

# Influence of Collagen Gel on the Orientation of Epithelial Cell Polarity: Follicle Formation from Isolated Thyroid Cells and from Preformed Monolayers

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**ABSTRACT** The influence of collagen gels on the orientation of the polarity of epithelial thyroid cells in culture was studied under four different conditions. (a) Isolated cells cultured on the surface of a collagen gel formed a monolayer. The apical pole was in contact with the culture medium and the basal membrane was attached to the substratum.

(b) Isolated cells embedded inside the gel organized within 8 d into follicles. The basal pole was in contact with collagen and the apical pole was oriented towards the interior of the follicular lumen.

(c) Cells were first organized into floating vesicles, structures in which the apical surface is in contact with the culture medium, and the vesicles were embedded inside the collagen gel. After 3 d, cell polarity was inverted, the apical pole being oriented towards the cavity encompassed by cells. Vesicles had been transformed into follicles.

(d) Monolayers formed on collagen gels as in a were overlaid with a second layer of collagen, which was polymerized in contact with the apical cell surface. A disorganization of the continuous pavement occurred within 24 h; cells attached to the upper layer of collagen and reorganized into follicles in the collagen sandwich within 4-8 d.

A similar process occurred when the monolayer was grown on plastic and overlaid with collagen, or grown on collagen and covered with small pieces of glass cover slips. No reorganization was observed between two glass surfaces.

In conclusion, first, a basal pole was always formed in the area of contact between the cell membrane and an adhesive surface and, second, the interaction of a preformed apical pole with an adhesive surface was not compatible with the stability of this domain of the plasma membrane. The interaction of the cell membrane with extracellular components having adhesive properties appears to be a determinant factor in the orientation and stabilization of epithelial cell polarity.

The formation of a polarized epithelial cell monolayer involves two types of events: (a) the formation of intercellular junctions, the most typical for epithelia being the tight junction (14, 36); and (b) cell polarization by an asymmetrical distribution of membrane components between the apical and basolateral domains (8, 21) and by a polar distribution of intracellular

organelles. Once established, the polarity must be maintained despite continuous turnover of molecules. In several epithelial systems, the polarized state is described at the morphological and molecular levels, but the mechanisms involved in the genesis and maintenance of cell polarity as well as the factors that take part in the determination of the orientation of the

polarity are almost unknown.

In vivo, the orientation of the cell layer is determined during morphogenesis. The basolateral surface is always in contact with the internal milieu from which cell nutrients are available. In monostratified epithelia, the apical membrane bounds a closed or opened cavity and the composition of the fluid in contact with the apical surface is specific for each tissue.

In vitro, isolated epithelial cells reorganize into polarized cell monolayers. The apical surface of the cell layer is in contact with the culture medium, whereas the basal pole is attached to the culture substratum. Under these conditions, as in vivo, one orientation of cell polarity is chosen and maintained.

In culture, isolated porcine thyroid cells reorganize into two types of structures that essentially differ in the orientation of the polarity of the cell layer (15–17, 28). When a thyroid stimulator (thyrotropin, prostaglandin E<sub>2</sub>, or dibutyryl cyclic AMP) is present in the culture medium, follicle-like structures are obtained. As in genuine follicles, the apical pole of cells faces the follicular cavity in which thyroglobulin accumulates. On the contrary, when cells are cultured in the absence of a stimulator, they organize into monolayers on a substratum or into vesicles (or cysts) in suspension (24, 35). In both monolayers and vesicles, the apical surface of the cell layer is in contact with the culture medium. With this orientation the reconstituted thyroid epithelium cannot accumulate thyroglobulin (which is still synthesized at a lower rate [5]), is no longer able to concentrate and to organify iodide, and responds poorly to thyrotropin stimulation (28, 33). The existence of two opposite orientations for cell polarity is also supported by the transepithelial potential difference: the apical side is always negative as compared to the basal side (34). This system appears especially favorable for the study of factors and mechanisms involved in the determination of the orientation of epithelial cell polarity. In addition, as two orientations can be obtained from isolated cells, the possibility of modifying cell polarity without dissociating the cells can be considered. This raises the question of the stability of the polarized state. Indeed, the inversion of cell polarity in rat thyroid follicles upon increasing the serum concentration in the culture medium has been recently reported (41).

In recent years, collagen gels (10) have been used as substrata for the culture of various epithelial cells. On these permeable and elastic supports, a better expression of specific differentiation characteristics and a higher sensitivity to hormonal stimulation have been reported for hepatocytes and mammary gland cells (12, 13, 37, 45).

We report here that isolated porcine thyroid cells form a monolayer when cultured on a collagen gel. In contrast, when the cells are cultured embedded inside the gel, which provides an isotropic environment (10, 50), they organize into three-dimensional follicle-like structures. This observation prompted us to investigate the effect of collagen on a preformed apical surface. Vesicles were embedded inside the gel and monolayers were overlaid with a layer of collagen gel. We observed that in both cases a reorganization of cells into follicles occurred. The orientation of cell polarity can therefore be inverted as a result of the interaction of collagen with the apical surface of polarized cells. Part of this work has already been presented in preliminary form (6, 31, 32).

## MATERIALS AND METHODS

### *Cell Isolation and Culture*

Epithelial thyroid cells were isolated from adult porcine thyroid glands as

previously described (method 2 in reference 35), except that the minced tissue was incubated in a Spinner salt solution without Ca<sup>2+</sup> and Mg<sup>2+</sup>, containing both trypsin (0.15%) and EGTA (3 mM). Isolated cells were finally suspended ( $2 \times 10^6$  viable cells/ml) in Eagle's minimal essential medium (MEM; Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.), supplemented with penicillin (200 U/ml), streptomycin (50 µg/ml), and newborn calf serum (1 or 10%; Gibco Laboratories).

Isolated cells were also obtained from thyroid cells maintained in monolayer on a plastic substratum, after a brief treatment with the same trypsin-EGTA solution (10 min, 37°C).

The cellular suspension was plated in 35-mm-diameter Petri dishes and incubated at 37°C in a 5% CO<sub>2</sub>/95% air, water-saturated atmosphere. The medium was changed 24 h after plating and then routinely every 2nd or 3rd d. Cells were observed daily with an inverted microscope (Wild M40).

### *Preparation of Collagen Solution*

Collagen was solubilized by stirring rat tail tendons for 48 h at 4°C in a sterile 1:1,000 (vol/vol) acetic acid solution (100 ml for 1 g of collagen). The resulting viscous solution was filtered through a sterile gauze and then centrifuged at 16,000 g for 1 h at 4°C. The supernate was our stock solution and can be stored at -20°C. The collagen concentration was ~0.4% (wt/vol).

### *Preparation of Hydrated Collagen Gels*

Collagen gels were prepared according to Elsdale and Bard (10). Ionic strength and pH were raised simultaneously, the collagen solution being maintained at 4°C to delay its polymerization. A pH of 7.2 and a convenient ionic strength were obtained by mixing 8 vol of the ice-cold collagen solution with 2 vol of a 1:1 mixture of a  $\times 10$  concentrated MEM with 0.125 N NaOH in 0.26 M NaHCO<sub>3</sub>. This cold gelation mixture was dispensed (50 µl/cm<sup>2</sup>) into tissue culture dishes and allowed to gel for a few minutes at room temperature.

The collagen-coated dishes were kept with the culture medium in the cell incubator until use. When the collagen gel had to be released from the plastic substrate, the collagen was polymerized in Falcon dishes that had not been treated for tissue culture.

### *Cell Culture on the Collagen Surface*

2 ml of the cell suspension was seeded into each collagen-coated dish. The cells adhered to the surface of the collagen layer, and their culture was performed as with conventional attached cells. Eventually, the collagen membrane, which was thick and elastic, could be released from the plastic dish and allowed to float in the culture medium, without damaging the cells grown on its surface.

### *Cell Culture in the Collagen Gel*

To embed isolated cells in the collagen gel, the following procedure was used. Isolated cells were suspended in serum-free MEM, and 2 ml of the cell suspension was seeded per collagen-coated dish. Cells were allowed to attach to the surface of the gel for 4–6 h. Under these conditions, they attached but did not spread and remained isolated. The medium and unattached cells were removed and a second layer of collagen was allowed to polymerize on top of the first gel, thus embedding the cells.

With this method, the cells were in a plane and observation of their behavior was therefore easier. Similar results were obtained with the method described by Yang et al. (50) for mammary gland cells, which did not allow direct observation of the reorganization process.

### *Embedding of Vesicles inside the Collagen Gel*

Isolated porcine thyroid cells were reorganized into vesicles after 4 d in suspension culture, as previously described (35). Vesicles, collected by centrifugation, were suspended in the cold collagen mixture ( $3 \times 10^7$  cells/ml of collagen). The suspension was added to collagen-coated dishes as a thin layer or as drops on the substrate. After gelation (5–10 min) in the incubator, the culture medium was added.

### *Overlaying of Monolayers with Collagen Gel*

Thyroid cell monolayers were obtained on collagen, plastic, or glass substrata. After 4–15 d, the medium was carefully drained and 0.5 ml of the cold collagen solution was spread on the cell layer. After 10 min in the incubator, gelation was achieved and fresh medium was added.

## Preparation for Electron Microscopy

Cultured cells were fixed in situ at room temperature for 1 h in 2% glutaraldehyde in 0.1 M sodium cacodylate pH 7.2, postfixed in 1% osmium tetroxide in 5 mM veronal buffer, and dehydrated in alcohol. Small pieces of the fixed and dehydrated collagen gel containing the cells were cut and embedded in Epon. Semithin sections, perpendicular to the cell layer, were stained with azure blue and observed with a Zeiss photomicroscope III. Thin sections were contrasted with uranyl acetate and lead citrate before they were examined in the Philips EM 200 or EM 301 electron microscope.

For scanning electron microscopy, the dehydrated samples were critical-point-dried with CO<sub>2</sub>, mounted on stubs, coated with gold-palladium (300 Å thick), and observed with a Jeol SM 35 at 15 kV.

## RESULTS

### Monolayer Formation on Attached Collagen Gels

Freshly isolated porcine thyroid cells, seeded at high density in collagen gel-coated dishes, were attached within 6–12 h and had formed a confluent monolayer after 24–48 h. Attachment occurred more rapidly in serum-free medium (3–6 h) and the delay in reaching confluence was mainly dependent on the initial cell density. Cells appeared polygonal and formed a continuous pavement (Fig. 1). The average cell density was  $\sim 3 \times 10^5$  cells/cm<sup>2</sup> but higher densities were reached near the center of the dish ( $7 \times 10^5$  cells/cm<sup>2</sup>). Observation of the monolayer by transmission and scanning electron microscopy (Figs. 2 and 3) showed that cells were polarized and that the apical surface was in contact with the culture medium. In thin sections, the apical pole, limited by junctional complexes, could be identified by the presence of microvilli (Fig. 3). The organization of the cytoskeleton (microfilaments and microtubules) was similar to that observed in thyroid cells in vivo (J. Gabrion, unpublished observations). The centriole at the base of a cilium was close to the apical membrane. The basal domain of the plasma membrane was in contact with the collagen fibrils. No basement membrane was synthesized under these culture conditions. SEM observation of the apical cell surface showed numerous microvilli and a single cilium on each cell (Fig. 2). The density of the microvilli was irregular and often higher at the cell margin. When cells were cultured on glass or plastic surfaces, they were more stretched and microvilli were less abundant (Fig. 2a). The apical surface was also in contact with the culture medium. Domes that were formed on nonpermeable supports, as observed with other epithelial cells (35), were never observed on collagen gels. Moreover, cells could not be released from collagen by trypsin or EGTA treatments that rapidly detached the monolayer from glass or plastic substrata. This suggests a stronger attachment of cells to collagen than to glass or to plastic.

### Monolayer Formation on Floating Collagen Gels

To facilitate intercellular interactions, to reduce the stretching of cells on the substratum and to allow nutrient access to both sides of the cell layer, the collagen gel was detached from the plastic surface 4 d after plating, when the monolayer was confluent. As described for other systems, a contraction of the floating collagen gel was observed (12, 45), which did not occur in the absence of cells. The speed and amplitude of this contraction depended on several parameters, including the thickness, the concentration of the gel, and the cell density. Under our standard conditions, the contraction was rapid during the 1st 2 h and then much slower. The total surface was four times smaller after 3 wk in culture. On the floating gel,

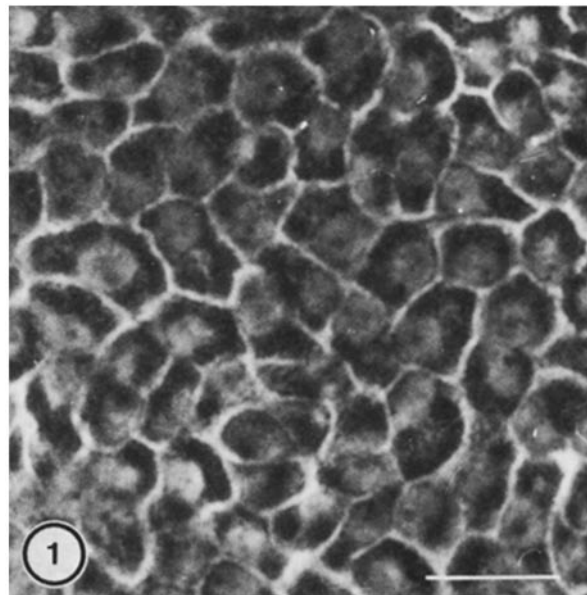


FIGURE 1 Thyroid epithelial cells organized into a monolayer on an attached collagen gel (phase-contrast microscopy). No reciprocal polarity is observed between cells. The zone of cellular apposition shows a clear space. Bar, 25  $\mu$ m.

only minor modifications of the cell layer were observed. The average cell size was smaller, resulting in higher cell density (Fig. 2b and c). On the apical poles, the density of the microvilli was higher. No important difference in the appearance of the cells was observed between the center and the periphery of the collagen membranes as described for mammary gland cells (12). A confluent monolayer was also formed within 24 h when the gel was released early after cell adhesion, before spreading, i.e., 5 h after the onset of culturing.

### Follicle Formation inside Collagen Gels

When seeded on the surface of a collagen gel, porcine thyroid cells formed a monolayer. The orientation of cell polarization appeared to be a consequence of the interaction of the cell membrane with collagen, which induced the formation of a basal pole. We have subsequently investigated the behavior of isolated porcine thyroid cells embedded inside the collagen gel. Cells were initially randomly distributed inside the gel (Fig. 4a). During the first 24–48 h, the cells moved and formed strands with one or several rows (Fig. 4b). Later, polarized structures appeared in increasing number (Fig. 4c and d). Rings of phase-dense granules bounded the cavities surrounded by cells. After 2 wk, almost all cells were organized into such structures (Fig. 4d). Observation of thin sections showed that the structures that formed inside the collagen gel had the characteristics of follicles (Fig. 5). The apical poles of the cells, characterized by the presence of microvilli and limited by the junctional complexes, were oriented towards the follicular lumen, which contained an electron-dense material.

The same organization was obtained with culture media containing 1 or 10% calf serum, but the formation of polarized structures, as observed by phase-contrast microscopy, was more rapid with high serum concentrations.

Isolated cells obtained by a trypsin-EGTA treatment of monolayers maintained on a plastic substratum had the same behavior as freshly isolated cells. They were able to form follicles when further cultured inside the collagen gel; the

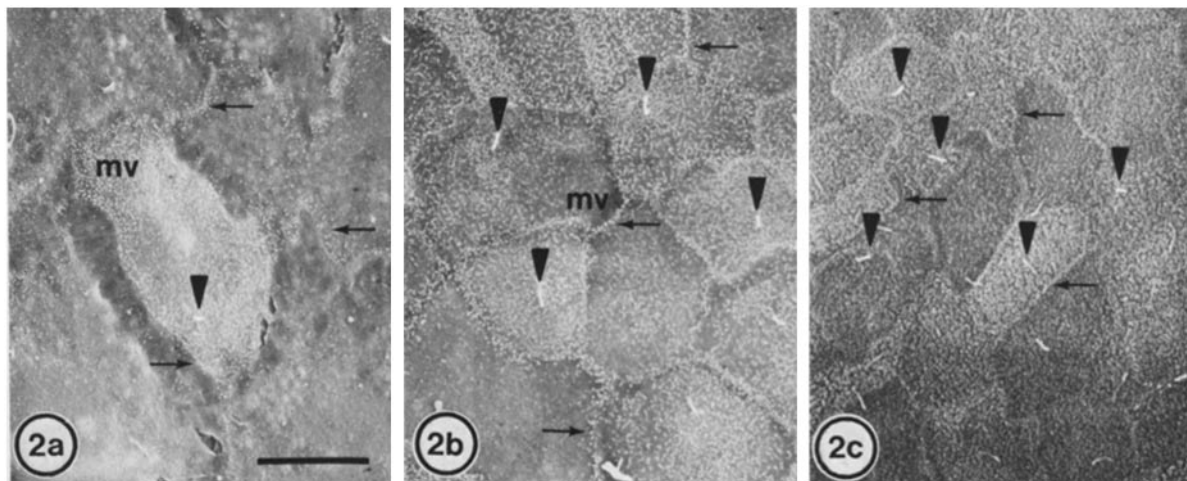


FIGURE 2 Apical surface of thyroid cells organized into a monolayer on tissue culture plastic (a), on an attached collagen gel (b), or on a floating collagen gel (c) (scanning electron microscopy). The apical poles of the cells characterized by the presence of microvilli (*mv*) and of one cilium per cell (▼) are in contact with the culture medium. The density of microvilli is heterogeneous from cell to cell but is always higher at the cell margin (→). On the collagen gel (b), the cell density is higher and apical microvilli are more abundant than on plastic. On the floating collagen membrane, the cell surface decreases with contraction of the gel. Bar, 10  $\mu\text{m}$ .

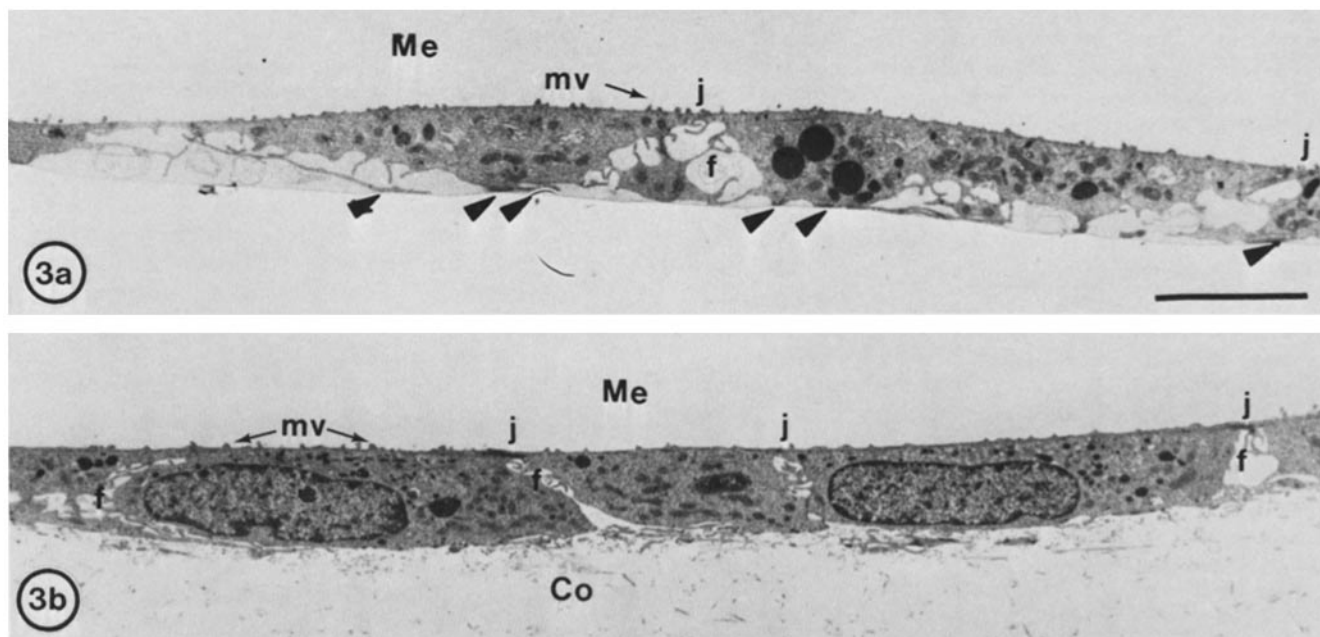


FIGURE 3 Ultrastructure of cells organized into a monolayer on tissue culture-treated plastic (a) and on an attached collagen gel (b). In both cases the apical membranes with microvilli (*mv* →) and limited by the junctional complexes (*j*) are facing the culture medium (*Me*). On plastic, the intercellular spaces are large, and long filopods (*f*) extend towards the substratum (▶). On collagen, the intercellular spaces are narrow and the filopods are shorter (*f*). A large portion of the basal membrane is in contact with the underlying collagen fibrils (*Co*). Bar, 5  $\mu\text{m}$ .

reorganization was even more rapid, because cells were already adapted to culture conditions and were less damaged by the isolation procedure.

When cells were embedded inside the collagen gel, the only possibility allowing cell polarization was the formation of a cavity inside the cellular aggregates where the apical pole could be formed, thus giving follicle-like structures.

#### Transformation of Vesicles into Follicles

We observed previously (35) that, when cultured in suspension, in serum-containing medium, isolated porcine thyroid

cells reorganized into vesicles (Fig. 6a). On a morphological basis these structures can be considered as inverted follicles because the apical surface of the cell layer was oriented towards the exterior (Fig. 7a and c). The effect of collagen on the external apical surface was investigated. Vesicles obtained after 4 d in suspension culture were embedded inside the collagen gel and further cultured. Continuous observation by phase-contrast microscopy of a given vesicle showed that the cells did not dissociate and that, after 3–4 d, intracellular phase-dense granules appeared bounding a central cavity (Fig. 6b). This suggested a change in the orientation of cell polarity. Observation of the ultrastructure in thin sections showed that after

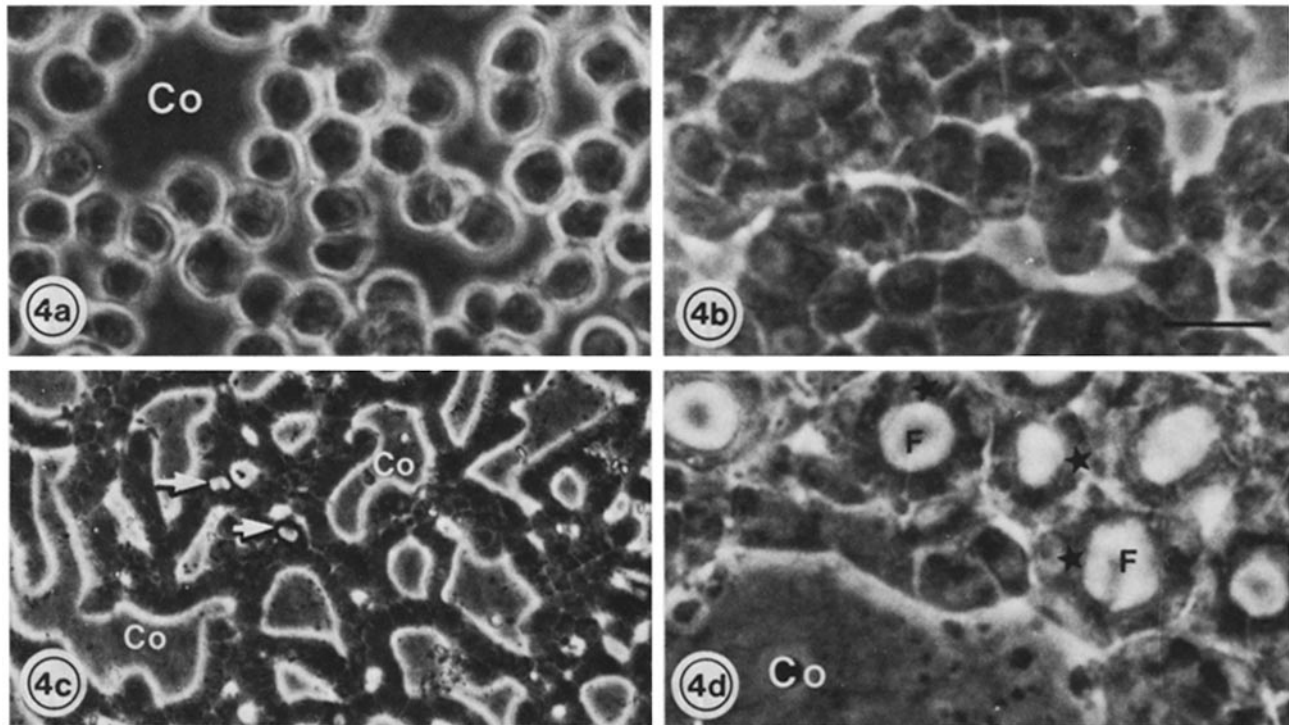


FIGURE 4 Time-course of the reorganization of isolated thyroid cells embedded inside a collagen gel (phase-contrast microscopy). (a) 15 min after being embedded, cells are isolated and uniformly distributed. (b) After 18 h in culture, cells are organized into strands and clusters. (c) After 4 d, follicular structures (→) are observed inside the trabeculae. Large areas of collagen (Co) are free of cells. (d) After 10 d, ~80% of the cells are involved in the formation of follicles. Dense granules (★) are apically concentrated, bounding the lumen (F). Bar: a, b, and d, 20  $\mu\text{m}$ ; c, 70  $\mu\text{m}$ .

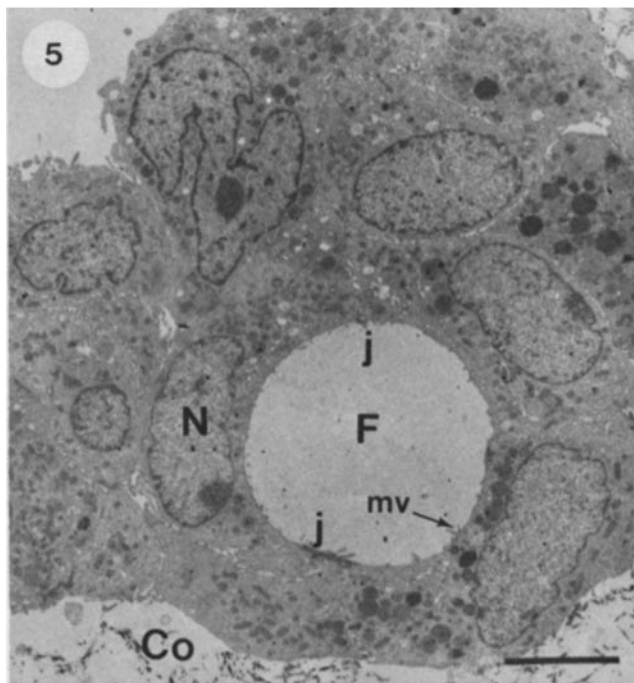


FIGURE 5 Ultrastructure of a follicle formed by isolated thyroid cells embedded inside a collagen gel after 14 d in culture. The apical poles of cells with microvilli (mv) bound the follicular lumen (F), which contains an amorphous electron-dense material. j, intercellular junctions; Co, collagen fibrils; N, nucleus. Bar, 5  $\mu\text{m}$ .

4 d the apical pole of the cells was oriented towards the interior of the cavity bounded by cells (Fig. 7b and d). Preliminary observations showed that evidence for the inversion of polarity

appeared 24 h after embedding the vesicles. During this inversion, intercellular junctions were always observed between adjacent cells. Contact with the collagen gel appears, therefore, to be incompatible with the stability of the apical domain of the cell membrane that was subsequently formed on the internal surface, thus transforming the vesicle into a follicle.

#### Transformation of Monolayers into Follicles

When vesicles were embedded inside the collagen gel, the inversion of cell polarity was not hampered by the initial presence of an attachment surface on the basal side of the cells. We have therefore studied the behavior of cells cultured as monolayers on collagen after covering their apical surface with collagen. Monolayers, after 4, 8, or 15 d in culture on an attached collagen gel, were overlaid with a second layer of collagen. A disorganization of the uniform monolayer occurred rapidly. Two different events took place simultaneously: (a) cells moved between the two layers of collagen and (b) polarized structures of various sizes and shapes appeared. Within the 1st 2 d, narrow clefts were observed between adjacent cells that showed symmetrical distribution of dense granules (Fig. 8a). These clefts progressively enlarged and within 8 d the majority of the cells were forming typical follicle-like structures (Fig. 8e). At the same time, in other areas of the dish, cells reorganized into elongated and branched tubules (Fig. 8b). In areas of high cell density the majority of cells were involved in circular follicles (Fig. 8c), whereas in areas of lower cell density, tubular structures were predominant (Fig. 8d). After 8–10 d, the new organization was stable and was maintained for weeks without further modifications. The observation of semithin (Fig. 9a) and thin (Fig. 9b) transverse sections confirmed the follicle-like organization of cells that were cultured between

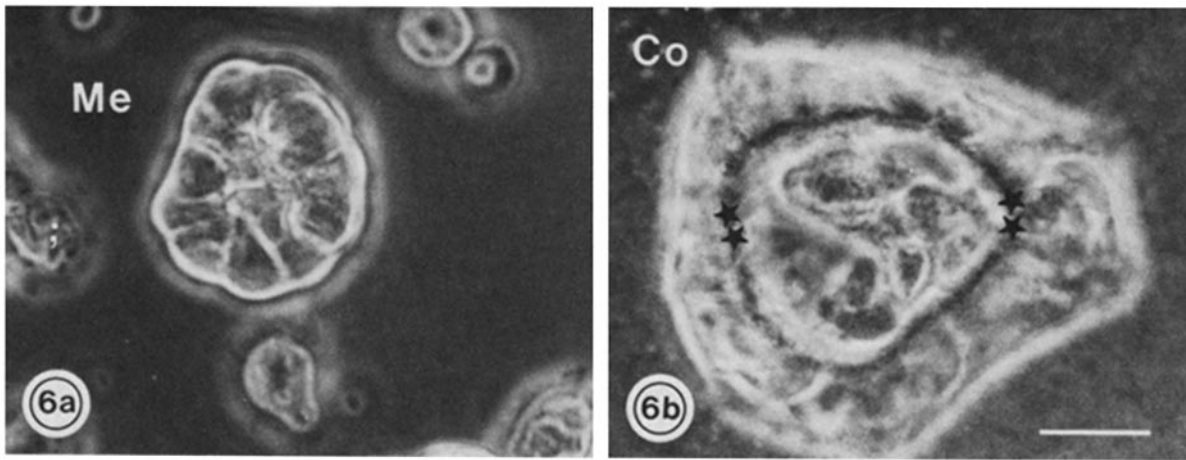


FIGURE 6 Transformation of floating vesicles into follicles (phase-contrast microscopy). (a) Floating vesicle formed by isolated thyroid cells cultured for 4 d in suspension. Me, culture medium; (b) Follicle observed 4 d after embedding a vesicle inside the collagen gel (Co). Apical dense granules (★) are concentrated around the lumen. Bar, 20  $\mu$ m.

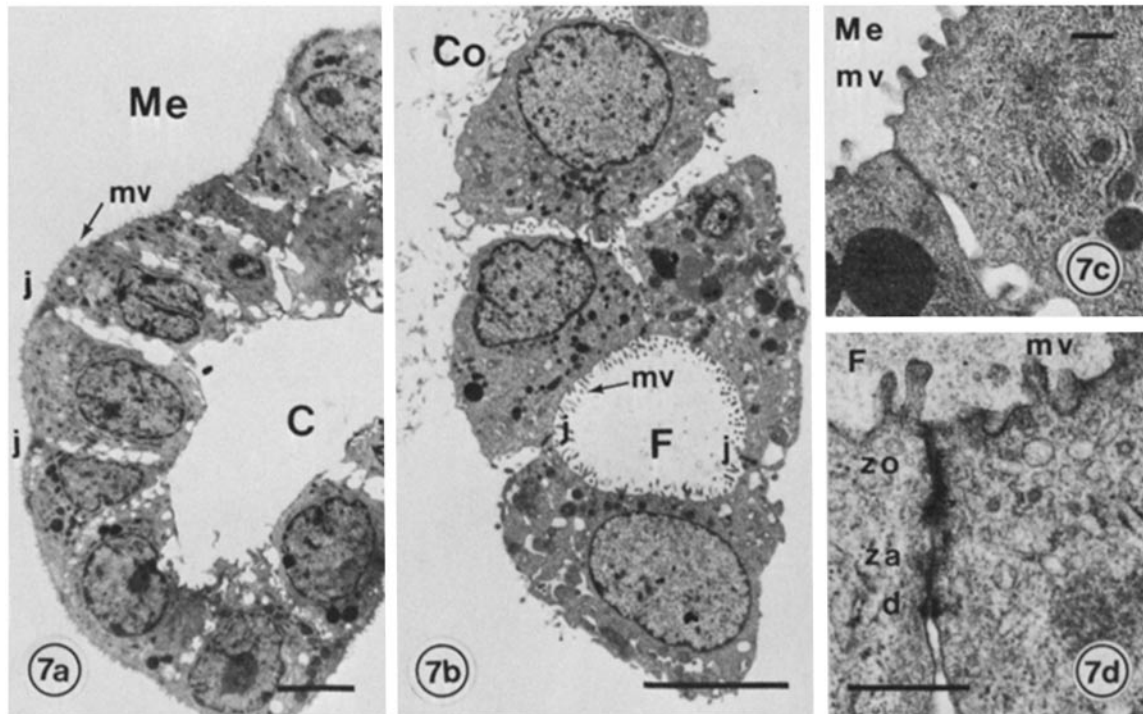


FIGURE 7 Transformation of floating vesicles into follicles. (a) Ultrastructure of a vesicle formed in suspension culture. The apical poles of the cells are oriented towards the exterior of the structure, in contact with the culture medium (Me). The basolateral membranes line an internal cavity (C). (b) Ultrastructure of a follicle formed 4 d after the vesicles were embedded inside the collagen gel. Cell polarity has been inverted, showing follicular structures. The apical poles are oriented toward the follicular lumen (F), which contains electron-dense material. The basal poles are in contact with the surrounding collagen fibrils (Co). (c) Intercellular junction between two cells organized into a vesicle in suspension. Me, culture medium. (d) Junctional complex between two cells organized into a follicle in the collagen gel. zo, Zonula occludens, identified as such at higher magnification; za, zonula adherens; d, desmosome; mv, microvilli; j, intercellular junction. Bars: a and b, 5  $\mu$ m; c and d, 0.5  $\mu$ m.

the two layers of collagen. About half of the cells were attached to the lower surface of the new collagen layer and formed closed structures with other cells that were still attached to the first layer of collagen. Some cells at the equatorial periphery of follicles were in contact with both collagen layers. Electron-dense material was observed in the follicular lumen. The reorganization of the monolayer occurred also in the presence of 1% serum and was faster when the collagen gel was allowed to float in the culture medium.

#### Effect of Other Adhesive Surfaces

The effects of other surfaces known to support or not support cell adhesion were also studied. The following situations were tested: (a) cells grown as a monolayer on plastic or glass were overlaid with a collagen gel, (b) small pieces of glass cover slip were allowed to settle on cell monolayers grown on collagen gel, (c) cells grown on collagen were covered with agarose, and (d) small pieces of glass were allowed to settle on monolayers

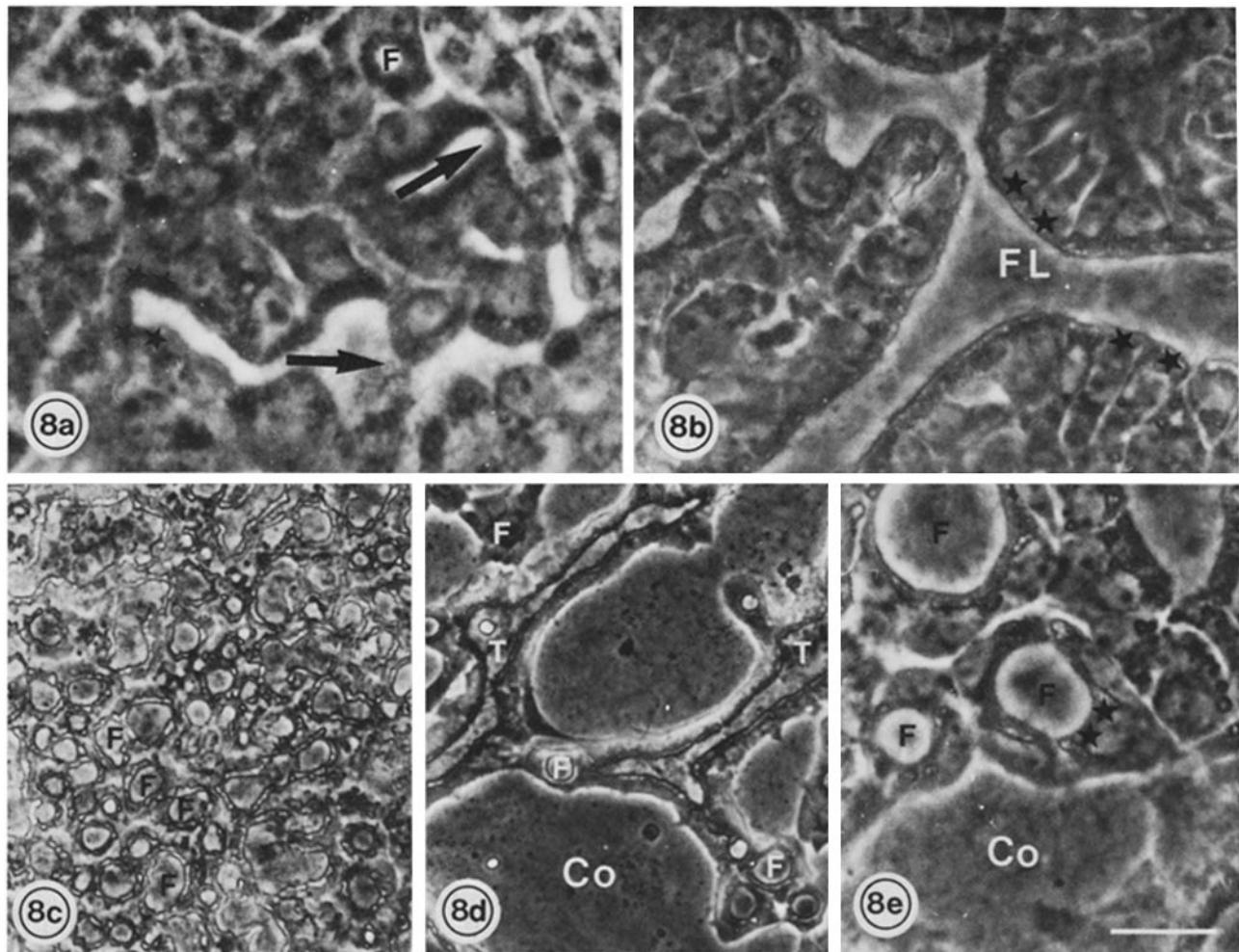


FIGURE 8 Time-course of the reorganization of thyroid cells at first organized into a monolayer, then overlaid with a collagen gel. (a) 24 h after being overlaid, the monolayer is disorganized. Circular (F) or elongated (→) polarized structures appear. (b) After 5 d, 80% of the cells are organized into polarized three-dimensional structures of various sizes and shapes. Dense apical granules (★) line the lumen (FL). (c–e) After 10 d, almost all the cells are involved in the formation of spherical follicles (F) or of elongated tubules (T). The proportion of these two types of structures depends on the locally high (c) or low (d) cell density. Co, collagen areas free of cells. Bar: a, b, and e, 20  $\mu$ m; c and d, 70  $\mu$ m.

grown on glass or plastic.

A reorganization similar to that described previously in the collagen sandwich occurred only under situations *a* and *b*, when collagen gel was present on one side of the monolayer. However, with asymmetric substrata (plastic-collagen or collagen-glass), the formation of follicles was slower. When small pieces of glass were used, reorganization did not take place in monolayer areas not covered with glass. When agarose, which did not support cell adhesion, was used to overlay the monolayer, no effect was observed. Between two glass or plastic rigid and impermeable surfaces, cells became spiked and did not survive.

Three different properties of the collagen gel appeared to take part in the reorganization process: adhesiveness, permeability, and elasticity. Optimal conditions for follicle formation were obtained when both substrata were adhesive, permeable, and elastic, i.e., when both were collagen gel layers. With other surfaces adhesiveness was required for both substrata, whereas permeability and elasticity were necessary only on one side of the monolayer.

## DISCUSSION

The following conclusions can be drawn from the present

results: (a) The interaction of a collagen gel with the plasma membrane of thyroid cells induces the formation of a basal pole, and the nature of the multicellular structures formed, monolayers on the collagen surface and follicles inside the gel, is a consequence of this interaction. (b) The collagen gel can destabilize a preformed structure when it interacts with its apical surface. A basal pole is formed in contact with the gel and cells subsequently reorganize into follicles. The addition of thyrotropin is not required for the formation of follicles that are stable for long periods of time, in contrast to what was previously reported when cells were cultured on plastic or glass (16, 17, 28). These conclusions are related to two different types of events: (a) the formation of polarized multicellular structures from isolated cells and (b) the stability of a preformed polarized structure. These aspects will be discussed successively.

## Relation of Cell Adhesion to Polarization

The mechanism of cell attachment on culture substrata (glass, plastic, or collagen) is not yet clarified. Molecules involved appear to depend on cell type (19). Collagen, fibronectin, and laminin are involved in fibroblast and epithelial cell adhesion on culture substrata (22, 23, 26, 46, 48). Among the

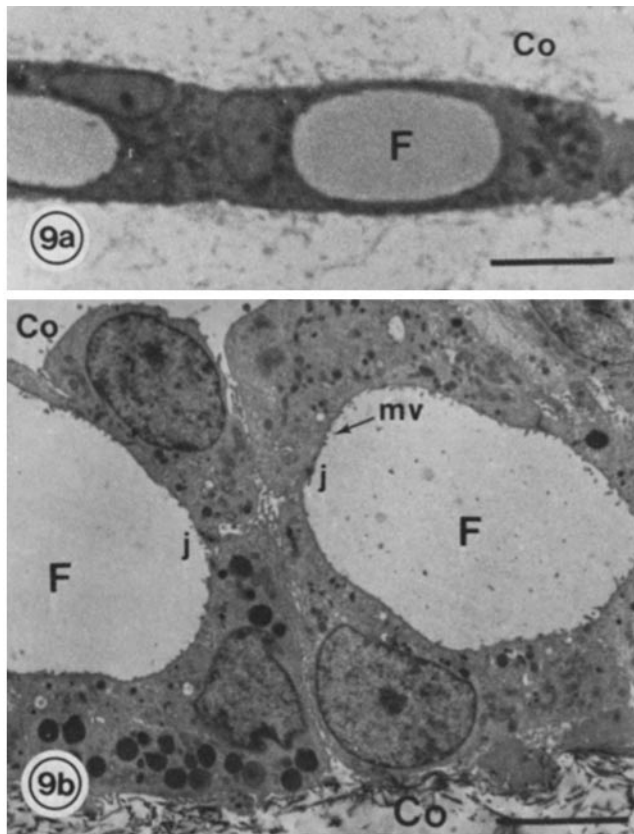


FIGURE 9 Follicles formed by cells previously organized into monolayers and overlaid with a collagen gel. (a) Transverse semithin section of follicular structures formed between the two collagen layers (Co), 7 d after overlaying the cell monolayer. Cells are attached to both collagen layers and encompass the follicular lumens (F) containing material stained with azure blue. (b) Ultrastructure of follicles formed between the two layers of collagen (Co) after 4 d in culture. The apical poles of the cells, characterized by the microvilli (mv) and by the position of the junctions (j), line the follicular lumens (F). Basal poles are in contact with the collagen fibrils. Bars: a, 10  $\mu$ m; b, 5  $\mu$ m.

various collagens, type IV, which is more specific for basement membranes (25), has been reported as a better substratum for epidermal and epithelial cells (39, 46). In the present study we used only type I collagen from rat tail tendon, which is able to produce gels under conditions compatible with cell viability (10). No comparison was made with other collagens.

The relation between epithelial cell attachment and polarization has never been extensively studied. In culture, the basal pole is always formed in contact with the substratum, but the reasons for this are unknown.

Comparisons can be made with other cell types. The initiation of polarization by clustering of surface molecules at the cell substratum contact is likely. Indeed such a phenomenon has been described for fibronectin binding sites on fibroblasts (20), for Fc and complement receptors on macrophages (38), and for asialoglycoprotein receptors on hepatocytes (49). Receptors for collagen have been described on the surface of fibroblasts (18). If similar processes occur with epithelial cells, the interaction with the substratum will induce a polarization of individual cells, subsequently orienting the polarity of the monolayer after the formation of cell-cell contacts. Information about the membrane-substratum contact is transmitted to the

cytoplasm, which becomes polarized. The cytoskeleton play a part in the coordination between polarization at the membrane and cytoplasmic levels (7).

Because polarization occurred on a floating collagen membrane, it appears that the existence of two distinct compartments limited by the cell layer is not required for the development and maintenance of morphological polarity.

Cell-cell contacts were required, because we observed that when cell density was low the cells that remained isolated did not survive, as previously reported for kidney epithelial cells (11).

When cells are embedded inside the gel, they must form aggregates to have a possibility for orientation. Under these conditions the generation of a cavity isolated from the environment is concomitant with cell polarization. The role of collagen is in the commitment to one orientation. A similar situation was described with mammary gland cells grown inside a collagen gel. They formed tubular structures, and their apical pole was oriented toward the lumen (1, 50). When cultured in suspension, in the absence of collagen, both thyroid gland and mammary gland cells reorganize into vesicles (12, 35).

### The Stability of the Polarized State

When thyroid cells are previously organized either into vesicles or into monolayers, the contact of their apical poles with the gel of collagen destabilizes the preformed apical domain and triggers cell reorientation and reorganization. An interaction must therefore occur between the apical membrane and the added collagen surface. Collagen appears as a new attachment surface. The apical pole of cells must therefore have, on its surface, binding sites that will interact with collagen or with a linker molecule such as fibronectin. These sites can be similar or dissimilar to those involved in the adhesion of the basal pole. The stability of the apical domain apparently requires that these sites remain free or at least not cross-linked. After their interaction with a suitable surface, cell polarity is no longer stable and cells reorganize to generate a new apical surface free of contact with an adhesive surface. When cells are forming vesicles, a simple mechanism can be proposed for the inversion of cell polarity. The number of "attachment sites" increases on the apical pole, which is now in contact with the collagen gel, and the inversion of cell polarity follows. In contrast, when cells initially form a monolayer, the process of reorganization appears to be more complex. The occupation of the apical attachment sites induces the disorganization of the monolayer, cell migration, and subsequent organization into follicles. Partial cell separation, which must occur before reorganization, implies the opening up of part of the intercellular junctions. A heterogeneity of the tight junctions has been described *in vivo* in chronically stimulated rat thyroid glands (47) and *in vitro* in monolayers of Madin-Darby canine kidney cells (4). If a similar situation exists in thyroid cell monolayers, some junctions might be disrupted allowing cell migration, and others might be maintained and involved in the initial formation of intercellular clefts and of small follicles. Cell movement is triggered by the contact of the cell's apical domain with collagen and stops when the new apical pole is formed inside the collagen-free follicular cavity. In embryos, the initiation of the migration of neural crest cells is related to the development of a fibrillar matrix on which cells move (9, 29, 40), and adhesive molecules have been implied in organogenesis (2, 43). The similarities between embryonic systems and thyroid cell reorganization are limited because in our case no true differ-



entiation occurs. Nevertheless our experimental system can be a useful model for the study of cell migration in relation to morphogenesis.

### Mechanism for the Determination and Stabilization of Epithelial Cell Polarity: A Model

Our observations made with both vesicles and monolayers show that external proteins, here collagen, can influence the polarized organization of the plasma membrane of epithelial cells and simultaneously play a part in the stability of this organization. The polarized state is destabilized when molecules that normally interact with the basal domain are experimentally put in contact with a preformed apical surface. This observation suggests that the asymmetrical distribution of molecules at the surface of an epithelial cell does not result only from an intracellular segregation of membrane elements en route to their respective location (30, 42). In fact, the specificity of proteins, which are considered as characteristic of the apical or basal domains, is never absolute.  $\text{Na}^+, \text{K}^+$ -ATPase, which is mostly located on the basal domain, is also present at low levels on the apical surface of kidney cells (27). A selection process might also operate at the membrane level, resulting from interactions with extracellular molecules that stabilize or destabilize proteins present at the cell surface. Collagen that stabilizes the basal pole acts as a destabilizer of the apical pole. The existence of molecules having an opposite effect, i.e., stabilization of the apical pole and destabilization of the basal pole, can be postulated. They remain to be characterized and might be present in serum that induces the transformation of follicles into vesicles (41). The two mechanisms, internal segregation and selection at the membrane level, are not mutually exclusive and can operate on different proteins. The asymmetry of the cell environment is therefore responsible, at least in part, for the stability of the polarized state. Stabilization of a local membrane differentiation by interaction with external elements occurs at the neuromuscular junction. The basal lamina present at the synaptic junction is responsible both for reinnervation of the original synaptic site in the absence of muscle cells and for reorganization of the subsynaptic membrane on regenerating muscle cells in the absence of nerves (3, 44).

A better knowledge of specific markers of the thyroid cell surface and of their interaction with basal or apical extracellular proteins is necessary to verify such a hypothesis and to evaluate the general character of these mechanisms.

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