

CELL ELONGATION IN THE CULTURED EMBRYONIC CHICK LENS EPITHELIUM WITH AND WITHOUT PROTEIN SYNTHESIS

Involvement of Microtubules

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ABSTRACT

Previous studies have shown that cells in the 6-day old embryonic chick lens epithelium elongate in tissue culture. In the present study, the time course of elongation during the 1st day of cultivation has been examined histologically. Cultured epithelia were also treated with cycloheximide or colchicine in order to determine if cell elongation depends on new protein synthesis and on the utilization of microtubules, respectively. In the first 5 hr of culture, the mean cell length increased from 11 μ to 21 μ . Subsequently, elongation was slower; the mean cell length was 28 μ after 24 hr in culture. Continuous exposure to cycloheximide did not inhibit the initial doubling of cell length, but did prevent further elongation. By contrast, colchicine inhibited elongation almost immediately. When added after the cell length had doubled, cycloheximide and colchicine each inhibited further elongation; the treated cells remained columnar. Radioautographic and electrophoretic tests showed that protein synthesis was not appreciably affected by colchicine, but was suppressed by cycloheximide. Electron microscopic examination revealed that microtubules oriented along surface membranes were present in epithelia cultured with serum alone and with cycloheximide, but not in those incubated with colchicine. These results indicate that the early stages of cell elongation in the cultured lens epithelium require an initial assembly and organization of preexisting microtubular elements and that continued elongation depends, in addition, on the *de novo* synthesis of protein, possibly microtubule protein.

INTRODUCTION

Cells in the excised lens epithelium of embryonic mice (15), chicks (25), and amphibians (17) elongate in tissue culture. Elongation of the cultured cells is associated with changes in protein synthesis (17, 29) and with the appearance of longitudinally

oriented microtubules (29). Similar changes in protein synthesis (16, 29, 42) and in microtubules (1, 6, 14, 21, 29, 30) have been observed during the formation of fibers in the intact lens. Recent studies in many different systems have correlated

longitudinal arrays of microtubules with elongated cells or cell processes (see, for example, 5, 8, 10, 13, 20, 22, 31, 37, 39, 44, 45, 49-51).

The present experiments were designed to determine if new protein synthesis and utilization of microtubules are necessary for cell elongation in the cultured 6-day old embryonic chick lens epithelium. A brief report of some of these findings has appeared earlier (28). Cycloheximide and colchicine were used to inhibit protein synthesis (7) and to dissociate microtubules (33), respectively. Cell length in cultured epithelia was measured from histological sections, protein synthesis was examined by radioautography and electrophoresis, and the presence and orientation of microtubules were monitored by electron microscopy. The results indicate that an initial assembly and organization of preexisting microtubular elements are required for the early stages of cell elongation, and that *de novo* synthesis of protein, possibly microtubule protein, is necessary for sustained cell elongation in the cultured lens epithelium.

MATERIALS AND METHODS

Obtaining and Culturing Embryonic Lens Epithelia

Fertilized eggs (from Truslow Poultry Farm, Chestertown, Md.) of White Leghorn chickens were incubated in a humid, air atmosphere at 37°C for 6 days. The lenses were surgically removed and placed into plastic culture dishes (Falcon Plastics, Oxnard, Calif., 60 mm × 15 mm) containing 5 ml of Ham's F-10 Medium (9) supplemented with 15% (v/v) fetal calf serum (Baltimore Biological Laboratories, Baltimore, Md.). Cycloheximide (Sigma Chemical Co., St. Louis, Mo., 20 µg/ml) or colchicine (Calbiochem, La Jolla, Calif., 2×10^{-5} M) was added to the medium when specified. In some instances, serum was omitted from the medium. The vitreous body and other adhering debris were removed from the lens with sharpened jeweler's forceps, and the fiber mass was separated from the epithelium, which was left, capsule down, on the surface of the dish. A square of 0.5-1.0 mm² was cut with a scalpel in the center of the epithelium, leaving the edges of the explant pushed into the surface of the plastic culture dish; the peripheral regions of the attached epithelia were trimmed away. One to five explants were placed in each dish, depending on the experiment. The epithelia were either fixed immediately or cultured for the times indicated at 37°C in an air environment,

saturated with water, and containing approximately 3% CO₂. Antibiotics were not used; contaminated cultures were discarded.

Paraffin Histology and Measurement of Cell Length

Before fixation, the medium was decanted and the explants, which remained attached to the culture dish, were rinsed twice with about 5 ml of saline G (32). All procedures were conducted at room temperature. The epithelia were fixed for 3 min with a modified Carnoy's fixative (3 parts absolute ethanol, 1 part acetic acid), stained with eosin in 95% ethanol, kept overnight in 70% ethanol containing a trace of eosin, dehydrated, cleared in xylene, embedded in Paraplast (Rezolin Inc., Chatsworth, Calif.), serially sectioned at 5 µ in a plane normal to the surface of the epithelium, and stained with Ehrlich's hematoxylin and eosin Y in 95% ethanol.

Cell length was determined with a calibrated ocular micrometer at a magnification of × 500. Mean values for cell length were obtained by averaging the individual values of cell length measured to the nearest 2.5 µ in the centers of five sections cut from the middle of the explants; the cells measured were spaced six to eight sections apart. The cell lengths were measured only in the central regions of the explants to ensure that epithelial cells located at the lens equator were not included. These are longer in the intact lens; thus their length represents elongation which has occurred *in vivo* and in culture. Furthermore, the edges of epithelia treated with cycloheximide were often detached from the culture dish and curled over so as to produce a double layer of cells at the periphery of the explants.

The variation in mean cell lengths is given as 2 standard errors (SE), which represents an interval, assuming a normal distribution for the means, that covers the true mean 95% of the time.

Radioautography

Explanted epithelia were labeled with 1 µCi/ml of L-2,3-valine-³H (Schwarz Bio Research Inc., Orangeburg, N. Y., 16.4 Ci/mmol) for 5 or 10 hr at 37°C in 5 ml of culture medium. Cycloheximide (20 µg/ml) or colchicine (2×10^{-5} M) was added where specified. In some experiments explants were cultured first for 8 hr and then labeled with L-2,3-valine-³H for 2 hr. The labeled epithelia were fixed and embedded as above, sectioned at 5 µ in a plane perpendicular to the surface of the epithelium, coated with NTB-3 liquid radioautographic emulsion (Eastman Kodak Co., Rochester, N. Y.), exposed at 4°C for 2 days, developed for 3 min in Dektol (Kodak), fixed, stained with hematoxylin, and photographed at a magnification of × 160 using 35 mm Panatomic X film (Kodak).

Electrophoresis of Labeled Proteins on Composite Polyacrylamide-Agarose Gels

For electrophoresis of proteins, 20 whole lens epithelia, rather than central regions of epithelia, were labeled with 200 $\mu\text{Ci/ml}$ of L-valine- ^3H (Schwarz Bio Research Inc., 0.161 Ci/mole) for 5 hr at 37°C in 0.25 ml of culture medium in polystyrene test tubes (12 mm \times 75 mm). Cycloheximide (20 $\mu\text{g/ml}$) or colchicine (2×10^{-5} M) was added where specified. After labeling, the radioactive medium was removed and the epithelia were washed twice in saline G. The last wash contained negligible amounts of radioactivity.

The labeled proteins were examined by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis as described by Shapiro et al. (40) and modified by Piatigorsky et al. (29). In brief, the labeled epithelia were lysed in 0.3 ml of 1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, and 0.1 M sodium phosphate at pH 7.1, and incubated for 3 hr at 37°C. The proteins were then dialyzed overnight against 0.1% sodium dodecyl sulfate, 0.1% 2-mercaptoethanol, and 0.01 M sodium phosphate at pH 7.1, and were examined by electrophoresis in composite 4% polyacrylamide-0.5% agarose gels, as given elsewhere (29). After fixation in 9% acetic acid and 45% methanol, the gels were frozen and sliced into 1 mm fractions. Each slice was dissolved in 30% hydrogen peroxide and assayed for radioactivity in a Beckman Scintillation Spectrometer (LS 233, Beckman Instruments, Fullerton, Calif.) using a toluene (Fisher Scientific Company, Pitts-

burgh, Pa.) scintillation fluid containing 8.5% solubilizer (BBS-3 from Beckman Instruments) and 3.3% Liquifluor (New England Nuclear Corp., Boston, Mass.) at an efficiency of about 30%.

Electron Microscopy

The preparative procedure was identical to that reported earlier (29). A total of 21 epithelia were incubated in media containing cycloheximide (20 $\mu\text{g/ml}$), colchicine (2×10^{-5} M), or serum alone for 5, 10, or 24 hr at 37°C. After culture, the epithelia were rinsed briefly in sterile saline G and immersed for 15 min in a 2% solution of glutaraldehyde containing 0.05 M Sørensen's phosphate buffer and 0.002% CaCl_2 . Then they were postfixed at 4°C for 1 hr in 2% OsO_4 with 3% sucrose and the same concentrations of buffer and calcium chloride, dehydrated in ethanol, and embedded in Epon. Sections perpendicular to the capsule surface of each epithelium were cut and stained as previously (29) for phase and electron microscopic studies.

RESULTS

Time Course of Cell Elongation in Cultured Lens Epithelia

The cell lengths were determined in epithelia examined histologically immediately after explantation and after 1, 2.5, 5, 10, and 24 hr of cultivation. The results (Fig. 1) showed that the

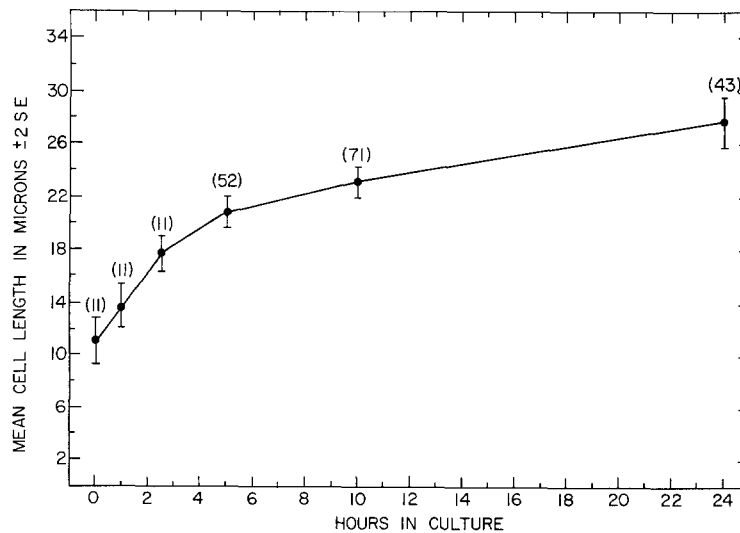


FIGURE 1 Time course of cell elongation. The cell length was measured from histological sections in lens epithelia fixed immediately after explantation or after cultivation for the times specified. Each vertical bar above and below the points represents ± 2 SE. The numbers of epithelia examined for each time interval of culture are shown in parenthesis.

cell length increased rapidly at first, changing from 11 μ initially to almost 21 μ in 5 hr of culture. Subsequently, there was a progressive decrease in the rate of elongation, and the increase in cell length occurred more slowly. After 24 hr of cultivation, the mean cell length had increased to approximately 28 μ .

The 2 se for mean cell length shown in Fig. 1 were calculated from a compilation of many different experiments. Often, the cell lengths in individual tests using several epithelia fell within the ± 2 se given. In other tests, the cell lengths in groups of epithelia cultured at the same time were tightly clustered, and these sometimes fell slightly above or below the 2 se given in the figure.

Effects of Cycloheximide and Colchicine on Cell Elongation in Cultured Lens Epithelia

The mean cell lengths in epithelia cultured in medium containing 15% serum, either alone or supplemented with cycloheximide or colchicine for 5, 10, and 24 hr, are given in Table I. The values for the control epithelia are taken from Fig. 1. Cycloheximide did not inhibit cell elongation for the first 10 hr of culture, during which time the cells doubled in length. In fact, the average length of the cells cultured with cycloheximide for 5 hr was slightly longer than that of cells cultured with serum alone. After 10 hr of culture in the presence of cycloheximide, however, further elongation of

the cells did not occur and there was even some decrease in cell length. In separate tests the cells did not elongate in the presence or absence of cycloheximide if serum was not added to the culture medium.

By contrast to the results with cycloheximide, colchicine inhibited cell elongation. The average cell length in epithelia cultured for 5 hr in the presence of colchicine was only slightly larger than the average length of the cells in epithelia fixed immediately after explantation. The mean cell lengths after 10 and 24 hr of culture with colchicine were not statistically different from the initial value. A similar result was obtained when epithelia were cultured for 10 hr in the presence of colchicine at a concentration of 2×10^{-7} M, rather than 2×10^{-5} M. The sections of epithelia cultured with colchicine at both concentrations showed cells in mitosis leaving the explants (illustrated below).

Colchicine also inhibited cell elongation in epithelia cultured in the presence of cycloheximide. The average cell length in ten explants cultured for 10 hr with both colchicine and cycloheximide was 16.4 μ ; this contrasts with 23.1 μ (see Table I) for the controls.

Tests were made of the ability of cycloheximide or of colchicine to inhibit elongation when added after the cultured cells had already doubled in length. In control epithelia, the initially cuboidal cells (Fig. 2 a) were progressively longer, as expected, after 10 hr (Fig. 2 b) and 20 hr (Fig. 2 c) of cultivation. The presence of cycloheximide (Fig. 2 d) or colchicine (Fig. 2 e) between the 10th and 20th hr of cultivation stopped further elongation of the epithelial cells. The mean cell length in the epithelia treated with cycloheximide was 22 μ , and that in the epithelia treated with colchicine was 21 μ . These values are in the same range as the mean cell length in the control epithelia cultured for only 10 hr, which was 23 μ . Colchicine was equally effective in inhibiting elongation when the concentration was lowered 100-fold. It is important to note that the epithelial cells treated with cycloheximide or with colchicine retained their columnar shape. Nonelongated mitotic cells leaving the epithelia were visible in the sections, especially in explants treated with colchicine.

A similar result was obtained in another experiment in which epithelia were precultured in serum alone for 21 hr followed by cultivation for 24 hr in the presence of cycloheximide or colchicine. In this experiment, however, the cells in the center of the

TABLE I

Cell Elongation in the Embryonic Chick Lens Epithelium Cultured in the Presence of Cycloheximide (20 μ g/ml) or Colchicine (2×10^{-5} M)

Time in culture	Mean cell length in microns ± 2 SE		
	Control*	Cycloheximide†	Colchicine‡
hr			
0	11.0 \pm 1.8		
5	20.9 \pm 1.1	25.0 \pm 0.9	14.1 \pm 0.6
10	23.1 \pm 1.1	22.1 \pm 1.8	12.2 \pm 1.4
24	27.9 \pm 1.8	17.2 \pm 1.5	12.0 \pm 1.9

* Control values of mean cell length are taken from the data given in Fig. 1.

† Cycloheximide values of mean cell length were determined after examination of the following number of epithelia: 5 hr, 28; 10 hr, 28; 24 hr, 12.

‡ Colchicine values of mean cell length were determined after examination of the following number of epithelia: 5 hr, 19; 10 hr, 28; 24 hr, 14.

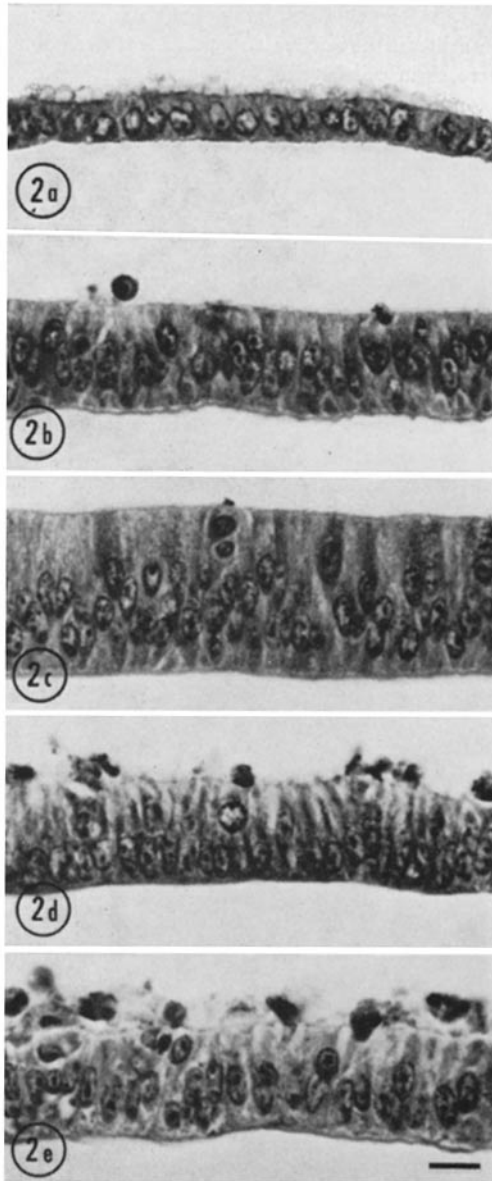


FIGURE 2 Effects of cycloheximide and colchicine on continued cell elongation and on maintenance of columnar cell shape in precultured lens epithelia. Nine epithelia were examined in each group in this experiment. (a) Fixed within 15 min after explantation. (b) Cultured for 10 hr in serum alone. (c) Cultured for 20 hr in serum alone. (d) Cultured for 10 hr in serum alone, plus an additional 10 hr with cycloheximide (20 $\mu\text{g}/\text{ml}$). (e) Cultured for 10 hr in serum alone, plus an additional 10 hr with colchicine (2×10^{-5} M). Mitotic cells and extruded nuclei are most numerous in the colchicine-treated epithelia. $\times 640$. Horizontal bar, 10 μ .

explant were somewhat less in length and more disorganized than after 10 hr of treatment with the inhibitors; the peripheral cells did not elongate further but remained columnar.

Several attempts were made to reverse the inhibition of elongation by colchicine. Partial success was obtained after treatment of the epithelia with colchicine at 2×10^{-7} M for 5 hr followed by an additional 10 hr of cultivation in medium supplemented only with serum. However, attempts to reverse the inhibition of elongation at higher concentrations of colchicine were unsuccessful.

Effects of Cycloheximide and Colchicine on L-Valine- ^3H Incorporation into Protein in Cultured Lens Epithelia

The effects of cycloheximide and colchicine on L-valine- ^3H incorporation into protein by cultured lens epithelia were examined qualitatively by radioautography and quantitatively by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels. Radioautographs of epithelia cultured with labeled valine for 5 hr are presented in Fig. 3. Epithelia cultured with serum alone (Fig. 3 a) and with colchicine (Fig. 3 c) had many exposed silver grains covering the sections, indicating that the cells incorporated appreciable amounts of L-valine- ^3H into protein. Mitotic cells were observed sloughing off the colchicine-treated epithelia (Fig. 3 c). The epithelia incubated in cycloheximide (Fig. 3 b) had relatively few exposed silver grains over the sections, which indicates that the cells incorporated much less L-valine- ^3H into protein. As noted above, the cells in epithelia cultured with serum alone and with cycloheximide were longer than those in epithelia cultured with colchicine.

Similar results were obtained when epithelia were labeled for 10 hr under the present conditions, and when epithelia were precultured for 8 hr followed by 2 hr of incubation with L-valine- ^3H . Thus, the incorporation of L-valine- ^3H into protein in the presence of colchicine continued for the duration of the experiment.

Quantitative tests utilizing scintillation counting confirmed the strong inhibition of protein synthesis by cycloheximide treatment and the continuation of protein synthesis after colchicine treatment. Three groups of 20 lens epithelia (one control group, one cycloheximide-treated group, and one colchicine-treated group) were labeled with L-valine- ^3H for 5 hr. The whole epithelia were dissolved

in sodium dodecyl sulfate, the proteins were reduced with 2-mercaptoethanol, and the samples were dialyzed exhaustively. In two experiments, colchicine-treated epithelia incorporated 83% and 99% as much label into nondialyzable materials as did control epithelia, while epithelia treated with cycloheximide incorporated less than 1% as much label as did the control epithelia. The effect of cycloheximide was not due to an inhibition of uptake of L-valine- ^3H since the epithelia treated with cycloheximide accumulated 78% as much dialyzable radioactivity as did the controls.

The polyacrylamide-gel electrophoretic pattern of the labeled proteins from control epithelia (Fig. 4 A) resembled that published previously (29); namely, a predominant peak of label corresponding to a protein with a molecular weight near 45,000 daltons was superimposed on a base line of

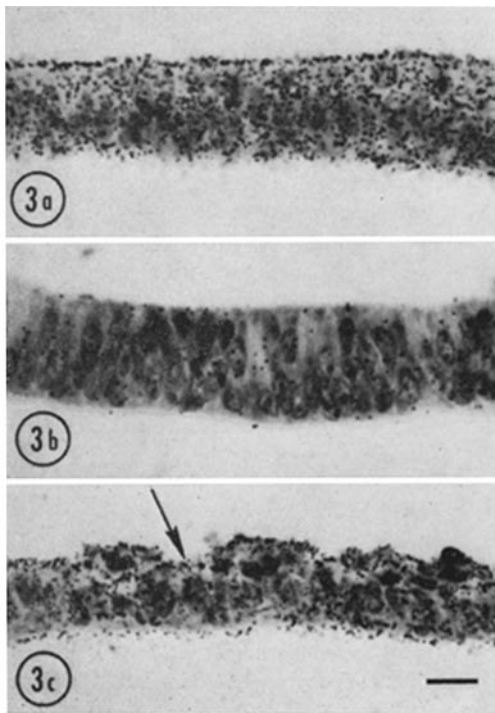


FIGURE 3 Radioautographs of lens epithelia labeled with $1\ \mu\text{Ci/ml}$ of L-2,3-valine- ^3H (specific activity $16.4\ \text{Ci/mmole}$) for 5 hr. 10 epithelia were examined in each group in this experiment. (a) Cultured in serum alone. (b) Cultured with cycloheximide ($20\ \mu\text{g/ml}$). (c) Cultured with colchicine ($2 \times 10^{-5}\ \text{M}$). On both sides of the arrow there are rounded mitotic cells leaving the epithelial surface. $\times 640$. Horizontal bar, $10\ \mu$.

polydispersed label. The present evidence indicates that the primary peak is largely delta crystallin (29). The quantitative and qualitative aspects of the electrophoretic pattern of radioactive proteins from colchicine-treated epithelia (Fig. 4 B) were

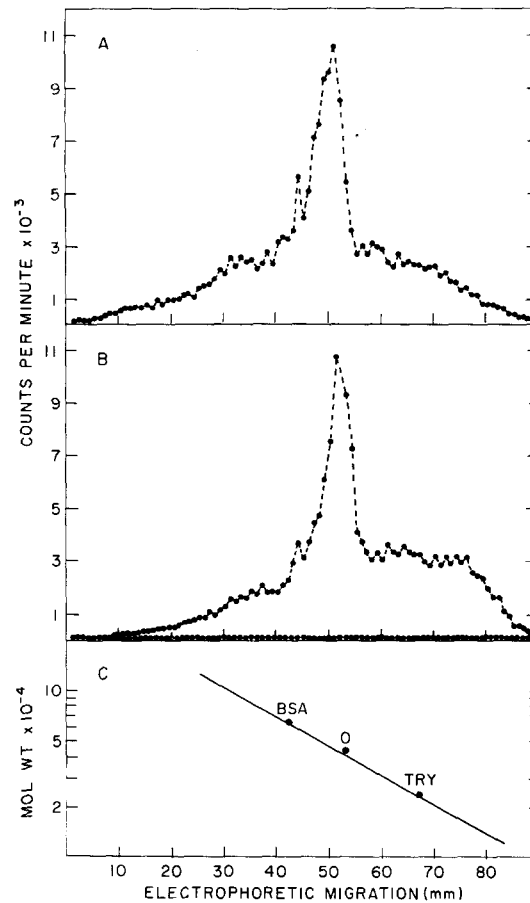


FIGURE 4 Effects of cycloheximide and colchicine on the electrophoretic patterns of proteins labeled in cultured lens epithelia. 20 epithelia were labeled with $200\ \mu\text{Ci/ml}$ of L-valine- ^3H (specific activity $0.161\ \text{Ci/mmole}$) for 5 hr with serum alone, 20 with cycloheximide ($20\ \mu\text{g/ml}$), and 20 with colchicine ($2 \times 10^{-5}\ \text{M}$); the proteins of each were examined by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels. The conditions of labeling and electrophoresis are given in Materials and Methods. One-fourth of each extract was layered on the gel; the counts per minute given on the ordinate represent the values actually obtained for the amount tested. (A) Labeled in serum alone. (B) Labeled with cycloheximide ($20\ \mu\text{g/ml}$), $\bullet\text{---}\bullet$; labeled with colchicine ($2 \times 10^{-5}\ \text{M}$), $\bullet\text{---}\bullet$. (C) Marker proteins of known molecular weights were electrophoresed in parallel with the labeled lens protein. BSA, bovine serum albumin; O, ovalbumin; Try, trypsin.

very similar to those from control epithelia. Approximately the same amount of radioactivity was recovered in proteins from the gel of colchicine-treated epithelia as from the gel of control epithelia. However, cycloheximide treatment of the epithelia virtually eliminated the incorporation of label into all proteins (Fig. 4 B).

Effects of Cycloheximide and Colchicine on Microtubules in Cultured Lens Epithelia

The presence of microtubules in control, cycloheximide- and colchicine-treated lens epithelia, fixed within 15 min after explantation or after cultivation for 2, 5, 10, and 24 hr, was investigated by electron microscopy (Fig. 5). The average cell lengths in the sections of epithelia prepared in Epon for phase microscopy were about 25% longer than the mean cell lengths in histological sections prepared in paraffin, where more shrinkage was apparent. However, the per cent change in cell length during elongation was the same.

We have described the fine structure of the cultured lens epithelial cells elsewhere (29) and thus will confine our attention to microtubules. The arrangement of microtubules in cells fixed immediately after explantation (Fig. 5 a) differed from those fixed after 5 hr of cultivation (Fig. 5 b). Initially, there were relatively few, randomly oriented microtubules in the cuboidal epithelial cells. After 5 hr of cultivation, the elongated cells contained many microtubules which were most numerous along surface membranes and were oriented longitudinally. Treatment of the cultured lens epithelia with cycloheximide did not affect the presence or arrangement of microtubules (Fig. 5 c). Microtubules were not observed, however, in cells treated with colchicine (Fig. 5 d). Instead, there was an abundance of filaments approximately 100 Å in diameter, which are probably breakdown products of the microtubules (48, 50). Similar tests with epithelia cultured for 10 and 24 hr gave comparable results.

The longitudinal arrangement of microtubules adjacent to cell membranes was already evident in epithelia cultured for 2 hr. When explants cultivated for 2 hr in medium supplemented with serum alone were maintained for an additional 22 hr in culture medium containing colchicine, the columnar shape of the cells was retained, although further elongation did not take place.

DISCUSSION

We have reported previously that cultured embryonic chick lens epithelial cells approximately double in length during the 1st day of cultivation (26, 29). The present study shows that much of this occurs within the first 5 hr after explantation. Subsequently, cell elongation proceeds more slowly. Recent studies show that the elongation can continue for at least a month.¹ The prompt and simultaneous elongation of the cells shows that elongation can occur during most of the cell cycle, since the cells are dividing nonsynchronously at the time of explantation (24, 27).

Our experiments with cycloheximide show that the early and rapid elongation can take place without protein synthesis, but that the slower and later occurring elongation is dependent on continued protein synthesis. Both the early and later phases of lens cell elongation appear to need microtubules, since the colchicine-treated cells lack microtubules and do not elongate. The modest increase in cell length in epithelia cultured with colchicine for 5 hr is probably due to the relatively slow dissociation of microtubules by interaction with colchicine (3, 4, 46). Experiments by others have shown that protein synthesis is also required for the maintenance of axonal outgrowth in neuroblastoma cells (38) and for cilia regeneration in *Tetrahymena* (34), both being processes which need microtubules as judged by their sensitivity to colchicine. A microtubule requirement for cell elongation in the cultured lens epithelium is consistent with the correlation between oriented microtubules and lens fiber formation in vivo (1, 6, 14, 21, 29, 30).

Less extensive tests with vinblastine sulfate (28) gave results similar to those with colchicine. Vinblastine prevented cell elongation in the cultured lens epithelia and disrupted microtubules, but did not inhibit protein synthesis. These experiments, then, support the conclusion that microtubules are necessary for the occurrence of lens cell elongation in tissue culture.

The inhibition of cell elongation by colchicine cannot be accounted for by mitotic arrest, since elongation is suppressed in nonmitotic cells. However, elongated cells can become rounded and arrested in mitosis when further elongation is prevented with colchicine. Mitotic cells are invariably

¹ Piatigorsky, Joram, and S. S. Rothschild. Manuscript in preparation.

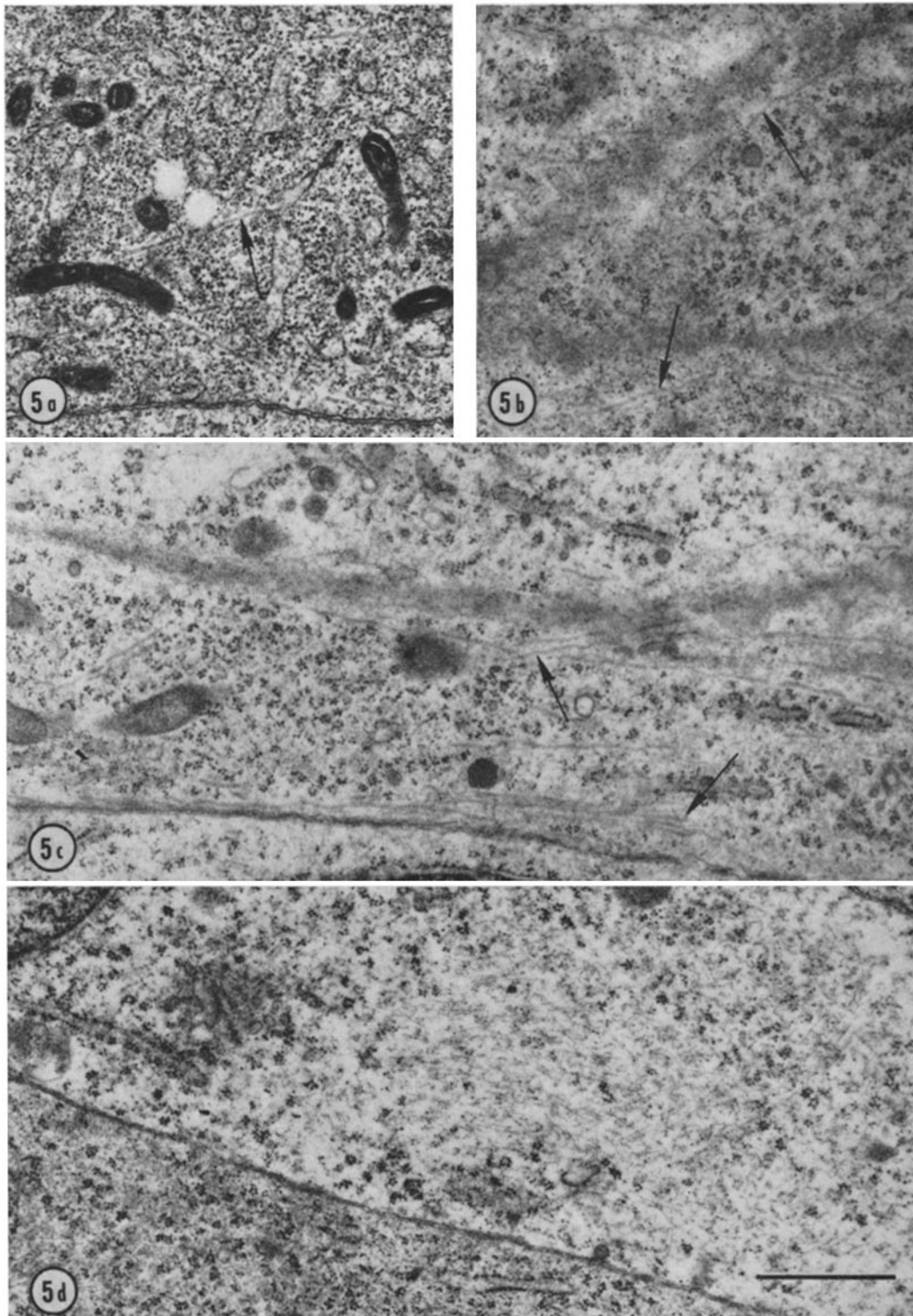


FIGURE 5 Microtubules (arrows) in lens epithelial cells. (a) Fixed within 15 min after explantation. Only occasional, randomly oriented microtubules measuring about 240 Å in diameter are observed. (b) Cultured for 5 hr with serum alone. Microtubules are present along surface membranes, which are sectioned obliquely. (c) Cultured for 5 hr with cycloheximide (20 µg/ml). Longitudinally oriented microtubules are abundant. (d) Cultured for 5 hr with colchicine (2×10^{-5} M). No microtubules are present, but there are numerous filaments approximately 100 Å in diameter. $\times 21,000$. Horizontal bar, 1 µ.

detached from the capsule and found at the free surface of the epithelium, which is reminiscent of interkinetic nuclear migration in the lens placode (21, 52). The progressive accumulation of cells in mitosis and the continuation of protein synthesis in the presence of colchicine indicate that the inhibition of cell elongation by colchicine is not due to cell death.

The fact that the cultured epithelial cells retain their columnar shape for at least 10 hr if treated with colchicine after the cells have elongated suggests that the microtubules have a more immediate role in the process of their elongation than in the maintenance of their elongate shape. A similar conclusion was reached by Tilney and Gibbins (45) for morphogenesis of primary mesenchyme in sea urchin embryos and by Pearce and Zwaan (21) for elongation of epithelial cells in the lens placode of chick embryos. It is likely that the close apposition of neighboring cells attached to the basement membrane helps retain the columnar shape of the cultured cells in the absence of microtubules.

Since elongation of the cultured epithelial cells apparently involves microtubules and can occur for 5–10 hr in the absence of protein synthesis, there must be a pool of microtubule subunits which can be utilized. Preliminary tests have shown that the 6-day old embryonic chick lens epithelium contains significant amounts of protein which can bind colchicine-³H at the time of explantation (Piatigorsky and Rothschild, unpublished); further experiments are in progress to determine quantitatively the amounts of colchicine-³H-binding protein present at different stages of cell elongation. The assembly of microtubules without protein synthesis from a precursor pool of microtubule protein has been demonstrated for regeneration of flagella in *Chlamydomonas* (35), regeneration of cilia in sea urchin embryos (2), reformation of axopodia in *Actinosphaerium* (44), assembly of cilia-like protein in *Tetrahymena* (43), and formation of axons in neurons (39, 51). There is also strong evidence that dividing cells have a pool of microtubule subunits which polymerize and depolymerize during the formation and disassembly of the mitotic apparatus (12, 18, 41). The mutual requirement for microtubules during cell elongation and division suggests an explanation for the inverse relationship between lens cell elongation and mitosis, which is observed both during lens fiber formation in vivo (11, 19, 23, 53) and during lens epithelial cell elongation in tissue culture (24, 27).

The mechanism by which microtubules promote cell elongation is not known. Discussions of this can be found elsewhere (5, 44, 45). It is likely that other factors cooperate with microtubules to alter cell shape. For example, membrane addition must be an integral part of cell elongation, at least during the increase in cell mass. In addition, a growing body of work implicates contractile protein for cell elongation (47). In preliminary tests, 5 μ g/ml of cytochalasin B, which is believed to interfere with the contractile function of microfilaments (36, 47), inhibited cell elongation in the cultured lens epithelium (Piatigorsky and Wollberg, unpublished). Thus, the possibility that microtubules function together with microfilaments during elongation of the cultured lens epithelial cells is presently being explored.

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REFERENCES

1. ARNOLD, J. M. 1966. On the occurrence of microtubules in the developing lens of the squid, *Loligo pealii*. *J. Ultrastruct. Res.* 14:534.
2. AUCLAIR, W., and B. W. SIEGEL. 1969. Cilia regeneration in the sea urchin embryo: evidence for a pool of ciliary proteins. *Science (Wash. D. C.)*. 154:913.
3. BORISY, G. G., and E. W. TAYLOR. 1967. The mechanism of action of colchicine. Binding of colchicine-³H to cellular protein. *J. Cell Biol.* 34:525.
4. BORISY, G. G., and E. W. TAYLOR. 1967. The mechanism of action of colchicine. Colchicine binding to sea urchin eggs and the mitotic apparatus. *J. Cell Biol.* 34:535.
5. BURNSIDE, B. 1971. Microtubules and microfilaments in newt neurulation. *Dev. Biol.* 26:416.
6. BYERS, B., and K. R. PORTER. 1964. Oriented microtubules in elongating cells of the developing lens rudiment after induction. *Proc. Natl. Acad. Sci. U. S. A.* 52:1091.
7. ENNIS, H. L., and M. LUBIN. 1964. Cycloheximide: aspects of inhibition of protein synthesis in mammalian cells. *Science (Wash. D. C.)*. 146:1474.
8. FISCHMAN, D. A. 1967. An electron microscopic

- study of myofibril formation in embryonic chick skeletal muscle. *J. Cell Biol.* **32**:557.
9. HAM, R. G. 1963. An improved nutrient solution for diploid Chinese hamster and human cell lines. *Exp. Cell Res.* **29**:515.
 10. HANDEL, M. A., and L. E. ROTH. 1971. Cell shape and morphology of the neural tube: implications for microtubule function. *Dev. Biol.* **25**:78.
 11. HANNA, C., and H. C. KEATTS. 1966. Chicken lens development: epithelial cell production and migration. *Exp. Eye Res.* **5**:111.
 12. INOUE, S., and H. SATO. 1967. Cell motility by labile association of molecules. *J. Gen. Physiol.* **50**:259.
 13. KARFUNKEL, P. 1971. The role of microtubules and microfilaments in neurulation in *Xenopus*. *Dev. Biol.* **25**:30.
 14. KUWABARA, T. 1968. Microtubules in the lens. *Arch. Ophthalmol.* **79**:189.
 15. MANN, I. 1948. Tissue culture of mouse lens epithelium. *Br. J. Ophthalmol.* **32**:591.
 16. McDEVITT, D. S., I. MEZA, and T. YAMADA. 1969. Immunofluorescence localization of the crystallins in amphibian lens development, with special reference to the γ crystallins. *Dev. Biol.* **19**:581.
 17. McDEVITT, D. S., and T. YAMADA. 1969. Acquisition of antigenic specificity by amphibian lens epithelial cells in culture. *Am. Zool.* **9**:1130.
 18. McINTOSH, J. R., P. K. HEPLER, and D. G. VAN WIE. 1969. Model for mitosis. *Nature (Lond.)*. **224**:659.
 19. MODAK, S. P., G. MORRIS, and T. YAMADA. 1968. DNA synthesis and mitotic activity during early development of chick lens. *Dev. Biol.* **17**:544.
 20. OVERTON, J. 1966. Microtubules and microfilaments in morphogenesis of the scale cells of *Ephestia kuhniella*. *J. Cell Biol.* **29**:293.
 21. PEARCE, T. L., and J. ZWAAN. 1970. A light and electron microscopic study of cell behavior and microtubules in the embryonic chicken lens using Colcemid. *J. Embryol. Exp. Morphol.* **23**:491.
 22. PERRY, M. M., and C. H. WADDINGTON. 1966. Ultrastructure of blastopore cells in the newt. *J. Embryol. Exp. Morphol.* **15**:317.
 23. PERSONS, B. J., and S. P. MODAK. 1970. The pattern of DNA synthesis in the lens epithelium and the annular pad during development and growth of the chick lens. *Exp. Eye Res.* **9**:144.
 24. PHILPOTT, G. W. 1970. Growth and cytodifferentiation of embryonic chick lens epithelial cells *in vitro*. *Exp. Cell Res.* **59**:57.
 25. PHILPOTT, G. W., and A. J. COULOMBRE. 1965. Lens development. II. Differentiation of embryonic chick lens epithelial cells *in vitro* and *in vivo*. *Exp. Cell Res.* **38**:635.
 26. PIATIGORSKY, J. 1969. Synthesis and processing of ribosomal RNA in the developing lens of the chick embryo. *Am. Zool.* **9**:1130.
 27. PIATIGORSKY, J., and S. S. ROTHSCHILD. Loss during development of the ability of chick embryonic lens cells to elongate in culture: inverse relationship between cell division and elongation. *Dev. Biol.* **28**:382.
 28. PIATIGORSKY, J., H. DEF. WEBSTER, and S. P. CRAIG. 1970. Ultrastructural and biochemical aspects of lens fiber formation *in vivo* and *in vitro*. *J. Cell. Biol.* **47**:158 a.
 29. PIATIGORSKY, J., H. DEF. WEBSTER, and S. P. CRAIG. 1972. Protein synthesis and ultrastructure during the formation of embryonic chick lens fibers *in vivo* and *in vitro*. *Dev. Biol.* **27**:176.
 30. PORTE, A., M. E. STOECKEL, and A. BRINI. 1968. Formation de l'ébauche oculaire et différenciation du cristallin chez l'embryon de poulet. Etude au microscope électronique. *Arch. Ophthalmol. Rev. Gen. Ophthalmol. (Anal.)*. **28**:681.
 31. PORTER, K. R. 1966. Cytoplasmic microtubules and their functions. *Principles Biomol. Organ. Ciba Found. Symp.* **308**.
 32. PUCK, T. T., S. J. CIECIURA, and A. ROBINSON. 1958. Genetics of mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. *J. Exp. Med.* **108**:945.
 33. ROBBINS, E., and N. C. GONATAS. 1964. Histochemical and ultrastructural studies on HeLa cell cultures exposed to spindle inhibitors with special reference to the interphase cell. *J. Histochem. Cytochem.* **12**:704.
 34. ROSENBAUM, J. L., and K. CARLSON. 1969. Cilia regeneration in *Tetrahymena* and its inhibition by colchicine. *J. Cell Biol.* **40**:415.
 35. ROSENBAUM, J. L., J. E. MOULDER, and D. L. RINGO. 1969. Flagella elongation and shortening in *Chlamydomonas*. The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. *J. Cell Biol.* **41**:600.
 36. SCHROEDER, T. E. 1969. The role of "contractile ring" filaments in dividing *Arbacia* eggs. *Biol. Bull. (Woods Hole)*. **137**:413.
 37. SCHROEDER, T. E. 1970. Neurulation in *Xenopus laevis*. An analysis and model based upon light and electron microscopy. *J. Embryol. Exp. Morphol.* **23**:427.
 38. SCHUBERT, D., S. HUMPHREYS, F. DE VITRY, and F. JACOB. 1971. Induced differentiation of a neuroblastoma. *Dev. Biol.* **25**:514.
 39. SEEDS, N. W., A. G. GILMAN, T. AMANO, and M. W. NIRENBERG. 1970. Regulation of axon formation by clonal lines of a neural tumor. *Proc. Natl. Acad. Sci. U. S. A.* **66**:160.
 40. SHAPIRO, A. L., E. VINUELA, and J. V. MAIZEL, JR. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-poly-

- acrylamide gels. *Biochem. Biophys. Res. Commun.* **28**:815.
41. STEPHENS, R. E. 1969. Factors influencing the polymerization of outer fiber microtubule protein. *Q. Rev. Biophys.* **1**:377.
 42. TAKATA, C., J. F. ALBRIGHT, and T. YAMADA. 1965. Lens fiber differentiation and gamma crystallins: immunofluorescent study of Wolffian regeneration. *Science (Wash. D. C.)*. **147**:1299.
 43. TAMURA, S. 1971. Synthesis and assembly of microtubule proteins in *Tetrahymena pyriformis*. *Exp. Cell Res.* **68**:180.
 44. TILNEY, L. G. 1968. II. Ordering of subcellular units. The assembly of microtubules and their role in the development of cell form. *Dev. Biol.* **2**(Suppl.):63.
 45. TILNEY, L. G., and J. R. GIBBINS. 1969. Microtubules in the formation and development of the primary mesenchyme in *Arbacia punctulata*. II. An experimental analysis of their role in development and maintenance of cell shape. *J. Cell Biol.* **41**:227.
 46. WEISENBERG, R. C., G. G. BORISY, and E. W. TAYLOR. 1968. The colchicine-binding protein of mammalian brain and its relation to microtubules. *Biochemistry.* **7**:4466.
 47. WESSELLS, N. K., B. S. SPOONER, J. F. ASH, M. O. BRADLEY, M. A. LUDUENA, E. L. TAYLOR, J. T. WREN, and K. M. YAMADA. 1971. Microfilaments in cellular and developmental processes. *Science (Wash. D. C.)*. **171**:135.
 48. WISNIEWSKI, H., M. L. SHELANSKI, and R. D. TERRY. 1968. Effect of mitotic spindle inhibitors on neurotubules and neurofilaments in anterior horn cells. *J. Cell Biol.* **38**:224.
 49. YAMADA, K. M., B. S. SPOONER, and N. K. WESSELLS. 1970. Axon growth: roles of microfilaments and microtubules. *Proc. Natl. Acad. Sci. U. S. A.* **66**:1206.
 50. YAMADA, K. M., B. S. SPOONER, and N. K. WESSELLS. 1971. Ultrastructure and function of growth cones and axons of cultured nerve cells. *J. Cell Biol.* **49**:614.
 51. YAMADA, K. M., and N. K. WESSELLS. 1971. Effect of nerve growth factor on microtubule protein. *Exp. Cell Res.* **66**:346.
 52. ZWAAN, J., P. R. BRYAN, JR., and T. L. PEARCE. 1969. Interkinetic nuclear migration during the early stages of lens formation in the chicken embryo. *J. Embryol. Exp. Morphol.* **21**:71.
 53. ZWAAN, J., and T. L. PEARCE. 1971. Cell population kinetics in the chicken lens primordium during and shortly after its contact with the optic cup. *Dev. Biol.* **25**:96.