TRANSEPITHELIAL TRANSPORT IN CELL CULTURE

A Theoretical and Experimental Analysis of the Biophysical Properties of Domes

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ABSTRACT Dissociated cells of transporting epithelia, when cultured on an impermeant substratum, form polarized monolayers frequently characterized by the presence of domes. If the assumption is made that the monolayer exhibits a uniform stretch modulus of elasticity and tension of cell-dish adhesion, T_a, then biophysical properties of the epithelium can be predicted. We have shown that for such epithelia, domes should (*a*) have circular bases, (*b*) be sections of spheres with a constant height to radius, h/r, ratio, (*c*) have a dome-wall tension, T_w, that is constant, and (*d*) have a dome volume that is a function of radius alone. Additionally, a Laplace equation derived for this geometry predicted the hydrostatic pressure from within to outside domes as a decreasing function of radius alone. By microscopy, domes had predominantly circular bases and were found to be sections of spheres with a constant height, h, to radius, r, ratio of 0.684. Using the Laplace equation derived for this geometry and measurements of ΔP and r, the tension of cell-dish adhesion, T_a, and dome-wall tension, T_w, were found to be constants of 6.60 and 7.08 torr, respectively. Combining the constants for T_a and h/r ratio, and the fact that domes are sections of spheres, ΔP and dome volume were shown to be known functions of radius alone. In addition, the modulus of elasticity of the epithelium was calculated to be 4.82×10^3 dyn/cm^2.

INTRODUCTION

Cells from transporting epithelia in cell culture may retain the phenotype of functionally and morphologically polarized cells with distinct apical and basolateral membranes separated by a region of intercellular junctional fusion. In cell culture a monolayer sheet forms with the fusion of epithelial cellular membranes at tight junctions. The planar epithelial sheet may be distorted by the formation of domes or blisters, localized regions of the monolayer sheet that are lifted off the culture dish. Since the epithelial sheet has been demonstrated to transport solutes and water (1, 2), it is reasoned that dome formation occurs when the cell layer on an impermeable substratum is pushed away from the culture dish by the accumulation of water beneath. The regions of the sheet that form domes are not inherently rigid and domes will collapse spontaneously or when punctured. Thus as dome formation proceeds, the epithelial tissue behaves as an elastic sheet that is distorted and lifted from the culture dish by the pressure of water as a result of transepithelial transport.

In the theoretical analysis of the biophysical parameters, two assumptions were made. (*a*) The monolayer exhibited a uniform tension of cell-dish adhesion, T_a; that is, throughout the monolayer, the force of cell-dish adhesion per unit surface area was considered constant; and (*b*) the monolayer had a uniform stretch modulus of elasticity, i.e., that the monolayer was uniformly compliant. These two assumptions were tested by measuring various biophysical parameters of the renal cell line, Madin-Darby canine kidney (MDCK), which forms a monolayer epithelial sheet in culture.

MATERIALS AND METHODS

Cell Culture

A clone was established in our laboratory from MDCK cell line, passage 60–66, and used exclusively in these experiments. The culture medium, Delbecco's modified eagle medium (KC Biological, Inc., Lenexa, KS), was supplemented with 10% newborn calf serum (Irving Scientific, Santa Ana, CA) 10 μgm/ml insulin (Sigma Chemical Co., St. Louis, MO), penicillin, and streptomycin. The cells were cultured at 37°C under 5% CO_2 and were passed by removal from the flasks by trypsin and ethylenediamine tetraacetic acid. For experiments the cells were plated into 35-mm dishes (Corning Medical and Scientific, Corning Glass Works, Medfield, MA).

Morphometrics

The diameters of domes were measured using an ocular reticle calibrated by ruler (American Optical Scientific Instruments, Warner-Lambert Co., Buffalo, NY) with 10-μm intervals. Measurements were made under an inverted phase microscope (Wild Heerbrugg Instruments Inc., Farmington, NY) with Hoffman modulation at 200X. The height of a dome was taken to be the vertical distance from the plane of focus in the monolayer adjacent to the dome to the plane of focus containing the cells

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at the peak of the dome, as measured by the scale on the fine focus. The calibration of this scale was confirmed by comparison with calibration beads of known diameter. Heights determined in this fashion were reproducible to ± 2 μm. Dome profiles were obtained by measuring the diameters of given domes in narrow focal planes at 20-μm increments from base to apex. For each focal plane, diameters were measured in two directions perpendicular to each other. By connecting the end points of sequentially higher diameters drawn on graph paper, two perpendicular profiles for each dome were obtained.

Cell Density

The cells in the culture dish were fixed in 2.5% glutaraldehyde, after washing three times with phosphate-buffered saline, then placed in 70% ethanol and washed twice in water prior to staining with hematoxylin. By this procedure the domes retained their original shape and size, and allowed counting the cells in the dome. Such cell counts were made from photographs that were taken at two or three focal planes per dome. Cell density was also determined for the planar region adjacent to the dome.

Pressure Measurements

The pressure within domes was measured by a servo-nulling micropressure device (W-P Instruments, Inc., New Haven, CT). The device was sensitive to 0.5% full scale and the output was 0.1 torr/mV. Pressure measurements were accepted if, before and after dome puncture, the baseline was invariant. The instrument also provided for simultaneous determination of the potential difference across the dome, which allowed an independent measure of the entry into the dome space and tightness of seal about the micropipette. At the time of pressure measurement the dome diameters were determined with a calibrated ocular reticle under a stereomicroscope with a magnification of 70.

THEORETICAL ANALYSIS

A Laplace Equation for Domes: Epithelia with a Constant Tension of Cell-Dish Adhesion, T_a, have Circular-based Domes

For slowly growing or stable domes, the force of distending the epithelium, F_d, can be taken as equal to the opposing force of cell-dish adhesion, F_a. A static analysis of dome forces can be undertaken. These forces can then be conveniently analyzed in the plane of the dish (Fig. 1). The force of distension, F_d, is the product of the hydrostatic pressure gradient from within to outside domes, and the area perpendicular to F_d, the area at the base of the dome in the plane of the dish. The opposing force of adhesion is the product of the tension of cell-dish adhesion, T_a, and the area over which it operates, the rim of cells at the base of the dome. Equating these forces yields

$$F_d = F_a, \tag{1}$$

$$\Delta P \cdot (\text{area of the base}) = T_a \cdot (\text{area of rim at base}). \tag{2}$$

The area of the rim of cells at the base of the dome can be taken as equal to some measure of the perimeter of the dome base times its wall width, w_d. Substituting and rearranging yields

$$\frac{\Delta P \cdot (\text{area of base})}{(\text{perimeter of base})} = T_a \cdot w_d. \tag{3}$$

If the tension of cell-dish adhesion, T_a, is constant throughout the monolayer, and for any given w_d, then the shape of the base maximizing its area per perimeter ratio, and thus minimizing the hydrostatic pressure within the dome, is that of a circle. Thus for such epithelia, the hydrostatic

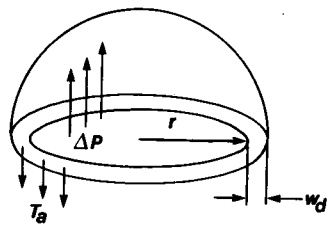

FIGURE 1 Where r_i is inside dome radius, w_d is wall width, ΔP the hydrostatic pressure from within to outside the domes, T_a the tension of cell-dish adhesion, and F_d and F_a, the forces of distension and adhesion. $F_d = \Delta P \cdot$ (area at the base of the dome), $F_a = T_a \cdot$ (area of rim cells at base of dome).

pressure within a dome provides a driving force to maintain a circular base. Substituting circular formulae into Eq. 2 yields

$$\Delta P \pi r_i^2 = T_a[\pi(r_i + w_d)^2 - \pi r_i^2], \tag{4}$$

rearranging

$$\Delta P = T_a\left(\frac{2w_d}{r_i} + \frac{w_d^2}{r_i^2}\right). \tag{5}$$

From electron microscopy, unstressed epithelial monolayers on various substrata typically have apical-to-basal widths in the 5–7-μm range (1–7). Dome-wall tension will act to further reduce the dome-wall width compared with the unstressed apical-to-basal widths. Since small domes have radii in the 50–70-μm range, it can be seen that w_d/r_i will be ~ 0.1 or less. Thus w_d^2/r_i^2 can be neglected and Eq. 5 becomes

$$\Delta P = \frac{2T_a W_d}{r_i}, \tag{6}$$

which is what would have been obtained if the area of the rim of cells at the base of the dome had been taken as equal to the product of its inside circumference, $2\pi r_i$, and its wall width, w_d. The error in doing so is maximal at small dome radii where w_d is large compared with r_i.

$$\text{Error} = \left(\frac{T_a\left(\frac{2w}{r_i} + \frac{w^2}{r_i^2}\right)}{\frac{2T_a w_d}{r_i}} - 1\right)10^2. \tag{7}$$

Substituting $w_d = 0.1\ r_i$, for small domes, we obtain

$$\text{Error} = 5\%. \tag{8}$$

The error is even less at larger radii where $w_d < 0.1\ r$. In fact, since the outside dome radius, r_o, is

$$r_o = r_i + w_d, \tag{9}$$

the radius in Eq. 6 need only be specified as r_i in small domes where r_o is maximally 10% > r_i i.e., $(w_d/r_i = 0.1)$, and even then only if the precision of measuring r_i will permit it. Thus in general

$$\Delta P = \frac{2T_a w_d}{r_i} \tag{10}$$

where r is the unspecified, inside or outside, radius of a dome. Further-

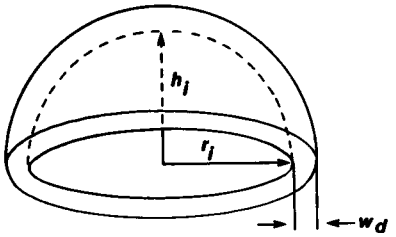

FIGURE 2 Inside height, radius, and W_d are shown. Outside height and radius, h_o and r_o are $h_o = h_i + w_d$ and $r_o = r_i + w_d$. For large domes $h_o \approx r_i$.

more

$$\log \Delta P = \log \frac{2T_a w_d}{r_i} \tag{11}$$

or

$$\log \Delta P = -\log (2T_a w_d) r_i. \tag{12}$$

Thus if hydrostatic pressures are measured in domes of varying radii, a plot of log ΔP vs. log r_i will be linear only if T_a is constant assuming, as it will be shown, that w_d does not vary with r_i. At r_i corresponding to log $\Delta P = 0$,

$$0 = \log \frac{2T_a w_d}{r_i}, \tag{13}$$

then

$$T_a = \frac{r_i}{2w_d}. \tag{14}$$

From substitution of r_i corresponding to log $\Delta P = 0$, T_a can be calculated. Thus, from hydrostatic pressures measured at varying radii, the tension of cell-dish adhesion, T_a, can be established, confirming or denying the original assumption that T_a is constant. To do so, however, requires a more precise knowledge of w_d other than that w_d is simply less than w_i, the unstressed apical-to-basal width, a point to be discussed below.

Domes are the Same Relative Section of Varying Sized Spheres; Dome-Wall Tension, T_w, and Dome-Wall Width, w_d, are a Function of the Dome Height-to-Radius Ratio

Moduli of elasticity, E, are defined as stress divided by strain, where strain is a deformation induced by a stress and is expressed as the ratio of the final to original configuration. A material under a load within its elastic limits will deform so as to minimize stress. The wall tension in a dome, T_w, is a stress that is induced by the distending fluid beneath the dome that stretches the epithelium from its original surface area on the substratum beneath the dome to the surface area of the dome itself. Given a dome formed from an epithelium with a constant stretch modulus of elasticity, and a wall tension within its elastic limits, the dome will assume the shape that minimizes its surface area compared with the distending volume; that is, it will conform to a section of a sphere.

From formulae of mensuration (8), the surface area of a section of a sphere is

$$SA = \pi(r^2 + h^2). \tag{15}$$

Where h and r are the height and radius of the section of the dome, unspecified as to whether they represent the height or radius to the inner or basal dome surface, or the outer or apical dome surface. The surface area beneath the dome from which the monolayer in the dome was recruited is πr^2, where r is similarly unspecified. Strain is thus

$$\text{Strain} = \frac{\pi(r^2 + h^2)}{\pi r^2} \tag{16}$$

$$\text{Strain} = 1 + \left(\frac{h}{r}\right)^2. \tag{17}$$

From the definition of the stretch modulus of elasticity, above, and wall tension, T_w,

$$E = \frac{T_w}{\text{Strain}} \tag{18}$$

$$E = \frac{T_w}{1 + \left(\frac{h}{r}\right)^2}, \tag{19}$$

Thus strain is a function only of the height to radius ratio h/r, and E is a function of h/r and T_w.

Fig. 3 shows that for a constant tension of cell-dish adhesion, T_a, wall tension, T_w, is a function of the angle ϕ formed between the cell monolayer and the dish at the point where the monolayer leaves the dish to form the dome

$$T_w = T_a(\sin \phi)^{-1}. \tag{20}$$

Substituting Eq. 20 into Eq. 19 and rearranging yields

$$\left[1 + \left(\frac{h}{r}\right)^2\right] \sin \phi = \frac{T_a}{E}, \tag{21}$$

if E and T_a are assumed to be constant, then their ratio is a constant, $k_{T_a/E}$,

$$\left[1 + \left(\frac{h}{r}\right)^2\right] \sin \phi = k_{T_a/E}. \tag{22}$$

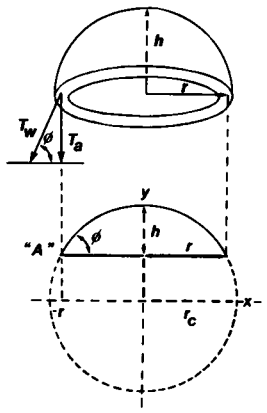

FIGURE 3 A vertical slice through a dome with the circle completed. ϕ is the angle the cells leave to form the dome. T_w and T_a are the wall tension and tension of cell-dish adhesion vectors. r_c and r are the radius of the circle and the unspecified radius of the dome, respectively, h is the unspecified height of the dome.

As ϕ increases from zero (absence of a dome) to 90° (a perfect hemisphere), sin ϕ increases from zero to one, and from Fig. 3 as ϕ increases, h/r increases. Thus $k_{T_a/E}$ is the product of two increasing functions of ϕ.

The relationship between ϕ and h/r is as follows. From Fig. 3, the tangent of ϕ is the slope of the circle at point "A" where $x = -r$. The equation of the circle is

$$x^2 + y^2 = r_c^2. \tag{23}$$

The slope at any point x is

$$\frac{dy}{dx} = -x(r_c^2 - x^2)^{-1/2}. \tag{24}$$

Substituting $-r$ for x at "A" and tan ϕ for the slope, we obtain

$$\tan \phi = r(r_c^2 - r^2)^{-1/2}. \tag{25}$$

From the formulae of mensuration (8) the relationship between r_c and r is

$$r_c - h = (r_c^2 - r^2)^{1/2}. \tag{26}$$

Rearranging yields

$$r_c = \frac{r^2}{2h} + \frac{h}{2}. \tag{27}$$

Substituting into Eq. 25 and rearranging yields

$$\tan \phi = \left[\frac{1}{4}\left(\frac{h}{r}\right)^{-2} + \frac{1}{4}\left(\frac{h}{r}\right)^2 - \frac{1}{2}\right]^{-1/2}. \tag{28}$$

Hence for any given ϕ, h/r is constrained to a single value; and since kT_a/E is the product of two increasing functions of ϕ, Eq. 22 will be true for one and only one ϕ and h/r.

Thus domes formed from an epithelial monolayer with a constant stretch modulus of elasticity, E, and tension of cell-dish adhesion, T_a, not only are sections of spheres, but maintain a constant h/r ratio and angle ϕ as they grow. That is, they are the same section of various sized spheres. Furthermore, if ϕ is constant, it can be seen from Eq. 20 that dome wall tension, T_w, is also constant regardless of dome diameter. If T_w is constant, then the magnitude of E and strain (Eqs. 17 and 19) can be calculated from h/r. Since the h/r constant can be determined for a large number of various sized domes, including domes large enough to ignore differences between inside and outside values of h and r (Fig. 2), then these equations with unspecified heights and radii can justifiably be used to calculate T_w, strain, and E.

If the constant for strain is known, a reasonable value for dome-wall width, w_d, can be calculated. Strain is defined in Eq. 17 as the ratio of the final to the original surface areas. The product of this ratio and the ratio of dome-wall width, w_d, to the unstressed apical-to-basal width, w_u, will closely approximate the ratio between the volume of the epithelial sheet in its stressed position in the dome, V_s, to what would have been its unstressed volume, V_u, had it still occupied the surface area beneath the dome

$$(\text{Strain})\frac{w_d}{w_u} = \frac{V_s}{V_u}. \tag{29}$$

If mitotic activity can be assumed to have added negligible material to the dome, then by conservation of mass, the volume of the epithelium will not have changed and $V_s/V_u = 1$ or

$$(\text{Strain})^{-1}w_u = w_d. \tag{30}$$

Since strain is constant, w_d is not expected to vary with increasing dome radii in a given epithelium, and from a knowledge of h/r and the unstressed apical-to-basal width as measured from electron micrographs, a value for w_d can be obtained. If this is substituted into Eq. 10, measurements of ΔP and r will yield T_a. Values of w_d expressed as percent w_u for various values of h/r are presented in Table I. Since from very flat to hemispherical domes w_d varies by about a factor of 2, and since from Eq. 10, T_a is inversely proportional to w_d, such an estimate is necessary for a calculation of T_a.

TABLE I

RELATIONSHIP BETWEEN h/r AND DOME-WALL WIDTH, w_d AS h/r INCREASES, THE STRAIN IN THE EPITHELIUM INCREASES FURTHER REDUCING w_d

h/r	w_d (percentage of w_u)
1.00	50
0.75	64
0.50	80
0.25	94

Volume of a Dome is a Function of Radius Alone

From formulae of mensuration (8) the volume of a section of a sphere is

$$V = \frac{\pi}{6}h^3 + \frac{\pi}{2}hr^2, \tag{31}$$

dividing by r^3 yields

$$\frac{V}{r^3} = \frac{\pi}{6}\left(\frac{h}{r}\right)^3 + \frac{\pi}{2}\left(\frac{h}{r}\right). \tag{32}$$

Thus if h/r is a known constant, V/r^3 must be constant, k_V, and

$$V = k_V r^3. \tag{33}$$

As discussed above, the value for h/r and hence k_V can be determined from measurements of many domes of varying sizes, including those large enough to ignore differences between inside and outside values of h and r. Thus k_V can be known with reasonable confidence. However, Eq. 33 also requires a precise knowledge of r to accurately predict volume, because r is cubed, e.g., a 10% error in radius would yield a 33% error in volume.

When radius is measured optically, it most likely reflects r_i, because the diameter of the dome base is determined while focusing on the monolayer adjacent to the dome, and the plane of focus includes the rim of cells at the dome base (Fig. 4). Similarly, height is taken as the distance between the two planes of focus, which include the cells at the dome apex and those in the adjacent monolayer, and so most likely reflects h_i. This is fortuitous because the volume of a dome is the volume beneath the inner or basal surface. Nevertheless simultaneous determinations of volume, radius, and perhaps height would be required to confirm the utility of Eqs. 31–33. If a

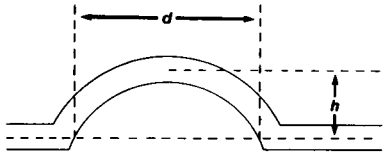

FIGURE 4 The plane of focus utilized when measuring dome heights and diameters optically is shown. The out-of-focus region where there are no cells is taken as the diameter. The distance between the two planes of focus is taken as the height, and most likely reflects inside height, h_i.

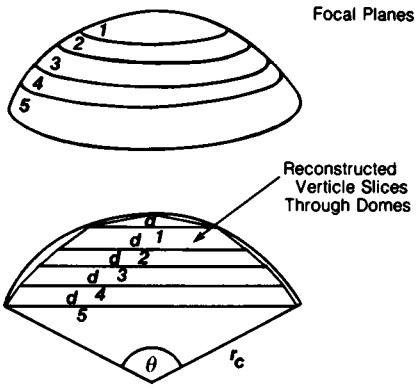

FIGURE 5 The method of reconstruction of dome profiles from diameters measured at sequentially higher levels in the dome. The area of a section of a circle is $A = \frac{1}{2} r^2 (\theta - \sin \theta)$.

trignometric substitution is used to solve Eq. 32 for h/r,

$$\frac{h}{r} = 2 \sinh \left(\frac{1}{3} \sinh^{-1} 0.955 \frac{V}{r} \right), \tag{34}$$

then such V and r determinations should predict the h/r constant as shown.

EXPERIMENTAL RESULTS

Domes are the Same Relative Sections of Various Sized Spheres, and Dome Volume is a Function of Radius

Domes in these cultures were predominantly circular at the base, with perpendicular diameters varying no more than 10%. Such a finding suggests, as previously determined, that the tension of cell-dish adhesion, T_a, is constant throughout the monolayer. For a variety of such domes, vertical profiles through domes were reconstructed on graph paper from dome diameters measured at sequentially higher levels from base to apex (Fig. 5). These vertical profiles described arcs that closely fit the arcs from a section of a circle. For any given dome, the same arc was a good fit to profiles constructed from diameter measurements made in both the east-west and north-south directions of the dome. The areas of these sections of circles were calculated by the formula and compared with the areas obtained by direct measurement of the profiles reconstructed on graphs (Table II). The areas calculated by these two methods differed from each other by no more than 3%.

TABLE II
COMPARISON BETWEEN THE AREAS OF THE RECONSTRUCTED PROFILES TO THE AREA OF THE SECTIONS OF A CIRCLE TO FIT BOTH PROFILES

Dome	N-S, E-W*	Area (from graphical reconstruction)	Area $A = \frac{1}{2} r^2 (\theta - \sin \theta)$	Maximum variation
		μm^2	μm^2	%
1	N-S	7,460	7,335	1.9
	E-W	7,500		
2	N-S	10,400	10,339	1.9
	E-W	9,900		
3	N-S	12,800	12,443	2.8
	E-W	12,800		
4	N-S	5,600	5,762	2.9
	E-W	5,600		
5	N-S	10,600	11,427	2.8
	E-W	13,000		

*N-S and E-W indicate diameter measurements made in the north-south and east-west directions of the dome.

The sections of circles that we have described lie in a vertical plane through the dome whose base lies in the horizontal directions. Thus, these domes are sections of spheres. And as previously derived, domes that are sections of spheres are composed of epithelia with a uniform stretch modulus of elasticity. If this is so, and if the tension of cell-dish adhesion, T_a, is constant, then domes in such an epithelial monolayer should have a constant height to radius, h/r, ratio. Heights and radii for various sized domes were measured and plotted against each other (Fig. 6) yielding a slope $h/r = 0.684$. Thus, these domes are not only sections of spheres, but the same relative section of various sized spheres, that is, they maintain a constant height to radius ratio of 0.684. As previously derived, the volume within a dome is

$$V = \left[\frac{\pi}{6} \left(\frac{h}{r} \right)^3 + \frac{\pi}{2} \left(\frac{h}{r} \right) \right] r^3. \tag{35}$$

Substituting $h = 0.684r$ yields

$$V = 1.242 r^3. \tag{36}$$

Thus volume is a known function of radius alone.

Tension of Cell-Dish Adhesion (T_a) is 6.60 torr, Dome-Wall Tension (T_w) is 7.08 torr, Hydrostatic Pressure Gradient (ΔP) is a Function of Radius Alone, and Modulus of Elasticity (E) is 6.42×10^3 dyn/cm^2

When confluent cultures of MDCK cells with domes are fed serum-supplemented media three times weekly, dome radii increase most rapidly in small domes, but typically no more than 5% per day. In microscopic fields followed for weeks many domes revealed remarkably stable structures. That is, the same domes were present and growing slowly at the same location for prolonged periods. This lack of dynamic activity reflects a relative equilibrium between the force distending the epithelial sheet, F_d, and the force of cell-dish adhesion, F_a. Thus the Laplace equation previously derived from a static analysis of these forces can

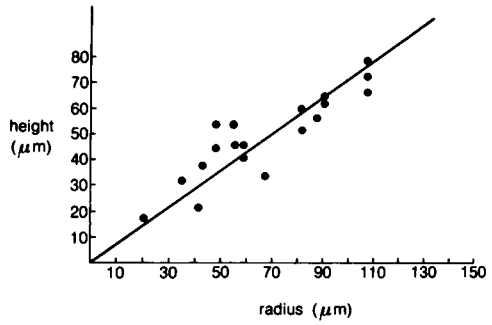

FIGURE 6 Heights and radii for various sized domes were measured and plotted against each other. A regression line passing near the origin yielded a slope of 0.684. Standard deviation from the slope is 0.01. The correlation coefficient is 0.975.

justifiably be used for these domes. From Eq. 10

$$\Delta P = \frac{2T_a w_d}{r},$$

where T_a is the tension of cell-dish adhesion and w_d is the dome-wall width. An estimate of dome-wall width can be obtained as previously shown (Eq. 30)

$$(\text{Strain})^{-1} w_u = w_d,$$

where w_u is the apical-to-basal width of the unstressed monolayer adherent to the substratum. From nine electronmicrographs in seven publications (1–7), the apical-to-basal widths, w_u, in unstressed MDCK monolayers averaged 6.9 μm. Substituting this, and strain as previously derived (Eq. 17) yields

$$\left[1 + \left(\frac{h}{r}\right)^2\right]^{-1} w_u = w_d. \tag{37}$$

Substituting $h/r = 0.684$ and $w_u = 6.9$ μm yields

$$4.7\ \mu = w_d, \tag{38}$$

or a wall width of ~70% of the initial width. The hydrostatic pressure gradient, ΔP, was measured in domes of various sizes. From these data (Fig. 7) and a w_d of 4.7 μm, the value of T_a as calculated by Eq. 14 was 6.60 torr. The slope of -1 (Fig. 7) confirms that T_a did not vary with the radius and ΔP was a function of r alone.

As previously derived (Eq. 20), dome-wall tension, T_w, is

$$T_w = T_a(\sin \phi)^{-1}.$$

Where ϕ is the angle at which the cells leave the dish to form the dome and is a function of h/r (Eq. 28),

$$\phi = \tan^{-1}\left[\frac{1}{4}\left(\frac{h}{r}\right)^{-2} + \frac{1}{4}\left(\frac{h}{r}\right)^2 - \frac{1}{2}\right]^{-1/2}. \tag{39}$$

Substituting $h/r = 0.684$ yields

$$\phi = 68.7°. \tag{40}$$

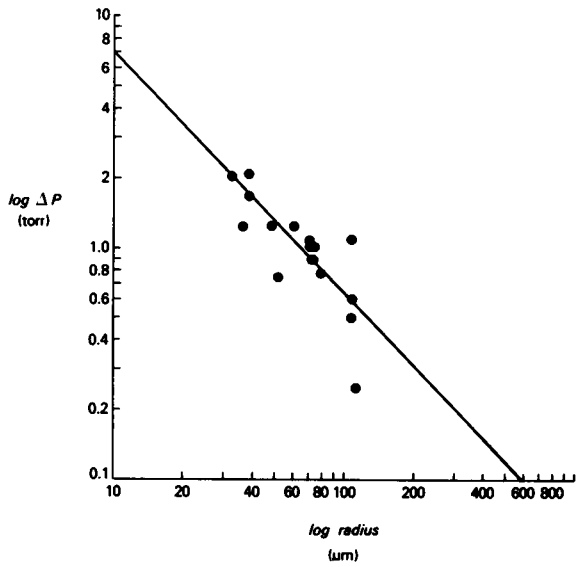

FIGURE 7 Measurements of ΔP for domes of various radii plotted as log ΔP vs. log r_i (Eq. 12). The slope was determined by least-squares regression, -1.03 ± 0.209 (standard deviation). From the r_i corresponding to log $\Delta P = 0$, the T_a was calculated to be 6.60 torr (Eq. 14).

Substituting T_a and ϕ into Eq. 20 yields

$$T_w = 7.08 \text{ torr}. \tag{41}$$

Dome-wall tension of medium-sized domes, $r \approx 70$ μm, is compared with other physiologic vessels (9) in Table III. In addition, T_a and w_d can be substituted into Eq. 3 yielding

$$\Delta P = \frac{6.60 \text{ torr} \cdot \mu}{r}. \tag{42}$$

Thus ΔP is a simple inverse function of radius.

As previously defined (Eq. 19) the stretch modulus of elasticity, E, is

$$E = \frac{T_w}{1 + \left(\frac{h}{r}\right)^2}.$$

But since T_w and h/r are constants of 3.08 torr and 0.684, respectively, E is a constant of

$$E = 4.82 \text{ torr}, \tag{43}$$

or

$$E = 6.42 \times 10^3 \text{ dyn/cm}^2. \tag{44}$$

As calculated, the stretch modulus of elasticity, E, assumes that the amount of material in the dome wall is identical to that of the adjacent planar monolayer if the dome were collapsed to its circular base. This may not be precisely the case. While the ratio of dome area to that beneath the dome remains invariant with radius (Eq. 17), the cell density of the dome in comparison with the adjacent cell layer decreases progressively as the dome radius increases.

TABLE III
COMPARISON OF DOMES TO OTHER PHYSIOLOGIC VESSELS*

	ΔP	Wall tension/ Wall thickness
	dyn/cm²	*dyn/cm*
Vena Cava	1.3×10^4	21,000
Venules	2.6×10^4	26
Capillaries	4.0×10^4	16
Domes	0.1×10^4	25

*Hydrostatic pressure gradients, ΔP. Wall tension divided by wall thickness in medium-sized domes compared with other physiological vessels as reported by Burton in reference 9.

This is despite reports (10) and our own unpublished observation of mitoses occurring in dome cells and the constant cell density in the confluent monolayer (11). When the ratio of the cell density of the adjacent planar monolayer to dome-cell density was analyzed as a function of dome radius, the least-squares regression yielded a slope of 0.0015 μm ($r = 0.772$, $N = 15$, $p > 0.01$ slope is not 0). Thus as the dome radius increases, the effect of this unexplained observation would be to decrease the modulus of elasticity, E, ~1.5% for each 10-μm increase in dome radius.

DISCUSSION

Two assumptions regarding the biophysical properties of polarized transporting epithelium in cell culture were made. The first was that the tension of cell-dish adhesion, T_a, was constant throughout the monolayer. For such epithelia, it was shown theoretically that the bases of the domes should be circular. The extent to which this is true for any epithelium, and equally important cell-substratum interaction, is the extent to which the assumption is valid. In addition, a Laplace relation was derived for circular-based domes that, with measurements of ΔP and r, would yield the actual magnitude of T_a at varying dome radii, provided that some knowledge of dome-wall width could be obtained.

The second assumption was that monolayer exhibited a uniform stretch modulus of elasticity, E, or that the monolayer was uniformly compliant. Combining the two assumptions it was shown that (*a*) such domes should be the same relative section of various sized spheres, i.e., that their height to radius ratio, h/r, should be constant, (*b*) that dome-wall width is a function of the h/r constant and the unstressed monolayer width, and (*c*) that dome volume is a function of radius alone. While probably not true for all combinations of epithelia and culture-dish substrata, such as those that are not monolayers or that form irregular, noncircular-based domes, morphometric analysis of the MDCK domes cultured under the conditions described above revealed circular bases, and sections of spheres with a constant height to radius ratio, h/r.

Thus, using measurements of ΔP as a function of radius, the h/r constant, and the Laplace equation derived, the actual magnitudes of the constants for cell-dish tension of adhesion, T_a, the dome-wall tension, T_w, and modulus of elasticity, E, were calculated. These constants reflect two features of dome growth. First the tension in the dome wall remains constant because unlike a cardiac ventricle or toy balloon, as the dome radius grows it incorporates more and more material into its structure by lifting more epithelium from the substratum. A second feature that limits dome growth is that transiently the tension of cell-dish adhesion must be overcome, which also transiently raises dome-wall tension and lifts cells from the dish resulting in dome growth and normalization of wall tension. Should the fracture strength of the cells and/or their junctions be exceeded, the dome would be expected to rupture and collapse.

From an understanding of these principles of dome formation, differences among cultured transporting epithelia can be determined. In addition, the mechanism by which agents or factors increase dome formation or growth can be elucidated; that is, whether they modify the transport function or tension of cell-dish adhesion can be directly determined and quantitated. Measurement of epithelial cell-wall tension or the stretch modulus of elasticity has not been possible before. As would be expected, the gossamer quality of an epithelial cell layer devoid of supporting mesenchymal elements would exhibit a modulus significantly lower than tissues containing elastin, 3×10^6 dyn/cm², or collagen at 1×10^9 dyn/cm² (9). The 6.42×10^3 dyn/cm² determined for the MDCK epithelial monolayer is very small in comparison.

The innovation of culturing transporting epithelial cells on a membrane support (1, 2) has permitted the experimental manipulation of cultured cells with techniques applied to sheetlike tissues, such as studies with the Ussing chamber for electrophysiological and isotopic flux measurements. The study of individual domes offers a new technical approach for the study of epithelial transport that extends the advantages of cell culture. The continuous propagation of epithelial cells remains a significant problem that limits the preparation of epithelial-cell layers on membrane supports if the numbers of cells isolated is limited and the cells do not proliferate. In the absence of proliferation, primarily isolated cells can be plated and from a few hundred epithelial cells, domes may form. From studies on individual domes it is possible to investigate epithelial transport on a small cell population. Of potentially great value is the application of the advances in chemical separation offered by reverse-phase high performance liquid chromatography and detection methods (12), and the sensitivity provided by techniques such as the electron microprobe (13). The opportunity to study transport by direct chemical analysis is a reality with the ability to sample fluids that are a pure aliquot of the transported solution undiluted by nontransported extracellular fluids.

This approach extends the variety of solutes whose transport can be studied as the investigator is not limited to only those solutes that are isotopically labeled or detectable by electrophysiological techniques.

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