells keep on multiplying during organ rudiment formation. *Thirdly,* the change in tissue shape usually begins with a condensation of the tissue (formation of a placode, plate, or similar structure) before folding of the cell sheet takes place. These movements of the whole cell population are accompanied by changes in the shape of the cells, which constitute the organ *Anlage.* Typically, cellular configurations change from cuboidal to columnar and wedge- or flask-like.

Current thinking has been focused on the last of these three points and it is commonly accepted that organelles within individual cells generate forces, which are responsible for the restructuring of the cells, that in turn determines organ morphogenesis. The assembly of cytoplasmic microtubules, perpendicular to the surface of prospective lens cells, shortly after the beginning of lens induction may account for the transformation of cuboidal head ectoderm into the tall lens placode (Byers and Porter, 1964). Cellular elongation by itself, however, does not appear sufficient to explain invagination. McKeehan (1951) suggested that contraction of the terminal bar network, located at the apical ends of the "palisaded" lens cells, could lead to invagination. Later, ultrastructural investigations demonstrated the presence of groups of microfilaments under the apical surfaces of lens placode cells (Porte et al., 1968; Wrenn and Wessells, 1969; Pearce and Zwaan, 1970). Wrenn and Wessells (1969) implied that the microfilaments are contractile and form the major vehicle for lens invagination. Obviously, if coordinated contractions of cell apices, which are tied together by junctional complexes, occur, the width of the top of the cells diminishes relative to the width of the base, and the entire rudiment is forced to curve. Similar findings on other developmental systems have led to the widely held opinion that microfilaments and microtubules are the agents generally involved in the control of cell shape and thereby, indirectly, of organ morphogenesis (Porter, 1966; Tilney, 1968; Wessells et al., 1971).

Yet, it appears likely that the regulation of such important parameters of differentiation as cell and organ form is multifactorial in nature. Other possibilities coming to mind almost immediately are the influences that tissue interactions, (differential) cell division, and cellular adhesion may exert on the molding of cell and organ architecture. These factors have received relatively little attention in recent years, mainly because the available data, taken at face value, appear to show that their morphogenetic effect is limited, or even negligible. McKeehan (1951), for instance, has reported that there is no localized augmentation of the mitotic rate and no change of average cell volume in the lens placode area. These findings, confirmed in our laboratory (Zwaan and Pearce, 1970, 1971; Hendrix and Zwaan, unpublished), have been taken as evidence that cell division cannot be the cause of the cell crowding that characterizes placode formation. Similarly, because isolated eye rudiments develop normally in culture, it has been argued that lateral pressure or other influences exerted by neighboring ectoderm are of no importance (Byers and Porter, 1964; Wrenn and Wessells, 1969; Pearce and Zwaan, 1970). Lens formation is not dependent on direct contact with the underlying optic vesicle (McKeehan, 1958; Muthukkaruppan, 1965), which seems to lessen the possible role of tissue interaction as a morphogenetic factor. It is small wonder that investigators in this area have concluded that intracellular mechanisms must be responsible for the

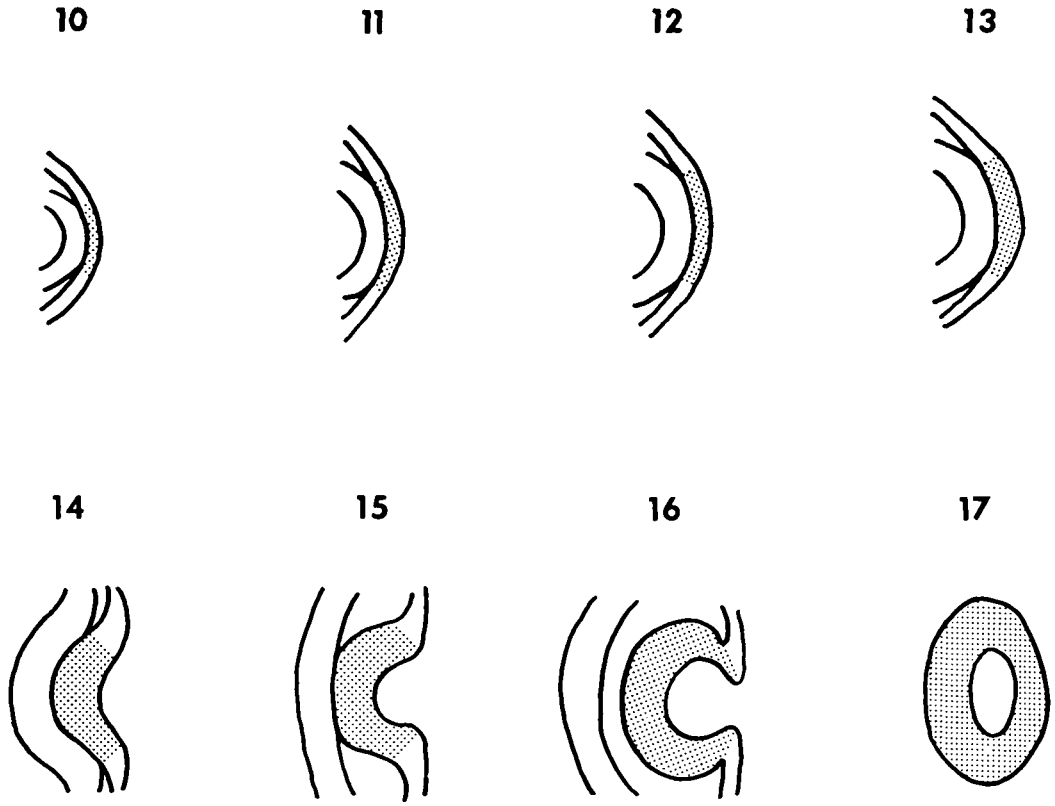

FIG. 1. Diagram of the stages of early development of the lens rudiment (shaded area) in the chicken embryo, with the Hamburger-Hamilton stages in which they usually occur. The period that we are concerned with begins in stage 10, when the tip of the outgrowing optic vesicle (unshaded; on the left) meets the lateral head ectoderm, at approximately 35 hr of incubation. It ends in stage 17, at around 58 hr, when the completed lens vesicle becomes detached from the surface ectoderm. Further details are given in the text.

initiation of lens invagination.

The outcome of recent experiments has forced us to reconsider this position, and we have now concluded that it is necessary to give serious consideration to the possibility that cell division and tissue interaction are the main agents in the determination of organ and cell shape. Here we review those data on morphogenesis of the eye lens that led us to this new hypothesis. More detailed information will be given in subsequent publications.

EARLY LENS MORPHOGENESIS

The period in which the basic architecture of the lens rudiment in the chicken embryo becomes established begins at about 35 hr of incubation and ends approximately 22 hr later. There is considerable variation of the times at which a certain level of lens development is reached. O'Rahilly and Meyer (1959) correlated eye development with the stages of embryonic development proposed by Hamburger and Hamilton (1951). Utilizing their and our own results, we calculated an average incubation time for each morphological stage. Lens rudiments of a certain stage were assumed to have reached the corresponding age, regardless of their actual incubation period.

In Figure 1 a schematic presentation is given of the stages of chick lens development. At stage 10 (35.5 hr average incubation time), the optic vesicle makes contact with the lateral head ectoderm, which at this time consists of low cuboidal cells held together by junctional complexes at

their apical poles and bordered by a fine basement lamina at the opposite poles. Intercellular spaces are very prominent. In stage 11 the structure of the lens placode shows essentially no change. In stage 12 (47 hr) the presumptive lens cells clearly are elongating. The tissue makes a more regular impression, because nuclei have become smooth and oval and are oriented with their long axis at right angles to the surface. They take up positions in the basal portions of the cells. McKeehan (1951) has coined the term "palisading" to describe this complex of changes. In stage 13 the cells reach the height of around 50μ, or five times the original value. Invagination rapidly follows in stage 14 (51.5 hr), and early lens formation is completed in stage 17 (58 hr), when the lens vesicle is freed from the surrounding head ectoderm.

We have found it convenient to define three phases of lens organogenesis. In phase I the optic vesicle has become apposed to the surface ectoderm, but the latter shows virtually no change. Phase II is characterized by palisading of the prospective lens cells, while in phase III invagination takes place.

VARIABILITY OF LENS CELL SHAPE

Many investigators have been struck by the very ordered and regular appearance of the elongated lens placode cells under the light microscope and have stressed the relevance of this observation for our understanding of the inductive interaction between the optic vesicle and the presumptive lens ectoderm (Weiss, 1950; McKeehan, 1951; Byers and Porter, 1964). This is the more true, because the structural reordering is the first sign of lens differentiation, preceding the onset of specific protein synthesis by several hours (Zwaan, 1968). Close inspection of the rudiment, however, reveals that cells can deviate considerably from their supposedly columnar shape, seemingly adapting to the shape of surrounding cells. One important reason for the irregularity in form is related to the replicative behavior of the cells.

TABLE 1. *Cell proliferation parameters for lens rudiment and head ectoderm of the chick embryo.*[a]

Parameter	Age (in hours)	Lens	Ectoderm
Generation time	36	8.8 hr	9.1 hr
	50	8.8 hr	8.9 hr
S - phase	36	4.8 hr	4.7 hr
	50	4.9 hr	5.0 hr
Mitosis	36	18 min	22 min
	50	24 min	30 min
G_1 - phase[b]	36	0	0.9 hr
	50	0	0
G_2 - phase	36	3.8 hr	3.2 hr
	50	3.8 hr	3.7 hr
Growth fraction	36	0.94	0.91
	50	1.00	0.96

[a] Based on data from Zwaan and Pearce (1970, 1971).

[b] The duration of this phase cannot be determined reliably; it is certainly very short, perhaps 10 min or less.

Earlier work (Zwaan and Pearce, 1970, 1971) has shown that the lens placode consists of a population of cells, which have a

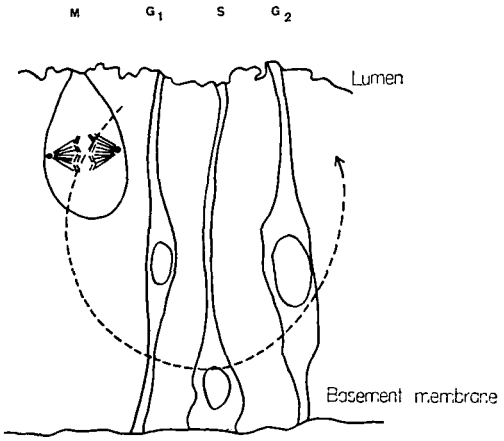

FIG. 2. Diagram of the cyclic changes in the shape of presumptive lens cells (stage 14) and in nuclear position in relation to the cell cycle. The cells, attached to each other at the lumen by junctional complexes, form a pseudostratified epithelium. During DNA synthesis (S) cells extend from the lumen to the basement membrane and their nuclei are located at the base. In the G_2 phase the nucleus migrates to the other pole of the cell and the cell itself contracts toward the lumen. Immediately after division (M) the daughter cells elongate again towards the basement membrane in the G_1 phase and the nuclei return to a basal position to initiate the next round of DNA replication.

cell cycle time of close to 9 hr, of which about 60% is taken up by the DNA synthetic phase (Table 1). These values remain the same for the bulk of the period studied here and do not differ significantly from the values for surrounding ectoderm. Furthermore, determination of the growth fraction indicates that virtually all cells are replicating. Cell loss, for practical purposes, is negligible. As is the case for many other epithelia (Sauer, 1937), prospective lens cells undergo interkinetic nuclear migration (Fig. 2), going through a cycle of elongation and contraction in relation to the cell cycle phases. Mitotic cells are rounded up next to the free surface of the ectoderm. In the G_1 phase, the daughter cells elongate towards the basement lamina and their nuclei migrate to the basal cell poles. When they have reached this position, DNA synthesis is initiated. In the G_2 phase, the process is reversed and the cells contract again towards the free surface of the tissue before entering the next division (Zwaan et al., 1969; Pearce and Zwaan, 1970).

Thus, for the largest part of the replicative cycle (during S and during portions of G_1 and G_2) the cells are elongated and "palisaded"; during M, which lasts only 20 min, they are rounded up and for the rest of the cycle their configuration is in between the two extremes. We have to conclude that, while *statistically* speaking lens placode cells are elongated, *individual* cells deviate considerably from this idealized shape.

CELL SHAPE PARAMETERS

Geometric laws dictate that the shape changes of lens placode cells during the cell cycle are accompanied by adjustments in cell volume and/or surface area. The elongation of the post-mitotic G_1 cell, for instance, can take place only if the surface area of the cell increases or its volume goes down. This seemed relevant for our study of factors involved in the regulation of cell and organ architecture. Consequently, from planimetric measurements on serial 0.5 or 1.0μ thick plastic sections, we calculated such parameters as cell and nuclear volume, cell surface area and surface/volume ratios for prospective lens cells in various developmental stages (Hendrix and Zwaan, unpublished). On the basis of their different morphology, cells could be classified according to cell cycle phase. It was found that, on the average, the volume of G_1 cells was only about one-quarter of that of mitotic cells, considerably less than expected. Their surface area, on the other hand, was half the surface of cells in division. During the S phase and G_2 phase, cell volume continuously became larger (Fig. 3). The surface area increase over the cell cycle first kept pace with this, but then lagged behind and even decreased at the end of G_2, when the cell was rounding up in preparation for mitosis. The surface to volume ratio was, for these reasons, very high in G_1 and decreased continuously throughout the cell cycle (Table 2). The cell volume loss in G_1 and the resulting high surface to volume ratio may give the cell the freedom to acquire a spindle-shape. In the G_2 phase, the opposite may occur, when the surface increase falls behind the volume increase, thus forcing the cell to become round again. Cone (1969) has described similar changes of surface to volume ratios with regard to cell cycle phase for fibroblasts in culture.

TABLE 2. *Average geometric parameters of stage 14 lens cells in relation to the cell cycle.*

Cell cycle phase	Cell surface area (μ^2)	Cell volume (μ^3)	Surface to volume ratio (μ^{-1})
M	482	884	0.55
G_1	245	213	1.14
S	408	452	0.91
G_2	500	667	0.76

It occurred to us that regulation of the amount of cell volume, apparently lost in G_1, could conceivably influence the degree of post-mitotic cell elongation. Therefore, the same parameters, calculated for cells of the invaginating lens rudiment, were determined for cells from stage 10 on. Examples of tracings of these are found in Figure 4. Much to our surprise, we found little, if any, change in cell volume or cell

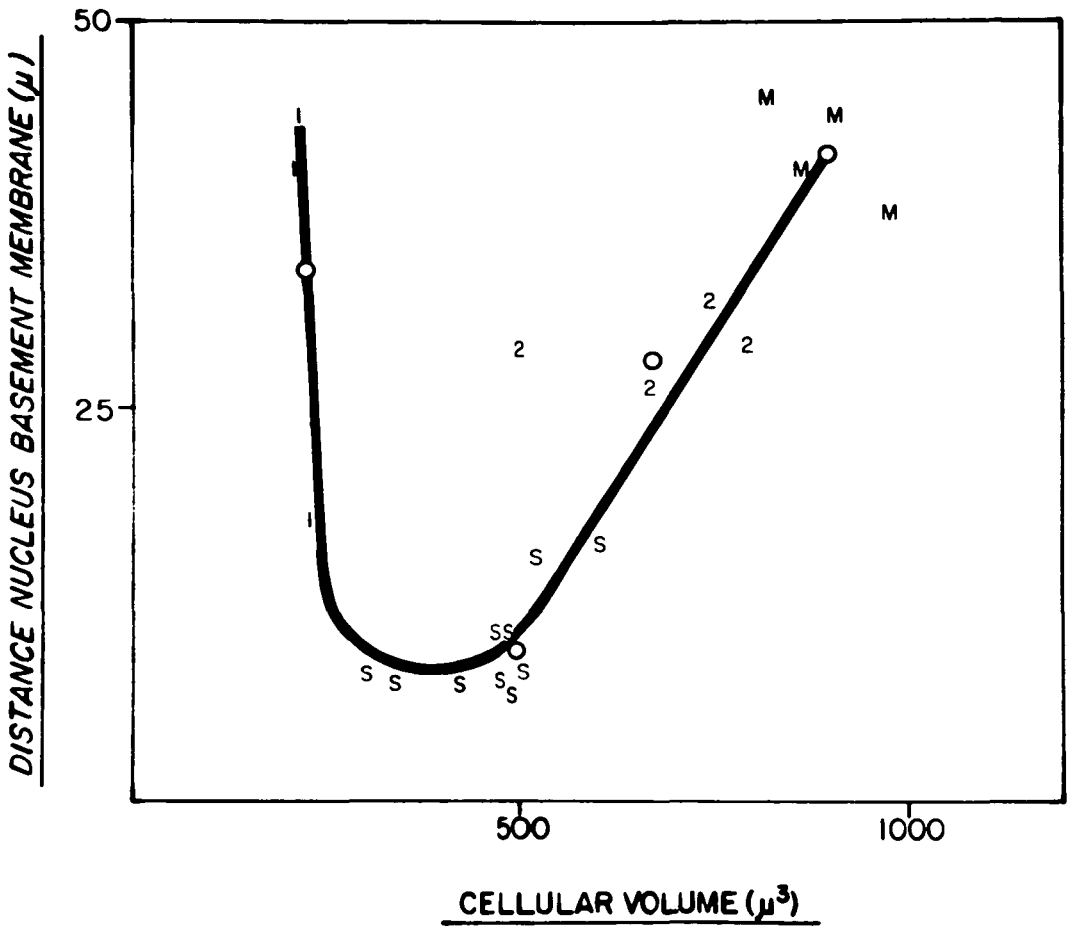

FIG. 3. Graph of the volume of individual cells of the invaginating lens rudiment in various phases of the cell cycle (1, S, 2, M for, respectively G_1, S-phase, G_2, and mitosis) against the position of their nuclei with regard to the base of the tissue. For the mitotic cells the center of the cell, rather than the nucleus, was chosen. Note that the volume of G_1 cells is considerably less than half of the volume of mitotic cells.

surface in relation to cell cycle phase from one developmental stage to the next. Apparently, the low cuboidal cell at an early stage loses as much volume in G_1 as the tall columnar cell at a later developmental stage. Closer inspection showed that the early cell seems cuboidal, but is actually highly irregular in shape. We had to abandon the idea that cell volume control as such directed cell shaping. Instead, we hypothesized that the high surface to volume ratio characteristic of the G_1 phase allows the cell to take up a variety of shapes, including the columnar one. Similarly, the diminishing of the ratio in G_2 limits the degrees of freedom and forces the cell towards a global form. Cell volume decrease in the G_1 phase *permits* the cell to elongate, but does not *instruct* it to do so. Other forces must be involved in this.

ARCHITECTURE OF THE LENS RUDIMENT

Analysis of early lens cell population kinetics (Zwaan and Pearce, 1971) indicates that the number of prospective lens cells must increase exponentially during the establishment of the lens vesicle. At the time of onset of lens induction, we counted slightly over 300 ectoderm cells in the area in contact with the optic vesicle. The population doubling time is close to 9 hr, and

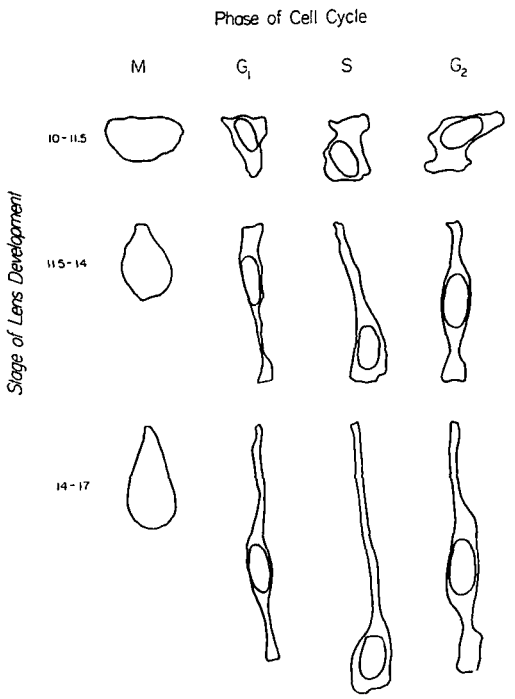

FIG. 4. Tracings of the morphology of prospective lens cells in consecutive developmental periods, as a function of cell cycle phase.

we can calculate that the organ contains almost 2000 cells when it detaches from the remainder of the surface ectoderm (Fig. 5). Actual cell counts, reported by McKeehan (1951), are very close to the theoretical curve, validating our approach.

The average volume of a lens cell remains the same from stage 10 to stage 17 (Table 3). If we neglect the possible contribution of the extracellular space to the organ volume, the latter must increase exponentially in exact parallel to the increase in cell number. Yet, the overall size of the rudiment does not appear to change dramatically.

These considerations led us to test the model depicted in Figure 6, which is based on the assumption that the lateral expansion of the lens rudiment is limited. In the first hours, after the optic vesicle has come into contact with the lateral head ectoderm, the rise in the volume of the lens *Anlage* is accommodated by circular lateral spreading of the cells. A second phase is initiated when, somehow, this spreading is inhibited. Because organ volume continues to increase, cells will be forced to elongate. Cellular elongation, in turn, obviously cannot go on ad infinitum, and therefore another shift in the distribution of the still growing volume has to take place: the cell plate buckles and invagination results. In brief, we propose that cellular replication within a confined territory may generate forces that are involved in the shaping of both whole organ rudiments and the cells constituting those rudiments. We have found it possible to build a simulated computer model that rather closely resembles actual lens organogenesis (Hendrix, Johnson, and Zwaan, unpublished) and, more to the point, have established that experimental data fit very well in the framework of our hypothesis.

The area of contact between optic vesicle and presumptive lens, as determined from measurements on serial sections, first grows gradually, then reaches a plateau, and finally declines again after invagination of the lens has begun (Fig. 7). The height of the lens cells (Fig. 8) and their packing (Fig. 9) show very little alteration as long as the contact area expands. When the latter becomes fixed, however,

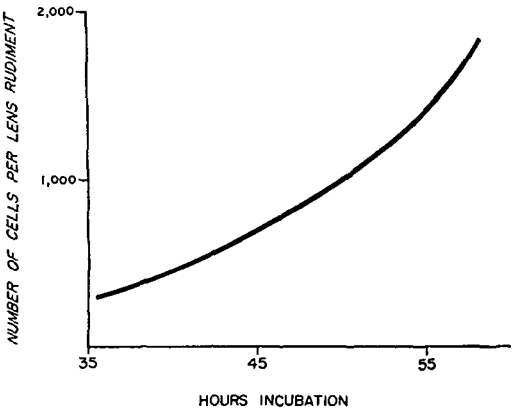

FIG. 5. Plot of the exponential increase of the number of presumptive lens cells over the period studied. Two sets of data were used to compute the curve: the initial number of cells was on the average slightly more than 300 per stage 10 rudiment and studies of cell population kinetics (Zwaan and Pearce, 1971) indicate a population doubling time of close to 9 hr. Actual cell counts (McKeehan, 1951) agree well with this theoretical curve.

TABLE 3. *Average geometric parameters for embryonic lens cells.*

Stage of development	Cellular surface area (μ^2)	Cellular volume (μ^3)	Surface/volume ratio (μ^{-1})	Intercellular space (% of organ volume)
10	376*	576	0.69*	30
14	444	546	0.84	17
17	488	585	0.87	12

* At this stage the surface of the presumptive lens cells shows numerous small irregularities, which necessarily are ignored in the light-microscopic measurements. The actual cell surface is underestimated by perhaps as much as 25%. Thus, more likely values for surface area and surface/volume ratio are, respectively, 470 and 0.81.

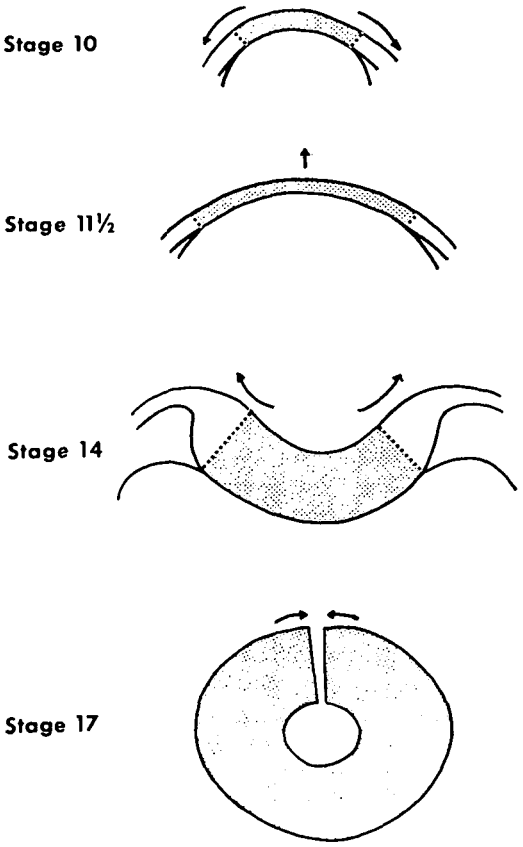

FIG. 6. Drawing of the proposed phases of lens morphogenesis. The arrows describe the directions in which the mass of the lens tissue expands to reach the next phase in development. In phase I (stages 10-11.5) the lens rudiment expands laterally in a circular fashion, with no change in cell morphology. Phase II (stages 11.5-14) is characterized by increase in cell height, together with a fixation of the lateral dimensions of the tissue. In phase III (stages 14-17) cellular elongation has reached a maximum and invagination begins. This phase ends in stage 17 (when the invagination has led to a rounded up vesicle) with the detachment of the lens rudiment from the surface ectoderm.

both of these parameters of lens cell architecture increase exponentially, exactly as predicted by the model. After the extent of the contact area diminishes again, cell height and cell density curves level off.

The acquisition of a wedge-shape by the cell still needs to be explained. As mentioned before, cells of the lens rudiment display interkinetic nuclear migration: during approximately three-quarters of the cell cycle the nucleus is located in the basal extremity of the cell. Because the nuclei are relatively large, constituting 30 to 50% of the total cell volume, their position is of major importance in the determination of cell shape. When the nucleus migrates to the cell base in G_1, a redistribution of cell mass is the unavoidable result and the cell has to become wedge-shaped. According to this line of thinking, the degree of "wedgeness" of the lens rudiment is a function of the excursion of the nucleus in relation to the replication cycle, which in turn depends on cell elongation. Figure 10 indicates that the "wedgeness" changes indeed show an inverse relation to the cell elongation curve.

Observations and theory agree well and it is not too difficult to think of further experiments, that could lend support to our ideas. For instance, Steding (1967) reported that by means of small metal rings pressed into the ectoderm he could inhibit lateral expansion of cells confined within the ring. He observed that the tissue within the restricted area exhibited changes very reminiscent of those seen during placode formation, such as increased cell density, cell elongation, and nuclear orientation. It is very intriguing that mesenchyme cells underneath the changed ec-

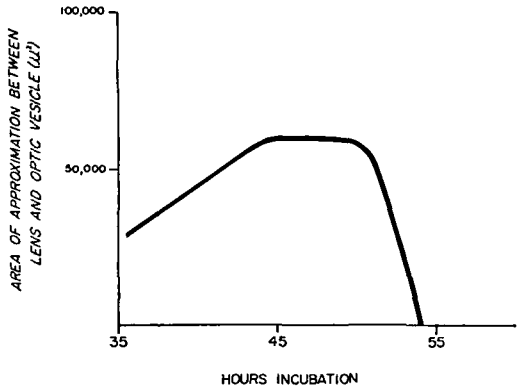

FIG. 7. The area of "contact" between lens rudiment and presumptive retina increases during phase I of lens morphogenesis, becomes stabilized during phase II, and diminishes again in phase III. In this figure (and in the following ones) actual data points have been omitted from the graph for the sake of clarity. The data will be presented in more detail in a subsequent publication (Hendrix and Zwaan, unpublished).

toderm also became condensed.

It is clear that a key element of our proposal is the inhibition of lateral rudiment expansion that indirectly leads to cell elongation. At present we can only guess at the causal factors involved, but one obvious possibility is that the extracellular matrix between lens rudiment and optic vesicle plays an important role.

THE EXTRACELLULAR MATRIX

Early electron microscopic work (Cohen, 1961; Hunt, 1961; Weiss and Jackson, 1961) made untenable the idea that lens induction is mediated by intimate cellular contact between inducing and induced tissue. Ectoderm and optic vesicle were shown to be lined by a basement membrane that remained present during the entire so-called "contact" period. As in other induction systems (Kallman and Grobstein, 1966), autoradiography after application of radioactive glucosamine shows very intense labeling of the interfacial matrix between lens and optic vesicle. It appears that both tissues contribute to the deposition of this extracellular material. It has been reported that in epitheliomesenchymal tissue, combinations of acid mucopolysaccharides and collagen are the constituents of the matrix. In the lens-optic vesicle system, however, neutral glycoprotein forms the bulk of the extracellular material.

Preliminary studies, utilizing microspectrophotometry after PAS-staining indicate a rise in the amount of glycoprotein when the contact area becomes fixed just prior

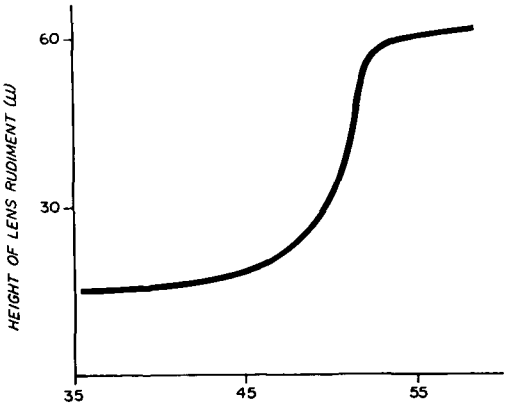

FIG. 8. The height of the lens rudiment, measured in the center, shows little change in phase I, increases dramatically in phase II and then levels off again in phase III.

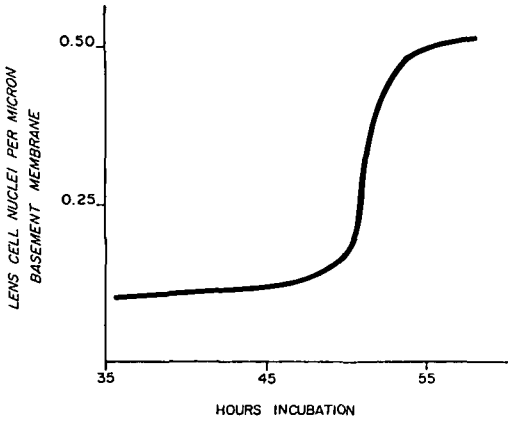

FIG. 9. The number of presumptive lens cells per standard area of basal surface of the organ, as reflected by the density of nuclei, changes during lens morphogenesis in exact parallel to the changes in cell height (Fig. 8).

to cell elongation. This could be due to increased synthesis or, an explanation that we currently favor, to restricted diffusion away from the cells as a result of the close approximation of the two producing tissues. It is suggestive to think that the in-

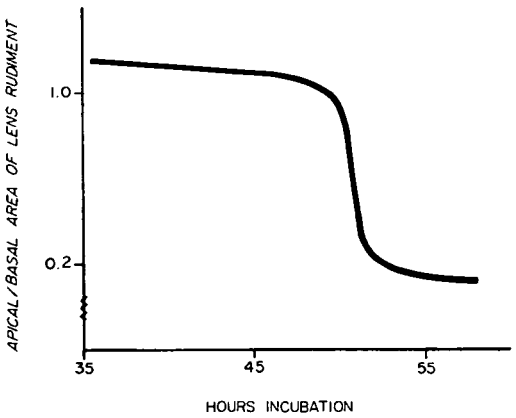

FIG. 10. The apical area of the lens rudiment is first somewhat larger than the basal area, a result of the slight curvature of the lateral surface of the embryonic head. The ratio of the two areas slowly approaches 1, then drops rapidly when invagination is initiated. The pattern is the opposite of that seen in Figures 8 and 9.

creased amount of extracellular matrix plays a role in the prevention of lateral expansion that characterizes early lens formation, but this remains to be proven.

OTHER CONSIDERATIONS

The theory of lens invagination presented above does not exclude the possibility that microtubules and microfilaments have a role in organ formation, perhaps not as stars of the play, but they could still act as supporting cast.

Elsewhere we have reported (Pearce and Zwaan, 1970), in agreement with earlier work of Byers and Porter (1964), that microtubules are primarily located in the top parts of elongated presumptive lens cells. They run parallel to the long axis of the cell from the apex down to the level of the nucleus, but they are rarely seen beyond this in the basal pole of the cell, even if the cell stretches out further than the nuclear position, as is the case in G_1 and G_2. We think, therefore, that the microtubules cannot directly be responsible for all of the cellular elongation and have proposed that their function is related instead to the nuclear movements in the G_1 phase of the cell cycle (Pearce and Zwaan, 1970). If the wedge-shape of the cells is a result of this nuclear repositioning (see also Langman et al., 1966), assembly of cytoplasmic microtubules after the completion of division is of obvious importance. It has been established that concentration of subunit pool and ionic conditions are determinants in the polymerization of microtubules, and it may be pertinent in this regard that the loss of cytoplasmic volume in G_1 leads to increased cytoplasmic density and, conceivably, to changes in ionic balance.

Apical microfilaments very frequently appear to originate and terminate in close association with junctional complexes (Wrenn and Wessells, 1969). Their thickening in relation to the narrowing of the cell apices can be interpreted as being reactive rather than active in nature. Perhaps they serve to anchor the cell-to-cell junctions, which must be of utmost importance in maintaining the integrity of the epithelium when it is undergoing the stresses undoubtedly accompanying complex morphogenetic movements.

The ideas expressed in this report can have validity for systems other than the developing eye lens. Preliminary studies indicate that invagination of the neural plate in the chicken embryo and the division of the optic vesicle into the presumptive neural and pigment cell stratum may well be explainable in similar terms.

Biochemical differentiation of the lens appears to be initiated only after major topographical reshuffling of the cell. Increase in bulk (ribosomal?) cytoplasmic RNA (McKeehan, 1956; Hunt, 1961) and ultrastuctural signs of nuclear activation (Zwaan, 1973) become noticeable when maximal cellular elongation has occurred. Specific lens proteins can be detected first at this time (Zwaan, 1968), their synthesis probably being initiated at the end of the S phase (Pearce and Zwaan, unpublished; Zwaan, unpublished). This agrees with the hypothesis that DNA synthesis may be required for a shifting of developmental pathways (Ebert and Kaighn, 1966). Finally, the lens rudiment becomes capable of self-differentiation (McKeehan, 1954).

In summary, we suggest that the following sequence of events takes place. The

establishment of contact between optic vesicle and ectoderm leads to a (quantitative) change in extracellular matrix. The ensuing limitation of lens territory, in combination with continuing cell replication, causes the presumptive lens cells to elongate, and when this reaches a maximum, differentiation begins. We do not propose that the morphological changes provide the specific stimulus for the initiation of cellular specialization, but it is attractive to think that tissue interaction and cell division set the stage for the subsequent cytodifferentiation along the lines of the discussed model.

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