THE GROWTH AND DIFFERENTIATION OF TRANSITIONAL EPITHELIUM IN VITRO

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ABSTRACT

The development of rat transitional epithelial cells grown on conventional nonpermeable surfaces was compared with development on permeable collagen supports. On glass or plastic surfaces, cells grew as expanding monolayer sheets. Once confluent, growth continued with a bilayer being formed in most areas and apical cells being continuously sloughed off. Although most cells were interconnected by desmosomes, and junctional complexes were formed, no other indications of differentiation were observed. After 2–3 wk of growth, division stopped and cell death ensued. In contrast, single-cell suspensions plated on collagen-coated nylon disks reassociated into multicellular islands and commenced growth. Mitoses were confined to the basal cells in contact with the permeable substrate. The islands developed into epithelial trilayers tapering to monolayers along spreading edges. Once the islands were confluent, stratification was completed and appeared similar to that observed in vivo. Germinal cells formed a basal lamina, and the upper layer was composed of large, flattened cells with an unusually thick asymmetrical plasma membrane on the apical surface. Electron microscopic and radioactive tracers demonstrated "leaky" zonulae occludentes with a restricted permeability to small molecules. The movement of urea was retarded in comparison to water. Unlike the slow turnover of adult epithelium in vivo, maturation and sloughing of apical cells were measurable. Transfer of cells could be effected and growth maintained for up to 4 mo. These results may indicate the necessity of a nutrient-permeable growth surface for the polarized differentiation of adult transitional epithelium.

KEY WORDS differentiation · transitional epithelium · in vitro

With the recent exception of keratinocytes (30, 37), it has not been possible to reproduce in vitro the normal architecture and differentiation of lining epithelia derived from isolated cells of adult mammals. Because of the microscopically thin nature of lining epithelia, this failure has precluded the type of direct biochemical studies that have become commonplace with larger tissue masses such as liver, muscle, or adipose tissue. Methods have been reported for the isolation and short-term culture of transitional epithelial cells as monolayers on Petri dishes (2, 5, 8, 11, 29, 38). However, transitional epithelium exists in vivo as a stratified tissue composed of three distinct layers of cells (14, 33, 41).

In this report, a culture method that allows the growth and stratification of transitional epithelium

is described. This method, which uses the permeable collagen-coated nylon disks developed by Cereijido et al. (6), is compared to cell culture on conventional Petri dishes.

MATERIALS AND METHODS

Isolation of Transitional Epithelium

Bladders were dissected from young male Fischer-344 rats (~200 g; Charles River Breeding Laboratories, Wilmington, Mass.), rinsed in Ham's F-12 medium (Grand Island Biological Co., Grand Island, N.Y.), and cut into quarters. Twelve quarters were put into 2.5 ml sterile F-12 containing 1 U/ml Type II collagenase (Worthington Biochemical Corp., Freehold, N.J.). After 5 h at 37°C in 95% air and 5% CO_2 without agitation, the pieces were gently aspirated 3–12 times through a wide-bore, 10-ml pipette to dissociate the epithelium from the underlying connective tissue. Detachment was monitored with a phase-contrast microscope. Remnants of the quarters were removed with forceps, leaving behind the fragmented, but large, epithelial pieces that had settled on the bottom and the smaller contaminants suspended in the medium. The suspension was aspirated off, and the epithelium resuspended to 10 ml with F-12, gently vortexed, and allowed to settle for 30 s before removal of the medium over the settled epithelium. This process was repeated three times and usually yielded a preparation sufficient for culture on nylon discs. These preparations were apparently quite pure because fibroblast growth was not observed. When complete homogeneity was desired, individual pieces of epithelium were removed with a breaking pipette under microscopic observation and transferred through several changes of F-12 before plating.

For single-cell suspensions, purified epithelium was placed in 1.5 ml of Ca^{++}- and Mg^{++}-free Hanks' balanced salt solution containing 0.05% trypsin and 0.02% EDTA obtained from Grand Island Biological Co. After incubation for 20 min at 37.5°C, the solution was diluted 10-fold with F-12 containing 20% fetal calf serum (Grand Island Biological Co.) and aspirated 20 times with a Pasteur pipette to break up clumps of cells. The cells were washed in F-12 by centrifugation at 500 *g* for 2 min before plating or determining cell number using a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.).

Preparation of the Collagen Support

Nylon disks 14 mm in diameter were cut from 103-μm-mesh Nitex (HC-103; Tetko Inc., Elmsford, N.Y.) and glued (SuperGlue, Rose Chemical Co., Detroit, Mich.) to 14-mm bouyant rings of plastic (i.d. = 12 mm, o.d. = 14 mm; height = 4 mm) cut from polyethylene vial stoppers (Rochester Scientific Co., Rochester, N.Y.). The nylon-mesh disks were coated with a layer of rattail collagen, cross-linked with 4% glutaraldehyde, and processed as described by Cereijido et al. (6). In most studies, cells were grown on the disk surface opposite the plastic ring. For permeability studies, disks were glued between two plastic rings, and cells were plated in the well formed by the ring considered to be the upper ring. The bottom ring was notched to allow diffusion of medium when it was placed on a Petri dish. The seams of the double wells were coated with Silicone Sealant (Dow Corning Corp., Midland, Mich.).

Culture of Transitional Epithelium

Cells were cultured at 37.5°C in a humidified 95% air, 5% CO_2 atmosphere in medium 199 and Ham's F-12 combined in a 1:1 ratio and supplemented with 5% fetal bovine serum, 1 μg/ml hydrocortisone, 1 μg/ml insulin, 100 U/ml kanamycin, 100 U/ml penicillin-streptomycin, and 1 μg/ml fungizone (all from Grand Island Biological Co.). The composition of the culture medium was determined empirically. Single-cell suspensions or pieces of tissue were plated on disks held above the adjusted level of medium in Petri dishes by the attached plastic rings. The collagen of the immobilized disks was wet by capillary action. 85,000–170,000 cells/cm^2 or equivalent pieces of tissue (~40–80 μg protein) were plated as a suspension in 25-μl drops of medium. After cell attachment (~24 h), the filters were floated by increasing the level of medium so that the support was kept approximately level with the air-liquid meniscus. Every 3 d the disks were placed in dishes with fresh medium. Transfer of tissue from mature cultures (7 d or older) was accomplished by incubating disks in serum-free Ham's F-12 containing 0.25% trypsin and 0.02 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES) buffer (pH 7.4) for 20 min followed by vigorous aspiration to release the tissue in large pieces, which were then plated at a 1:4 split.

For experiments with Petri dishes, single-cell suspensions or pieces of tissue equivalent to 275,000–380,000 cells/cm^2 (~130–180 μg protein) were inoculated. The medium was changed every 3 d.

In growth studies, incorporation of [*methyl*-^{3}H]thymidine (20 μCi/mmol, New England Nuclear, Boston, Mass.) and protein (16) was determined using 0.2 N NaOH digests of TCA-insoluble cell fractions. For permeability studies using the double wells, ^{3}H-water (25 mCi/g), [^{14}C]sucrose (4 mCi/mmol), [*methoxy*-^{3}H]inulin (413 mCi/g), and [^{14}C]urea (5 mCi/mmol) were obtained from New England Nuclear.

Microscopy

Cells growing on disks or dishes were placed in 3% glutaraldehyde-2% formaldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature. The disks were cut away from the flotation rings and into strips 2-mm wide. After 3 h of fixation, tissues were rinsed in buffer overnight at 4°C and postfixed for 1 h in 1% osmium tetroxide in 0.1 M phosphate buffer. Strips were dehydrated through a graded series of ethanol to propylene oxide and flat-embedded in Epon (Shell Chemical Co., Houston, Tex.) (23). Cells on dishes were dehydrated to absolute ethanol and embedded *in situ*.

For electron microscopic tracer studies, disks were fixed for 30 min in 1% lanthanum nitrate in 0.75% glutaraldehyde and 0.1 N cacodylate buffer (pH 7.4) (35). Lanthanum was present in all subsequent solutions for processing through 50% alcohol (6, 35). Alternatively, only the apical surfaces of tissues grown in double wells were exposed to the lanthanum solution for 30 min, after which time normal fixation and processing took place.

Thin sections were cut with a diamond knife on a Porter-Blum MT-1 ultramicrotome (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.), mounted on 300-mesh copper grids, and stained with a saturated aqueous solution of uranyl acetate followed by lead citrate (36). Sections from lanthanum-treated tissues were not stained. The specimens were viewed with a Phillips 300 electron microscope.

RESULTS

Transitional epithelium lining the urinary bladder of mammals is composed of a basal germinal layer,

an intermediate layer of slightly larger cells, and an apical layer encompassing the lumen (Fig. 1). The differentiated apical cells are characterized by an unusually thick (~120 Å) lumenal plasma membrane (Fig. 2). The asymmetry of this membrane is due to plaques of particles protruding out of the lumenal half of the bilayer (7, 41). Underlying the lumenal membrane is a network of cytoplasmic filaments and discoidal vesicles composed of membrane plaques. Discoidal vesicles serve as a cytoplasmic pool of lumenal membrane, allowing for variations in surface area during expansion-contraction cycles of the bladder (27). Junctional complexes appear typical (Fig. 2) and have been shown in the rat to be composed of tight junctions (zonulae occludentes) containing four to six sealing strands (31).

Isolation of Epithelium

After a 5-h exposure to collagenase as described in Materials and Methods, the epithelium was detached from the underlying dense connective tissue (Figs. 3 and 4). The connective tissue appeared more diffuse after the enzyme treatment but remained intact. The lumenal layer of cells, which appeared to be loosely attached to the intermediate cell layer (Fig. 4), was dissociated from the epithelium proper during subsequent washing. The final tissue isolate was composed almost completely of basal and intermediate cells (Fig. 5). Approximately 256 ± 12 μg protein were present in the tissue isolated from the bladder of each 200-g rat. Dissociation of the epithelium into single cells with divalent, cation-free, trypsin-EDTA solution as described in Materials and Methods yielded ~539,000 ± 68,400 cells/bladder. Neither trypsin alone nor EDTA alone dissociated the epithelium into single cells.

Growth of Transitional Epithelium on Petri Dishes

If single-cell suspensions or pieces of isolated tissue were plated at a density of ~130–180 μg protein/cm^2 on glass or plastic Petri dishes, the plating efficiency varied between 13 and 29% as judged by measurement of cell protein attached by 36 h. The epithelium grew as expanding sheets from islands of reassociated single cells or from pieces of tissue (Fig. 6). Within 1 wk, an epithelial monolayer covered the dish—yet no density-dependent inhibition of growth was apparent. Cells continued to divide, forming a bilayer of cells in many areas, with apical cells being continuously sloughed off. The rate of growth and sloughing was such that within 24 h the unattached cells obscured the underlying growing cells (Fig. 7). The sloughed cells were dead as judged by trypan blue uptake and by their pycnotic appearance.

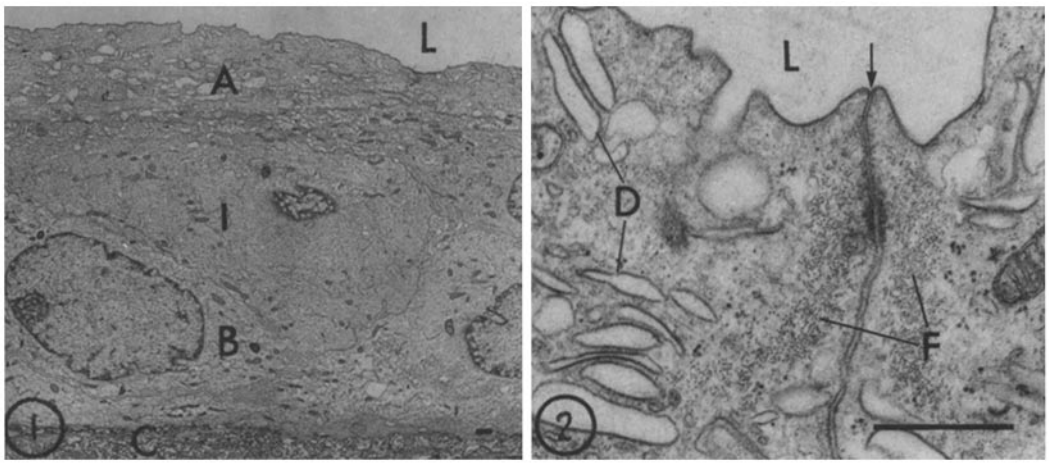
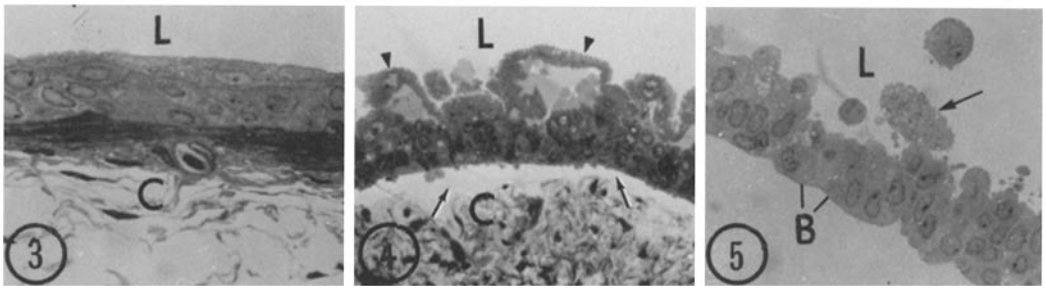


FIGURE 1 Electron micrograph of rat transitional epithelium lining a distended urinary bladder. Basal cells (*B*) are attached to the underlying collagen network (*C*). The large flattened apical cells (*A*) face the lumen (*L*). *I*, intermediate cells. × 3,600. (The bar in the lower right corner of all electron micrographs corresponds to 0.5 μm.)

FIGURE 2 Electron micrograph of a junctional complex (arrow) between apical epithelial cells. Transversely sectioned filaments (*F*) are associated with the complex. *D*, discoidal vesicles; *L*, lumen. × 37,500.



FIGURES 3–5 Light micrographs demonstrating the isolation of epithelium. In Fig. 3 (× 435) intact epithelium is demonstrated. *L*, lumen; *C*, connective tissue. In Fig. 4 (× 200) the epithelium has detached from the connective tissue (*C*) leaving a space (arrows) after 5-h exposure to collagenase. Note the detachment of apical cells (arrowheads) from the epithelium proper. In Fig. 5 (× 400) a single apical cell (arrow) remains loosely attached to the former lumenal surface (*L*) of isolated epithelium. Basal cells (*B*) remain intact.

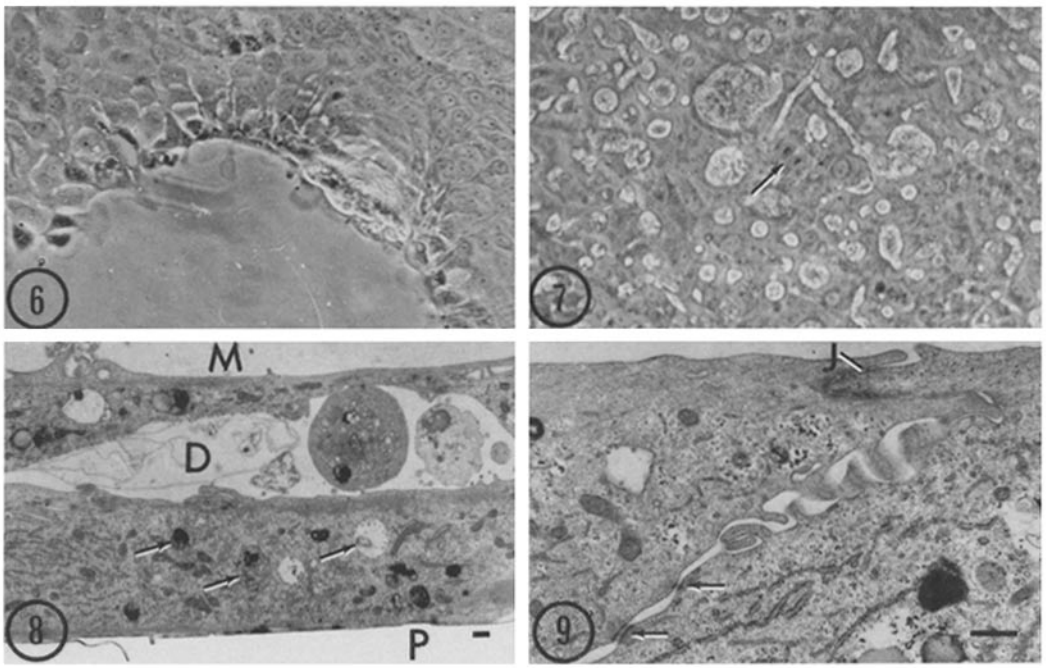


FIGURE 6 Phase-contrast micrograph of epithelium growing on a Petri dish 3 d after inoculation. × 160.

FIGURE 7 Phase-contrast micrograph of epithelium on a Petri dish 8 d after inoculation. The phase-contrast refractile entities are single cells and groups of cells sloughed off during the preceding 24-h period. The arrow indicates a dividing cell. × 160.

FIGURE 8 Electron micrograph of a bilayer of confluent cells growing on a Petri dish (*P*) for 8 d. Cellular debris (*D*) is often present between cells, and autophagic vacuoles (arrows) are common. *M*, medium surface. × 4,125.

FIGURE 9 Electron micrograph of the same specimen shown in Fig. 8. *J*, junctional complex between apical cells. The arrows indicate desmosomes. × 12,500.

Cell debris was often observed in spaces between cells, and autophagic vacuoles containing lamellate forms were common (Fig. 8). Junctional attachments joined the apical regions of lateral membranes of most, but not all, adjacent cells in contact with the medium, and desmosomes were present (Fig. 9). The apical membranes of cells were not differentiated and measured ~85 Å in width.

Cell growth slowed and stopped between 11 and 15 d after plating, and by 16–21 d few living cells remained attached. If cells were plated at a density <~90 μg protein/cm^2, little if any growth was observed. In our culture system, transfer of homogeneous primary cultures grown in Petri dishes has not been accomplished reliably, although we used a variety of techniques, including those previously published (2, 5, 11, 29, 38).

Growth of Transitional Epithelium on a Permeable Collagen Support

As with Petri dish cultures, no basic differences were observed between cultures derived from plating single-cell suspensions and those derived from pieces of isolated tissue. The following data apply equally to both preparations.

From 43 to 80% of the cellular protein applied to collagen-coated disks attached within 24 h. Although a minimum inoculum of ~20 μg protein/cm^2 was needed to observe cell growth, 40–80 μg protein/cm^2 was routinely plated to ensure both cell growth and development of a stratified epithelium. Pieces of tissue or islands of reassociated single cells flattened and expanded over the surface. The centers of islands developed into trilayers and tapered to a bilayer or a monolayer along the spreading edge. Mitotic figures were routinely observed in basal cells. Cell division continued until the islands were confluent, and protein mass increased until a trilayer covered most of the surface (Fig. 10). A bilayer was observed in some areas.

After trilayer formation (145 ± 6 μg protein/cm^2), mitosis of cells slowed but continued with concomitant maturation and replacement of sloughed apical cells. The dynamic maintenance of the stratified tissue structure was confirmed by simultaneous [^{3}H]thymidine incorporation, cellular protein measurements (Fig. 10), and by microscopic monitoring (Figs. 11 and 12). [^{3}H]Thymidine incorporation/μg cellular protein dropped considerably with the gradual completion of trilayer formation on about day 7 and fluctuated

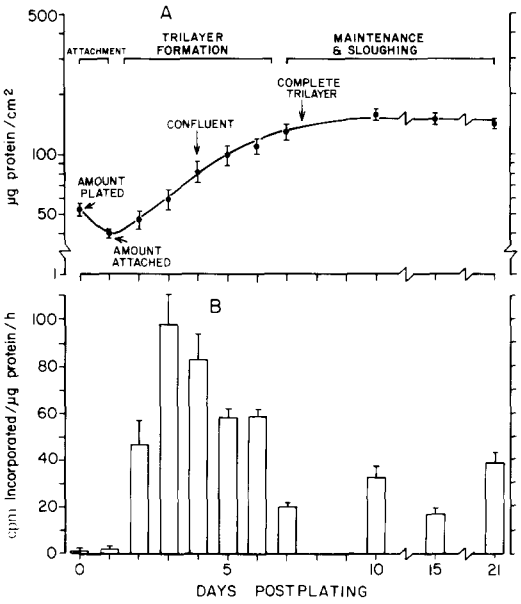


FIGURE 10 A comparison of cellular protein increase (*A*) and [^{3}H]thymidine incorporation (*B*) during the formation and maintenance of stratified transitional epithelium in vitro. Single-cell suspensions were isolated, plated and cultured on disks as described in Materials and Methods. At the indicated times, disks were removed and placed in growth medium with 1 μCi/ml [*methyl*-^{3}H]thymidine (20 Ci/mmol) for 1 h, drained, and transferred through two 10-min incubations in serum-free medium. Cellular protein (16) and radioactivity were determined on TCA-insoluble fractions solubilized in 0.2 N NaOH. Values are the means of three determinations ± SEM.

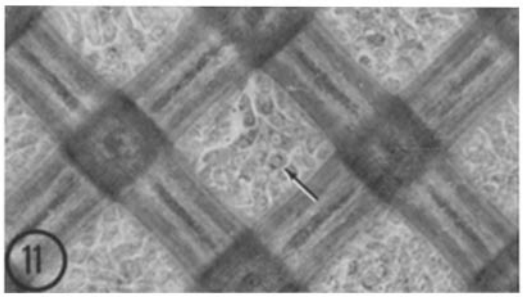
variably thereafter. When calculated as a function of surface area, the average rate of thymidine incorporation during trilayer formation on days 2–6 (5,387 ± 701 cpm/cm^2 per h) was 26% greater than the average rate observed during maintenance of the completed trilayer on days 7–21 (3,976 ± 583 cpm/cm^2 per h). Thus, a pool of dividing cells, diluted by the accumulation of nondividing cells in the trilayer, persisted throughout the culture period. Cultures bave been maintained, with or without transfer, for up to 4 mo.

The appearance of fully developed cultures was remarkably similar to tissue fixed in vivo (cf. Fig. 12 and Fig. 3, Fig. 13 and Fig. 1). Small cells attached to the permeable substrate and large, flat, lumenal-like cells formed the apical layer. Intermediate cells were usually, but not always, present. Cells were joined together by desmosomes, and in other areas a spacing of ~200 Å between adjacent

cells was usual. Junctional complexes between apical cells were present (cf. Fig. 2 and Fig. 14).

A network of cytoplasmic filaments underneath the apical membrane appeared to be attached to it and to the junctional complex. The apical membrane, which corresponds to the lumenal membrane in vivo, was thick and asymmetrical as is its ~120-Å counterpart. It was, however, up to ~280-Å thick. This extraordinary width was a result of an electron-dense coating that was continuous with the outer leaflet of the membrane (Fig. 15). It is not known whether this coating represents an abnormal configuration of the particles observed in vivo (8, 41). Small microvilli were observed on some surface cells. Round and discoidal vesicles composed of thickened membrane were occasionally observed in the cytoplasm. Basal cells formed a continuous basal lamina (Fig. 16).

When lanthanum was applied to only the apical surface of trilayers grown in a double-well system, a dense precipitate formed along that surface (Fig. 17). However, no penetration of the heavy metal through tight junctions was noted. In contrast, when en bloc exposure of the tissue-covered disks was carried out (Fig. 18) lanthanum penetrated into all intercellular spaces and formed a dense precipitate along the apical surface. Tight junctions varied from being completely unstained to containing lanthanum trapped in from one to four areas (Fig. 19), indicating the presence of up to



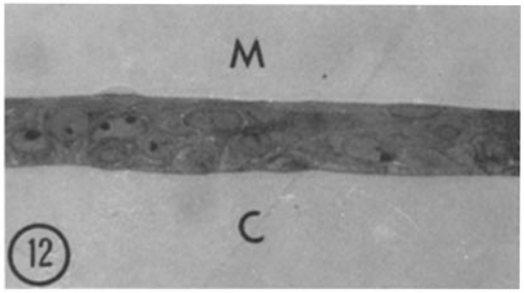
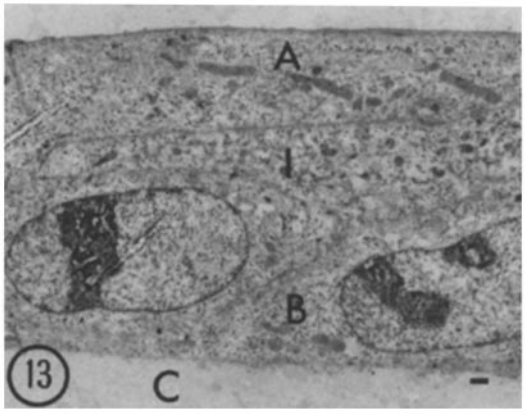


FIGURE 11 Phase-contrast micrograph of a trilayer growing on a collagen-coated disk 10 d after inoculation. The arrow indicates a dividing cell. × 160.

FIGURE 12 Light micrograph of a transverse section through a trilayer growing on a collagen-coated disk for 13 d. Note the large flat cells in contact with the medium (*M*) and the smaller basal cells attached to the collagen (*C*). × 600.



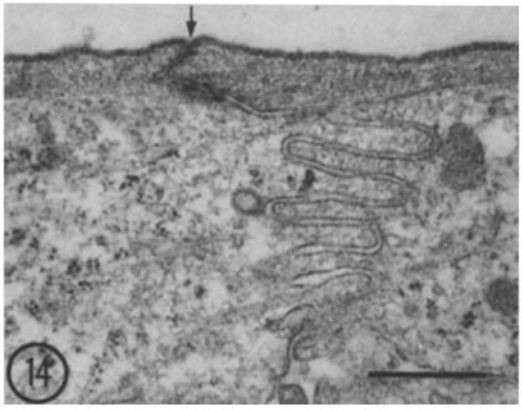


FIGURE 13 Electron micrograph of a section from the same preparation shown in Fig. 12. The apical (*A*), intermediate (*I*), and basal (*B*) cells can be distinguished. *C*, collagen support. × 4,500.

FIGURE 14 Electron micrograph of the same specimen shown in Fig. 13. The arrow indicates a junctional complex between apical cells. Note the associated filaments. × 31,000.

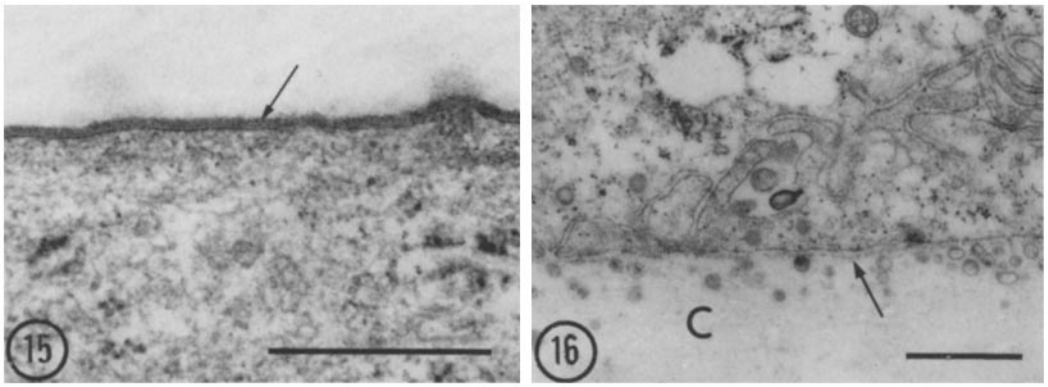


FIGURE 15 Electron micrograph demonstrating the ~280-Å thick apical plasma membrane. This specimen was prepared exactly as the micrograph shown in Fig. 13. × 60,000.

FIGURE 16 Electron micrograph of basal cells in the same specimen shown in Fig. 15. The arrow indicates illustrating the basal lamina. *C*, collagen. × 32,000.

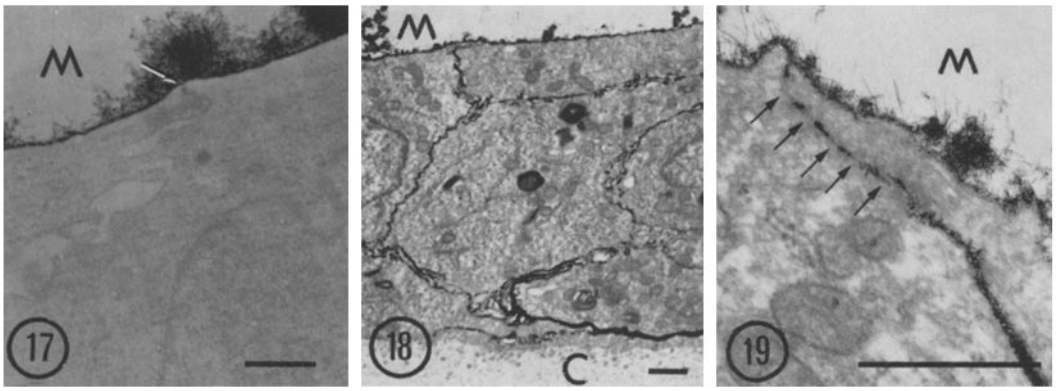


FIGURE 17 Electron micrograph of the apical surface of an unstained section of an 18-d trilayer grown in a double well. Only the apical surface was exposed to lanthanum. Although precipitate can be seen along the apical surface, no penetration through the junctional complex (arrow) was observed. *M*, medium surface. × 20,000.

FIGURE 18 Electron micrograph of an unstained section of an 18-d trilayer treated with lanthanum en bloc. The metal was trapped in all paracellular spaces and precipitated along the apical membrane. *M*, medium surface, *C*, collagen. × 10,000.

FIGURE 19 Electron micrograph of a tight junction of a specimen similar to the one shown in Fig. 18. The lanthanum has penetrated between at least five sealing strands (arrows). Note the dense precipitate along the apical surface bordering the medium (*M*). × 50,500.

five "leaky" sealing strands (9). In confirmation of this observation, [^{14}C]sucrose and [^{3}H]inulin were observed to slowly penetrate trilayers from the apical side of double wells along a hydrostatic gradient (Fig. 20 A). The passage of [^{14}C]urea through the trilayers from the apical surface was consistently retarded in comparison to water (Fig. 20 B).

DISCUSSION

This report demonstrates that growth and a considerable degree of differentiation of adult transitional epithelium can be achieved when cells are cultured on a permeable, as opposed to a nonpermeable, growth surface. That a high degree of development occurred in the absence of mesen-

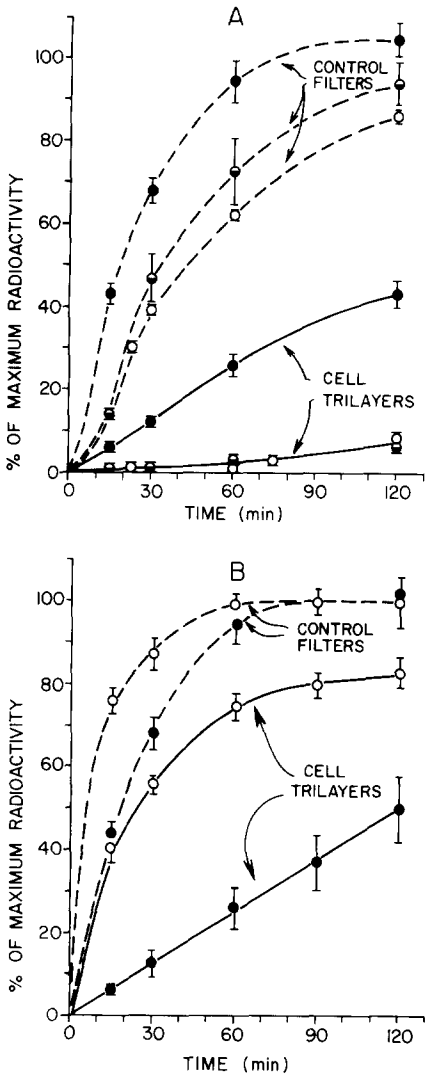


FIGURE 20 Permeability of the apical surface of cultured trilayers to radioactive inulin (○), sucrose (◒), and urea (●) (*A*) or water (○) and urea (●) (*B*). Trilayers that had been growing on discs in a double well for 16 d or cell-free collagen filters were positioned in a 35-mm Petri dish containing 2 ml of continuously mixed growth medium so that the cell-free surface of the collagen support of the lower well was bathed in medium just below the level of the upper well. 100 μl of medium containing 440,000 dpm of ^{3}H-water (25 mCi/g), 460,000 dpm of [*methoxy*-^{3}H]inulin (413 mCi/g), 360,000 dpm of [^{14}C]sucrose (5 mCi/mmol), or 600,000 dpm of [^{14}C]-urea (5 mCi/mmol) were placed in the upper well and mixed every 5 min by gentle aspiration. At the times indicated, 50 μl was removed from each dish and counted in Liquiscint (National Diagnostics Inc., Parsippany, N.J.), in a Packard Tri-Carb (Packard Instrument Co., Inc., Downers Grove, Ill.). Results are presented as the percent of potential maximum radioactivity entering the lower well. Values are the means of three determinations ± SEM.

chymal elements other than the glutaraldehyde cross-linked collagen may indicate that adult epithelium is programmed to stratify and differentiate exclusive of these elements—given the appropriate growth surface and nutritional support. However, a mesenchymal role cannot be ruled out because the trilayers grown in vitro are not completely identical to those observed in vivo. Nonetheless, the growth system described may offer a method for determining the factors needed for complete differentiation.

Michalopoulos and Pitot (24) and their colleagues (25, 26, 39) have observed that primary cultures of adult rat hepatocytes will maintain a high degree of morphological and physiological differentiation when cultured on floating collagen gels. In contrast, hepatocytes plated on Petri dishes with or without a collagen coating have a lower viability and retain less differentiated structure and function (3, 4). Similarly, Emerman and Pitelka (12) have succeeded in maintaining morphological differentiation of mouse mammary epithelial cells on floating collagen membranes for up to 1 mo in culture. As with hepatocytes, dissociated mammary epithelial cells plated on glass, plastic, or collagen-coated Petri dishes do not maintain their differentiated state but rather form flattened monolayers (1, 10, 16, 32). Michalopoulos and Pitot (24) have suggested that the flexibility of the floating membranes allows hepatocytes to form a more native, cuboidal differentiated shape, as evidenced by the fact that the collagen gels contract in the presence of a confluent monolayer of cells but do not in the absence of cells. Emerman and Pitelka (12) report similar observations with confluent monolayers of mammary epithelium. The collagen supports used in our study were rigidly cross-linked with glutaraldehyde to nylon-mesh disks, which were glued to supporting rings. Therefore, no shrinkage or flexing of the support occurred during the growth and development of the cultured transitional epithelial cells. Thus, normal cell shape changes and the stratification of the transitional epithelium could not be attributed to a flexible support. It has also been suggested that floating cultures, being located at an air-liquid meniscus, have improved access to oxygen supply (24). However, when transitional epithelial cells

were grown in double wells for permeability studies, medium was present above and below the collagen disks. Under these conditions, trilayers formed, indicating that access to gases was not a critical factor in our results.

The possibility remains that the cross-linked collagen itself, being a natural substrate of epithelium, affected the differentiation of the transitional epithelium. It is well recognized that in embryonic systems collagen is an important component of the epithelial-mesenchymal interactions that allow differentiation. There is little question that plating efficiency of transitional epithelial cells is greater on collagen (43–80%) than on glass or plastic (13–29%). However, the question remains as to whether a chemical effect of the collagen support, its permeability to nutrients, or a combination of these factors was responsible for allowing the stratification of transitional epithelium. Under normal in vivo conditions, all lining epithelium is polarized, with the site of attachment also being the site of metabolic exchange. The apical surface of lining epithelium faces a nonnutritive environment, which, in the case of transitional epithelium, is urine. Mature trilayers of cultured epithelium in double wells with sterilized rat urine placed on the apical surface and with growth medium bathing the collagen support maintain stratification and suffer no ill effects.[1] Thus, permeability of the collagen support would seem to be an important consideration. In support of this observation, Leighton and his colleagues (18, 19) have reported that primary cultures of embryonic chick skin (18) and rat transitional epithelium derived from minced explants (19) stratify on collagen supports enclosed in a cellulose sponge chamber. Under these conditions, fresh nutrients are available to the cells only through the collagen support. Their results, however, are complicated by the presence of mesenchymal cells. Similarly, Misfeldt et al. (28) and Cereijido (6) have observed that the renal-derived MDCK epithelial cell line (17, 34) forms a morphologically and electrically polarized transporting monolayer when cultured on a permeable support. Taken together, these results may indicate that in vitro, as in vivo, most epithelia require a permeable growth surface to allow full development to occur.

Hepatocytes (24) and mammary epithelial cells (12) do not actively grow when cultured on a collagen support. This may be contrasted to our findings with growing transitional epithelial cells. The mitotic index of adult Fischer-344 rat transitional epithelium in vivo is very low (0.01–0.02%, reference 42). Nevertheless, rapid growth occurs during wound healing (13, 15, 21). The rapid growth observed in our study was similar to wound healing only in that the available surface area was quickly covered by the epithelium. The continued growth, maturation, and sloughing of cells in the mature trilayer, although slower than in the developing trilayer, was not comparable to the inordinately slow turnover measured in vivo; the reason for this is not known.

The lanthanum trapping data were consistent with the freeze-fracture study of Peter (31), demonstrating that tight junctions of rat transitional epithelium contain four to six sealing strands. Thus, both in vivo and in the culture system, the junctions could be classified as intermediate (9). Lewis and Diamond (20) have reported that the electrical resistance of rabbit bladder epithelium is extremely high, indicating very "tight" junctions. Barring species differences, our observation that the cultured epithelium is slightly permeable to some small molecules may indicate abnormal discontinuities of the sealing strands. The retarded passage of urea, but not water, through the trilayers is an interesting observation because most cells are freely permeable to urea (40). This finding may indicate that the atypical apical surface membranes of the cultured trilayers have differentiated to a degree sufficient to have the chemical properties of the specialized lumenal membranes observed in vivo.

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