RAPID COMMUNICATIONS

Epithelia Suspended in Collagen Gels Can Lose Polarity and Express Characteristics of Migrating Mesenchymal Cells

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ABSTRACT This study of epithelial-mesenchymal transformation and epithelial cell polarity in vitro reveals that environmental conditions can have a profound effect on the epithelial phenotype, cell shape, and polarity as expressed by the presence of apical and basal surfaces. A number of different adult and embryonic epithelia were suspended within native collagen gels. Under these conditions, cells elongate, detach from the explants, and migrate as individual cells within the three-dimensional lattice, a previously unknown property of well-differentiated epithelia. Epithelial cells from adult and embryonic anterior lens were studied in detail. Elongated cells derived from the apical surface develop pseudopodia and filopodia characteristic of migratory cells and acquire a morphology and ultrastructure virtually indistinguishable from that of mesenchymal cells in vivo. It is concluded from these experiments that the three-dimensional collagen gel can promote dissociation, migration, and acquisition of secretory organelles by differentiated epithelial cells, and can abolish the apical-basal cell polarity characteristic of the original epithelium.

Epithelium is the tissue that lines body surfaces. The cells are polarized with respect to the outside or free surface, with Golgi zones usually in the apical cytoplasm. Specializations such as microvilli and junctional complexes distinguish the apical and lateral surfaces from the basal surface, which is attached to extracellular matrix in the form of a basal lamina (13). In the early embryo, certain epithelia give rise to mesenchymal cells that invade extracellular matrices and lose the apical, lateral, and basal specializations that characterized the parent epithelium. Their attenuated cell bodies and bipolar or stellate shapes distinguish them from closely apposed, polygonal epithelial morphology, as does the ability to migrate with a three-dimensional extracellular matrix.

The epithelial and mesenchymal phenotypes, once formed, appear to be stable. If epithelial cells are grown on top of collagenous matrices in vitro, they develop a flat basal surface next to the matrix (14–16), whereas mesenchymal cells (16) and presumptive mesenchymal cells (3) invade underlying extracellular matrix. Adult thyroid (7) and normal and malignant mammary epithelial cells (20), embedded within collagenous matrices in vitro, organize into polarized epithelial structures with basal surfaces contacting the collagen gel and apical poles facing a central cavity. Cells cloned from a mammary tumor cell line, however, can form pointed outgrowths of loosely associated elongated cells that invade into collagen gels from surface monolayers (2).

To study further the stability of the epithelial phenotype, especially as regards surface polarity, we suspended a number of different epithelial tissues within gelling solutions of colla-

gen. To our surprise, we found that adult lens epithelium and a number of embryonic epithelia that do not form mesenchyme in vivo routinely give rise to cells that separate from the tissue explant and migrate individually into the surrounding collagen gel. The migrating cells assume a bipolar morphology and fine structure almost indistinguishable from that of mesenchymal cells in collagen gels (1, 19). In this paper, we report the behavior of corneal, notochordal, limb, and lens epithelia, and endothelial cells, suspended in collagen gels, and describe in more detail the behavior in gels of adult and embryonic anterior lens epithelium, which can be isolated as a pure population of simple cuboidal epithelial cells resting on an intact basal lamina.

MATERIALS AND METHODS

Collagen Gel Preparation

Epithelia were also cultured on the upper surface of collagen gels prepared by pipetting 0.5 ml of gelling solution (see above) onto the tissue culture dish and allowing it to polymerize at 37°C for 30 min. The intact lens epithelium isolated as described below was placed on the surface of the gel, incubated 1–2 h (37°C) in a small volume of medium, then covered with complete medium for the duration of the culture period.

Cell and Tissue Isolation

Notochords were isolated from posterior trunk of 2-d-old (stage 13) chick embryos with 1% trypsin (Difco Laboratories, Detroit, MI) in HBSS. There was no mesenchymal cell contamination because no mesenchyme is present in the posterior trunk region (6, 8). Limb bud ectoderm was isolated from 5-d-old chick embryos by the method of Erick and Saunders (10), which permits epithelium to · be separated completely from limb mesoderm. Corneal epithelium was isolated with trypsin/collagenase as reported by Meier and Hay (15), which routinely yields pure epithelial tissue.

Both embryonic and adult avian lens epithelia were dissected with lens capsule intact as pieces 1 mm in diameter from the anterior portion of the lens by the method of Piatigorsky (17). Embryonic avian lens cells were also dissociated from the lens capsule by incubating tissue fragments in a solution of 0.2% collagenase (Sigma Chemical Co., St. Louis, MO) in HBSS for 30 min followed by 0.05% trypsin (type I, Sigma Chemical Co.) plus 0.02% EDTA in HBSS without Ca^+ and Mg^{++} for 15 min at 37°C. The dissociated cells were centrifuged for 5 min, washed in complete medium and repelleted. This pellet was then pipetted into the gelling collagen.

Corneal endothelial cells were isolated from adult bovine eyes and maintained in Dulbecco's modified Eagle's medium H-16 (DME) supplemented with 10% FCS, 10 mM glutamine, 2.5 μg/ml fungizone, 50 μg/ml gentamycin, and 100 ng/ml purified brain fibroblast growth factor (FGF, added every other day; a gift from Dr. D. Gospodarowicz, University of California, San Francisco), as previously described (11, 12). Cultures were passaged every week with a split ratio of 1 to 64, and cells were used between the 5th and 30th generations. Cells were suspended in collagen gels by first dissociating them from the plastic dish with trypsin-EDTA in HBSS without Ca^+ and Mg^{++}, centrifuging in complete DME medium and pipetting the pellet into the gelling collagen. Cultures were maintained in complete medium without FGF.

Light and Electron Microscopy

Live cultures of tissues suspended within collagen gels were observed and photographed with inverted phase-contrast and Nomarski microscopes. For transmission electron microscopy (TEM), tissue or cultures were fixed for 30 min in 2% paraformaldehyde and 2.5% glutaraldehyde in cacodylate buffer (0.1 M and postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4, at 4°C for 30 min. They were stained en bloc in 1% uranyl aetate, dehydrated, and embedded in Spurr (D.E.R. 736 embedding kit, Tousimis Research Co., Rockville, MD). Thin sections were cut on a Sorvall MT2-B ultramicrotome (Dupont Instruments-Sorvall Biomedical Div., Newtown, CT) and stained with lead citrate (0.2%); thick sections were stained with 1% toluidine blue in 1% sodium borate.

RESULTS

To study the relationship of epithelial polarity to contact of epithelial cell surfaces with collagenous matrices, we suspended a number of different epithelial tissues and dissociated cells within gelling solutions of type I collagen. When placed within a native collagen gel, enzyme isolated embryonic notochord (Fig. 1A) and limb ectoderm (Fig. 1B) both give rise within 36 h to elongated bipolar cells that break free of the explant and migrate as individual cells within the three-dimensional collagenous matrix. Embryonic corneal epithelium suspended in collagen gels form mesenchymelike cells in the same manner (data not shown). On the contrary, isolated corneal epithelium grown on top of collagen gels remain epithelial (15). Notochordal (6), limb (10), and corneal (15) epithelia are easily isolated free of contaminating mesenchyme, under the conditions described here (6, 10, 15). Trypsin-isolated notochordal epithelia cultured on plastic substrata have been reported to send out individual, flattened cells onto the substratum (6), even though the original explant is free of mesenchyme.

The ability of epithelial cells to transform to mesenchymelike

cells is not restricted to embryonic tissues. Adult anterior lens epithelia isolated by dissection with the basal lamina (lens capsule) intact gives rise to individual cells that migrate radially away from the explant (Fig. 1C). Confluent monolayers of adult bovine corneal endothelial cells, which exhibit morphological features of corneal endothelium in vivo (11, 12), were dissociated and suspended as a cell pellet within the gel. These endothelial cells from adult corneas also acquire the morphology of bipolar mesenchymal cells migrating within the extracellular matrix (Fig. 1D); they form epithelium, however, when grown on top of collagen.

Adult and embryonic anterior lens epithelia were used as model tissue to study, in more detail, the cellular events that accompany epithelial-mesenchymal transformation within collagenous lattices. This tissue was dissected as a pure population of simple cuboidal cells attached to their basal lamina, the lens capsule (17). Figs. 1 E–J illustrate by phase-contrast microscopy the temporal sequence of cellular events that follow suspension within a collagen gel of freshly isolated 12-d-old chick embryonic anterior lens epithelium on its capsule. At 4 h after suspension in the collagen gel (Fig. 1E), the tissue shows no morphological alterations. By 38 h (Fig. 1F) and 48 h (Fig. 1G), these peripheral cells can be seen elongating. By 62 h (Fig. 1H), numerous spindle-shaped cells extend from the explant into the surrounding gel and a few cells are completely detached from the explant. The number of bipolar cells that have migrated free of the explant increases by days 4 and 5 (Figs. 1I and J, respectively).

The morphology of these lens-derived, mesenchymelike cells is better visualized with Nomarski optics. An adult lens explant after 9 d of incubation within the gel is seen in Fig. 2A. A cell, free of the lens capsule, has an extended cell process (pseudopodium) on its leading edge (p, Fig. 2A) and a cell process trailing behind. Migrating cells have an appearance resembling that of mesenchymal cells, including an elongated cell body and fine filopodia (arrow, Fig. 2B) that extend from the leading pseudopodium. If embryonic lens epithelia are first dissociated from the capsule with collagenase and trypsin-EDTA, and the cell pellet placed within the gel (Fig. 2C and D), migrating epithelial cells also form that are mesenchymelike. The cells are bipolar and spindle-shaped with long cell processes, pseudopodia and fine filopodia (Fig. 2C and D). Thus, epithelial cells are able to invade a three-dimensional collagen matrix as elongated, bipolar cells, hitherto believed to be a unique property of mesenchyme and malignant cells.

Light micrographs of sections of plastic-embedded cultures show that the migrating cells derive from the apical surface of lens epithelium suspended in collagen (as, Fig. 3A and B), whereas the anterior lens epithelium cultured on the surface of collagen gels maintains the integrity of its apical surface (as, Fig. 3C). When lens epithelium is suspended within a gel, it is only in areas of close contact of the cell surface with collagen fibrils that cells disperse and migrate (arrows, Fig. 3A) into the matrix. If the collagen gel does not contact the epithelium (asterisk, Fig. 3A), the apical surface remains smooth. When the lens explant is placed on top of the gel, the epithelial cells migrate away from the explant as a monolayer of contiguous cells over the surface of the gel. Cells at the leading edge of the migrating cell sheet have flattened stellate shapes (not shown). With time, the cells acquire a cuboidal shape (Fig. 3C) and deposit a newly synthesized basal lamina between their basal surfaces and the collagen gel. The cuboidal cells in the area of the original explant elongate, acquiring the morphology of mature fiber cells as described by Piatigorsky (17).

Ultrastructural analysis of adult lens epithelium within the collagen gel (Fig. 4) reveals with better resolution, the surface changes associated with elongation of cells into the gel and alterations in the fine structure of the cytoplasm consistent with increased secretory activity. Freshly isolated adult lens epithelium has a smooth apical or free surface (*fs*, Fig. 4*A*) with occasional microvilli. The gap junctions that linked anterior to posterior lens epithelium in vivo are broken during the isolation. Junctional complexes and gap junctions link lateral surfaces of the anterior lens cells. The basal surface, which is in contact with the lens capsule, is flat and contains a microfila-

ment-rich cortex, as is typical of other epithelia in contact with basal laminae (15). The ground cytoplasm appears fibrillogranular (*fg*. Fig. 4*A*), presumably due to the presence of lens crystalline proteins. The granular endoplasmic reticulum is moderately well developed, mitochondria are small, and the Golgi zones of the cells are not prominent. Free polyribosomes are seen in the cytoplasm, nucleoli are small, and the nuclear chromatin shows areas of condensation (arrows, Fig. 4*A*).

In contrast to the relatively smooth apical surface of freshly isolated lens epithelia, the apical surfaces of cells in contact with collagen fibrils of the gel display prominent filopodia (*f*,
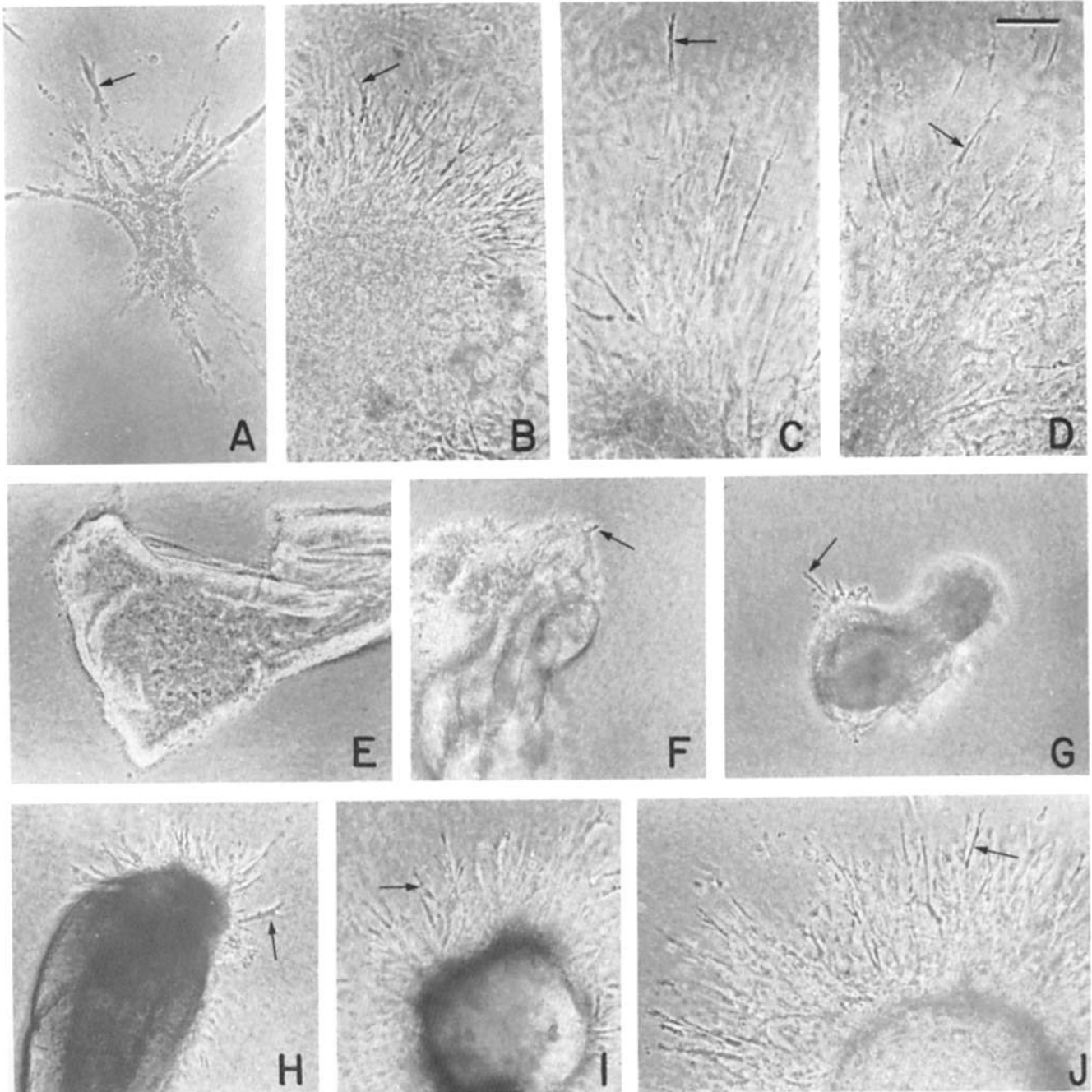

FIGURE 1   Phase-contrast micrographs showing bipolar cells (arrows) originating from several embryonic and adult epithelia (*A–D*) and steps in the origin of bipolar cells from embryonic lens epithelium (*E–J*) suspended within collagen gels. (*A*) Notochord from 2-d chick embryo cultured for 5 d in a gel. (*B*) Chick embryonic limb ectoderm after 4 d of culture in a gel. (*C*) Adult chick lens explant after 5 d in a gel. (*D*) Dissociated cultured bovine corneal endothelial cells after 9 d. To illustrate steps in this transformation, chick embryo lens epithelia were fixed and photographed (*E*) 4 h, (*F*) 38 h, (*G*) 48 h, (*H*) 62 h, (*I*) 4 d, and (*J*) 5 d after suspension in collagen gels. The elongating cells (arrows) are becoming mesenchymal in appearance. Bar, 100 μm. × 100.
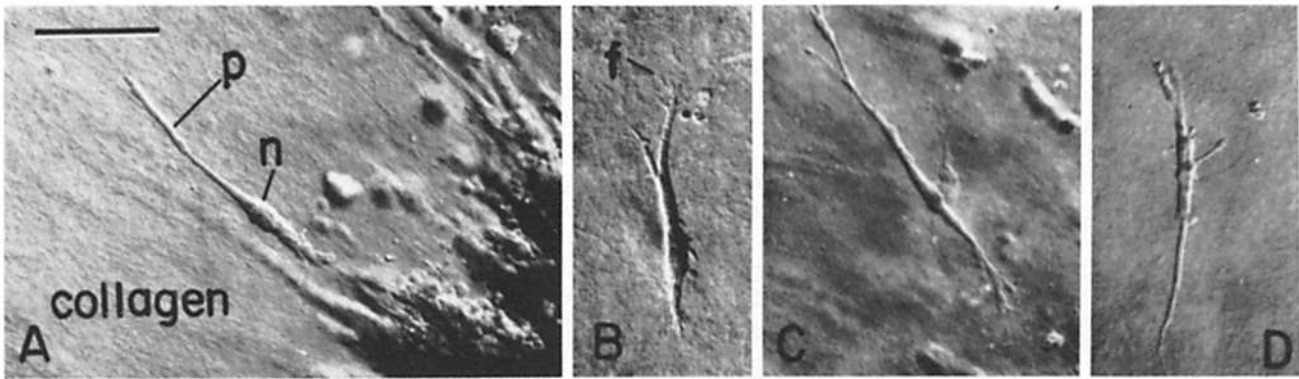
FIGURE 2 Nomarski light micrographs showing at higher magnification, the bipolar shape, prominent pseudopodia (as at *p*), and filopodia (as at *f*) of the mesenchymelike cells that derive from epithelia suspended in collagen gels. (*A* and *B*) Intact adult lens epithelium cultured for 9 d. *n*, nucleus. (*C* and *D*) Pellet of dissociated embryonic lens epithelial cells cultured for 11 d. Bar, 50 μm. × 320.
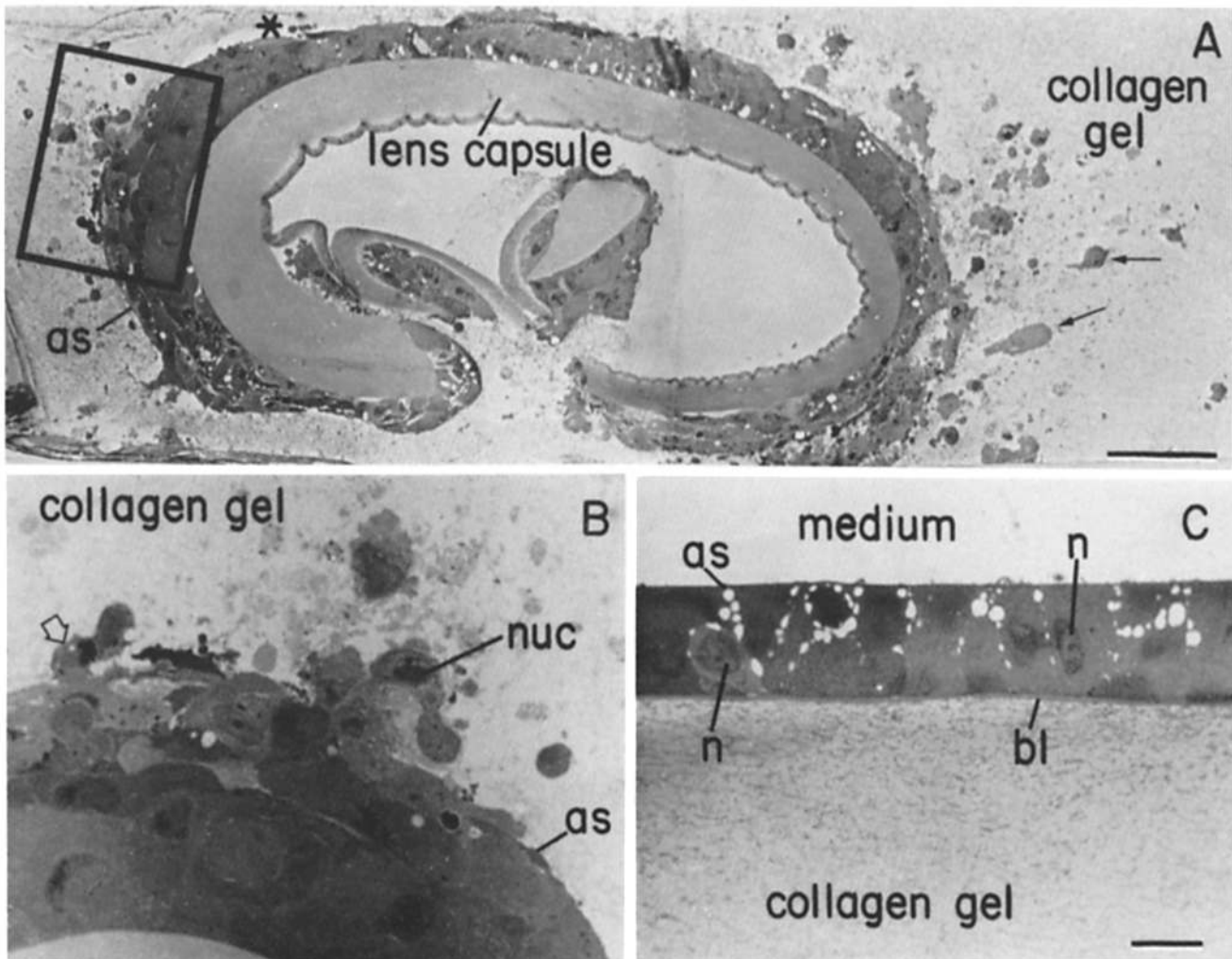

FIGURE 3 Light micrographs of thick (1 μm) sections of plastic embedded lens epithelia cultured within a collagen gel for 9 d (*A* and *B*) or on the surface of a collagen gel for 7 d (*C*). The area in the rectangle in *A* is shown at higher magnification in *B*. The simple cuboidal lens epithelium suspended in collagen multilayers (*A* and *B*). Cells protrude (open arrow, *B*) from the apical surface (as) and migrate into the gel (arrows, *A*). In sections, the migrating cells are cut in various planes; they are actually elongate in shape (Fig. 2). The apical surface not in contact with collagen (*, *A*) remains smooth. Nuclei of lens cells in gels enlarge and acquire prominent nucleoli (*nuc*, *B*). On the surface of collagen gel, in contrast, the epithelium (*C*) forms a simple cuboidal monolayer with a smooth apical surface (*as*) and secretes a basal layer (*bl*) with the morphology of lens capsule as viewed in the electron microscope. Nuclei (*n*) do not contain prominent nucleoli. In *A*, the lens capsule appears thicker than in vivo due to slight obliqueness of section plane, and also to swelling in vitro. (*A*) Bar, 50 μm; ×, 300. (*B* and *C*) Bar, 10 μm; × 1000.

Fig. 4B) that extend into the gel. Larger pseudopodia also project into the collagen gel. The epithelium of the adult anterior lens embedded in collagen soon becomes multilayered (Fig. 3B) due to cell division and the peripheral layer of cells in contact with collagen fibrils seems to dissociate and to develop new secretory organelles. Cells that maintain contact with the lens capsule have a fibrillogranular, organelle-poor cytoplasm, and remain epithelial in appearance (lower right, Fig. 4E). The elongating cells, even while still maintaining cell-cell contacts with the basal cells, develop numerous large mitochondria, well developed Golgi zones with dilated lamellae, granular endoplasmic reticulum, and prominent nucleoli (Fig. 4E). Nuclear chromatin condensations disappear (n, Fig. 4E). Thus, concomitant with the elongation and acquisition of a bipolar morphology, ultrastructural features characteristic of mesenchymal cells appear in the lens cells.

In freely migrating bipolar cells (Figs. 4C and D), the ultrastructure of the cytoplasm comes further to resemble that of mesenchymal cells. The prominent filopodia are organelle-free (f, Fig. 4C). Cell contacts with passing cells occur, but are not as extensive as in epithelia. The cell body is organelle-rich with large mitochondria, well developed Golgi zones, many free polyribosomes, and long parallel segments of granular endoplasmic reticulum (er, Fig. 4D). The nuclear chromatin does not contain condensations, and nucleoli are well developed. The cytoplasm of migrating lens epithelial cells loses the fibrillogranular appearance that characterizes cells rich in crystalline lens proteins. Intermediate filaments and microfilaments are now abundant in the cytosol (fil, Fig. 4D). Surface specializations characteristic of epithelia (microvilli, junctional complexes, basal laminae) are not present, nor do these elongated cells within collagenous lattices ever have ruffling lamellipodia characteristic of cells on two-dimensional surfaces in vitro. These results show that differentiated epithelia, when placed within native collagen gels, are able to give rise to individual cells that migrate through the three-dimensional lattice with a morphology virtually indistinguishable from that of mesenchymal cells in vivo.

DISCUSSION

In the very young embryo, epithelia are known to give rise to mesenchymal cells (13, 14) that have no free surface and are able to migrate freely within a three-dimensional extracellular matrix. Such epithelial to mesenchymal transformations, however, are not known to occur in definitive embryonic tissues or in the adult. The present study shows that environmental conditions in vitro can dramatically alter the epithelial phenotype, cell shape, and polarity as defined by the presence of clearcut basal and apical surfaces. Both adult and embryonic epithelia, when placed within gelling solutions of collagen, can give rise to cells that separate from the tissue explant and migrate into the surrounding collagen gel as elongated, individual cells with prominent filopodia. Ultrastructural changes accompanying epithelial cell elongation and migration are consistent with an increase in secretory activity. However, biochemical analyses will be required to determine whether these cells are synthesizing proteins typical of mesenchymal cells.

Our results were unexpected because previous studies of epithelial cells in vitro have suggested that the epithelial phenotype is relatively stable. When epithelial junctional complexes are disrupted, components of the free surface can migrate to the basolateral surface (18), but when isolated epithelial cells are allowed to attach to a planar substratum, apical-basal polarity is reestablished (4, 5). Previous studies by Chambord et al. (7) have shown that exposure of the outer, apical cell surface of thyroid cysts to suspension in collagen gel results in an apparent reversal of cell polarity so as to create a free surface inside the cyst, which now resembles a follicle. In our study, however, the apical surfaces of lens epthelial cells were found to respond to suspension in collagen gel by extending filopodia into the three-dimensional matrix. Epithelia under the conditions studied here migrate as individual cells into the gel, dissociating from their neighboring cells rather than maintaining their junctional complexes and forming epithelioid structures that preserve cell polarity. It would be interesting to suspend thyroid epithelium within collagen gels to see if it can give rise to mesenchymelike cells in this situation.

Both intact tissue explants with a basal lamina and epithelium isolated without a basal lamina give rise to mesenchymelike cells under the conditions described here. In the case of the enzyme-isolated epthelial sheets (corneal, notochordal, and limb epithelia) and pellets (endothelium, lens), it was difficult to judge whether the migrating cells arose from the former apical or basal side of the tissue. The cuboidal lens epithelium, however, could be isolated with its basal lamina (lens capsule) intact by dissection because of the absence of mesenchyme in the area and the fact that the lamina is thick. In this case, it was clear that it is the apical surface of the epithelium that interacts with adjacent collagen fibrils. It is tempting to conclude that membrane components characteristic of the apical surface are altered in the presence of collagen and that the apical surface comes, in composition and motility, to resemble the leading end of a mesenchymal cell under these circumstances.

The cells that dissociate from the lens epithelium alter their basolateral as well as their apical surfaces. The extensive gap junctions between former lateral surfaces disappear and intercellular contacts between migrating cells seem random and unstable. The migrating cells derived from the adult lens epithelium may not have possessed real basal surfaces, for this tissue multilayers in the process, with basal cells remaining epithelial and attached to lens capsule whereas the superficial cells move away. Experiments to mark and follow apical and basolateral membrane components are needed to reveal the degree of turnover and/or membrane flow that occurs during these epithelial-mesenchymal transitions.

Our experiments suggest that many, if not all, epithelial cells have the capacity to elongate and to invade extracellular matrix. They raise the question that what might be the normal constraints that operate to control epithelial polarity in vivo. One such constraint may be the fact that apical surfaces are normally protected from contact with collagen. It is the basal epithelial cell surface that becomes disrupted during "normal" epithelial-mesenchymal transformations in the embryo (3, 13). These transformations are predetermined during morphogenesis, in the sense that they occur at specific times in specific epithelia, e.g., formation of primary mesenchyme from epiblast (13), derivation of cushion mesenchyme from endocardium (3), migration of neural crest from neural tube (8). It does not seem sufficient merely to remove basal lamina from the basal surface of embryonic epithelia to alter basal surface properties along these lines. For example, enzyme-isolated ventral neural tube grown on top of collagen gels maintains epithelial morphology; neural tube with dorsal epithelium present, however, gives rise to neural crestlike cells even on top of a collagenous matrix (8).
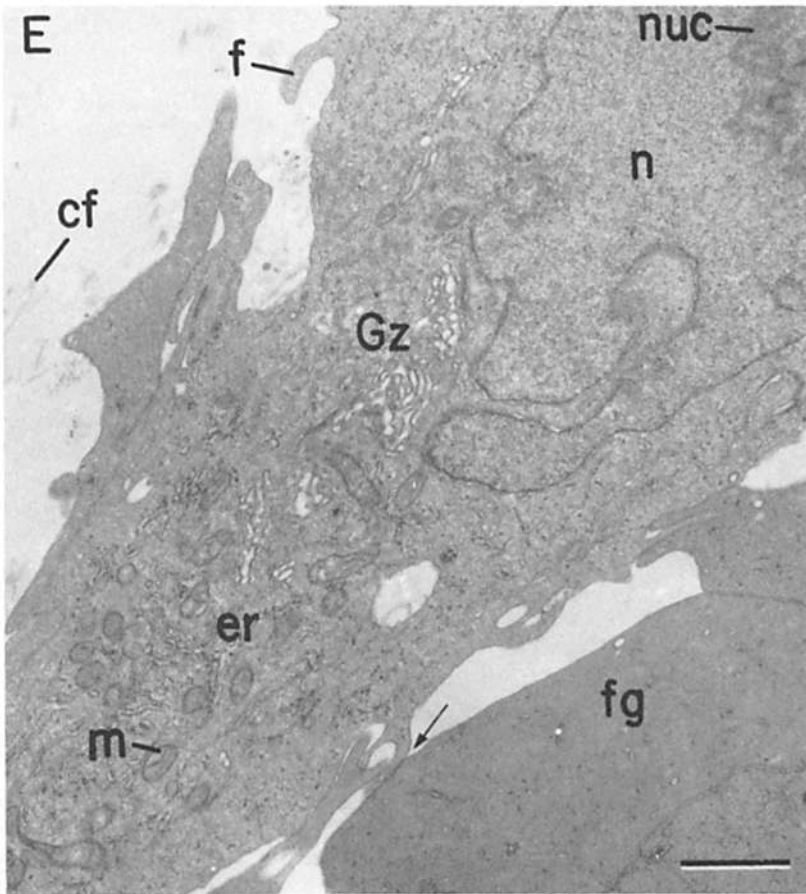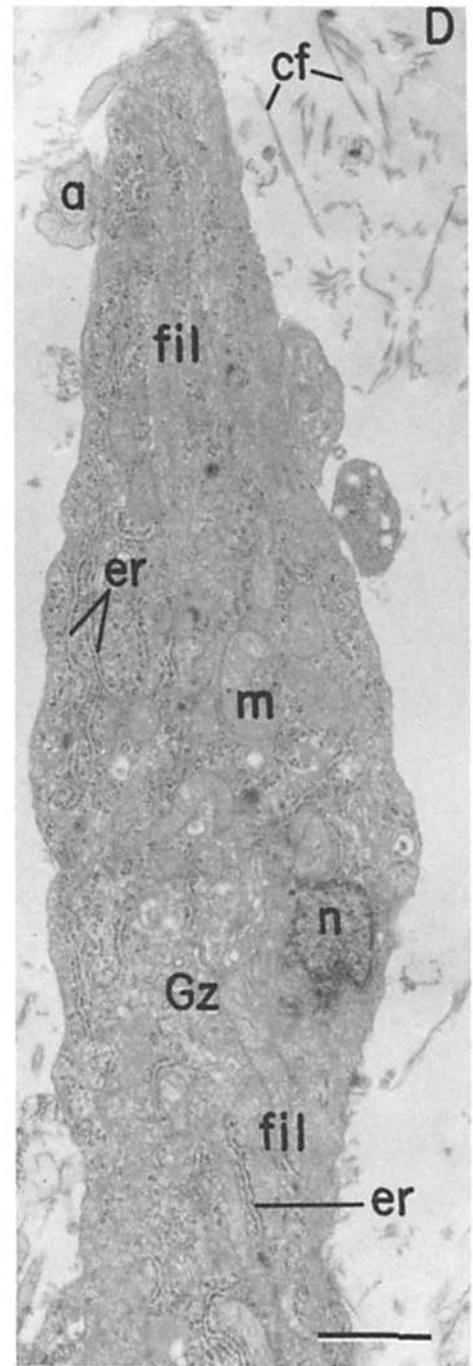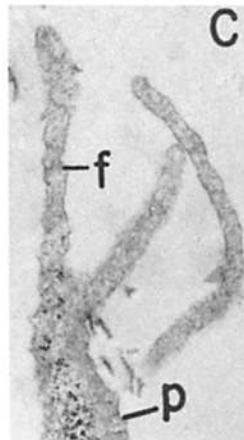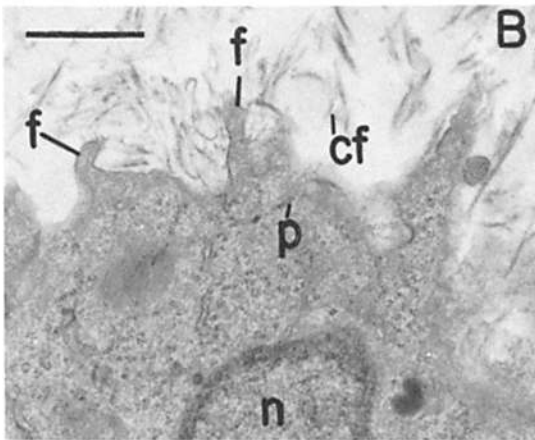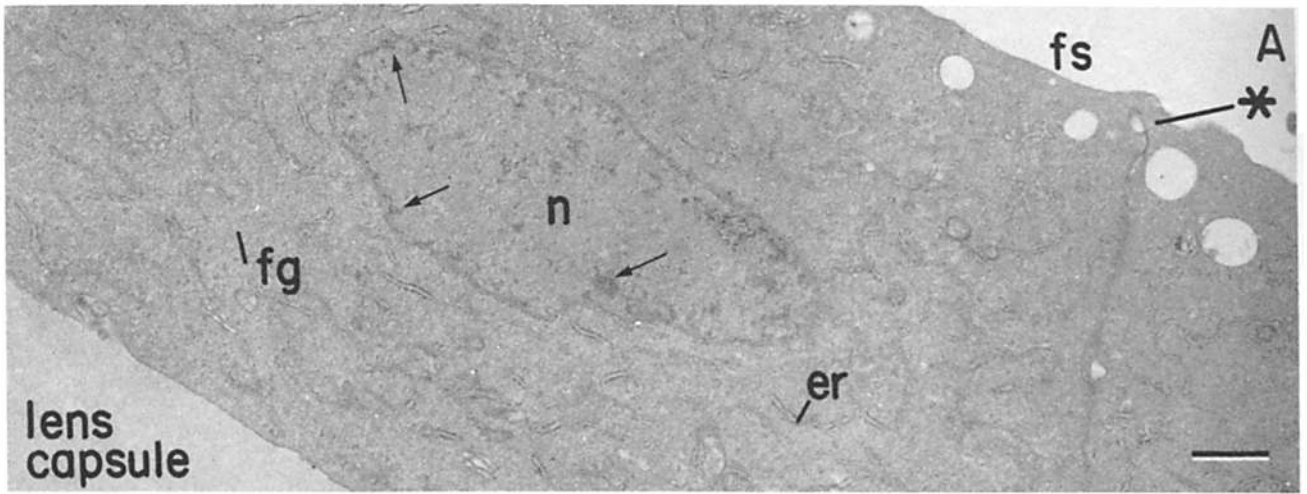
Future study of embryonic and adult epithelial tissues under various environmental conditions should provide information that will help in establishing the rules that govern epithelial-mesenchymal transformations. The capacity of normal adult tissues, other than those studied here, to give rise to mesenchymelike cells is unknown, but could be an inherent property of all epithelial cells that might manifest itself during pathological conditions.

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FIGURE 4 Ultrastructure of steps in the transformation of adult lens epithelium to mesenchymelike cells. (*A*) Freshly isolated epithelium is attached to lens capsule has a smooth free surface (*fs*) with junctional complexes between the cells (as in the region of the asterisk). Nucleus (*n*) contains heterochromatin clumps (arrows) and cytoplasm is fibrillogranular in appearance (*fg*) with short cisternae of granular endoplasmic reticulum (*er*). (*B*) When this tissue is immersed in collagen for 5–9 d (*B–E*), the free surface in contact with collagen extends filopodia (*f*) that touch collagen fibrils (*cf*). *p*, polyribosome; *n*, nucleus. (*C*) Filopodia (*f*) can be seen to be well developed on the leading pseudopodium (*p*) of a freely migrating cell. (*D*) The freely migrating cells have elongated cell bodies, with cytoplasm rich in granular endoplasmic reticulum (*er*), mitochondria (*m*), and filaments (*fil*). *Gz*, Golgi zone; *a*, process of an adjacent cell; *cf*, collagen-fibrils; *n*, nucleus. (*E*) A cell extending from the apical surface is seen to contain prominent Golgi zone (*Gz*), granular endoplasmic reticulum (*er*), mitochondria (*m*) and filopodia (*f*). The euchromatic nucleus (*n*) has a prominent nucleolus (*nuc*). The cytoplasm of an adjacent cell that is still attached to the lens capsule has fibrillogranular cytoplasm (*fg*) like that of the lens *in situ*. Bar, 1 μm. *A*, × 10,500. *B*, *C*, × 16,000. *D*, × 14,700. *E*, × 13,600.