Normal urinary bladder transitional epithelium (urothelium) contains a permeability barrier to water and solutes. Electron microscopy studies show that three structural elements of the plasma membranes of superficial urothelial cells, i.e., asymmetrical unit membrane plaques and interplaque membrane at the luminal surface and tight junctions at lateral surfaces, provide the actual barrier. In spontaneous transitional cell carcinomas in humans and in similar ap-
FANFT obtained from Saber Laboratories, Morton Grove, Ill. This drug produces a high incidence of urothelial tumors with a clinical course similar to the disease in man (2, 25). FANFT was administered ad libitum as 0.2% of a powdered Charles River rat chow for 25 weeks. Twenty-six to 61 weeks after the initiation of FANFT feeding, rats were anesthetized by i.p. injection of 0.5 to 1.0 ml of Nembutal, 50 mg/ml (Abbott Laboratories, North Chicago, Ill.). The urinary bladder was then exposed and immediately covered externally with a 2% glutaraldehyde fixative in 0.1 M cacodylate buffer, pH 7.4. Simultaneously, the bladder was inflated through the urethra with buffered glutaraldehyde at a constant pressure of 50 mm Hg. The urethra was ligated when the bladder was fully inflated. The bladder was excised and placed in fixative for an additional 15 min. It was then opened, and representative pieces of epithelia were removed for study. Thirty-week-old Fischer rat controls as well as several adult BALB/c mice, all having been maintained on standard diets, were anesthetized, and bladder specimens were prepared as outlined above.

Human Urinary Bladder. Studies on human urinary bladder ultrastructure are particularly difficult because of the nature of standard cystoscopy procedures which must be used to obtain human bladder specimens. During cystoscopy, the bladder is filled either with distilled water or a nonelectrolyte solution, such as glycine. These unphysiological solutions undoubtedly introduce artifacts (30) and, therefore, must be carefully considered in an interpretation of ultrastructure (32). Further, cystoscopies are generally performed on patients who are suspected of having a disease in the urogenital system, since urologists are understandably reluctant to cystoscopy asymptomatic patients. In general, so-called "control" specimens in this type of study are from patients with disease in the genitourinary tract but whose bladder urothelium appears normal by light microscopic histopathological criteria. We feel justified in using cystoscopy specimens as controls in this study, since the membrane structures under consideration are known to be stable and to resist artifactual modification.

Human tumors were obtained at cystoscopy from 10 patients with Grade I or II transitional cell carcinoma of the urinary bladder. Controls consisted of biopsies obtained at cystoscopy from 4 adult humans. These were from 2 patients with incomplete urethral obstruction and from 2 patients with benign prostatic hyperplasia and relatively mild urethral obstruction (Fig. 16). The cystoscopy and biopsy procedures were performed very rapidly using standard surgical methodology which included filling the bladder with distilled water. Histopathological evaluation of the biopsy specimens by light microscopy showed essentially normal epithelium. So-called "water artifact" (30) was judged to be minimal (Fig. 16). The identity of superficial cells in the electron microscope was based on location and the presence of tight junctions, since tight junctions in normal urothelium are present exclusively at the lateral margins of superficial cells. In addition to these biopsies, 2 specimens of human bladder were obtained at autopsy from postnatal infants who died from unrelated causes and who had grossly normal appearing urothelium at autopsy and by light microscopy evaluation. All specimens were diced into 1- to 2-mm cube blocks in Karnovsky’s paraformaldehyde (17) and fixed for 1 hr.

Thin-Section Electron Microscopy. All human, rat, and mouse bladder samples for thin-section electron microscopy were rinsed briefly in 0.1 M cacodylate buffer, pH 7.4; postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4; dehydrated with graded ethanol solutions; and embedded in Epon 812. Thin sections were prepared with diamond knives and were double stained with uranyl acetate and lead citrate.

Freeze-Fracture Electron Microscopy. Specimens for the freeze-fracture technique were prepared according to methods described elsewhere (23, 32). In brief, glutaraldehyde-fixed, 1-cm tissue blocks were soaked overnight in 20% glycerol-0.1 M cacodylate. They were then quenched in Freon 22 cooled with liquid nitrogen. Replicas were prepared at −100° in a Balzers Model BAF 301 freeze-etch device. All thin sections and freeze-fracture replica specimens were examined by transmission electron microscopy using either a Philips Model 300 or Philips Model 301 electron microscope.

SEM. For SEM, postfixed specimens were rapidly dehydrated through ascending ethanol and amyl acetate solutions and then dried in a Denton DCP-1 critical point drying apparatus (Denton Vacuum Inc., Cherry Hill, N. J.). The tissues were cemented to specimen stubs, coated in a vacuum with gold, and photographed either in a JEOL Model JSMU-3 or an ETEC Autoscan SEM microscope at an accelerating voltage of 25 kV.

Results

Luminal Membrane in Normal Rat Bladder. Superficial cells line the luminal surface of normal Fischer rat bladder. SEM demonstrates that the luminal faces of individual cells are polygonal in the plane of the bladder wall. Single seam-like folds are frequently observed at the boundaries between adjacent luminal faces (16) (Fig. 1). These are similar to those reported by Noack et al. (24) in expanded Wistar rat bladder. A characteristic SEM feature of the luminal cell surface is a complex system of microridges (16) (Fig. 2).

In all thin-section studies on control urothelium and tumors in humans and rats, luminal membrane was identified by its proximity to tight junctions. This is especially important in studies of human urothelium, since it is possible for superficial cells to slough during cystoscopy or with processing for electron microscopy. The morphology of thin-sectioned luminal membrane in Fischer rat urothelium is essentially identical to that reported elsewhere for other rat strains (14, 18). The luminal membrane has concave plaques that appear as troughs when viewed in profile (Fig. 7). The plaques appear as a triple-layered AUM approximately 11 to 12 nm in thickness. The outer leaflet is thicker than the inner one but is less well defined. The plaques appear to be rigid, but they are joined by flexible "hinges" of symmetrical interplaque membrane measuring 8 to 9 nm in thickness. The combination of rigid and flexible membranes results in prominences that apparently correspond to the microridges. When microridges are present, asymmetrical membrane may form the lateral walls, but at the crest only symmetrical interplaque membrane is observed (Fig. 8).

The cytoplasm of superficial cells contains many dilated
fusiform vesicles of which some are found in close proximity to Golgi complexes and others are in contact with the luminal membrane (12, 18). These vesicles are often partially lined by AUM plaques with the thick leaflet facing inward. At the poles of each fusiform vesicle, segments of unthickened symmetrical membrane link the plaques together. Fusiform vesicles that bear plaques are frequently encountered in deeper (intermediate) cells, but the plasma membranes of these cells are symmetrical.

**Luminal Membrane in Control Human Bladder.** The superficial cells of the control adult human bladders are smaller and more variable in size than are those of rat bladder. SEM of the luminal surface reveals that, although the texture of the luminal surface is smooth, many irregular furrows and ridges are present. Microvilli are uniform, and many are present at the surface, but the number varies from cell to cell (Fig. 14).

Thin sections of normal bladder urothelium, obtained from human infants at autopsy, reveal many AUM plaques measuring 11 to 12 nm thick at the luminal surface (Fig. 17). There are numerous fusiform vesicles in the periphery of the cytoplasm (Fig. 18). On the other hand, luminal membranes of our adult human control bladders are not usually thicker than 10 to 11 nm (Figs. 19 to 21). Although AUM plaques are encountered in the urothelium of the adult controls (Fig. 21), they are very infrequent, although umbrella (superficial) cells are present and appear fully differentiated in the light microscope (Fig. 16).

A surface “fuzzy” coat (i.e., glycocalyx) is often present on the luminal surface of adult human bladder (22), especially where the membrane profile displays gently rounded contours (Fig. 19). In areas where the profile is more angular or the membrane thickness more pronounced, a glycocalyx is less likely to be observed. Sometimes the luminal membrane profile in humans appears to have a reversed appearance may be the result of tonofilaments, closely apposed to the inner leaflet and running parallel to the membrane face. When transversely sectioned, the cross-sectioned filaments are indistinguishable from the inner leaflet (Fig. 19), but when the plane of section is tangential, tonofilaments are seen (not illustrated).

Vesicles are often present in the cytoplasm of superficial cells near the luminal surface (Fig. 23). They are usually quite round but occasionally are fusiform (Fig. 18). We have been able to demonstrate a clear association between these vesicles and the Golgi apparatus in human bladder, a finding also reported by others (9).

**Luminal Membrane in Rat Tumors.** Luminal surfaces of Fischer rat bladder tumors are found to be composed of many cell faces that vary greatly in size and shape (Fig. 3). Microridges are occasionally encountered, but they are generally obscured by masses of pleomorphic microvilli (16, 25) (Fig. 4). Between adjacent faces, there are enlarged seam-like folds (Fig. 5) that often appear split by an intercellular cleft (Fig. 6), thus forming paired ridges. These folds and ridges overlie the tight junctions at the lateral surfaces of neighboring urothelial cells.

Thin sections of rat bladder tumors reveal many cell surface projections extending into the lumen. Although a small percentage of the surface area of the luminal membrane does retain the thickened rigid appearance of typical AUM plaques (Fig. 12), the luminal surface of the most superficial tumor cells consists exclusively of interplaque membrane (Figs. 11 and 13). The ultrastructure of luminal membrane profiles of normal superficial cells differs at straight and sharply bent regions (Fig. 8). On the other hand, profiles of tumor luminal membranes that are devoid of AUM appear the same at gently contoured and acutely angulated regions (Fig. 13). Luminal membranes of tumors generally appear thin and flexible and are often associated with a conspicuous glycocalyx (Fig. 11).

Large numbers of vesicles are present in rat urothelial tumor cells. Some of these vesicles are fusiform, but unlike the fusiform vesicles of normal rat bladder, they often appear collapsed and are not observed to coalesce with the luminal membrane (Figs. 9 and 10). Other vesicles have round profiles consisting of symmetrical membrane (Fig. 9).

**Luminal Membrane in Human Tumors.** The superficial cell surfaces of human bladder tumors are shown by SEM to appear similar to the controls, except that the microvilli are generally more numerous and somewhat pleomorphic (Fig. 15).* The ultrastructure of thin-sectioned luminal membranes in tumors is similar in every biopsy we have examined, regardless of histopathological grade of the tumor. Their morphology also resembles that of the luminal membranes usually present in control biopsies (compare Figs. 19 and 22). The luminal membrane of tumors is of uniform thickness throughout its length and is usually covered with a well-formed glycocalyx. We have not observed asymmetrical plaques associated with either the luminal membrane or the surfaces of numerous round vesicles present in the cytoplasm of human tumor cells.

Freeze-fractured luminal membrane of human transitional cell carcinomas resembles the interplaque membrane in normal human urothelium. It differs from the normal luminal membrane in that there are many more microvilli. These are often freeze fractured near their base (Fig. 24). The luminal membrane in noninvasive tumors can be distinguished from plasma membrane at the surfaces where there is cell-cell apposition by the relatively low numerical density of intramembrane particles on the protoplasmic fracture face of the luminal membrane.

**Tight Junctions in Normal and Malignant Fischer Rat Bladders.** Tight junctions (zonulae occcludentes) are continuous belt-like regions of intimate contact (union) between plasma membranes of adjacent cells. These junctions, which girdle bladder superficial cells near their luminal faces, consist of a network of branching intramembrane fibrils that are visualized by freeze-fracture electron microscopy (21) (Fig. 25). In urothelium of normal Fischer rats, the network has a width of 3 to 5 strands (Fig. 25). In FANFT-induced tumors examined 26 weeks after initiation of FANFT feeding (i.e., 1 week after FANFT is withdrawn from the diet), tight junctions are often focally attenuated to a single strand (Fig. 26). We have examined replicas of invasive transitional cell carcinomas obtained 61 weeks *Because of preparative artifacts in human specimens, a definitive statement on occurrence of pleomorphic microvilli cannot be made at the present time.
after initiation of FANFT feeding and find that in these
tumors the tight junctions are frequently discontinuous (not
illustrated). Our thin-section data on control and tumor
material corroborate the freeze-fracture observations.
These discontinuities were interpreted as showing that the AUM
plaques and intemplaque membrane have not been studied individually, it can nevertheless be assumed that
each component has, as a minimum, the permeability char-
acteristics of the entire intracellular barrier, since both oc-
cupy a significant fraction of the membrane surface area.
The possibility remains that plaque regions may be rela-
tively impermeable compared to interplaque regions, but
the physiological significance of the differences is probably
trivial (4). Observations on human bladder urothelium, pre-
sented in the current study, provide additional evidence that
AUM-plaque membrane does not endow the urothelium with
unique permeability characteristics. In normal human bladder
urothelium obtained from infants, AUM plaques are
prominent and occupy the majority of the urothelial surface
area, as previously described for other species (11, 18, 27).
However, in bladder specimens obtained from adults with
mild or minimal urinary obstruction, and with histopatho-
logically "normal" urothelium, AUM plaques are rarely en-
countered. The vast majority of the luminal surface mem-
brane consists of interplaque symmetrical unit membrane.
Although we have not measured urothelial permeability in
these particular patients, it is well known that the permea-
bility barrier is intact in comparable patients. Absence of the
AUM plaques in these specimens essentially rules out a
specific permeability barrier function for the plaques (5).
Staehelin et al. (28) have proposed an alternative function
for the plaques. These authors suggest that AUM plaques
serve as an attachment site for cytoplasmic filaments (28).

In the transitional cell carcinomas of humans and rats,
luminal surfaces of the superficial tumor cells share a simi-
lar ultrastructure. In each species, the surface membrane
has a symmetrical unit membrane appearance and, on
some cells, a conspicuous "fuzzy" coat (i.e., glycocalyx).
Many, surface projections including pleomorphic mi-
cro villi extend into the bladder lumen. We have no data on
the permeability characteristics of the luminal membrane of
the superficial tumor cells. However, it is noteworthy that
there is no evidence in the literature that membrane perme-
ability per unit area to either water or small ions is altered by
changes in the contour of membranes, by the formation of
pleomorphic microvilli, or by the overdevelopment of the
cell surface glycocalyx. Therefore, alterations in the ultra-
structure of the tumor luminal membrane cannot be related
to alterations in transurothelial permeability at the present
time. On the other hand, there is strong evidence that al-
terations in zonulae occludentes junctions can account for
the altered permeability in transitional cell carcinomas (4)
as has previously been reported for a number of other types
of tumors (19, 20, 29, 34, 35).

In normal epithelium, extracellular bypass diffusion of
small molecules is blocked by zonulae occludentes cell-to-
cell junctions (tight junctions) (7, 10), and it is generally
held that functional differences between various tissues
stem in part from structural differences in these junctions
(1, 7, 8). Claude and Goodenough (1) have classified epithe-
lia ranging from "very leaky" to "very tight" on the basis of
measurements of electrical resistances across the epithe-
lium. They have correlated the number of interconnected
strands located within the membranes at these intercellular
junctions with transepithelial resistance to electric current,
a measure of bypass diffusion. In our study, we have shown
that in both human and rat urinary bladder, tight junctions

Discussion

A coherent and continuous sheet of superficial cells lines
the excretory system from the renal pelvis to the urethra and
encloses the urinary space within a semipermeable barrier
to diffusion. There are 3 major structural elements of the
permeability barrier, all of which are components of the
plasma membrane of urothelial superficial cells. Two of the
barrier elements, AUM plaques and interplaque membrane,
are present at the luminal surface of urothelial superficial
cells (6, 11–14, 18, 24, 28, 31). The 3rd component is zonu-
lae occludentes ("tight"), intercellular junctions that are
structurally specialized zones located at the apical-lateral
surfaces of these superficial cells. They form a continuous
belt-like region completely encircling the cells (3, 21, 26,
27). The luminal membrane elements retard the intracellular
(transcellular) diffusion of water across the epithelium,
whereas the tight junctions provide a barrier to intercellular
(paracellular or bypass) diffusion of water across the blad-
der wall (1). Tracer studies have suggested that the barrier
may be complex and that the different substances may
penetrate the barrier at different locations; for example,
water may pass through the intracellular barrier, and so-
dium may pass through the intercellular barrier (5).

It has been widely speculated that the AUM plaques may
endow the intracellular barrier with special permeability
properties. Early support for this concept came from thin-
section and negative-stain electron microscopy observa-
tions that were interpreted as showing that the AUM
plaques covered most or all of the luminal surface of su-
perficial cells (13). The results of recent freeze-fracture studies
challenge this concept. Staehelin et al. (28) have shown that
in species in which AUM plaques are particularly prominent,
the plaques occupy less than 80% of the luminal surface with
interplaque membrane accounting for the remainder of the
surface area. Although the permeability characteristics of
the AUM plaques and interplaque membrane have not been
generally have 3 to 5 intramembrane strands (Figs. 25 and 27). According to the criteria of Claude and Goodenough (1), it might be expected that these junctions could account for relatively tight seals between cells.

The tight junctions between superficial tumor cells are remarkably similar in moderately low-grade (Grade I and II) human transitional cell carcinomas, as reported previously (34, 35), and in the FANFT-induced transitional cell carcinomas harvested at 26 weeks. In tumors of both species, the tight junctions are focally attenuated and often have only a solitary strand (Figs. 26 and 28). Attenuation of the tight junctions to a single strand can account for an increase in epithelial permeability (1). In higher-grade human tumors and in invasive FANFT-induced tumors harvested at 61 weeks, the intramembrane fibrillar network of the tight junctions is frequently discontinuous (i.e., they become maculae or fasciae ocludentes). This may further increase permeability. Similar observations have been made in intracranial germinomas (29). On the basis of our findings and the results of other studies (for reviews see Refs. 21 and 27), it is reasonable to conclude that transepithelial leakiness in bladder tumors can be explained on the basis of tight junctional attenuation.

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References

Fig. 1. Scanning electron micrograph of the luminal surface of a distended urinary bladder from a normal Fischer rat. The polygonal faces of superficial cells are covered with irregular folds. Narrow, seam-like elevations (arrow) are frequently observed at boundaries between the superficial cell faces. x 2,500.

Fig. 2. Luminal surface of a distended normal Fischer rat bladder at high magnification. The honeycombed network of microridges covering a superficial cell is demonstrated. x 11,000.

Fig. 3. Scanning electron micrograph of the surface of a Fischer rat FANFT-induced bladder tumor. The superficial cell faces are irregular in size and in some areas are separated by seam-like elevations. Long slender microvilli (arrows) are prominent on faces of underlying cells recently exposed by sloughing superficial cells. x 3,500.

Fig. 4. Surface of FANFT-induced Fischer rat bladder tumor. Pleomorphic microvilli cover the luminal membranes of several superficial cells. Microridges are rare. x 5,500.

Fig. 5. Detail of seam-like elevation between bladder tumor cells of a Fischer rat. In tumors the seams usually appear wider than in bladders of control animals. x 7,500.

Fig. 6. Split seam in Fischer rat bladder tumor. The seams are partially or entirely split along the midline by a cleft. x 9,000.

Fig. 7. Thin section of normal Fischer rat bladder urothelium. Two superficial cells are joined together by a junctional complex (upper left). The profile of the luminal surface has a scalloped appearance and includes asymmetrical membrane plaques. Cytoplasmic vesicles, some of which are fusiform in shape, frequently appear in superficial cells. x 65,000.

Fig. 8. Sagittal section of a microridge in normal mouse bladder. Rigid asymmetrical plaque membrane (12-nm thick) is located at the lateral walls of the ridge. At the crest of the ridge only flexible symmetrical membrane (8-nm thick) is found. Arrows, regions where the plaques join interplaque membrane. x 102,000.

Fig. 9. Superficial cell of a FANFT-induced Fischer rat bladder tumor. Microvilli are prominent, and the contour of the luminal membrane profile is generally less angular than that found in controls. Collapsed fusiform vesicles are prominent in the cytoplasm. x 36,000.

Fig. 10. Detail of collapsed fusiform vesicles in a FANFT-induced Fischer rat tumor. Vesicle walls contain AUM plaques. x 100,000.

Fig. 11. Detail of a Fischer rat bladder tumor. The luminal membrane (8-nm thick) is symmetrical throughout its length and is covered with a delicate glycocalyx. x 115,000.

Fig. 12. Detail of Fischer rat bladder tumor. The luminal membrane has a scalloped appearance and includes asymmetrical membrane plaques. x 115,000.

Fig. 13. Sagittal section of a microribbon from a FANFT-induced Fischer rat bladder tumor. The luminal membrane, approximately 9 nm thick, is uniform in appearance throughout its length. x 102,000.

Fig. 14. Scanning electron micrograph of the luminal surface of a control human bladder. Many ridges and furrows are present on the surfaces of the cells, but the surface appears smooth in areas not covered with microvilli. Numbers of microvilli vary greatly from cell to cell. x 3,250.

Fig. 15. Scanning electron micrograph of human bladder luminal cells. Although smooth areas at the surface are visible, most regions of the cell surfaces are covered with microvilli. The microvilli are usually more numerous in human tumors than in controls. Compare with Fig. 14. x 5,000.

Fig. 16. Light micrograph of Epon-embedded, toluidine blue-stained, typical control urothelium. This is a cytology specimen from a patient with bladder neck constriction. The epithelium is contracted and slightly dysplastic. "Water-artifact" (30) is minimal. x 345.

Fig. 17. Luminal membrane of postnatal human bladder obtained at autopsy. Asymmetrical membrane plaques have an appearance similar to those found in normal rat urothelium. x 115,000.

Fig. 18. Fusiform vesicles (FV) in superficial cell of postnatal human bladder. The vesicles, located near the luminal membrane (LM), bear AUM with the thick leaflet facing inward. x 115,000.

Fig. 19. Superficial cell of control bladder from a patient with benign prostatic hyperplasia. The luminal membrane is approximately 9 nm in thickness and is covered at the free surface with a delicate glycocalyx. At some regions, the inner leaflet of the membrane appears thicker and more electron dense than does the outer leaflet, but plaques are not observed. x 175,000.

Fig. 20. Superficial cell of control bladder from a patient with bladder neck constriction. The symmetrical luminal membrane is about 10 nm in thickness. x 185,000.

Fig. 21. Superficial cell of control bladder from a patient with benign prostatic hyperplasia and very mild obstruction. Transversely sectioned luminal membrane (between arrowheads) appears to be an asymmetrical plaque; the inner leaflet has greater density than does the outer leaflet and in some regions is thicker as well. x 160,000.

Fig. 22. Superficial cell of patient with noninvasive transitional cell carcinoma (Grade I to II). The luminal membrane is covered with a glycocalyx and in some areas shows reversed asymmetry (compare with control, Fig. 19). Thin sections of all the human bladder tumors we examined have luminal membranes with a similar appearance. x 185,000.

Fig. 23. Luminal surface of adult human bladder obtained from a patient with incomplete urethral obstruction. A cytoplasmic "seam" projects into the lumen (upper right) where a tight junction (TJ) joins 2 superficial cells. Tight junctions may provide support for the prominences which frequently appear in the SEM as seam-like folds between luminal faces of superficial cells. A heterogeneous population of vesicles is present in the cytoplasm of the cells. x 45,000.

Fig. 24. Freeze-fracture replica of a superficial tumor cell in a patient with transitional cell carcinoma (Grade I). The luminal membrane displays numerous cross-fractured pleomorphic microvilli. No asymmetrical membrane plaques are observed. Intramembrane particles are more numerous on the lateral surface (from Ref. 35). x 68,000.

Fig. 25. Freeze-fractured tight junction in a normal Fischer rat bladder. The junctions consist of an interconnected fibrillar network that is 3 to 5 strands in depth. x 96,000.

Fig. 26. Tight junction in a FANFT-induced bladder tumor of the rat. At the right, the full complement of junctional strands is present. Elsewhere the network is narrowed and is focally attenuated to a single strand (between arrows). x 96,000.

Fig. 27. Freeze-fractured tight junction in normal human bladder. The tight junction is 3 to 5 strands deep. x 96,000.

Fig. 28. Freeze-fractured tight junction of patient with transitional cell carcinoma (Grade I). The junction is focally attenuated to a single strand (between arrows). x 96,000.
F. B. Merk et al.
Ultrastructure of Bladder Permeability Barrier
Malignant Transformation of Urinary Bladder in Humans and in
N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide-exposed Fischer
Rats: Ultrastructure of the Major Components of the
Permeability Barrier

Frederick B. Merk, Bendicht U. Pauli, Jerome B. Jacobs, et al.