Genet. Res., Camb. (1975), **25**, *pp.* 79–82
With 2 plates
Printed in Great Britain

SHORT PAPERS

Failure of growth regulation of the lens epithelium in strains of fast-growing chicks

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(*Received* 17 *April* 1974)

SUMMARY

Two strains of poultry selected for high growth-rate were found to have an anomalous lens morphology indicating a failure of the normal process of growth regulation. The implications for lens fibre differentiation are discussed, as are the implications for genetic selection for growth-rate.

INTRODUCTION

It is known that stringent genetic selection over a number of generations for a trait such as high growth-rate eventually leads to reduced fitness, while the rate of response in the desired character to selection diminishes. In recent years, stringent selection of animals of economic importance has been based on growth-rate and on viability; thus lines of animals, especially in poultry, have been established which have growth-rates far in excess of the normal (Clayton, 1972).

In the course of work on the crystallins of the chick, several lines of birds have been used. Of these, two unrelated lines of chicks, both strongly selected for high growth-rate, were found with abnormalities of the lens epithelium of a kind not hitherto reported in ophthalmic literature, and which appear to be caused by an unregulated and continuing growth of the lens epithelium. One of these lines (designated here as Hy-1) was originally derived mainly from Cornish game birds and has been strongly selected for not less than 20 years. The second (designated here as Hy-2) is an F_1 between two lines originating mainly from White Rock fowl, each of which has been selected separately for a similar period of time to Hy-1.

METHODS

Lenses of day-old chicks of various strains were fixed in Bouin's fixative. Paraffin sections were cut, and stained with haematoxylin and eosin. The lens shown in Plate 2E was processed by freeze-substitution, sectioned, treated with fluorescent antisera to lens proteins and photographed by ultraviolet microscopy before being stained with haematoxylin and eosin as in Plate 2F.

RESULTS AND DISCUSSION

The normal lens of the day-old chick has an anterior epithelial layer (Plate 1A) with mitosis confined to a germinal zone just anterior to the equator of the lens. Behind the germinal zone the cells elongate, forming the annular pad, and then differentiate into fibres, moving inwards into the body of the lens as they do so (Hanna & Keatts, 1966) and enclosing the mature fibres formed at an earlier stage of development.

Genetic cataracts of the lens have been rarely reported for birds (Krehbiel, 1972): no

other type of genetic lens defect has been described. In the chick strains reported here, at present referred to as hyperplastic 1 (Hy-1) and hyperplastic 2 (Hy-2), the defect occurs in all lenses so far examined. It is variable in expression but always involves an increase in the number of epithelial cells, which remain as a coherent sheet. (Genetically determined hyperproliferation of the lens epithelium has been reported in mammals, by Cogan & Kuwabara (1962), Clayton & Campbell (1968), Zwaan & Williams (1969), and Pierro & Spiggle (1970). In all these cases the cells form nodules or masses of undifferentiated cells and are never organized as continuous sheets.) The excessive growth in Hy-1 and Hy-2 appears to originate in the germinal zone (Plate 1 B) and in more extreme cases the excess epithelium is pushed between the fibres below and the capsule and original anterior epithelium above, forming multiple-layered (Plate 1 C, D)) or buckled epithelia (Plate 1 E, F). Rarely, parts of this growth appear to consist of strings of nuclei with little or no visible cytoplasm around them (Plate 2 A, B). The outermost epithelial layer remains normal but the cells of the inner layers may partially differentiate into short fibre-like cells which elongate perpendicular to the plane of the epithelial sheet and are therefore not oriented with respect to the body of the lens (Plate 1 E, F). Although lens fibres are thought to be normally induced in lens epithelial cells by proximity to the retina, these fibres are on the corneal side of the lens, which is incapable of forming fibres either in normal or rotated lenses (Coulombre & Coulombre, 1963). Thus the normal *in vivo* stimulus to differentiation appears to be replaced by some other stimulus in those cells which are in contact with others on their upper and lower surfaces as well as laterally. Okada, Eguchi & Takeichi (1971) find that groups of cells in a fully confluent tissue culture of chick lens epithelium differentiate into fibres in the absence of retina or other inducing tissues. Thus the nature of cell-to-cell contact may be important for fibre differentiation, and there is evidence from the behaviour of lens epithelial cells from the

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PLATES 1 AND 2

All lenses shown in Plates 1 and 2 are from day-old chicks and are stained with haemotoxylin and eosin, except for Plate 2 E. The orientation of the lenses are as follows: anterior face of the lens towards top left of photograph in Plates 1 A–E and 2 A–C and F, to centre in 1 F and G, top right in 2 D and E and bottom in 1 H.

PLATE 1

(A) The mitotic zone (germinal zone) of the lens epithelium and the anterior pad region of a normal lens from a control strain.

(B) Lens from strain Hy-2, showing the minimal degree of hyperplasia found. The epithelium is doubled back in the region of the mitotic zone.

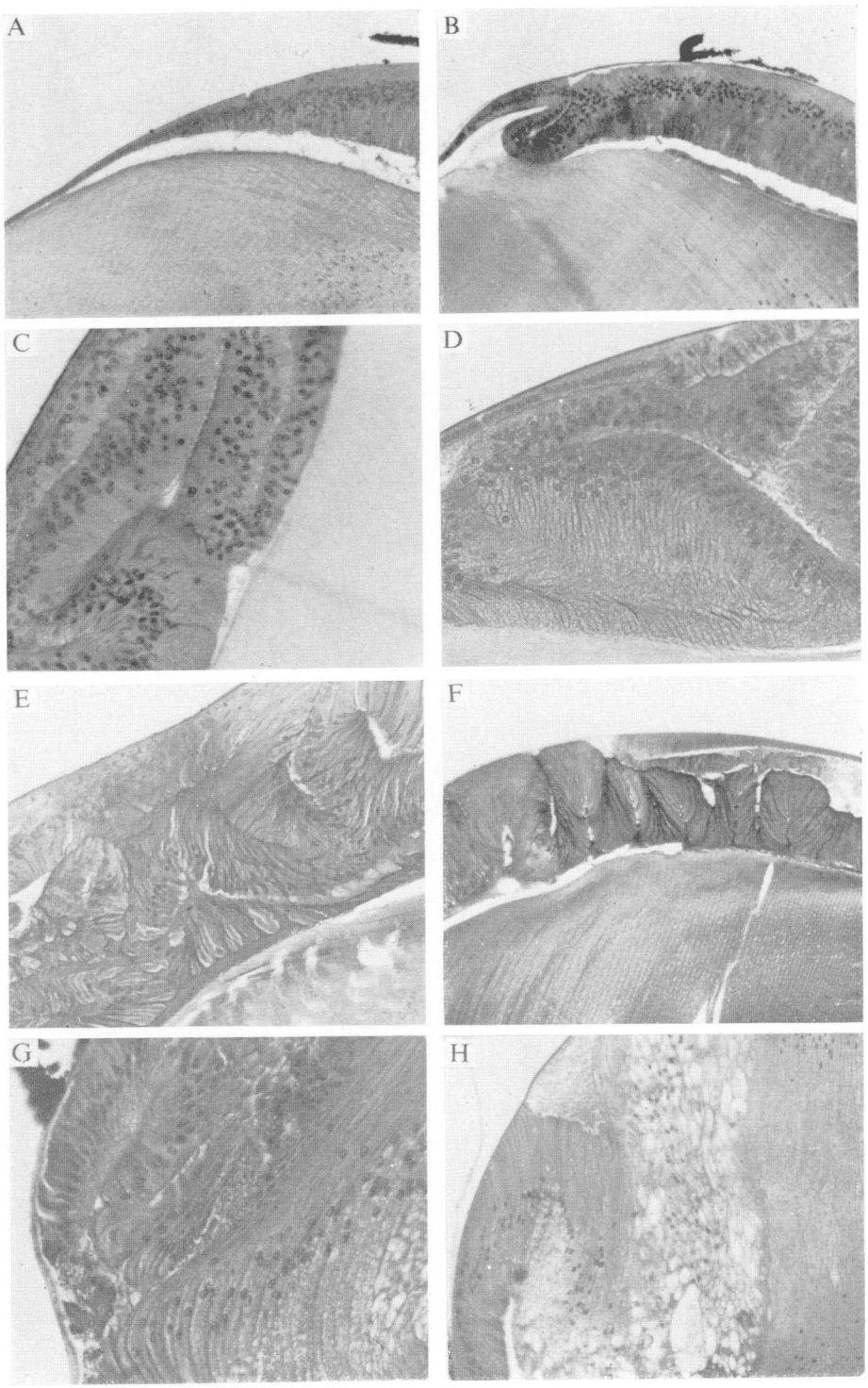
(C) Hy-2 lens in the central anterior region, showing where the multiply-folded epithelium from the periphery meets in the mid-zone. The outermost epithelial layer is normal. The four inner folds show some cellular elongation.

(D, E) Hy-2 and Hy-1 respectively, showing stages in the formation of fibre cells from the hyperplastic epithelium.

(F) The buckled epithelium has differentiated into short fibre cells over the anterior face of the lens. A section of normal outer epithelium is visible.

(G) Annular pad and germinal zones of Hy-2 showing cataractous transformation of the hyperplastic tissue.

(H) The zone of transformation of annular pad cells into cortical fibres (Hy-1). The distortion of the lens bow (the 'line' formed by the nuclei of elongating and young fibres cells) is visible (cf. Plate 2C). The most recently formed fibres, adjacent to the annular pad, are swollen anterior to the nuclei, while the young cortical fibres further in are degenerate over their whole length. Older (more central) fibres are relatively undamaged.



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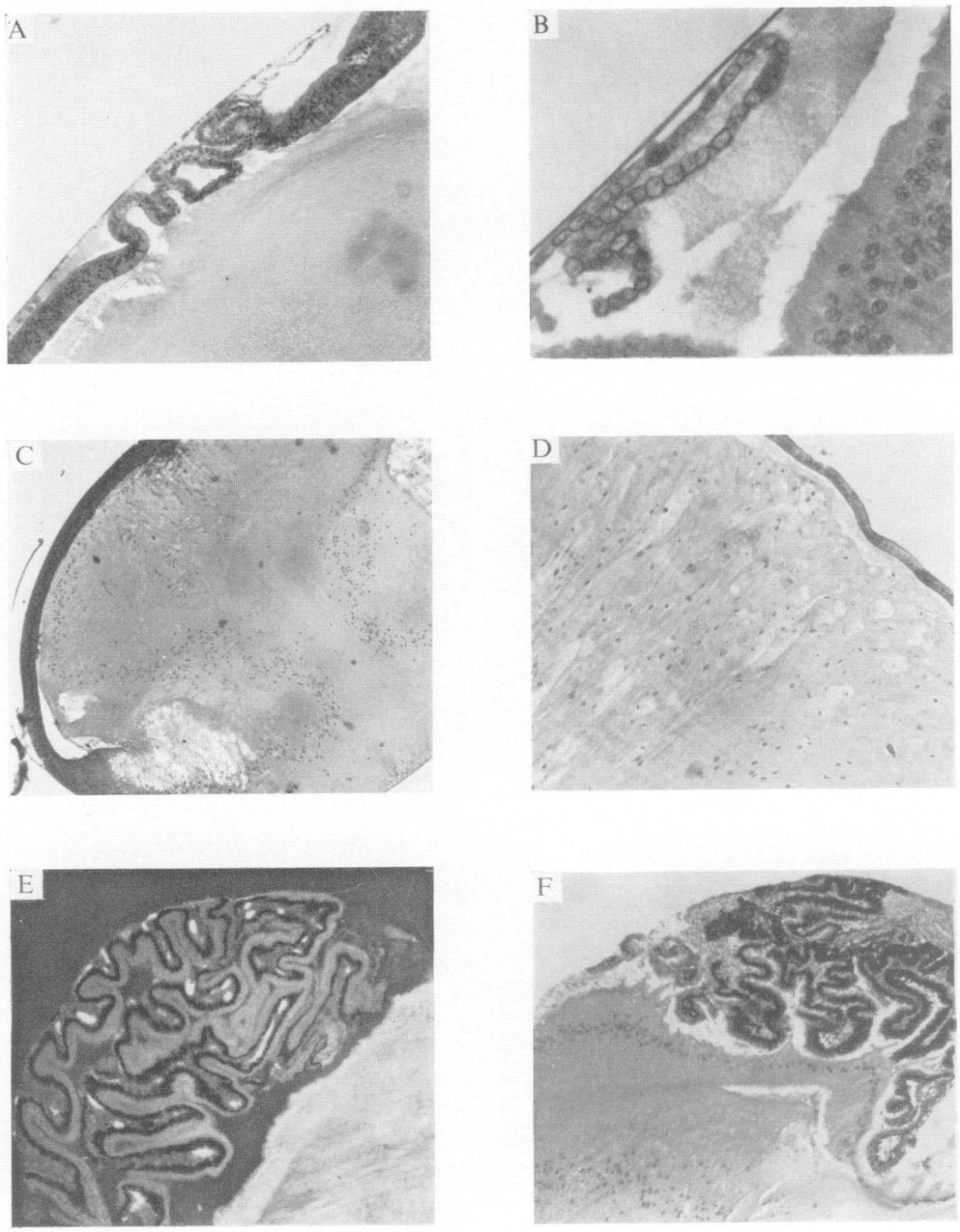
(*Facing p.* 80)

https://doi.org/10.1017/S0016672300015457 Published online by Cambridge University Press



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https://doi.org/10.1017/S0016672300015457 Published online by Cambridge University Press

Hy-1 strain compared to epithelial cells from non-broiler strains that these cells have modified cell-surface properties. They are strongly mutually adhesive and have a higher intrinsic growth rate and propensity to precocious *in vitro* differentiation (Eguchi, Clayton & Perry, in preparation). Further evidence of anomalous cell-surface properties in strain Hy-1 is provided by the rare occurrence of growths in which lens and iris tissue mutually interpenetrate each other (Plate 2E, F).

The presence of a normal lens body in strains Hy-1 and Hy-2 indicates an absence of developmental anomalies over the early embryonic period, when the incidence of mitosis in the entire lens epithelium is normally high (Modak, Morris & Yamada, 1968). In normal lenses, the anterior epithelium gradually ceases mitosis and only the cells in the germinal zone continue to divide. Thus the anomaly in these strains seems to be in a failure to regulate the mitotic rate in the germinal zone and a failure to contract the mitotic area down to the normal dimensions. Autoradiographic studies show that nuclei in any part of the Hy-1 epithelium may still be able to sythesize DNA (Clayton, Eguchi, Truman, Perry, Jacob & Flint, in preparation). Differences between normal strains and the Hy-1 and Hy-2 strains in the relative rates of synthesis of the specific proteins are also observed (Clayton, Truman & Hunter, in preparation).

These two lines, and several other lines of chicks selected for growth rate, have been observed to have the occasional cataract, especially ring cataracts (Plates 1E, 2C, D). In these cases the fibre nuclei persist. Nuclei become pycnotic in young fibres in the normal chick lens and are lost from the older central fibres after fragmentation of the DNA (Modak & Perdue, 1970; Persons & Modak, 1970). In these cataracts the nuclei are displaced posteriorly in the cortical fibres and advance to an extreme anterior position in the central fibres (Plate 2D). Thus this persistence seems to imply either failure of full fibre maturation or a deficit of nucleases. It would appear then that the processes of growth and differentiation are disturbed in these lenses, and there is other evidence that some fast growing birds may be susceptible to such disturbances.

Spontaneous tumours are rare in very young chicks of non-broiler lines but are observed in broiler birds of less than 10 weeks old at an apparently 50-fold increase in incidence (Campbell & Appleby, 1966); these tumours were classified as embryonal, hamartomatous or teratomatous. Almost half of their series were in skin and subcutis, liver and nervous tissue. They suggested that growth and differentiation were possibly showing signs of incoordination in these intensively selected, intensively fed birds. Selection pressure for growth-rate has continued since this work was published and mean performance values are higher today (G. A. Clayton, personal communication). While the system of rearing may contribute to accelerated growth post hatching, and therefore be significant for post-hatching tumours, this is unlikely to account for the eye anomalies

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PLATE 2

(A) Hy-1. Here the additional growth has given rise to a distorted sheet of epithelium and a continuous strip of nuclei or cells with minimal cytoplasm.

(B) Higher magnification of part of the lens in (A).

(C) Cataractous lens with persistent lens bow, suggesting incomplete fibre differentiation. The cataractous zones include a small anterior polar region and the youngest cortical fibres.

(D) A more fully cataractous lens, in a third strain; with a tendency to cataract.

(E, F) A lens from Hy-1 showing mutual invasion of lens and iris. (E) shows part of the iris with inclusions of lens epithelial cells which are brightly fluorescent with labelled antiserum to lens proteins. Part of the lens is to the right.

(F) The same lens, showing the penetration of iris into the body of the lens and fingers of lens between the folds of the iris.

described here which arise *in ovo*. The eye is one of the fastest growing structures in early development and may therefore be a particularly sensitive indicator of incipient dysgenesis.

I am grateful to Mr J. Archibald and Mr A. Gristwood of Ross Poultry Ltd., and to Dr T. C. Carter of the Poultry Research Centre for day-old chicks of various strains. I would like to thank Mr A. Burns, Dr J. C. Campbell, Dr J. G. Campbell, Dr G. A. Clayton, Dr G. Eguchi, Dr D. E. S. Truman and Dr P. A. L. Wight for useful discussions, Mr A. Gillies and Mr D. Easton for histology, and Miss V. Andrews for processing the lens in Plates 2E and F. The work was supported by the Cancer Research Campaign.

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