MORPHOMETRIC ANALYSIS OF THE TRANSLOCATION OF LUMENAL MEMBRANE BETWEEN CYTOPLASM AND CELL SURFACE OF TRANSITIONAL EPITHELIAL CELLS DURING THE EXPANSION-CONTRACTION CYCLES OF MAMMALIAN URINARY BLADDER

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ABSTRACT

The flow of membrane between the cytoplasm and the lumenal surface during the expansion-contraction cycle of urinary bladder was estimated by stereological examination of electron micrographs of urothelial cells from guinea pigs, gerbils, hamsters, rabbits, and rats. The quantitative data obtained allowed an approximation of the surface area, volume, and numbers of lumenal membranelike vesicles and infoldings per unit volume of cytoplasm. Depending upon the species, ~85 to ~94% of the membrane surface area translocated into and out of the cytoplasm was in the form of discoidal vesicles. The remainder was accounted for by infoldings of the lumenal plasma membrane. The density of vesicles involved in transfer of membrane was quite similar in all the species examined, except guinea pigs which yielded lower values. In contrast, the densities of the total cytoplasmic pools of discoidal vesicles potentially available for translocation varied greatly among the different species. In general, species of animals with a highly concentrated urine had a greater density of discoidal vesicles than species with a less concentrated urine. This correlation may indicate an authentic relationship between lumenal membranes and the tonicity of urine, such as increased membrane recycling or turnover with increasingly hypertonic urine; or it may signify the existence of some other, more obscure relationship.

KEY WORDS urinary bladder - lumenal plasma membrane - discoidal vesicles

Discoidal vesicles can be found in the apical cells of the transitional epithelium lining the urinary bladder of a variety of mammals (3, 8-10). The vesicles, which appear fusiform when sectioned transversely, are composed of two apposing plaques identical to those seen in the plasma membrane covering the cell's lumenal surface. The lumenal membrane is made up of the concave plaques which are interconnected by smooth inter-plaque regions (8-10). It has been proposed that the hexagonal arrays of particles within the plaques serve as anchors for an underlying filamentous network, thereby preventing rupturing of the specialized membrane during expansion of the bladder (1, 10).
During contraction of the urinary bladder, the mucosa and submucosa are thrown into folds, thereby decreasing the volume of the lumen. It has been postulated that the surface area of luminal plasma membranes is decreased by the pinching off of adjacent plaques to form discoidal vesicles (3, 9, 10). However, other studies have indicated that infolding of the membrane may also be responsible for the rapid change in surface area (4, 13).

In this report, we present the results of stereological observations upon the volume, surface, and numerical densities of discoidal vesicles in the cytoplasm of apical transitional epithelial cells of five mammals—gerbils, guinea pigs, hamsters, rabbits, and rats. The contribution of cytoplasmic infoldings to the total amount of internalized luminal membrane in contracted urinary bladders also was assessed. Our data show that interchange of discoidal vesicles between the cytoplasmic pool and the luminal membrane and, to a lesser and more variable extent, infoldings of the luminal membrane, are involved in surface area fluctuations during the expansion-contraction cycles of the bladder. In addition, our results indicate a significant variation in the size of the cytoplasmic pool of discoidal vesicles among the different mammals that may be positively correlated with the degree of hypertonicity of the urine.

MATERIALS AND METHODS

Preparation of Tissue

**ANIMALS:*** Adult female New Zealand white rabbits (3-4 kg, White Pine Rabbity, East Douglas, Mass.); adult male Caesarean-delivered Sprague-Dawley rats (250-300 g, Charles River Breeding Laboratories, Wilmington, Mass.); adult female Tum:(MON) strain mongolian gerbils (55-70 g, Tumblebrook Farms, West Brookfield, Mass.); adult female NIH Hartley albino guinea pigs (450-500 g, Elm Hill Breeding Laboratories, Chelmsford, Mass.); and adult male LVG Golden Syrian hamsters (60-90 g, Charles River Breeding Laboratories) were utilized in this study. All animals were given standardized laboratory chow specific for their species and water ad lib.

**EXPANDED BLADDERS:** All animals were sacrificed by cervical dislocation and only animals with at least partially distended bladders were used. Each bladder was exposed in situ and the urethral end was clamped with a hemostat. With the use of a syringe and an 18- or 24-gauge needle (depending upon bladder size), the bladder was fully distended, if not already so, by injecting minimal essential medium (MEM, Grand Island Biological Co., Grand Island, N. Y.) at 37°C. When fully expanded, the MEM was withdrawn while being simultaneously replaced with fixative (3% glutaraldehyde-2% formaldehyde in 0.1 M phosphate buffer at pH 7.4 and 37°C) injected with another syringe. Care was taken to maintain the bladders in the full state of distention by injecting and withdrawing fluids at a constant rate. At the same time, fixative was applied to the external surface of the bladder. After 10 min, pieces of the bladder were cut with fine scissors, transferred to a Petri dish containing fixative, cut with razor blades into small blocks containing ~9 mm² of luminal surface area, and placed in fresh fixative at room temperature for 3 h. After the desired period of fixation, tissues were rinsed overnight at 4°C in the buffer and postfixed for 1 h at room temperature in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). The specimens were dehydrated through a graded series of ethanol to propylene oxide, and flat-embedded in Epon (5).

Thin sections were cut with a diamond knife on a Porter-Blum MT-1 ultramicrotome (DuPont Instruments-Sorvall, DuPont Co., Wilmington, Del.), mounted on 300-mesh copper grids, and stained with a saturated aqueous solution of uranyl acetate followed by lead citrate (11). A thin layer of carbon was evaporated onto the sections before viewing with a Philips 300 electron microscope. The magnifications of electron micrographs were calibrated with a carbon grating replica containing 28,700 lines per inch.

**CONTRACTED BLADDERS:** Within 10 min after normal micturition, animals were sacrificed by cervical dislocation, and the collapsed bladders were dissected out and fixed according to the following modification of Luft’s ruthenium red en bloc technique (cf. reference 2). The bladders were placed in Petri dishes containing 1.5% glutaraldehyde-0.01% ruthenium red in 0.1 M cacodylate buffer (pH 7.4), cut into small tissue blocks, and placed in fresh ruthenium red-containing fixative for 4 h at 4°C. After this period, the tissue was rinsed overnight at 4°C in 0.1 M cacodylate buffer, and postfixed in 1% osmium tetroxide-0.01% ruthenium red in 0.1 M cacodylate buffer (pH 7.4) for 3 h at room temperature. The specimens were dehydrated, embedded, and viewed as described above for expanded bladders. Using this technique, only those surfaces in contact with the lumen of the bladders were stained by ruthenium red.

**Sampling**

Three fully expanded and three normally contracted bladders from each mammalian species were used. 20 tissue blocks were prepared from each bladder and three blocks were randomly selected for sectioning. Five grids with four sections on each grid were prepared from each block. The four sections on each grid were separated from the preceding and succeeding series of sections by ~30 μm. One micrograph was taken of the most central section on each grid where the luminal cell layer was discernible. The remaining sections served as an insur-
ance against section defects. Random sampling of the potential fields of view was ensured by positioning the grid so that the edge of the fluorescent viewing screen coincided with the edge of the grid's copper bar where the left edge of the normally oriented lumenal surface intersected the grid bar. The final position of the field was obtained by moving the grid bar along the fluorescent screen to that point at which the maximum possible length of lumenal membrane surface was oriented in the photographic field. By use of this technique, 15 random micrographs were obtained from each bladder. Thus, 45 random micrographs were obtained from the expanded and the contracted bladders of each species.

Morphometric Nomenclature

The following definitions of compartments of cells composing the apical layer of transitional epithelium were accepted for the purpose of this study:

(a) Cytoplasm included the intracellular space from the lumenal membrane to the basal membrane of each apical cell. Lateral membranes and their accompanying extracellular spaces were included as part of the cytoplasm when they appeared in the field. Lumenal spaces, cytoplasm of cells composing the underlying epithelial layers, and nuclei were excluded.

(b) In contracted bladders, cytoplasmic infoldings of membrane attached to the lumenal surface were delineated by drawing a straight line across the presumptive neck of the infolding as illustrated in Figs. 2 and 4. The lumenal spaces within the infoldings were included as part of the cytoplasm in order to stereologically determine the volumetric density of the infoldings.

(c) Discoidal vesicles were considered to be those vesicles that were bounded by a lumenal-like membrane and appeared to be composed of two plaques. Such vesicles usually assumed a fusiform shape in the sections when cut at a normal angle. Multiplaque vesicles which were composed of lumenal-like membrane were not included in the analysis.

Stereological Procedures

The stereological procedures used were based upon the methods for morphometric analysis described by Weibel et al. (15). Micrographs recorded on 8.1 x 10 cm DuPont Graphic Arts Film (DuPont Instruments, Wilmington, Del.) at a primary magnification of 5,500 were projected on a screen at a final magnification of 42,790. The projected image was superimposed upon an extended multipurpose test system with 168 test points and 84 lines, each 3.5 cm in length, arranged in an equilateral triangular network with a lattice period of 3.5 cm (cf. reference 14). The sides of the square test area measured 42 cm. At the constant magnification used, each test line corresponded to 0.8179 /u.m, and the potential maximum test field corresponded to 96.3 /u.m².

The volumetric densities of discoidal vesicles (V₁) or infoldings (V₂) of the lumenal membrane were estimated by counting the number of test points enclosed within profiles of the appropriate structures (P₁ or P₂), as compared to the total number of test points within the cytoplasm of apical cells (P₃):

$$V₁ = \frac{P₁}{P₃}$$

The values obtained were dimensionless fractions that could be multiplied by any arbitrary volume of cytoplasm for comparative purposes. In this paper, the volumes were expressed as /u.m³ per /u.m³ of cytoplasm.

The surface densities of the membranes of either discoidal vesicles (S₁) or infoldings of the lumenal membrane (S₂) were determined from counts of the intersection points (I₁ or I₂) of the membranes with test lines. Since the total length of the test lines (L₃) could be determined by multiplying the number of test lines within the cytoplasm by 0.8179 /u.m, the surface density was given by the following equation (15):

$$S₁ = \frac{2I₁}{L₃}$$

Actual surface area of membrane in a given volume was determined by multiplying surface density times unit volume. The surface areas were expressed as /u.m² per /u.m³ of cytoplasm.

The numerical density (N₁) of discoidal vesicles was estimated from counts of profiles in a frame subdivided into 1- /u.m², as in the method of Weibel and Gomez (14). Vesicles were counted in the same test area previously used for surface and volumetric analysis.

$$N₁ = \frac{1}{β₁} × \frac{N₃}{V₃} × K$$

where N₃ was the number of profiles per /u.m³. The shape-dependent coefficient β₁ was assumed to be 1.25. This was based upon the observation that the usual shape of a discoidal vesicle was similar to an extremely short cylinder where the diameter was much greater than the length. However, since the edges of the two plaques composing the vesicles were in fact connected, the shape was closer to a pancake-like, flattened sphere. The Axial ratio of the profiles were <2. Thus, the coefficient β₁ was determined to be 1.25 from the graph presented by Weibel and Gomez (14). The size distribution was assumed to be normal and the corresponding size distribution coefficient K was set at 1.07 (14). V₃ was the volumetric fraction determined in Eq. 1. The final numerical data were expressed as number of vesicles per /u.m³ of cytoplasm.

Urine Osmolality

Individual animals were placed in wire observation cages with a sheet of aluminum foil underneath the bottom screen. Immediately after voluntary micturition, the urine was retrieved and placed in a capped vial. The
the osmolality of the fresh samples was determined by diluting each sample five-fold in glass-distilled water and analyzing duplicate 200-μl volumes with a calibrated Fiske Automatic Osmometer (model 130, Fiske Associates, Inc., Uxbridge, Mass.). Samples were collected from four to six animals of each species over a 3-day period. The results were the means of these determinations, and the variation of the range of all the values obtained for each species did not exceed ±8%. The mean osmolalities obtained were: guinea pig, 712 mosmol; rabbit, 1,605 mosmol; gerbil, 2,385 mosmol; and hamster, 2,712 mosmol. The measurements for rat and hamster were somewhat lower than previously published values of 2,400 mosmol (12) and 3,000 mosmol (6), respectively. A value of 1,500 mosmol has been reported for rabbit (7).

RESULTS

Representative micrographs of rat and gerbil transitional epithelial cells from fully expanded as well as fully contracted urinary bladders are illustrated in Figs. 1–4. In comparing the cytoplasms of the contracted and the expanded states in each species, an increase in the number of discoidal vesicles is apparent in the apical cells of contracted bladders. This visual perception is confirmed by the morphometric data summarized in Table I and discussed below.

In the micrographs of the distended state, apical cells appear squamous. The network of the ~60 Å tonofibril-like filaments previously described by Staehelin et al. (13) can be seen (Figs. 1, 3, and 5). When sectioned along the longitudinal axis, the filaments appear to be oriented approximately parallel to the lumenal surface, running between the lateral poles of the cells. The intense condensations of filaments at the junctional complexes of the cells are also distinct (Figs. 1, 3, and 5). The lateral plasma membranes between flattened apical cells are usually thrown into folds, while the junctions of the basal plasma membranes with the apical plasma membranes of the underlying intermediate cells are relatively unfolded (Figs. 1 and 3).

In contrast, apical cells from contracted bladders demonstrate several morphological changes which presumably represent adjustments in response to the compressive forces of contraction (Figs. 2, 4, and 6). The cells are somewhat cuboidal in shape, and the filaments are re-oriented, extending throughout the cytoplasm of the cells. The orientation of the filaments now appears to be random (Fig. 6). At the junctional complexes, the masses of filaments are usually bent, no longer being parallel to the lumenal surface. The lateral plasma membranes bordering the heightened apical cells in the contracted state demonstrate an apparent diminution in the amount of folding (Fig. 4). On the other hand, the basal-apical membrane junctions between luminal and intermediate cell layers which demonstrated little folding in the expanded state, usually are quite folded in the contracted state (Fig. 2a, inset).

The mean surface, volumetric, and numerical densities of discoidal vesicles in the cells of fully contracted and fully expanded bladders are compared in Table I. By subtracting densities of the "residual pool" of discoidal vesicles (i.e., vesicles...
TABLE I Densities of Discoidal Vesicles in Apical Transitional Epithelial Cells of Expanded and Contracted Mammalian Urinary Bladders

| Animal  | Density/μm² | Contracted (total pool density ± SEM) | Expanded (total pool density ± SEM) | Δ* | % Change² |
|---------|-------------|---------------------------------------|-------------------------------------|----|-----------|
|         |             | (residual pool density ± SEM)         | A/total pool density x 100          |    |           |
| Hamster |             | 3.8804 ± 0.1970 2.9129 ± 0.1843 0.9675 25.0 |
|         | Surface (μm²) | 0.2883 ± 0.0132 0.2258 ± 0.0140 0.0625 21.7 |
|         | Volumetric (μm³) | 8.4902 ± 0.5264 6.7400 ± 0.4661 1.7502 20.6 |
|         | Numerical (μm³) | 4.0500 ± 0.2643 2.9370 ± 0.1992 1.1130 27.5 |
| Gerbil  |             | 0.3298 ± 0.0206 0.2340 ± 0.0171 0.0958 29.1 |
|         | Surface (μm²) | 6.5160 ± 0.4496 5.1475 ± 0.3189 1.3685 21.0 |
|         | Volumetric (μm³) | 0.2583 ± 0.0123 0.1834 ± 0.0100 0.0749 29.0 |
|         | Numerical (μm³) | 2.9904 ± 0.2089 1.8542 ± 0.1514 1.1362 38.0 |
| Rat     |             | 5.3430 ± 0.2245 3.4797 ± 0.2507 1.8633 34.9 |
|         | Surface (μm²) | 1.5754 ± 0.1021 0.7024 ± 0.0553 0.8730 55.4 |
|         | Volumetric (μm³) | 0.1582 ± 0.0097 0.0758 ± 0.0056 0.0824 52.1 |
|         | Numerical (μm³) | 3.6317 ± 0.1729 1.6617 ± 0.1235 1.9700 54.2 |
| Rabbit  |             | 0.0803 ± 0.0055 0.0476 ± 0.0035 0.0327 40.7 |
|         | Surface (μm²) | 0.8925 ± 0.0666 0.5695 ± 0.0354 0.3230 36.2 |
|         | Volumetric (μm³) | 1.6014 ± 0.0796 1.0625 ± 0.0814 0.5389 33.7 |

* Δ = Total pool density minus residual pool density.
† % Change = Δ/total pool density x 100.

Left free in the cytoplasm of apical cells of fully expanded bladders from densities of the "total pool" of discoidal vesicles (i.e., vesicles in the cells of fully contracted bladders), it is possible to estimate the relative densities of vesicles involved in translocation of membrane between the cytoplasm and the luminal surface. As shown in Table I, the absolute values of the densities of vesicles involved in the changes between contracted and distended states (referred to hereafter as Δ) were quite similar in all of the species examined, except guinea pigs which demonstrated a lower Δ. However, in guinea pigs the size of the "total pool" of discoidal vesicles was very small, being approximately equivalent to the Δ of the other species examined.

In contrast to the slight variations in Δ, the percent of changes of the total pools of discoidal vesicles represented by Δ varied dramatically from species to species. This, in turn, was due to the large variations in densities of the total cytoplasmic pools of vesicles among the different species examined. These variations, which can be seen in Table I, will be examined more closely later on in Results.

The use of three different parameters for the

Figure 3 An electron micrograph of transitional epithelial cells of an expanded gerbil urinary bladder. Apical transitional epithelial cells from expanded gerbil bladder demonstrate characteristics similar to those from rat bladder shown in Fig. 1. These properties include: (a) an orientation of groups of cytoplasmic filaments (F) that are approximately parallel to the luminal surface; (b) an association of the filaments with the junctional complex (arrow); (c) highly folded lateral membranes (LM); and (d) an unfolded border composed of the basal plasma membrane of the apical cells and the apical plasma membrane of the intermediate cells (arrowheads). Note the discoidal vesicles (D) composing the residual pool described in the text. L, lumen. × 11,275.

Figure 4 An electron micrograph of apical transitional epithelial cells of contracted gerbil urinary bladder stained en bloc with ruthenium red. Apical cells from contracted gerbil bladders appear similar to those from rat bladders shown in Fig. 2. Ruthenium red-stained infoldings of luminal membrane (LM) are observed. An increase in the number of discoidal vesicles (D) seems apparent relative to the expanded state. The filamentous network (F) is reoriented to a seemingly random pattern and can be seen throughout the cytoplasm of the field shown. Note the unfolded appearance of the lateral membranes (LM) between adjacent apical cells. I, infolded membrane; arrow, junctional complex. × 11,275.
measurements of discoidal vesicles provided a means for determining the consistency of the morphometric data obtained. When \( \Delta \) was expressed as the percent of change of the total pool of discoidal vesicles, the percentages of the three parameters obtained showed a range of variation \( \leq 9\% \) for each species (Table I). Thus, independent of whether densities were determined as surface area, volume, or numbers, the values were relatively uniform within a given species.

To determine the contribution of infoldings of the lumenal membranes to the internalization of surface area during contraction, the surface and volumetric densities of ruthenium red-stained infoldings were analyzed. Figs. 2 and 4 are micrographs of transitional epithelium from contracted bladders stained en bloc with ruthenium red (2). Serial sections demonstrated that the use of ruthenium red allowed infoldings to be clearly distinguished from other internalized membrane even though the connections of the infoldings to the cell surface were not always present in a given section. In Table II, the densities of infoldings are compared to those of discoidal vesicles. Since many of the random micrographs showed little or no infolding, the standard errors of the mean densities of infoldings were quite large. Notwithstanding, we feel that the data do allow a good approximation of the amount of infolding. The absolute values of the infoldings in all the species demonstrated that a relatively small density of membrane was internalized into the cytoplasm by this mechanism (Table II).

The volumetric densities of infoldings consti-

| Animal | Density/\( \mu m^2 \) | Lumenal membrane infoldings | Discoidal vesicles | Total lumenal membrane internalized | % Infoldings |
|--------|-----------------|---------------------------|-------------------|-----------------------------------|-------------|
| Hamster | Surface (\( \mu m^2 \)) | 0.0624 ± 0.0265 (3.3)\*  | 0.9675 (15.1) | 1.0299 | 6.1 |
|         | Volumetric (\( \mu m^3 \)) | 0.0186 ± 0.0070 | 0.0625 | 0.0811 | 22.9 |
| Gerbil  | Surface (\( \mu m^2 \)) | 0.1303 ± 0.0598 (4.3) | 1.1130 (11.6) | 1.2433 | 10.5 |
|         | Volumetric (\( \mu m^3 \)) | 0.0303 ± 0.0140 | 0.0958 | 0.1261 | 24.0 |
| Rat     | Surface (\( \mu m^2 \)) | 0.2000 ± 0.0696 (8.8) | 1.1362 (15.2) | 1.3362 | 15.0 |
|         | Volumetric (\( \mu m^3 \)) | 0.0225 ± 0.0075 | 0.0749 | 0.0974 | 23.1 |
| Rabbit  | Surface (\( \mu m^2 \)) | 0.0616 ± 0.0327 (3.8) | 0.8730 (10.6) | 0.9346 | 6.6 |
|         | Volumetric (\( \mu m^3 \)) | 0.0160 ± 0.0093 | 0.0824 | 0.0984 | 16.3 |
| Guinea pig | Surface (\( \mu m^2 \)) | 0.0244 ± 0.0151 (4.6) | 0.3230 | 0.3474 | 7.0 |
|         | Volumetric (\( \mu m^3 \)) | 0.0053 ± 0.0040 | 0.0327 (9.9) | 0.0380 | 14.0 |

* The SEM's are extremely high because micrographs displaying no infoldings were scored as zero.
† The ratios of surface density to volumetric density (\( \mu m^2 \) per \( \mu m^3 \)) are given in parentheses.
‡ \( \Delta \) was taken from Table I.
§ Total lumenal membrane internalized = mean lumenal membrane infoldings plus \( \Delta \).
∥ Total lumenal membrane infoldings = total lumenal membrane internalized × 100.

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**Figure 5** A junctional complex between apical transitional epithelial cells of an expanded gerbil urinary bladder. In this section the junctional complex (arrow) and the lumenal plasma membrane (PM) are seen at slightly oblique angles. The close association of cytoplasmic filaments with the junctional complex, as well as with the lumenal plasma membrane, can be seen. Note the approximately parallel arrangement of most of the filaments with respect to the lumenal surface. L, lumen; LM, lateral membranes; D, discoidal vesicles. × 31,400.

**Figure 6** An electron micrograph of the lumenal surface of an epithelial cell of a contracted rat urinary bladder. The lumenal surface (L) of the bladder runs from left to right as indicated by the double arrow line in the upper-right corner. Portions of cytoplasmic filaments sectioned at right angles to (arrowheads) or along (arrows) the longitudinal axis can be observed. Note the variety of angles at which the filaments are arranged with respect to the lumenal plasma membrane (PM). × 37,500.
tuted considerably larger percentages of the total amounts of membrane internalized as compared to the surface densities of infoldings due to the splayed nature of the infolded membrane. Thus, the surface density values represented a more meaningful measure for the comparison of the actual amount of membrane internalized by infoldings and that internalized by discoidal vesicles. This comparison, illustrated in Fig. 7, graphically demonstrates the lesser role played by lumenal membrane infolding during contraction of the bladder.

As described before, the variations in the densities of the total cytoplasmic pools of discoidal vesicles between species were quite large. For example, in terms of numerical density, values ranged from ~1.6 vesicles per μm² of cytoplasm in guinea pigs to ~8.5 vesicles per μm² of cytoplasm in hamsters. If the volumetric, surface, and numerical densities are plotted against the osmolality of urine of each species, as shown in Figs. 8-10, respectively, a striking positive correlation is observed. The correlation was due to variations in the “residual pool” of discoidal vesicles found in the cell cytoplasm of fully expanded bladders, since the differences between the total pool of vesicles found in cells of contracted bladders and the residual pool were quite similar in all of the species except guinea pigs.

**DISCUSSION**

The morphometric data presented in this report demonstrated that pinching off of and reattachment of discoidal vesicles played the primary role in fluctuations of the surface area of lumenal membranes of apical transitional epithelial cells during the contraction-expansion cycle of the urinary bladders of several different mammals. This

![Figure 7](image7.png)

**Figure 7** Comparison of estimates of surface densities of lumenal membrane internalized as discoidal vesicles (∆ from Table I) and as infoldings during contraction of the urinary bladder.

**Figures 8-10** Volumetric (Fig. 8), surface (Fig. 9), and numerical (Fig. 10) densities of total pools and residual pools of discoidal vesicles of various mammals in relation to urine osmolality.
finding was in agreement with the observations of Hicks (3), as well as Porter et al. (9, 10), who described the uptake of ferritin into the vesicles by the pinching off of two adjacent plaques during contraction, and who hypothesized that this phenomenon occurred as part of a mechanism designed to decrease the surface area of the lumenal membrane during contraction of the bladder. The amount of membrane involved in the translocation process (Δ of Tables I and II) was considerable, averaging ~1.02 μm² per μm³ of cytoplasm in rabbits, rats, gerbils and hamsters, and ~0.32 μm² per μm³ in guinea pigs. In terms of volume, these vesicles constituted an average increase or decrease in the cytoplasm of the various species of ~8%, except in guinea pigs where a change in cytoplasmic volume of ~3% occurred. The estimations are probably slightly conservative since small round vesicles which appeared to be composed of one plaque and multiplaque vesicles composed of more than two plaques were not taken into account in this study. The contribution to the membrane internalization process by infoldings of the lumenal membrane was minor, accounting for from ~6 to ~15% of the total surface area of membrane introduced into the cytoplasm during contraction.

It has been demonstrated that the dense network of ~60 Å cytoplasmic filaments underlying the apical surface is tightly attached to the plaques of the lumenal membrane (1, 13). The attachment of filaments to discoidal vesicles also has been reported (3). The network of filaments, which in the cells of expanded bladders bears some similarity to the terminal webs of columnar epithelia, seems to be interconnected with the junctional complexes and desmosomes of the cells. Staehelin et al. (13) have proposed that the lumenal membrane-filament interaction serves to stabilize and strengthen the membrane, preventing its rupture during expansion of the bladder. In addition, these authors proposed that the structural cross-linking and interlocking of adjacent plaques leads to an orderly infolding of the membrane surface during contraction—with infolds being responsible for the major adjustments of surface area.

Our present data indicate that while infolding does participate in the surface area changes, the major process involved in internalization of membrane proceeds a step further—with the formation of discoidal vesicles from infolded plaques of the lumenal membrane. By comparing the ratios of surface to volumetric densities for discoidal vesicles involved in the internalization process to those for infoldings, using the data of Table II, it can be calculated that an average of ~12.6 μm² of membrane is present per μm³ of discoidal vesicles, while only ~4.9 μm² of membrane is present per μm³ of infoldings. Thus, discoidal vesicles contain about 2.6 times the amount of membrane per unit volume as do infoldings.

On the basis of the concept that discoidal vesicles are the important components of luminal membrane storage, we propose the hypothetical model of membrane translocation illustrated schematically in Fig. 11 and described below. The proposed model is a modification of the membrane-filament interaction model originally suggested by Staehelin et al. (13).

When the bladder is fully expanded, the filamentous network of each apical transitional epithelial cell is stretched between the junctional complex encircling the cell and desmosome of the...
lateral and basal membranes. The web of filaments is anchored to the particles composing the plaques of lumenal membrane, which in turn are cross-linked to each other by the filamentous network as described by Staehelin et al. (13). During voiding of the bladder, the force provided by the contracting musculature encircling the organ compresses the epithelia causing each apical cell to change from a flattened squamouslike shape to a cuboidal shape. The lateral membranes and basal membranes of the cell accommodate to the change in shape by unfolding and folding, respectively. During this transition, the filamentous web with its attached membrane plaques is compressed and displaced throughout the cytoplasm. Thus, the structural interlocking of the filamentous web leads to an orderly infolding of membrane, followed by pinching off of adjacent plaques to form discoidal vesicles, and displacement of the vesicles into the cytoplasm along with the reoriented reticulum of filaments. Conversely, as the bladder distends with urine, the hydrostatic pressure of the fluid forces the apical cells to undergo a transition back to the flattened shape. As this occurs, the filament network with its attached discoidal vesicles is once again stretched between the junctional complexes. During the stretching, the discoidal vesicles are lifted to the lumenal surface by the filament-membrane complex, fusing with the lumenal membrane and expanding its surface area. The actual fusion and pinching off of vesicles presumably would occur at particle-free interplaque regions as previously suggested (13).

In the model postulated, the reorientation of the network of ~60-Å filaments is due to forces extrinsic to the filaments. That is, the change in configuration from predominantly parallel to the lumenal surface in the distended condition to primarily vertical or oblique to the surface in the collapsed condition is due to the compression of the contracting musculature concomitant with the voiding of the bladder. Conversely, the hydrostatic pressure of urine filling the bladder reverses the process. Thus, the spatial rearrangement of the meshwork could be regarded as a passive phenomenon dependent upon the structural interlocking of the filaments and their attachments to the junctional complex and desmosomes of each apical cell.

In a like manner, the pinching off from and reattachment to the lumenal membrane of the plaques of discoidal vesicles may be thought of as a physical consequence of the movement of the attached filamentous network and the state of the lumenal membrane. In this view, the pinching off of plaques to form vesicles would be due to the "pull" of the filaments upon the infolding plaques during the transition in shape of the apical cells from squamous to cuboidal. Conversely, the reintegration of discoidal vesicle plaques with the stretched lumenal membrane would occur to reduce the surface tension of the lumenal membrane during expansion of the bladder.

The role of the filamentous network in moving discoidal vesicles should be clearly recognized as a working hypothesis based upon the limited evidence available.

One of the most intriguing results of this morphometric study was the observation that despite the relative similarity, among the different species, of the absolute amounts of membrane involved in translocation, the sizes of the cytoplasmic pools of discoidal vesicles varied greatly. The different pool sizes demonstrated a striking positive correlation with the osmolality of urine in the different animals. The reason for the increase in the amount of residual lumenal membrane stored as discoidal vesicles with increasing urine concentrations is not known. It is possible that the observed correlation may indicate a direct relationship (perhaps in an evolutionary sense) between the discoidal vesicle pool size and the tonicity of urine. For example, highly concentrated urine may be more deleterious upon the structural integrity of lumenal membranes than less concentrated urine. The large amount of "extra" membrane available in the cytoplasm of species with extremely hypertonic urine would allow for a constant recycling and alternation of membrane plaques with each expansion-contraction cycle. In this manner, the period of exposure of individual plaques to the urine would be shorter than in an animal with a smaller vesicle pool size.

Apart from the recycling process, or perhaps in concert with it, the varying pool sizes also may reflect variations in the rates of turnover of lumenal membrane. That is, a large discoidal vesicle pool size may be a consequence of a high rate of flux of vesicles into and out of the pool due to synthesis and degradation. Porter and Bonneville (8) have suggested that the internalization of lumenal membrane is part of a membrane-turnover process. The not uncommon presence of autophagic vacuoles containing discoidal vesicles seem to be evidence for some degradation of
lumenal membrane (8-10).

Other equally attractive and possibly spurious interpretations of the correlation could be presented, since as yet no conclusion is possible. The five species utilized in this study were rodents. It remains to be seen whether or not the discoidal vesicle pool sizes in transitional epithelial cells from other mammalian orders follow the correlation with urine concentrating ability.

This work was supported by Biological Research Support grant RO5712 and by grant CA 21612 from the National Cancer Institute, Department of Health, Education, and Welfare.

Received for publication 14 July 1976, and in revised form 8 February 1978.

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