The role of intraocular pressure in the development of the chick eye

V. Pigmented epithelium

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The pigmented and neural portions of the retina develop as separate layers and are free to slide over one another during the first half of development. Despite the fact that they follow different schedules of mitosis, cell death, and cell hypertrophy, these layers correspond in area throughout development. Descriptive and experimental findings in the chick embryo suggest the following explanation. After closure of the choroid fissure, vitreous substance accumulates in the vitreous cavity, resulting in rapid growth of the eye. Tensile forces thus generated at the level of the retina have little effect on the neural retina, which increases in area relatively independently of these forces. The pigmented epithelium, however, increases in area only in response to these forces. The expansion in area of the pigmented epithelium is, in large part, the result of cellular hypertrophy. Since the growth of the individual cells is dictated by the rate of expansion of the vitreous body, the pigmented epithelium is, at any stage of development, very closely tailored in its area to the size of the eye and of the neural retina.

During the development of the vertebrate eye the external layer of the optic cup gives rise to the pigmented epithelium of the retina, while the internal layer forms the neural portion of the retina. The pigmented epithelium develops as a simple, low columnar layer interposed between the neural retina and the choroid vascular bed. Because of its location, the pigmented epithelium functions as an avenue of exchange between the neural retina and the vascular compartment of the choroid coat. The presence of numerous microvesicles in its cells probably reflects this role. The pigmented layer functions mechanically to anchor the neural retina in place by the interdigitation of its cell processes with the rods and cones. Pigmented epithelium can also serve a number of optic functions. In most diurnal vertebrates the synthesis of melanin within the cells of this layer assures the absorption of light which has traversed the neural retina, prevents its reflection back on to the retina, and thus increases visual acuity. In many nocturnal species, on the other hand, cells of this layer are differently modified to form a tapetum. This scatters light back upon the retina, thus increasing sensitivity at the expense of visual acuity. Pigmented epithelium also has a photomechanical role. Movement of the pigment granules along the pigmented epithelial cell processes helps to adapt the retina optimally.

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for vision in bright or in dim light. This layer also has regenerative capacities. During the earlier stages of its development it is capable, under certain circumstances, of regenerating neural retina. In some of the tailed amphibia it retains the ability to regenerate neural retina throughout the life of the animal (see Stone for extensive summary). Under certain conditions pigmented epithelium can form lens tissue (see Reyer and Stone for extensive summaries), as well as iris. All of these capacities of the pigmented epithelium are affected by whether or not the contact between it and the neural retina is maintained.

With the exception of minor and transient mismatching of the areas of these two layers very early in the development of some species, it is notable that their areas do correspond rather exactly throughout development. This obtains despite the fact that the pigmented epithelium and neural retina follow different patterns and schedules of mitosis, cell death, and cellular hypertrophy. Previous work suggests that it is the pigmented epithelium which adjusts to the neural retina, not vice versa. Thus, when a small indwelling drainage tube is introduced through the eye wall, the vitreous body does not accumulate, and the tangential forces which normally operate on the developing wall are absent. Consequently the eye fails to increase appreciably in size even though cellular differentiation follows a normal time course. Under these conditions the neural retina continues to increase in area, due largely to the addition of new cells by mitosis at its margin. Because the neural retina continues to grow while the eye fails to grow commensurately, the neural retina becomes thrown into folds which become more extensive as development proceeds. The pigmented epithelium, however, remains unfolded and has an area which is appropriate to the actual size of the eye. The most satisfactory interpretation of these results is that the increase in size of the eye is dependent upon the increase in size of the vitreous body. Following closure of the choroid fissure, the vitreous substance accumulates in the closed vitreous cavity at such a rate that the neural retina, which is actively increasing its area, cannot become folded. It is probable that this mechanism even keeps the neural retina under modest tension during its development. The pigmented epithelium, on the other hand, does not increase its area independently but does so largely in response to tangential forces generated in it by the burgeoning vitreous body. Because of this its area matches the size of the eye and the area of the neural retina throughout development.

Since this layer is a simple epithelium, its area at any time is a function of the number of cells it contains and the area

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*The term 'hypertrophy is used throughout not in its pathologic sense but to denote simple increase in cell size.*

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**Fig. 1.** Semilog plot of the area of the pigmented epithelium as a function of age. Each point represents the mean of measurements on 10 specimens. The standard deviations are represented by the vertical lines.
of the individual cells. The number of cells, in turn, changes as a function of mitosis and cell death. Therefore, the next step in the analysis of this problem must be to ask in what ways the mechanical tensions discussed above influence the change in number of cells in the pigmented epithelium (mitosis, cell death), or affect the rate at which individual cells change in size (hypertrophy).

We have not yet quantitated the contributions of mitosis and cell death to the area of the pigmented epithelium, nor have we determined the extent to which these processes are influenced by mechanical tension. However, the experiments which we report indicate that the hypertrophy of individual pigmented epithelial cells contributes importantly to the increase in area of the pigmented epithelium, and this hypertrophy in turn is markedly influenced by the mechanical forces which are discussed above.

Materials and methods

Embryos of a strain of White Leghorn domestic fowl were studied. They were raised at 37.5° to 38° C. in a forced draft incubator. All ages cited in the text are given in days from the onset of incubation. The details of methods used in the several studies will be given at appropriate places with the results.

Results

Area of the pigmented epithelium as a function of age and eye diameter in untreated eyes. Groups of 10 eyes were collected daily between the fifth and sixteenth days of incubation. After cleaning each eye of its adnexa its diameter was measured to the nearest 0.01 mm. with a calibrated filar ocular micrometer. The eye wall was radially incised, freed of lens and vitreous body, and laid flat upon a microscope slide. A tracing was made of the outline of the eye wall and of the outer limit of the ciliary zone with a calibrated camera lucida fitted to a dissecting microscope. Planimetric measurements of the areas of these enlarged images enabled us to calculate the actual area of the pigmented epithelium. The pigmented epithelium undergoes a rapid increase in area prior to the eighth day, and thereafter increases in area at a much slower rate (Fig. 1).

Size of pigmented epithelial cells as a function of age. To evaluate the contribution of the hypertrophy of individual cells to the change in area of the pigmented epithelium, it is important to know the

![Graph](https://via.placeholder.com/150)

Fig. 2. The number of cells per unit area as a function of eye diameter (the superimposed age scale applies only to the untreated cases). Both the untreated eyes collected over a range of different ages (solid circles) and the eyes which were intubated on the fourth day and collected on the twelfth day of incubation (crosses) fall along essentially the same locus. This indicates clearly that the size of the individual pigmented epithelial cells is most importantly effected by the rate of growth of the eye, and little or not at all by chronological age. The value for a spontaneous microphthalmic eye from a 12-day embryo is represented by the solid triangle.
manner in which these cells change in area and in volume as a function of age. We found it simpler and more accurate to determine the reciprocal of cell area, i.e., the number of cells per unit area, than to measure the areas of single cells.

Groups of 10 or more eyes were collected at daily intervals between the fifth and sixteenth days of incubation and were cleaned of surrounding tissues. After the diameter of the eye was measured, a patch was cut from the eye wall and immersed in saline in order to float the neural retina free from the specimen. In the interests of uniformity only the posterior pole of the eye was used in making our determinations. Since the pigmented epithelial patch still adhered to the remaining layers of the eye wall, distortion was minimized. The specimen was placed in saline on a microscope slide with the pigmented epithelium uppermost, and a cover slip was floated over the specimen. Photomicrographs of the central portion of each specimen were made by transillumination. Under these conditions each cell is crisply outlined on the negative. A dissecting microscope was used to count the number of cells in each frame. Since the area which the frame represented on the specimen was known, the number of cells per unit area in the pigmented epithelial sample could be readily calculated.

The number of cells per square millimeter is in the neighborhood of 14,000 on the fifth day, diminishes rapidly until the eighth or ninth day, and thereafter decreases more slowly. Thus, since the surface area of the average pigmented epithelial cell is the reciprocal of the number per unit area, the surface area of the individual pigmented epithelial cell increases rapidly up to the 8th day and thereafter more slowly. This pattern parallels the change in size of the eye and the change in total area of the pigmented epithelium (Fig. 2).

With the exception of certain large binucleate cells the areas of the pigmented epithelial cells appear to be highly uniform. The majority of the cells are hexagonal in outline, some are pentagonal, and a few have seven or more sides (Fig. 3).

**Experimental manipulation of eye size.** To determine to what extent the observed cellular hypertrophy is dependent upon...
tangential forces generated by the expanding vitreous body, we altered the rate of accumulation of vitreous substance during the course of development by introducing drainage tubes through the eye wall (method in Coulombre'). The drainage tubes were allowed to remain in the eyes of different animals for different periods of time following the operation. As a result of this procedure the experimental eyes grew at different subnormal rates. On the twelfth day, when the experiment was terminated, we obtained a series of eyes which were all of the same age and had followed essentially the same timetable of differentiation, but which showed a range in size from normal to very small. Using the techniques described above, we determined the diameter of each of these eyes and the number of pigmented epithelial cells per unit area in the fundus. Fig. 2 is a scattergram in which the concentration of pigmented epithelial cells is plotted as a function of eye diameter. Values obtained from this group of experimental eyes as well as values obtained from the normal series of untreated eyes are plotted. Points from both of these groups fall together in a way which indicates that the number of pigmented epithelial cells per unit area is high in small eyes and becomes progressively lower as the size of the eye increases, regardless of such factors as age and state of differentiation. The concentration of cells in the pigmented epithelium of a single spontaneous microphthalmic eye (solid triangle on the graph) was appropriate to its size.

Binucleate cells. In the course of this investigation we observed binucleate cells scattered throughout the pigmented epithelium. These cells are much larger in surface area than their uninucleate neighbors. They are not present, however, in sufficiently large numbers to shift significantly the mean values for the numbers of pigmented epithelial cells per unit area. The possible significance of these binucleate cells must await a more extensive study of their frequency, their spatial distribution, and the times during development at which they occur. They do not appear to represent cells in the terminal stages of mitosis.

The distribution of mitotic figures in the pigmented epithelium. Our initial experiences with the role of mitosis do not permit us to make quantitative statements concerning the mitotic rate as a function of age, nor are they calculated to yield information concerning possible diurnal variations in mitosis. They do reveal, however, the spatial distribution of mitotic figures in the pigmented epithelium as a function of age, and also permit rough approximations of the degree of mitotic activity. Because they have some bearing on the present report these observations are outlined here.

Fresh untreated eyes, collected at daily intervals between the fourth and twelfth days of incubation, were studied. The eye walls were cut into segments from which the pigmented epithelium was dissected free and laid flat upon the surface of an albuminized microscope slide. After fixation for about 10 minutes in Carnoy's fluid the specimen was rehydrated, bleached with a 0.5 per cent solution of calcium hypochlorite, washed in water, and stained with Harris's hematoxylin. Microscopic examination of these stained whole mounts permitted us to map the location of mitoses in the pigmented epithelium.

Even as early as the fourth day mitoses are absent in the fundus but are widespread and numerous in the equatorial and more anterior portions of the pigmented epithelium. Thereafter they become progressively more confined to the margin of the optic cup. The number of mitotic figures decreases sharply between the fourth and eighth day. The only region in which it proved difficult to make accurate observations was a very narrow band at the margin of the optic cup. However, it appears that some mitotic activity goes on in this region up to at least the twelfth day of incubation. These findings are of interest in connection with the studies re-
ported above. The region of the fundus which we utilized for the study of cell size was free of mitotic activity during the stages which were employed.

Discussion

In the eye, as in no other organ, the several tissues must be shaped to exacting standards, and must bear precise geometric relationships one to another if optic and photoreceptive functions are to be possible. Exacting tolerances must be met at the gross level, at the cellular level, at the level of the organelles, and even at the molecular level. At the gross level several factors have been identified which help to coordinate the growth patterns of the tissues of the eye so that it becomes appropriately shaped for its optical functions.

The results of the present study, taken together with those of previous investigations, make possible a partial reconstruction of the probable sequence of events in the morphogenesis of the chick eye. While it can be assumed that the general outline of this sequence obtains in the development of the eyes of other vertebrates the details of similarity and difference require further study. Following closure of the choroid fissure vitreous substance accumulates between the lens and the presumptive neural retina in the vitreous cavity. As soon as the vitreous chamber becomes a closed compartment, the wall of the eye is brought under tension. From this time until the eighth day the eye increases rapidly in size. Following the eighth day the growth of the eye is at a markedly slower rate. During a relatively brief period of time the presumptive pigmented epithelium, which is initially low columnar or cuboidal, assumes a low cuboidal aspect. By a mechanism as yet unknown, tensile forces in the plane of the pigmented epithelium bring about an increase in area of individual pigmented epithelial cells as development proceeds. This contributes importantly to the increase in the area of the entire pigmented epithelium. Thus, the pigmented epithelium, at any stage in development, is very closely tailored in its area to the size of the eye.

It is, of course, possible that the increase in exposed surface area of the individual cells represents nothing more than a redistribution of existing cytoplasm to form a broader but flatter cell, with no real increase in cell volume (hypertrophy). To determine whether, in fact, a genuine cellular hypertrophy occurs during development, it is necessary to know how the thickness of the pigmented epithelium varies with age. In a previous study it was shown that the pigmented epithelium not only does not decrease in thickness from the fourth day onward but actually increases, especially at the time when the pigmented epithelial processes appear at about 15 days of incubation. Thus, the cells of the pigmented epithelium do undergo a true hypertrophy of considerable proportions as development proceeds.

The fact that mitosis becomes progressively more restricted to the anterior portions of the pigmented epithelium has interesting consequences. A tangible record of this time-space distribution of mitosis is preserved in the gradation of cell area from very large in the fundus to relatively small near the ora serrata.

One further aspect of this problem deserves mention. The pigmented epithelium and the neural retina must be free to slip over one another during the period between the fourth and fifteenth day during which they are in differential growth. That they are free to slip over one another is attested by the fact that they readily separate in freshly dissected eyes prior to the fifteenth day. Beginning on the fifteenth day of incubation pigmented epithelial cell processes begin to appear, first in the fundus, later at the equator, and finally at the ora serrata and, by interdigitating with the developing rods and cones, lock together the pigmented epithelium and the neural retina. From the fifteenth day onward it is increasingly difficult to separate the pigmented epithelium and the neural...
retina in freshly dissected specimens; it very quickly becomes impossible to do so without some damage to one or both layers. Thus, whatever mechanisms operate to insure that the pigmented epithelial processes will not develop prior to the time at which the neural retina has completed its marginal growth, it is a fact that they do not appear before the appropriate time.

The functional importance of the pigmented epithelium has been given increasing recognition. Studies of its developmental history afford richer opportunities for understanding its constitution and function than do investigations of the adult stage alone. Because the pigment layer is, in a sense, a two-dimensional sheet, a study of the population dynamics of its cells is greatly simplified.

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REFERENCES