Abnormalities in the differentiation and cellular properties of hyperplastic lens epithelium from strains of chickens selected for high growth rate


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SUMMARY

Similar morphological abnormalities of the lens of the eye of two unrelated strains of chicks, both of which had been selected for high growth rate, were found to be associated with epithelial cells showing marked deviations from the normal in cell surface properties, mitotic rate, capacity for differentiation, and in DNA, RNA and protein metabolism. The relationship between these modified properties and the observed morphology is discussed.

INTRODUCTION

In any study of the processes of cellular differentiation the investigation of genetic modifications may be expected to throw light on the interrelationships between cellular events and the specific syntheses involved. Such a system is provided by the lens of the chick eye, in which many aspects of normal differentiation have already been described. Ontogenic changes in cellular events, such as the incidence of mitoses and the progress towards the development of the differentiated end product (the lens fibre) have been investigated (Hanna & Keatts, 1966; Modak, Morris & Yamada, 1968). The distribution of nucleic acid syntheses in cells at different stages of differentiation has been studied by these authors, and also by Reeder & Bell (1965), by Persons & Modak (1970) and Modak & Persons (1971). The identification of the specific proteins (crystallins) synthesized in the chick lens, their subunit composition and the changes in the crystallin composition as the animal ages have been studied by numerous authors and reviewed by Clayton (1970, 1974). Among the problems still outstanding are the roles of mitosis and cell position in differentiation, the nature of...
the signal required to produce fibre differentiation from a lens epithelial cell, whether a specific external signal is required, and whether the response is always the same. Rotation of the embryonic lens in situ indicates that proximity to the retina is required for fibre differentiation (Coulombre & Coulombre, 1963; Genis-Galvez & Ruano, 1965; Genis-Galvez & Castro, 1971; Eguchi, 1967), and experiments with explanted lenses also suggest that retinal factors may be important (Takeichi, 1970). However, explants of lens epithelium into culture medium are capable of showing some signs of differentiation in the presence of serum proteins (Philpott, 1970; Piatigorsky & Rothschild, 1971; McDevitt & Yamada, 1969; Okada, Eguchi & Takeichi, 1971; McLean & Finnegan, 1974), and Craig & Piatigorsky (1973) have observed in vitro differentiation even in the absence of serum proteins. The spatial relationships of lens epithelial cells to each other in culture may be important since Okada et al. (1973) observed that lentoids will only arise in confluent cultures and McLean & Finnegan (1974) found that the curvature of an explanted epithelium determines whether fibre differentiation is possible.

It is the purpose of this report to present preliminary observations on the mitotic rate, metabolism of macromolecules, and cell behaviour indicating a modification of cell surface properties in two unrelated lines of chicks, which have been selected for high growth rate, and both of which show a tendency to unregulated hyperplasia and abnormal differentiation of the lens epithelium in vivo (Clayton, 1975).

METHODS

Birds. Day-old chicks of various strains, including the two strains with epithelial hyperplasia (Hy-1 and Hy-2, Clayton, 1975) and three control lines were obtained from Sterling Poultry Products Ltd., Ratho, Midlothian and the Poultry Research Centre, Edinburgh.

Histology. Lenses for sectioning were fixed in Carnoy's fixative or lanthanum acetate–trichloroacetic acid, embedded in paraffin, sectioned at 4 μm and stained in haematoxylin and eosin.

Autoradiography. Lenses were explanted directly into medium 199 + 10% foetal calf serum and labelled with radioactive tracers as described by Clayton, Truman & Campbell (1972). After 1–3 h incubation in one of the following: 75 μCi [³H]thymidine/ml; 75 μCi [³H]uridine/ml, 400 μCi [³H]proline/ml or 140 μCi [³H]amino acid mixture/ml (Radiochemical Centre, Amersham, Bucks) the lenses were washed in unlabelled medium, fixed, embedded and sectioned as above, placed on gelatin-coated microscope slides and coated by dipping in liquid emulsion (Ilford L 4) diluted 1:1 with distilled water. After 4–14 days of exposure, the autoradiographs were developed and stained with haemotoxylin and eosin.

Metabolic turnover. Lenses were explanted into labelled medium as described above, incubated, washed and homogenized, before processing. In some
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experiments with [3H]uridine the lenses were transferred after labelling to medium containing 20 µg/ml of unlabelled uridine. For estimation of gross incorporation of uridine, thymidine or [14C] amino acids, an aliquot of the homogenate was precipitated with trichloroacetic acid, filtered and washed before scintillation counting. Uridine incorporation into different cell fractions was estimated: the homogenate was centrifuged at 10000 g for 10 min giving a pellet (crude nuclear fraction), and the supernatant was used to prepare polysomal and soluble fractions (Clayton, Truman & Hannah, 1974). The crude nuclear fraction was washed by centrifugation through 2 M sucrose at 60000 g for 20 min. Incorporation of labelled amino acids into soluble proteins and cell membranes was estimated after centrifugation of the homogenate at 10000 g for 10 min to yield a soluble supernatant and a pellet which was extracted three times with 8 M urea to yield an insoluble membrane fraction (Maisel, personal communication).

Cell culture. The lens epithelial cells of Hy-1 as well as the normal strain were cultured in vitro. The epithelial cells were dissociated by incubation of isolated lens epithelia from day-old chickens in 0.05 % EDTA in Ca²⁺ and Mg²⁺-free Hanks saline (CMF) for 10 min at room temperature, and subsequently in 0.25 % trypsin in CMF for 40–60 min at 37 °C. Small clumps of cells were eliminated by centrifugation of cell suspension at 500 rev/min for 4 min. Final cell suspensions consisting mainly of single cells were then inoculated with a cell density of 1 x 10⁶ in 3.5 cm Falcon plastic culture dish with 1.5 ml of nutrient medium (EF) which consists of Eagle minimum essential medium supplemented with 6.0 % foetal calf serum (GIBCO control No. 00059) (Okada, Eguchi & Takeichi, 1971). The medium was replaced every 2 days. All the cultures were maintained at 37 °C in humidified atmosphere of 95 % air–5 % CO₂. To establish clonal cell cultures, 100–500 freshly dissociated single cells were inoculated in 6.0 cm Falcon plastic dishes with 3.5 ml of the mixture of EF, and EF conditioned with embryonic neuro-retinal cells at high density, with a ratio of 4:1 (Okada et al. 1973). The whole process of mass culture of lens epithelial cells from Hy-1 as well as the normal strain was recorded synchronously by time-lapse cinematography as described below. Detailed methods for cell culture will be given in a separate paper (Eguchi, Clayton & Perry, 1975, in press).

Time-lapse cinematography. Dissociated cells from hyperplastic and normal chick lens were filmed simultaneously with two Wild M 40 inverted microscopes joined by a comparison tube. Phase contrast was used during early and active phases of cell growth, while optical shadow casting (adapted from Hlinka & Sanders, 1970) was used during lentoid formation. Frame intervals of 30 and 60 sec were used during plating out and early phases of cell growth, while 120 sec (occasionally 90) was used during preconfluence, confluence, aggregation, and lentoid formation.

Immunofluorescence. Four antisera were used: (i) to total chick crystallins, (ii) to bovine α-crystallin, (iii) to chick anodal β-crystallin and (iv) to chick cathodal β-crystallin. These were prepared as described in Clayton & Truman.
R. M. CLAYTON AND OTHERS (1974), conjugated either to rhodamine B<sub>200</sub> or to fluorescein isothiocyanate as described in Nairn (1964) and applied to cell cultures which had been washed free of medium in Hanks salt solution, fixed briefly in methanol at −20 °C and stored dry at −20 °C before use. They were examined by u.v. microscopy.

Electron microscopy. Cultures 11−12 days after plating were rinsed in physiological saline and fixed in a solution of 3% glutaraldehyde in 0·1 M sodium cacodylate buffer at pH 7·1. After 1 h at room temperature they were rinsed in buffer and post-fixed in buffered 1% osmium tetroxide for 1½ h at 4 °C. They were then dehydrated in a graded series of alcohols and infiltrated with an Araldite−Epon mixture and embedded attached to the culture dishes. Sections were stained with alcoholic uranyl acetate and lead citrate and examined in an AEI EM 6B electron microscope.

RESULTS

Growth rate

Evidence from in vivo morphology. The number of epithelial cells which are present in the lens of the day-old chick of both strains Hy-1 and Hy-2, while variable, is always much greater than in normal birds. This increase is associated with a folding of the epithelium over the anterior face of the lens between the capsule and the fibre body. It is especially marked in the equatorial region of the lens. Some of the epithelial cells are partly differentiated into short fibres (Fig. 1). The mass of fibres of the lens body, however, is not diminished, suggesting that recruitment of fibres from epithelium is not impaired. It seems therefore that the increased mass of epithelial cells is attributable to their high rate of replication.

Evidence from cell culture. The epithelial cells of Hy-1 under conditions of culture have a rate of increment during the log phase of growth which is approximately twice that of normal epithelial cells (Fig. 2). The number of mitoses in an actively growing culture over the identical period of time (between the 4th and 6th days of culture) and a visual field of identical dimensions containing initially the same number of cells has been counted by analysing the frames of a time-lapse film with split field. The figures were 33 and 18 mitoses in 24 h for Hy-1 and normal respectively. Clones set up from single epithelial cells of Hy-1 fall into two classes: those in which the increase in cell number is similar to that of clones from normal lenses and those in which the growth rate is increased (Fig. 3). More complete data of the results in cell culture will be published elsewhere (Eguchi et al. 1975).

Cell surface properties

Evidence from morphological studies. The multiple-layered epithelium of the Hy-1 and Hy-2 strains dissects off more readily from the lens than does the single-layered epithelium of normal birds but the layers tend to adhere together. It would appear from the morphological arrangement of these epithelial cells
Fig. 1. The anterior aspect of the lens of a day-old chick of strain Hy-1.

(A) The epithelium has formed three layers, the innermost of which has folded up above the fibres of the lens body and has itself begun to differentiate into short fibre-like cells. × 320.

(B) Showing a multilayered epithelium anterior to the fibre mass. The outermost layer is normal in appearance; the inner ones are all composed of elongated cells. In some areas gaps appear between layers while in others the layers are closely apposed. × 320.

(C) Showing the intrusion into the lens of iris tissue. In some areas there are gaps between lens and iris. In the mid-right and upper left of the figure there is close apposition of the two tissues. × 160.

(D) Higher power, showing intrusion of iris tissue into the lens. × 320.
Fig. 2. Cell growth in primary mass cultures. Lens epithelium from normal and Hy-1 chick strains were cultured. Lentoid bodies first appeared in the culture at the point indicated by L.

Fig. 3. Clonal growth of individual single cells dissociated from lens epithelia of normal (left) and Hy-1 (right) strains of chick.
FIGURE 4

Frames from a split-field time-lapse film showing successive stages in the aggregation of dissociated lens epithelial cells after plating. Each frame is divided horizontally, the upper half showing normal and the lower half Hy-I cells. (A) 1 h, (B) 2 h 50 min, (C) 7 h 20 min, (D) 19 h 40 min, (E) 22 h after setting up cultures (A and B, 30 sec/frame, C–E, 60 sec/frame).
Fig. 5. Cells in the early stages of spreading of an epithelial culture treated with fluorescein-conjugated antiserum to total lens proteins. Of four cells which are in contact with each other only the one with greatest area of contact (4) shows positive fluorescence.

Fig. 6. Lentoids formed in cell culture. The lentoid is in the centre of the figure surrounded by confluent epithelial cells. The culture has been treated with fluorescein-labelled antiserum to anodal β-crystallin (A) and to cathodal β-crystallin (B). The antiserum to anodal β-crystallin labels a considerable area of the lentoid and some of the surrounding epithelial cells while those further away remain unlabelled. Antiserum to cathodal β-crystallin mainly labels the extreme periphery of the lentoid and some epithelial cells around it. × 64.
that they do not show contact inhibition on their upper and lower surfaces (Fig. 1B). Mutual invasiveness of the lens and iris has occasionally been observed in Hy-1 (Fig. 1C, D). No such anomaly has been previously reported, suggesting that contact inhibition between these two tissues normally occurs but that this may be diminished in the Hy-1 strain.

Evidence from cell culture. Cells from the lens epithelium of Hy-1 show only 50% of the plating efficiency of normal cells (Fig. 2) and large aggregates are rapidly formed when isolated epithelial cells are sown in a culture dish. Fig. 4 shows a still from a time-lapse record of normal and Hy-1 lens epithelial cultures: aggregate formation in Hy-1 cells occurs both earlier and more massively than in cells from normal birds.

When epithelial cells in culture were treated with antibodies to total crystallins the most intense fluorescence was observed in cells which were in contact with others over most of their periphery (Fig. 5). Condensates in confluent cultures, and lentoids formed at a later stage, both in normal and in Hy-1, show differential localization of $\beta$ crystallins, the anodal $\beta$ crystallins being located in the central region of the condensate or lentoid and cathodal $\beta$ crystallins in the peripheral region (Fig. 6).

Metabolism of the lens

DNA synthesis. Thymidine incorporation into lenses of Hy-1, Hy-2 and control birds is shown in Table 1. In day-old lenses, both hyperplastic strains incorporate more thymidine than normal, and the autoradiographs (Fig. 8) reveal the following: (i) more nuclei are labelled in Hy-1 and Hy-2 than normals, (ii) labelled nuclei may occur in clusters in the hyperplastic strains, (iii) labelled
nuclei may be found in any of the layers of a multilayered epithelium in the hyperplastic strain but the intensity of labelling is greater in the outermost layers, (iv) hyperplastic epithelial cells which show signs of differentiation into fibres have not been observed to show thymidine uptake, (v) labelled nuclei may be found occasionally in the elongating cells of the annular pad, a region normally considered to be entering the terminal phase of differentiation.

Table 1. [$^3$H]thymidine incorporation, 3- h pulse

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<th>counts/min/lens</th>
<th>% of normal</th>
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<tr>
<td>Normal</td>
<td>5198</td>
<td>100</td>
</tr>
<tr>
<td>Hy-1</td>
<td>9174</td>
<td>155</td>
</tr>
<tr>
<td>Hy-2</td>
<td>17965</td>
<td>304</td>
</tr>
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</table>

Fig. 8. Autoradiographs of Hy-1 lenses labelled with [$^3$H]thymidine. A high proportion of nuclei show incorporation. (B) Showing that the outer layer, which is folded inwards at the top of the field is active, while the inner layer is not. (A) Shows that in some lenses active nuclei may be found in all layers. × 640.

RNA synthesis. The incorporation of [$^3$H]uridine into the lenses of normal, Hy-1 and Hy-2 strains in organ culture is shown in Table 2. The Hy-1 strain consistently showed a higher total rate of uridine incorporation in all experiments and showed relatively little variability. However, Hy-2 was very variable, showing values for total incorporation falling on either side of the normal controls, depending on the batch of chicks taken. The degree of variability of the hyperplastic strains in this respect is still being investigated. When the distribution of
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radioactivity between different cellular fractions was investigated at various times after a 1-h pulse of \([^{3}H]\)uridine in organ culture, the pattern of RNA synthesis again distinguished strains Hy-1 and Hy-2 from each other and from the normal lenses (Fig. 9). The initial incorporation was principally into the

Table 2. \([^{3}H]\)uridine incorporation into total RNA, 3-h pulse (mean value of four experiments)

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<thead>
<tr>
<th></th>
<th>counts/min/lens</th>
<th>% of normal</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>31995</td>
<td>100</td>
</tr>
<tr>
<td>Hy-1</td>
<td>44793</td>
<td>140</td>
</tr>
<tr>
<td>Hy-2</td>
<td>32635</td>
<td>102</td>
</tr>
</tbody>
</table>

Fig. 9. Time course of uridine incorporation into cell fractions prepared from lenses of normal and hyperplastic (Hy-1 and Hy-2) strains of chick. Lenses were incubated in \([^{3}H]\)uridine for a pulse of 1-h duration and were transferred to unlabelled medium for the times indicated. Incorporation was measured in nuclear (N), polysomal (P) and supernatant (S) fractions.

nuclear fraction in all strains with Hy-1 showing much greater activity than the normal, and Hy-2 less (Fig. 9). Passage of labelled material out of the nucleus was more rapid in Hy-1, with a corresponding increase in the rate of labelling of polysomal and soluble fractions. The proportion of cytoplasmic (polysomal and soluble) to nuclear counts in the three strains and following different durations of chase is shown in Fig. 10. This clearly shows the relatively low
proportion of cytoplasmic radioactivity which is initially shown in strain Hy-1, followed by a high rate of transfer of labelled RNA to the cytoplasm compared with the normal lenses. Lenses of strain Hy-2 birds were intermediate between Hy-1 and normal with respect to these parameters. Autoradiographs (Fig. 11) show that while uridine incorporation in normal birds occurs in epithelium and annular pad and, to a lesser extent, in young fibre cells, in Hy-1 and Hy-2 an

![Graph showing time course of transfer of radioactive RNA from nucleus to cytoplasm.](image)

Fig. 10. Time course of transfer of radioactive RNA from nucleus to cytoplasm. Incorporation into cytoplasmic (polysomal + supernatant) fractions is expressed as a proportion of that in the nuclear fraction at intervals after labelling with a 1 h pulse of [3H]uridine into lenses of normal and hyperplastic (Hy-1 and Hy-2) strains of chick.

abrupt change in the intensity of labelling of the cell sheet is shown in lenses with multilayered epithelium. Layers close to the surface are always more active than deeper layers, while if the epithelial sheet is folded or doubled back, those regions of it which are folded outwards are more active in uridine incorporation than those which are folded inwards. The discontinuities of uptake of uridine do not appear to be due to limited penetration of the precursor to the deeper-lying cells of the lens, since other low-molecular-weight precursors, such as
thymidine and proline, may be incorporated in epithelial cells further from the surface (Figs. 8, 13).

*Protein synthesis.* The incorporation of amino acids in lenses of the three strains is compared in Table 3. This shows that lenses of Hy-1 are more active in soluble protein synthesis than are lenses of normal birds and that all of these strains also differ in the synthesis of cell membrane material. The distribution of radioactivity among the several crystallin subunits has been reported (Eguchi et al. 1975) and is also the subject of a more detailed paper (Clayton, Truman & Hunter, in preparation). In the folded epithelium, layers comprising

![Figure 11](image_url)

**Table 3.** [3H]amino acid incorporation into soluble protein and membrane fraction in 3-h pulse

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<tr>
<th></th>
<th>counts/min/lens</th>
<th>% normal</th>
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<tbody>
<tr>
<td><strong>Soluble protein</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>19848</td>
<td>100</td>
</tr>
<tr>
<td>Hy-1</td>
<td>24290</td>
<td>122</td>
</tr>
<tr>
<td>Hy-2</td>
<td>15988</td>
<td>80</td>
</tr>
<tr>
<td><strong>Membrane fraction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1231</td>
<td>100</td>
</tr>
<tr>
<td>Hy-1</td>
<td>1409</td>
<td>114</td>
</tr>
<tr>
<td>Hy-2</td>
<td>1046</td>
<td>85</td>
</tr>
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cuboidal cells label more densely in autoradiographs than hyperplastic layers whose cells have begun to elongate (Fig. 12).

**Proline incorporation.** Autoradiographs show that the most active proline incorporation is found in the anterior epithelium both in normal and in hyperplastic lenses. Hyperplastic lenses show marked heterogeneity, the incorporation varying sharply as the epithelium folds. The areas of high and low incorporation do not necessarily bear a relationship to the distance from the capsule and therefore do not seem to be attributable to problems of accessibility of the tracer (Fig. 13).

![Fig. 12. Autoradiographs of lenses labelled with [3H]amino acid mixture. (A) Normal lens showing highest level of incorporation in the anterior epithelium, gradually diminishing over the annular pad. (B) Hy-1 lens. The outer of the two epithelial layers is more active in incorporation, but a loop of cells pushed inwards from this layer is also very active, suggesting that distance from the surface cannot be the sole consideration in the regulation of incorporation. × 320.](image)

**Electron microscopy.** The structure of a proportion of the cells in the lentoid bodies has been shown to correspond closely with *in vivo* cortical fibres (Okada et al. 1973). A comparison of the lentoid bodies in Hy-1 cultures with those in normal cultures indicated that in the former differentiation had proceeded further in that they contained more cells displaying the features of cortical fibres (Eguchi et al. 1975). Other ultrastructural features of the cultures warrant attention in order to supplement the data in this report.

The differentiated cells, characterized by a homogeneous cytoplasm and absence of organelles (Fig. 14A), generally occupied the central regions of the lentoid bodies. They formed close contacts with each other over extensive areas where the intercellular space was approximately 10 nm in width. On either side and below they were surrounded by cells which were comparatively
Fig. 13. Autoradiographs of lenses labelled with [3H]proline. (A) Normal lens. Incorporation is high in the anterior epithelium and low in the annular pad. (B) Hy-1 lens. Low-power view showing buckled epithelium with extremely irregular distribution of incorporation. (C) High-power view of Hy-1 lens showing individual variation in the intensity of incorporation amongst neighbouring cells in the epithelial sheet. (D) Hy-1 lens, showing a small group of annular pad cells in which the nuclei have become displaced to the inner end of the cells. These cells incorporate much less than their neighbours. A and B × 320, C and D × 640.
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undifferentiated and similar in appearance to epithelial cells in early phases of outgrowth. These peripheral cells contained small, spherical mitochondria, small amounts of endoplasmic reticulum and traces of Golgi elements dispersed in a moderately compact cytoplasm (Fig. 14B). Similar unmodified cells, but with less compact cytoplasm, formed the uppermost layers in those regions remote from the lentoid bodies where the cultures were more than one cell layer in depth. Throughout the culture the lowermost layer, adjacent to the substratum, consisted of fibroblast-type cells (Fig. 4C) possessing well-developed systems of the organelles already described, sub-membranous arrays of microfilaments and electron dense bodies which are probably autophagic structures. The fibroblasts closely resemble the cells described in non-differentiating cultures of calf lens epithelial cells (Van Venrooij, Groenveld, Bloemendal & Benedetti, 1974). Cells with characteristics intermediate between these three general categories also occurred in various regions.

An extracellular space containing quantities of fibrillar material separated the fibroblast layer from the lentoid body proper (Figs. 14D, 15) except at the centre of the body where cell continuity with the substratum was maintained. The space was up to 8 μm in depth immediately below the lentoids and tapered off beyond their margins. Although the fibrils were mainly confined to the sub-lentoidal cavities some were also observed amongst the cell sheets between the lentoids. The fibrils probably represent a form of collagen that is normally incorporated into the lens capsule and has been shown to be secreted by lens epithelial cells in culture (Grant, Kefalides & Prockop, 1972).

No differences in structure were detected between the two strains apart from the larger size of the Hy-1 lentoid bodies with correspondingly larger pools of fibrillar material.

DISCUSSION

During the normal process of development of the chick the incidence of mitoses in the lens epithelium falls steadily, especially from the central anterior epithelium, until mitosis is confined to an annular zone (the germinal zone) (Hanna & Keatts, 1966; Modak et al. 1968). After mitosis in the germinal zone the cells enter a region where they start to elongate (the annular pad) and then become fibres in the body of the lens. The accretion of new cells by mitosis must

FIGURE 14
Electron micrographs illustrating the structural characteristics of the cells in 12-day cultures of lens epithelial cells from normal and Hy-1 strains. (A) Differentiating fibre cells with peg-like interdigitations at the centre of a lentoid. Polysomes are scattered in the compact, homogeneous cytoplasm. (B) A comparatively undifferentiated cell at the periphery of a lentoid is distinguished by the presence of mitochondria and endoplasmic reticulum. (C) A fibroblast in the region between the lentoids. It contains prominent elements of the Golgi apparatus and endoplasmic reticulum. (D) Accumulations of extracellular fibrils below a lentoid. × 30000.
As Fig. 14. A section perpendicular to the substratum (bottom left), to show the layer of extracellular fibrillar material (F) between the lowermost layer of the fibroblast cells and the overlying cells at the periphery of a lentoid. × 19000.
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be related to the rate of accretion of fibres, which are normally formed from cells in the terminal state of differentiation, posterior to the germinal zone, in such a manner that the epithelial layer always remains as a single smooth cover on the anterior face of the lens. This process appears to be universal in the vertebrate lens (Hanna & O'Brien, 1961; Mikulicic & Young, 1963; Papaconstantinou, 1965; Hanna, 1965; Modak et al. 1968).

It has been pointed out elsewhere that a frequently observed conformation of the day-old lens of Hy-1 and Hy-2 is that of a hyperplastic epithelium on a more or less normal lens body. Thus the earliest stages of development, when the mitotic rate in the normal chick is high and involves the whole epithelium, are unaffected and it would seem that the abnormality mainly affects the later stages when the incidence of mitosis normally drops (Clayton, 1975).

The strains Hy-1 and Hy-2 are genetically unrelated: Hy-1 is an inbred strain and Hy-2 the F₁ between two inbred strains. Both have been subjected for several years to especially stringent selection for high early growth rate and both have responded by a failure to regulate mitosis in the lens epithelium (Clayton, 1975).

This failure to regulate mitosis appears from autoradiography to involve the retention of a capacity for mitosis in the epithelium, except for those cells differentiating into fibre like cells. The apparent uptake of [³H]thymidine into occasional nuclei in the annular pad region is unlikely to be associated with cell division in vivo, although slightly elongated annular pad cells can round up and grow in tissue culture (Eguchi, unpublished). It may, however, indicate possible endoploidy in some cells. Evidence to this point is conflicting: Modak et al. (1968) and Persons & Modak (1970) found no evidence of DNA synthesis in the annular pad, but Hanna & Keatts (1966) and Kvinikhidze (1966) reported DNA synthesis in some of the elongated cells below the germinal zone.

There is some evidence in the literature that the rate of cell multiplication may be affected by the genotype and a little evidence suggesting that cell surface modifications may be associated with mitotic rate. Stone, Freedman & Fregin (1964) studied the genetic composition of the population of red cells in non-identical cattle twins. Such twins are red cell chimaeras, each twin having a similar mixture of cells of both genotypes, but with the proportions changing over a period of time. One of the two genotypes is always the 'successful' one, irrespective of the genotype of the host: this implies that the efficiency of replication of the cell lines is different and that the rate is directly related to the genotype of the stem cells.

Similar findings were reported for lymphocytes in AKR-CBA mouse chimaeras by Tuffrey, Barnes, Evans & Ford (1973), the AKR cells always coming to predominate. Of three possible explanations for this phenomenon, that of an intrinsic difference in the rate of cell multiplication is offered as the most likely.

In this paper we present evidence that lens cells from the two strains of chicks
have an intrinsic difference in mitotic potential and in cell surface properties. In the absence of Mendelian information we cannot yet say whether these are two manifestations of the same gene.

However, even if cell surface differences and differences in mitotic behaviour occur together it may be necessary to exercise caution before assuming a connexion between them. A genetic difference in the rate of multiplication of spleen cells from mice selected for high and low levels of antibody production was demonstrated by Biozzi et al. (1971), who transferred these lines of cells into identical irradiated recipients. In this case, while the lines differed in their H_2 loci, there is no evidence that these markers were specifically associated with the growth rates observed. Ede & Agerbak (1968) showed that homozygous talpid^a (ta^a) chick limb mesenchyme cells have different surface properties from cells of normal chicks when compared in cell culture. There is also a difference in mitotic behaviour between cells of these genotypes (Ede & Flint, 1972), but the evidence here points rather to differential sensitivity to trypsinization and probably also to different stages of chondrogenesis.

The metabolic studies reported here show that the increased rates of cell division in Hy-1 and Hy-2 do not appear to be mediated by the same mechanism, since the rates of thymidine and of uridine incorporation differ markedly between them; the rate of synthesis of the different classes of RNA in different cell fractions also distinguishes these two lines. The most striking distinction between these strains lies in the rate of transfer of RNA from nucleus to cytoplasm. Studies of the levels of incorporation of amino acids into each of the isolated crystallin subunits show that the synthetic profiles are distinct (Eguchi et al. 1975) as is the response of each subunit to actinomycin-D (Clayton, Truman & Hunter, in preparation).

The differences between these strains in the regulation of nucleic acid and protein synthesis appear to be complex. It is hoped to study these peculiarities of metabolism further by comparing lenses of different ages and under conditions which favour or inhibit mitosis.

The propensity of the epithelium to show precocious differentiation in culture and to differentiate into short fibre-like structures on the anterior face of the lens in vivo, points to a capacity in these cells for a ready entry into the terminal differentiated state: either they are abnormally responsive to the appropriate signal or they are offered such a signal abnormally readily. Nevertheless, it is not excluded that a signal to differentiate is generated by the proximity to the iris of cells just posterior to the zone of maximum cell multiplication, and that, although these cells are then pushed into a more anterior position on the lens by further cell division, the exposure to a retinal signal has been sufficient. However, the occurrence of cells undergoing DNA synthesis in other regions of the epithelium, and especially in association with local epithelial loops, suggests that not all the excess epithelium in these lenses is generated in the zone near the iris. Only epithelial sheets which show cellular elongation or which show
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cataractous changes fail to incorporate thymidine. Since lentoids do not form in cultures of lens epithelium until confluence is achieved (Okada et al. 1971) and Hy-1 cells form aggregates very rapidly after dissociation, their precocious differentiation into lentoids compared to epithelium from normal lenses (this paper, and Eguchi et al. 1975) may be explained in terms of triggering of fibre formation by extensive cell contact. In support of this hypothesis is the observation that cells in culture show an increase in the concentration of crystallins after contact with other cells has been achieved around most of the periphery, while in vivo the epithelial cells on the anterior face of the lens which show elongation are those which have contact with other epithelial cells on all surfaces, the outermost layer always remaining undifferentiated. Similarly, observations with the electron microscope on cell cultures show that the closely associated cells at the centre of the lentoids have differentiated into fibres, whereas those in contact with the culture dish have reverted to a generalized fibroblast cell type. This massive cellular contact is in its turn probably made possible by altered cell surface properties, as evidenced by the changed plating efficiency, rapid formation of cellular aggregates in vitro, and the mutual invasiveness of lens and iris which is sometimes observed in vivo. Invasion of lens by iris or iris by lens, which may occur together in Hy-1 (Clayton, 1975) are abnormalities not hitherto reported in ophthalmic literature.

Consequent upon the abnormal morphology associated with these modified cell properties, epithelial cells occur in atypical positions. This appears to affect their metabolism, as shown by the pattern of incorporation of uridine, amino acids and proline in autoradiographs. The activity of a cell is not always similar to that of its sister cells on either side in the continuous epithelial sheet, but may differ sharply as the sheet folds and appears to be more related to its position relative to the fibres underneath and the capsule above. Some of this metabolic microheterogeneity in the cell population may persist, since clones established from single epithelial cells of normal lens have fairly similar growth rates in the early stages of cell culture, while those established from single cells of Hy-1 are of two kinds: clones with growth rates within the same range as those from normal lenses and those with a growth rate which is considerably higher (this paper, and Eguchi et al. 1975). Microheterogeneity in the cell population associated with cell position may be a general feature of lens cells. It appears to arise in cell cultures of lens epithelium with respect to the cell types, characterized by electron microscopy, and to anodal and cathodal β-crystallins from both normal and Hy-1 lenses in condensates in the confluent sheet. Pigmented epithelium of the chick retina can transform in cell culture giving rise to lentoids (Eguchi & Okada, 1973). Such lentoids also show the same arrangement of β crystallins as do those formed from lens epithelium (Eguchi & Clayton, unpublished).

The ultrastructural studies confirm the difference in the rate of differentiation between normal and Hy-1 epithelial cells in culture: the Hy-1 cells, however, appear to be structurally normal.
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