

The Effects of Steroids on the Human Lens Epithelium

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Summary

A study of anterior capsules from cataractous lenses revealed that there are marked abnormalities in epithelial structure associated with cataract. In certain cases a distinctive reticulated pattern was observed in whole mounts of the anterior capsule and of these a higher number than expected were from patients receiving steroid medication. In tissue culture experiments it was found that the presence of steroids in the growth medium (10 μ M prednisolone) adversely affected the growth of human lens epithelial cells.

These observations are consistent with the hypothesis that steroid-induced cataracts are the result of effects on anterior lens epithelial cell function.

Steroids are thought to induce cataract.¹ This study was undertaken to test the hypothesis that steroids cause cataract by interfering with the normal functioning of the anterior lens epithelium.

Material and Methods

Human anterior capsules with adherent epithelial cells were obtained from clear, normal human lenses removed from fresh (<48 hours post mortem) donor eyes used for corneal transplantation. Lenses showing any cataractous changes were discarded.

Anterior capsules from cataractous lenses were obtained during extracapsular cataract surgery. Pre-operatively, data concerning the patient's medical history were collected, and details of steroid medication (if any) recorded.

The patient was examined by slit-lamp biomicroscopy and the morphology of the cataract recorded. The type and extent of the cataract were classified as described by Karim, Jacob and Thompson.²

Surgery was performed using a standardised technique by the same surgeon in all cases. A superior *ab externo* incision was made of partial

thickness at the limbus and the anterior chamber filled with Healonid through a small paracentesis. Capsulotomy was performed using a bent 23-gauge Brunswick needle. The lens capsule was divided by multiple small perforations just within the line of insertion of the zonules. The central capsule was removed in one piece with a pair of plain blocked forceps, the circular capsule (approximate diameter 6 mm) was then placed in Dulbecco's phosphate buffered saline.

The specimen of anterior capsule was spread on a poly-l-lysine coated microscope slide and fixed with Karnovsky's fixative (diluted to physiological osmolarity). After air drying, the specimen was stained with toluidine blue, washed with distilled water and dehydrated through graded alcohols finishing with xylene. The specimen was then mounted (DPX) and cover-slipped.

For tissue culture, normal lens epithelial tissue was obtained from human donor eyes used for corneal graft surgery within 48 hours of death of the donor. The eye was bisected equatorially behind the ora serrata and the lens removed by cutting the suspensory ligaments. Details of the explant technique and method of cell counting have been described previously.³ The explants were cultured

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in medium E199 with fetal calf serum (FCS), 50 per cent for the first 10 days, reduced to 36 per cent on day 10, 25 per cent on day 13, 10 per cent on day 16 and thereafter the cells were re-fed every three days with E199+10 per cent FCS. No antibiotics or antimycotics were used. The cultures were placed in a humidified, gassed (5 per cent CO₂) incubator and maintained at 37 degrees C. The outgrowth of cells was measured at specified intervals using a calibrated eyepiece graticule and phase contrast optics, and the number of cells determined as described previously.³

Results and Discussion

In the normal human lens the cells of the anterior epithelium exhibit a regular, hexagonal morphology with no visible intercellular spaces under light microscopy. The uniformity of both the cellular array and the degree of uptake of stain were most marked (Fig. 1). The density of cells increased from 4,450 cells/mm² in the central region to 5,500 cells/mm² in the equatorial region, reflecting the radial decrease in cell size.² In epithelia from normal lenses no significant changes were observed with ageing over the age range 23 to 84 years.

However it should be mentioned that some "normal" lenses from donors over 70 years old, with no history of visual impairment, had cortical opacities off the visual axis. The epithelia from these lenses exhibited the morphological features (often only peripherally) associated with cortical cataract (see below).

Distinct abnormalities in cell morphology, size and density have been observed in cataractous epithelia;² in particular in those cataracts involving cortical and/or posterior subcapsular opacities. Karim *et al.*² reported a significant decrease in cell density in these groups of cataractous epithelia due to the extensive appearance of enlarged cells. Figure 2 shows a whole mount of the epithelium from a lens with posterior subcapsular cataract (PSC). There is evidence of cell swelling, a disturbance to the regular hexagonal array and a non-uniformity of staining. Enlarged, non-staining cells were observed in 45 per cent of the cataractous epithelia studied by light microscopic examination of toluidine blue stained whole mounts. The absence of the basophilic stain toluidine blue in these

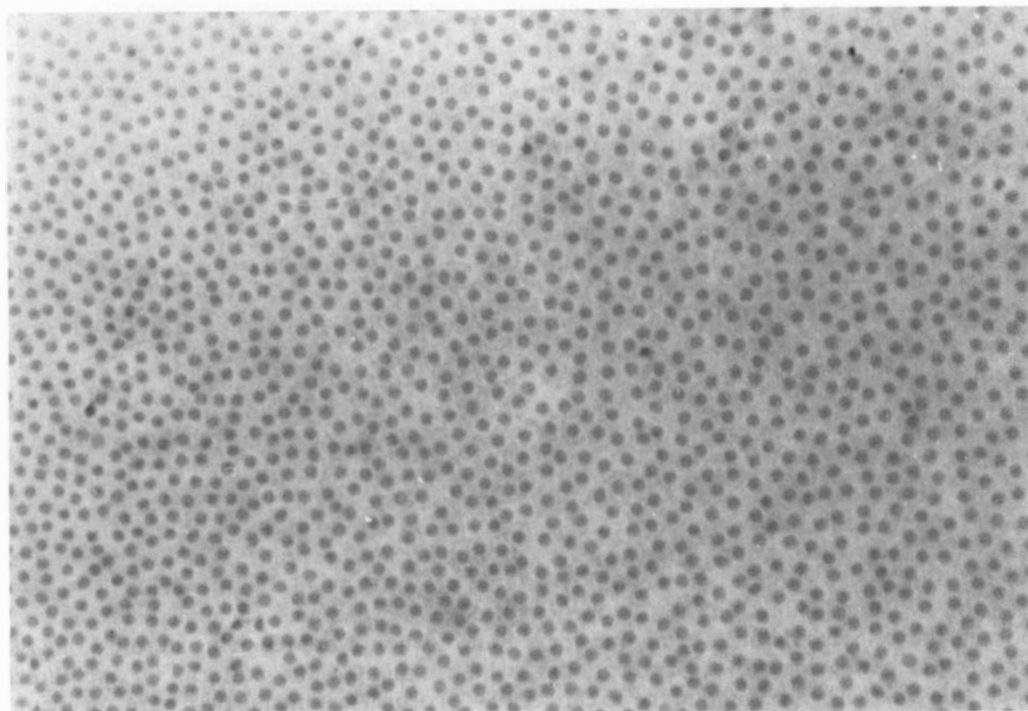


Fig. 1. Whole mount of an anterior capsule from a normal human lens, stained with toluidine blue, from a 63 year old donor eye (field width 1.1 mm).

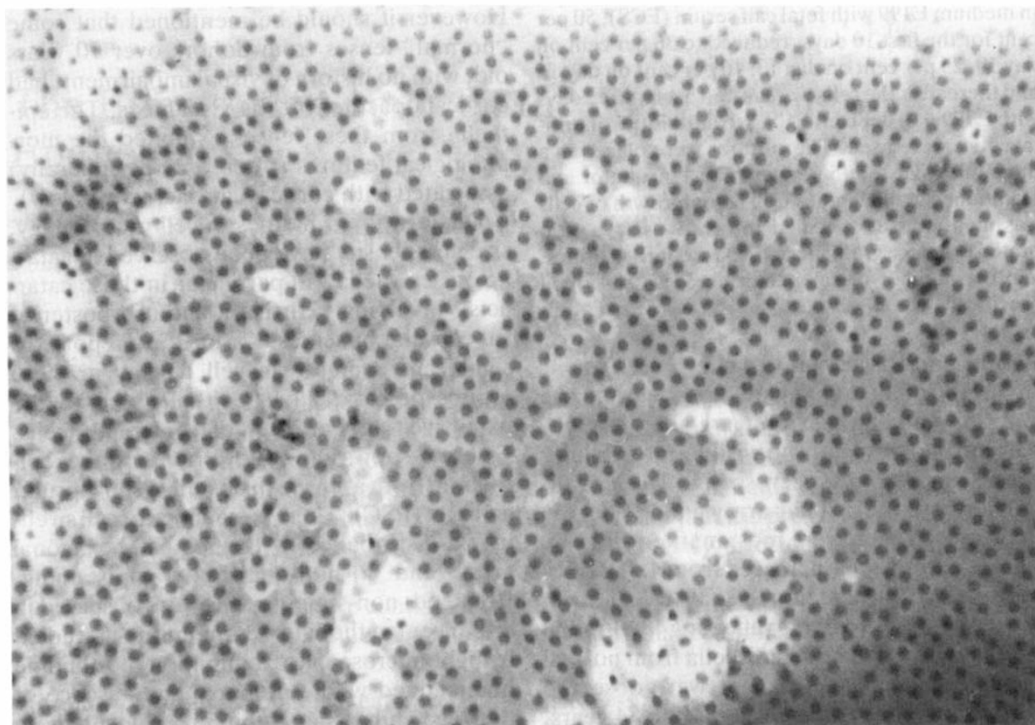


Fig. 2. Whole mount of a capsule from a lens with cortical and posterior subcapsular cataract, stained with toluidine blue (field width 1.1 mm).

cells indicates the loss of cytoplasmic components and this may be due to the permeabilisation of the cell membrane either caused by or resulting in cell swelling. The disruption of the regular hexagonal pattern was observed in most cataractous epithelia and was often confined to small regions within the epithelium, there being no apparent correlation between loss of this regular hexagonal pattern with cataract type. Often accompanying the appearance of enlarged cells, were intercellular clefts and vacuoles. The 13 normal lenses studied exhibited none of these characteristics.

Of the 41 cataractous epithelia studied thus far, 15 exhibited a distinctive reticulated pattern. This pattern, two examples of which are illustrated in Fig. 3, is the result of extensive intercellular uncoupling. Gaps appear between the cells, sometimes extending for some distance, leaving fibrous projections connecting cells across the gap. Of the 15 capsules exhibiting this pattern, a higher number than expected (11) were from patients receiv-

ing steroid medication. The proportion, 11/15, of those lens epithelia exhibiting the reticulated pattern to those cataract patients receiving steroids, compares with 4/26 for those exhibiting the reticulated pattern in the non-steroid group. On the basis of the chi-squared test there was a significant correlation ($P = 0.0002$; $\chi^2_1 = 13.77$) between the appearance of the reticulated pattern and the taking of steroids.

The relationship between posterior subcapsular cataract (PSC) and steroid medication has been well established^{4,5} (and see Ref 1 for a review). In our study, seven patients were classified as having pure PSC, three were receiving steroids and all exhibited the reticulated pattern shown in Fig. 3. PSC was more commonly associated with nuclear sclerosis and there were nine such mixed cataracts, four of whom were receiving steroids, one of whom exhibited the pattern. Of the three PSC+cortical cataracts, two were receiving steroids and both exhibited the pattern. The largest group of cataracts were those from

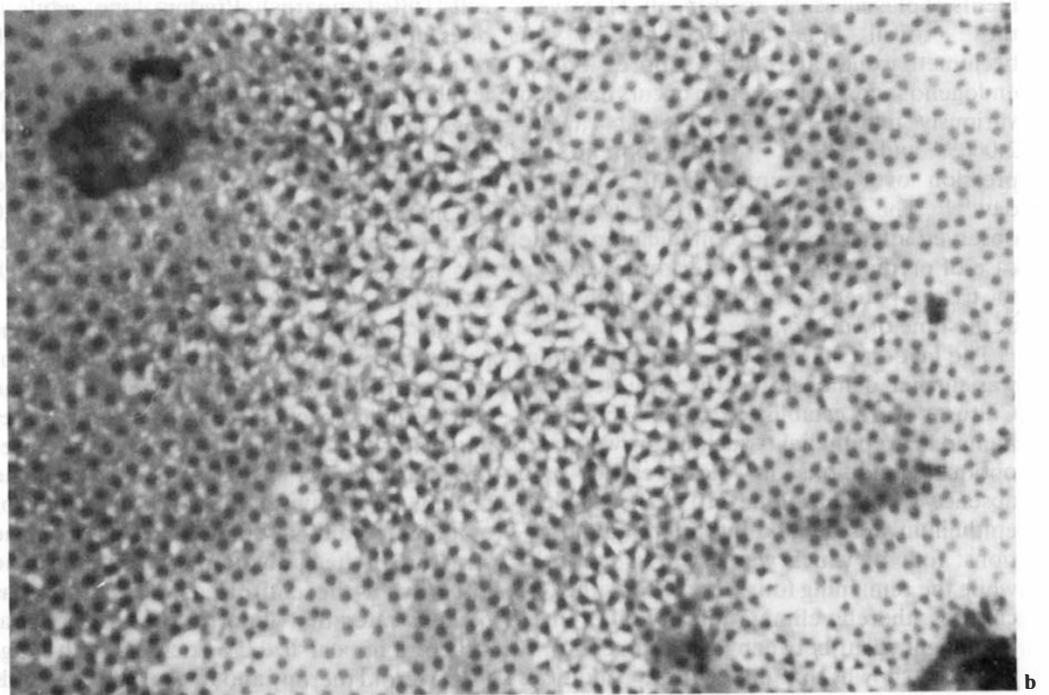
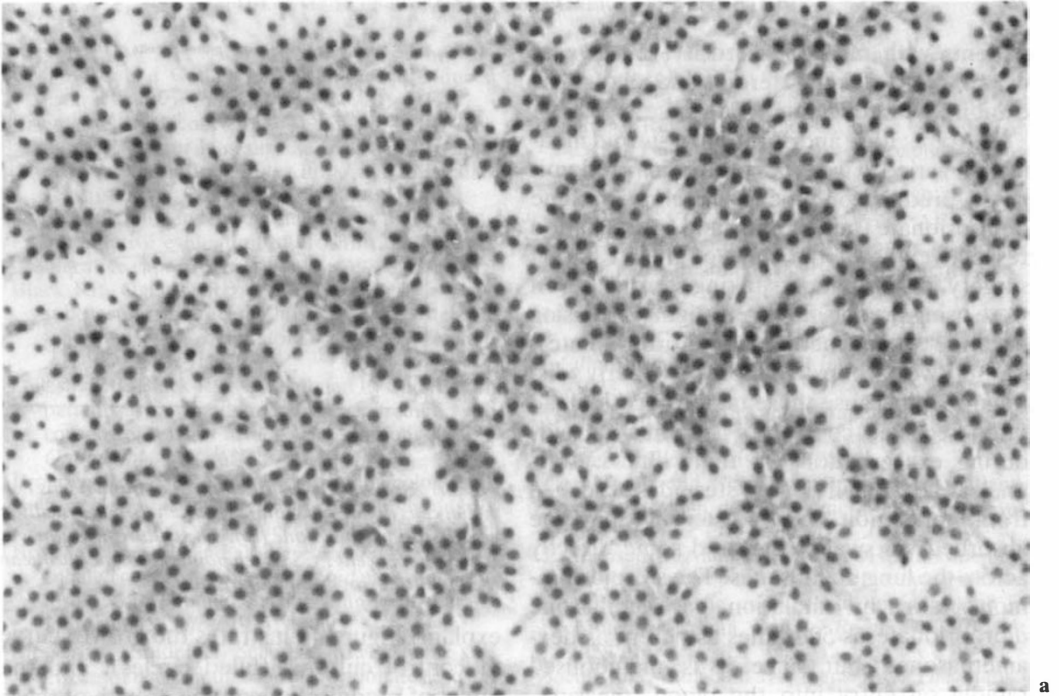


Fig. 3.

- (a) Whole mount of an anterior capsule from a lens with posterior subcapsular cataract. Note the reticulated pattern. This patient had a three year history of steroid medication. Stained with toluidine blue (field width 1.1 mm).
- (b) Whole mount of an anterior capsule from a steroid cataract illustrating another manifestation of the reticulated pattern shown in Fig. 3(a). Stained with toluidine blue (field width 1.1 mm).

patients with nuclear sclerosis (16), five of whom were receiving steroids and all of these exhibited the reticulated pattern.

The type, dose and duration of steroid medication varied. Two types of steroids predominated—prednisolone and beclomethasone dipropionate (taken orally and by inhalation respectively). At the time of their cataract surgery, of our patients taking steroids seven were using prednisolone and eight were using becotide (beclomethasone dipropionate). These two groups cannot be clearly distinguished because those taking becotide had often received courses of systemic steroids during exacerbations of their illness. It is unclear how much (if any) steroid is absorbed from inhalation. Some steroid may be absorbed via swallowing, and some directly across the lung membranes. The steroid commonly taken by inhalation, beclomethasone dipropionate, is 500 times as potent as dexamethasone,⁶ but since it is metabolised rapidly on entry into the blood, measurements of plasma levels are difficult. However, the critical *per diem* dosage of beclomethasone at which suppression of endogenous cortisol secretion occurs has been variously reported as 400,^{7,8} 800,⁹ 1,600,¹⁰ 2,000^{11,12} and 3,000 μg .¹³ Others report no suppression over the range 400 to 2,000 $\mu\text{g}/\text{day}$.^{14,15} The reticulated pattern was first observed after one year of medication in the case of prednisolone and after three years in the case of patients receiving beclomethasone at the time of surgery.

The fact that steroids effect the epithelium was further demonstrated by tissue culture. Cultures were established from explants of human lens epithelial tissue and grown under optimal conditions in the presence and absence of prednisolone (10 μM). The lens epithelium was divided into eight equal sections, four of which were used as controls, while the remaining four were used as the test cultures, thereby eliminating any differences that might occur between lenses (e.g. age). It has been shown³ that under these conditions the cells begin exponential growth after a lag period of 2–3 days. They undergo 4–6 population doublings before cell proliferation ceases. At this point the outgrowth extends some 5 mm from the edge of the original

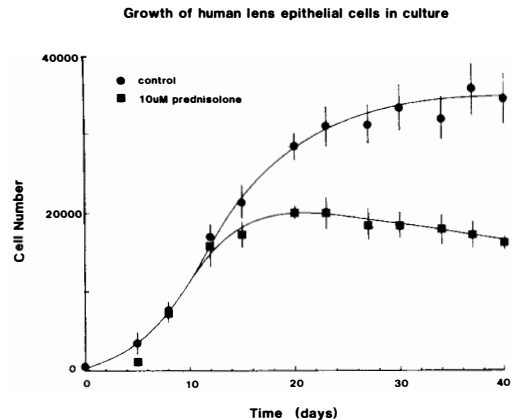


Fig. 4. Growth curves for cultures of human lens epithelial cells in normal culture medium (●) and medium with 10 μM prednisolone (■). The culture medium was E199+10 per cent FCS. Each point represents the mean (\pm standard error) of 4 cultures.

explant. Figure 4 illustrates the growth curve of control cultures (filled circles), and the effect of prednisolone on the growth of these cells (filled squares). Prednisolone, while it does not appear to effect the initial growth rate, causes the curve to plateau at a lower level; the cells undergo 4.6 population doublings compared to 5.4 under control conditions. And, further to this, prednisolone causes the cells to senesce more rapidly and the number of cells begins to decline after 20 days, presumably due to cell death. This does not happen in control cultures until much later.

The epithelium is responsible for lens homeostasis and this involves the control of the state of hydration of the fibre cells and the extracellular space.⁶ The transport of solutes and water that facilitate this process requires the existence of tight junctions (zonulae occludentes) between the cells of the anterior epithelium and anything that disturbs the structure of the epithelium will jeopardise the integrity of the entire lens. This study has demonstrated that abnormalities in epithelial structure appear to be associated with long-term steroid therapy and further, that steroids adversely the growth of these cells in culture.

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