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DNA synthesis in the embryonic chick lens epithelium is arrested after experimental lens rotation

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Summary. Using autoradiographic technique, we have studied DNA synthesis in normal embryonic chick lens epithelium and after experimental lens rotation.

Analysis of the autoradiograms clearly demonstrates that when the lens primordium was rotated 180°, so that lens epithelium was placed facing the interior of the optic cup, the lens epithelial cells completely stop DNA synthesis. This fact suggests that some retinal and vitreal factors are responsible for differentiation and replicative capacity of the lens epithelial cells.

Key words: Chick lens epithelium, (^{3}H) thymidine labelling, Autoradiography, Lens rotation

Introduction

The lens of the eye represents an ideal model for analysing the sequence of embryological processes of growth differentiation and morphogenesis (Spemann, 1901; Mckeehan, 1951; Jacobson, 1958; Rabaey, 1962; Papaconstantinou, 1967; Modak et al., 1968; Zwaan et al., 1969). This model has been studied by different technological approaches both *in vivo* and *in vitro* under normal and experimental conditions. *In vitro* studies have paid much attention to those retinal and mesenchymal factors which are responsible at a molecular level for the inductive influences that affect the lens mitotic behaviour (McAvoy and Fernon, 1984; McAvoy and Chamberlain, 1989) and differentiation (Beebe et al., 1980).

Of the experimental conditions *in vivo*, lens rotation represents a suitable experiment for altering the normal close spatial relationships of lens cells and which are modified under the inductive influence of retinal tissues. These experiments have shown that the primitive lens epithelial cells when oriented towards the optic cup rapidly start elongation (Coulombre and Coulombre, 1963; Eguchi, 1967; Yamamoto, 1976) and the macromolecular specific synthesis of crystallin proteins (Genis-Gálvez et al., 1968; Genis-Gálvez and Castro, 1971). However the replicative capacities of the lens epithelial cells in experimental lens rotation have never been studied.

We clearly demonstrate here that when the lens epithelium is reoriented toward the vitreous and neural retina, the lens epithelial cells completely stop DNA synthesis, and then elongate and differentiate into lens fibres.

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Materials and methods

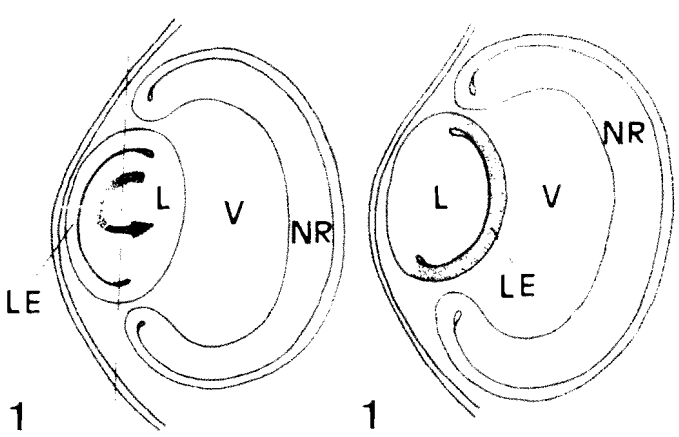
The experiments were performed on twenty White Leghorn chicken embryos at 72 hours of incubation (Fig. 1). The lens primordium was rotated 90° or 180° so that the lens epithelium was placed facing partially or totally the interior of the optic cup under direct influence of the retina. Six hours after lens inversion, the embryos were injected with 0.02 ml (^{3}H) thymidine. In four control animals, the thymidine was injected at 78 hours of incubation. Furthermore the lens primordium of another four control embryos was rotated 360°, to show that the effect of the experimental procedure does not have any influence on the incorporation of tritiated thymidine. The thymidine was supplied by the Radiochemical Centre, Amersham, England and has a specific activity of 1000 µCi/ml; thus each embryo received 20 µCi. The embryo was killed 24 hours after lens rotation, fixed with Carnoy's fixative and embedded in paraplast. Material was serially sectioned at 5 µm in a plane perpendicular to the surface of the epithelium and mouted on slides with egg albumin. Deparaffinized sections were stained with haematoxylin-eosin and dried in an incubator at 37 °C. The sections were then covered with an Ilford K2 emulsion and stored at 4 °C for 20 to 30 days in light-tight boxes containing silica gel as a drying agent. After storage the films were developed in Kodak D-19, and the sections dehydrated, cleared in cedar wood oil and mounted on DPX.

Results

We have studied the autoradiograms in twenty experimental and eight control embryos. Analysis of the autoradiograms clearly demonstrates that after lens rotation, the number of DNA-synthesizing lens epithelial and annular pad cells decreases dramatically (Fig. 2). Only about 2% of all lens population cells have nuclei labelled with thymidine 24 hours after lens reversed position (Fig. 2b). The percent of nuclei labelled in normal conditions in 23.11% of all lens population cells (Fig. 2a). The fibre cells, transferred by rotation to an anterior location, also show complete absence of mitosis and labelled nuclei (Fig. 2b). The results are summarized in Table 1 and Fig. 4.

Discussion

As was reported previously (Genis-Gálvez, 1971), our experimental material showed that when the lens



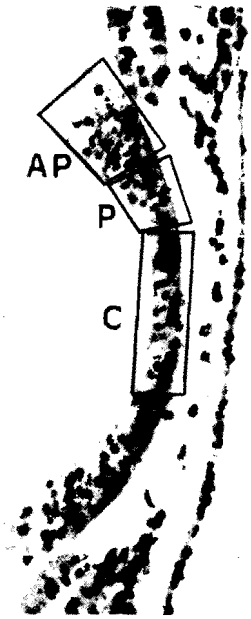


Fig. 1. Experimental procedure of lens rotation in chick embryo at 72 hours of incubation. **a.** Normal position of the lens (L) and lens epithelium (LE) with respect to the vitreous (V) and neural retina (NR) tissues. The arrow shows the direction of rotation. **b.** As a result of 180° lens rotation the lens epithelium (LE points) is now facing the vitreous (V) and neural retina (NR).

Fig. 2. Autoradiographs of control and experimental chick embryo lens at 96 hours of incubation. **a.** The control autoradiogram shows numerous labelled nuclei dispersed in each region of epithelium (central epithelium, C, peripheral epithelium, P) and annular pad (A.P). **b.** The experimental autoradiogram shows only two labelled nuclei within central and peripheral epithelium (arrows) and four labelled nuclei on the annular pad (arrowheads).

Fig. 3. Autoradiograph of chick embryo lens at 96 hours of incubation whose lens primordium was rotated 90° at 72 hours. Only the sector of epithelium which is outside the inductive influence of the neural retina has numerous labelled nuclei. In this case the vitreous shows a haemorrhage provoked during rotation of the lens (HV).

Table 1. Distribution of DNA-synthesizing and total cell populations in central and peripheral, annular pad and the entire lens of normal and experimental chick embryos of 4 days of age.

	TOTAL	LABELLED	%
Central and peripheral epithelium			
Normal	6329	2160	34.12
Experimental	5112	128	2.50
Annular pad			
Normal	15211	2820	18.53
Experimental	12840	241	1.87
Entire lens			
Normal	21540	4980	23.11
Experimental	17952	369	2.05

epithelium was reoriented toward the neural retina, the lens epithelial cells rapidly started to elongate and differentiate into lens fibres.

Our results agree with those reported *in vivo* (Hanna and Keatts, 1966; Modak et al., 1968; Persons and Modak, 1970; Zwaan and Pearce, 1971) and in tissue culture (Philpott, 1970; Piatigorsky and Rothschild, 1972) suggesting an inverse relationship betweeen lens cell elongation and mitosis. We believe that, although lens epithelial cells retain their initial genetic information, the expression of both processes of differentiation and replicative capacity are strongly dependent on the spatial position of cells with respect to retinal and vitreal tissues. In the last ten years, basic fibroblastic growth factor (Richardson and McAvoy, 1990) and other retinal and vitreal factors such as lentropin (Beebe et al., 1987), have been proposed as being responsible for differentiative synthesis of specific crystallins and also for lens epithelial cell morphogenetic elongation. These factors could also be indirectly responsible for the arrest of mitotic activity. Thus, when the lens primordium was rotated 90°, only the sector of the lens which was closer to the neural retina lost the capacity of DNA synthesis (Fig. 3).

These data shed light on some mechanisms which might regulate cellular proliferation in relation with the processes of differentiation and morphogenesis.

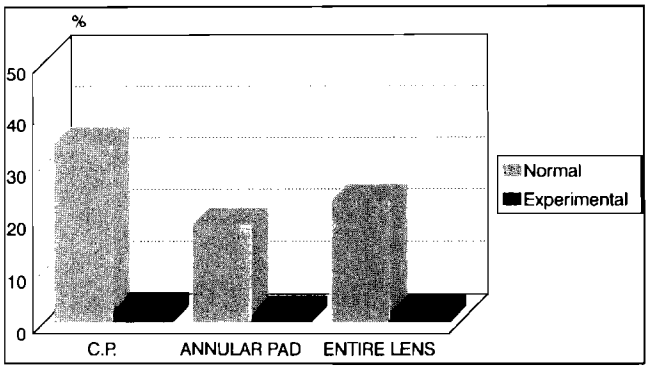


Fig. 4. Percentage of DNA-synthesizing cell populations in central and peripheral (C.P.) epithelium, annular pad and the entire lens.

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