of virus particles based on morphology such as that recently devised (*7*).

The preparations of the blood of normal people and of four of the leukemia patients revealed many particles in the same size range as virus particles. These might be mistaken for virus particles unless criteria, dependent on the observance of one of the types of morphology (*7*) now known to be associated with negatively stained virus particles, are considered. In no negatively stained preparations from the blood of these people were particles seen that exhibited any of the known types of virus-particle morphology.

It might be questioned whether the particles we observed could be the same as those occasionally seen by Dmochowski and others (*3*) in thin sections of leukemic cells and tissues of man. That both are covered with a membrane and in the same size range is in support of this possibility. Moreover, the particles we observed may be of the same general type as those described by de Harven and Friend (*13*), who, by means of a negative staining technique, found rare particles, approximately 1000 Å in diameter, resembling myxoviruses, in the blood of mice with Friend leukemia.

Since passenger viruses can be present in malignant tissue and since the method used in this study discloses fragile virus particles that might not be seen by other methods it would be unwise to attempt to draw any conclusions about whether the particles described here are directly related to the disease in which they were observed in two instances. However, even if the described particles are not directly related to the disease in which they were found, their presence in this disease poses a sufficiently interesting problem to alert those studying clinical leukemia (*14*).

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23 September 1963

Lens Development: Fiber Elongation and Lens Orientation

Abstract. *When the lens of the 5-day chick embryo is surgically reversed so that its epithelium faces the neural retina the elongation of those lens cells which have already differentiated is arrested and the epithelial cells differentiate into a new set of lens fibers. This internal reorganization, together with a reversal in polarity at the lens equator, results in a complete reversal of the polarity of the entire lens.*

This study demonstrates that the differentiation of lens epithelial cells into lens fibers is not simply a function of their age but depends importantly on their location within the eye. The lens of the embryonic eye of the domestic fowl begins as a placode of palisaded cells between the 10 and 20 somite stages. It is induced in head ectoderm by the tip of the optic vesicle (prospective neural retina) (*1, 2*). The placode invaginates during the 21- to 25-somite stage and separates from the surface by the 35- or 36-somite stage to form the hollow lens vesicle (see *2–4*).

Simultaneously the cells of the vesicle lying toward the vitreous body elongate, approach the anterior epithelium, and obliterate the lens cavity (*4, 5*). The primitive lens thus formed has a simple cuboidal epithelium toward the cornea, and an ellipsoidal fascicle of lens fibers located behind the epithelium and continuous with it at the equator. As early as 1907 Le-Cron (*6*) recognized that the presence of the optic cup is necessary for the maintenance of a lens at this stage. The mitosis which contributes to the growth of the lens is restricted to the epithelium. At the equator the differentiation of the epithelial cells is signaled by their elongation to form meridionally oriented lens fibers which are added to the body of the lens in concentric layers.

The factors which control the elongation of lens fibers during normal development, as well as the factors which confine the zone of incipient differentiation to the equator, have not yet been identified. However, during regeneration of the lens from the dorsal iris in adult amphibians (*7*) the lens fibers develop in that part of the lens which faces the neural retina, even when the iris has been surgically reversed, back to front, before the onset of lens regeneration. The observation that the lens was oriented appropriately to the eye rather than to the iris was interpreted to indicate the presence of a factor from the posterior half of the eye which promotes elongation of the posterior cells of the lens.

We have dealt with a similar phenomenon during normal lens development in the chick embryo. The specific questions we asked were: If they are appropriately stimulated can all of the cells of the lens epithelium, and not just those near the lens equator, form lens fibers? Does the position of lens cells relative to the neural retina determine whether or not they will form lens fibers? Once lens fiber elongation has been initiated is it autonomous? Can the polarity of the equatorial zone and of the lens as a whole be reversed?

To answer these questions we studied histogenetic changes in the embryonic chick lens after it had been surgically reversed so that the lens epithelium faced the vitreous body. The embryo was exposed for surgery by cutting a window through the shell and shell membrane, making an incision through the chorion at the edge of the allantois, and opening the amnion over the right eye. The lens enclosed within its capsule was removed intact from the right eye at 5 days of incubation through an incision at the dorsal corneal limbus in 22 embryos. The same lens, or a comparable one from a 5-day donor, was reinserted into the eye after being reversed.

Separate experiments demonstrate that after this procedure the eye wall can heal in as little as 4 hours. While the incisions did not heal in a few specimens this did not affect the sequence of changes in the lens (see Fig. 1*A*). At 2-day intervals after the

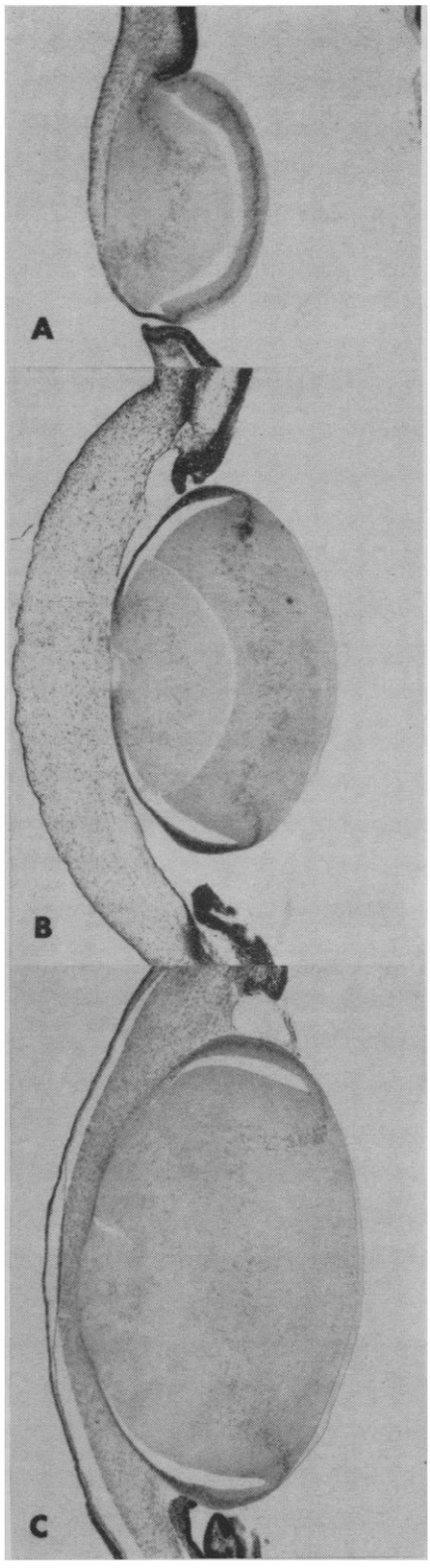

Fig. 1. Axial sections of chick-embryo lenses that had been surgically reversed at 5 days of incubation so that the epithelium faced the vitreous body. *A*, 2 days postoperatively. *B*, 6 days postoperatively. *C*, 10 days postoperatively. All × 40.

operation, groups of animals were killed and the eyes were fixed in Zenker's or in Carnoy's fluid, embedded in paraffin, and sectioned to a thickness of 7 μ in planes parallel to the optic axis. The sections were stained either with hematoxylin and eosin or with Azure-B.

As early as 2 days after the operation all of the cells in the former lens epithelium were measurably elongated. As time elapsed their elongation became more pronounced (Fig. 1). The sequence of cytological changes in the former epithelium was characteristic of that at the equator of a normal undisturbed lens and produced a new body of lens fibers posteriorly. The fibers which had been laid down prior to the operation were now situated anteriorly and ceased to elongate as soon as the lens was reversed. They remained as a subcapsular nest of organized lens fibers located on the lens axis toward the anterior chamber. A narrow ring of lens epithelium was left at the equator of the lens. The polarity of this remnant became reversed by a shift of the center of mitotic activity from the former epithelial side of the equator to that portion which now faced anteriorly. If the cells produced by this mitotic ring moved toward the neural retinal side they became lens fibers which were added to the new body of fibers. If these cells moved toward the cornea they formed simple cuboidal lens epithelium.

Thus, the lens becomes reversed in its organization, reconstitutes fibers posteriorly, and rebuilds an epithelium anteriorly. The rebuilt epithelium does not, even at 15 days, completely cover the old lens fibers which are trapped anteriorly, and the mass of old fibers touches the capsule over a small area. All 22 operated specimens had followed the same sequence of changes with remarkable uniformity.

We interpret these results as follows. (i) At 5 days (and, on the basis of other experiments, at other embryonic ages) all of the lens epithelial cells are competent to form lens fibers if they are appropriately stimulated. (ii) From the outset lens cells elongate to form lens fibers if they face toward the neural retina, and fail to do so if they face toward the cornea. (iii) Similarly when lens fibers are transferred from a posterior to an anterior location by reversing the lens they cease to elongate. Thus the conditions necessary to initiate the elongation of a lens fiber are also necessary to maintain its elongation. (iv) The polarity of the equatorial zone of the lens is determined by factors extrinsic to the lens and can be reversed at least as late as 5 days of incubation in the chick embryo.

These observations could be explained by a cell-differentiating factor for the lens originating in the posterior segment of the eye, or by a factor inhibiting lens-cell differentiation and originating anteriorly, or by both. Other results (*8*) indicate that the neural retina is the source of a factor which promotes lens differentiation. It may be significant that it is the presumptive neural retina which initiates palisading of ectodermal cells to form the lens placode. Indeed, the process of palisading is followed without any gap in space or time by the process of lens-fiber elongation. Both palisading and lens-fiber elongation are initiated and maintained for a prolonged period by the presumptive neural retina. It may be that both processes are identical and that the inductive interaction between neural retina and lens continues for a much longer period than we have hitherto suspected.

Whatever the physical basis of the phenomenon we have described, it clearly provides a mechanism for continually adjusting the geometric alignment of the lens to that of the rest of the optical system of the eye during development (*9*).

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