SURFACE ULTRASTRUCTURE OF THE EPITHELIA LINING THE NORMAL HUMAN LOWER URINARY TRACT

J. NEWMAN AND R. M. HICKS

From the Bland-Sutton Institute and School of Pathology, Middlesex Hospital Medical School, London W1P 7LD

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Summary.—The finding of cells with pleomorphic microvilli in urinary sediments has been proposed as an indicator for urothelial neoplasia. Recently, in addition to such cells, others with less bizarre, non-pleomorphic microvilli have also been found in urothelial cancers, and these cells are similar in appearance to others detected in the urinary sediments of healthy people. When using scanning electron microscopy as a diagnostic tool, these cells are a possible source of confusion. The entire lower urinary tracts from people free of urothelial neoplasia have therefore been examined to delineate the normal surface appearance of all cell types which could appear in the urine. There are 4 predominant cell types: the large, flat squamous cells of the urethral meatus which have abundant microridges; cells with mucus-coated, short, stubby microvilli lining the urethra and renal papilla; immature urothelial cells with chains and ridges of bleb-like processes in the ureters and bladder; and, also in the ureters and bladder, mature urothelial cells with microridges or ruffles.

The lining epithelia of the normal urethra and renal papilla may thus contribute cells with non-pleomorphic stubby microvilli to urine sediments, which cannot be differentiated by scanning electron microscopy alone from similar cells derived from urothelial neoplasms. However, the normal complement of cells lining the adult lower urinary tract does not include any with prolific, long, pleomorphic microvilli such as characterize transitional-cell carcinomas of the urothelium.

The sub-structure and histology of the urothelium lining the human urinary bladder and ureters has been the subject of a number of reports (Fulker, Cooper and Tanaka, 1971; Lloyd-Davies and Hinman, 1971; Kumagai, 1975; Hanna et al., 1976; Kjaer et al., 1976; Newman and Hicks, 1977; Hayakawa, 1978; Tannenbaum, Tannenbaum and Carter, 1978; and Nelson, Croft and Nilsson, 1979). Where adequate care has been taken to preserve the tissue and avoid postmortem loss of superficial cells, these studies have confirmed the similarity between human urothelium and that of other species (reviewed by Hicks, 1975). The remainder of the lower urinary tract, namely the lower part of the kidney and the urethra, have been less systematically studied either in man or experimental animals.

Recently, the potential value of the scanning electron microscope as a diagnostic tool for examining exfoliated cells in urinary sediments has been realized (Jacobs et al., 1977; Hicks, Newman and Beilby, 1978; Croft and Nelson, 1979; Croft, Nelson and Nilsson, 1979). The presence of numerous pleomorphic microvilli on the surface of the exfoliated cells has been used as a marker for neoplasia and preneoplasia since these surface features are known to characterize neoplasia of the urinary bladder both in man (Fulker et al., 1971; Newman and Hicks, 1977; Tannenbaum et al., 1978; Knowles et al., 1980), and in experimental animals (Hicks, Ketterer and Warren, 1974; Hicks, 1976; Hicks and Wakefield, 1976; Hodges, Hicks and Spacey, 1976; Jacobs et al., 1976). From time to time, cells with variable numbers of shorter and less pleomorphic microvilli are found in urine...
samples from apparently normal individuals and it seemed possible that these were normal cells derived from parts of the lower urinary tract other than the bladder or ureters. Such cells may confuse or obscure the accurate diagnosis of malignant disease in the urinary tract especially as the degree of pleomorphism of the microvilli in human bladder tumour cells is variable (Tannenbaum et al., 1978). This study was undertaken to locate the tissue origin of these microvillous cells found in some normal urine sediments and to characterize them more carefully. The observations reported here on the entire human lower urinary tract extend published reports on the structure of the renal pyramid, fornices and pelvis in the rat (Carroll et al., 1974; Khorshid and Moffat, 1974; Silverblatt, 1974; Burke, 1976), man (Hueckner, Frenzel and Skoluda, 1975; Hayakawa, 1978), and of the urethra of various rodents (Shehata, 1974), dog (Lloyd-Davies, Lee and Hinman, 1971; Lloyd-Davies, Hayes and Hinman, 1971; Mooney and Hinman, 1974) and man (Lloyd-Davies and Hinman, 1971; Hakky, 1979). The ultrastructural findings are intended to provide a baseline reference for identification of normal cell types which may exfoliate into the urine and thus enable more accurate diagnosis of malignant or premalignant conditions to be made by scanning electron microscopy of urine sediments from patients at risk for bladder cancer.

MATERIALS AND METHODS

This report is based on the study of 3 female and 6 male urinary tracts obtained post mortem from patients free from urinary tract disease, and 2 male upper urinary tracts removed at surgery for renal cell adenocarcinoma. The ages of the males ranged from 1 day to 66 years and the females were 37, 56 and 59 years old. The interval between death of the patient and postmortem removal of the tissues varied from 2 to 12 h. At postmortem examination, a block of tissue of the external urethral meatus was taken before removal en bloc of the whole renal tract. The urethral meatus was then ligated and the urinary tract inflated with phosphate-buffered formalin at pH 7.3 for approximately 15 min. Tissue blocks were taken from each anatomical region for light, scanning and transmission electron microscopy and fixed further as appropriate. The operative specimens were immediately opened and tissue blocks from the various anatomical regions taken and treated as for postmortem tissue.

Tissues for light microscopy were fixed for a further period in phosphate-buffered formalin and then prepared in the standard way for paraffin sectioning at 2 μm and were in the first instance stained with haematoxylin and eosin and, where indicated, with specific stains. The tissues for scanning electron microscopy were further fixed in 1% osmium tetroxide buffered to pH 7-3 with phosphate buffer, dehydrated through graded alcohols to Arcton, critically point-dried, gold-coated and then examined in a Jeol SEM 35 at 20 Kv. The tissues for transmission electron microscopy were fixed for 1 h in phosphate-buffered osmium tetroxide, dehydrated through graded alcohols and embedded in Spurr resin or in Epon. Thin sections were contrast-stained with lead and uranyl salts before being examined in a Jeol 100 B or Phillips 200 transmission electron microscope.

RESULTS

The gross anatomy and histology of the lower urinary tract in both male and female specimens were in accordance with previously published reports such as can be found in any standard histology textbook. Only the surface features of the epithelial cells in contact with the urine are described in detail here.

Pyramid to fornix

At the tips of the renal papillae, the collecting ducts open into the calyces in the area cribrosa as ducts of Bellini (Fig. 1). The tips of these collecting tubules are lined by a single layer of tall columnar cells (Fig. 2) which continue over the surface of the pyramid as far as the fornix as a 2-3-cell-thick epithelium. At the fornix the epithelium is gradually transformed into a transitional-type epithelium, or urothelium (Fig. 3). Scanning electron microscopy of the pyramid reveals 2 distinct regions in the area cribrosa, Zones A and B (Fig. 4). In Zone A, at the tips of the papilla in the region of the duct
FIG. 1.—Section through the renal pyramid showing the papillary ducts opening into the calyx in the area cribosa. Wax-embedded section. H. & E. × 170.

FIG. 2.—The distal end of a papillary duct lined by tall columnar epithelium which changes to a multilayered columnar epithelium over the area cribosa of the pyramid (arrow). Wax-embedded section. H. & E. × 750.

FIG. 3.—Section through the epithelium covering the fornix. This is a 3–4-cell-thick transitional epithelium with large, flat superficial cells. Epon-embedded section. Toluidine blue. × 1300.

FIG. 4.—The surface appearance of the area cribosa reveals 2 distinct regions, Zones A and B. In Zone A, around the openings of the papillary ducts (arrow) the surface cells are dome-shaped, regular in size and bulge into the lumen. In Zone B, the surface cells are flat, polygonal and their cell boundaries are prominent. Scanning electron micrograph. × 675.

FIG. 5.—Higher magnification of the surface of cells in Zone A. The cells are dome-shaped and bulge into the lumen and do not have prominent cell borders. Their free surfaces are covered by variable numbers of short, bleb-like microvilli and some cells possess a single cilium. Scanning electron micrograph. × 11,750.

FIG. 6.—Other cells in Zone A, which project into the lumen as if about to desquamate, have a deeply wrinkled or plicated surface. Scanning electron micrograph. × 6750.
FIG. 7.—The cells in Zone B are variously sized, polygonal in shape, and covered by variable numbers of short, globular, bleb-like microvilli. Scanning electron micrograph. × 1045.

FIG. 8.—A higher magnification of typical surface cells in Zone B showing the short, globular microvilli and wrinkles or indentations in the cell surface. Scanning electron micrograph. × 5700.

Fig. 9.—Interspersed between the more regular cells in Zone B, there are a number of multiciliated cells, 2 of which are shown here. Scanning electron micrograph. × 5700.

Fig. 10.—Thin section through the bleb-like microvilli on the surface of a cell in Zone B of the area cribosa. Despite postmortem deterioration of the tissue, it can be seen that a mucoid coat is associated with the surface membrane. Transmission electron micrograph. × 42,750.

Fig. 11.—This field illustrates the surface appearance of the fornix, which is covered by large, pavement-like surface cells with prominent cell boundaries. The luminal face of these cells is covered with a mixture of small, bleb-like microvilli which appear singly or arranged in chains; in some cells these have fused to form microplicae which give the surface of the cell a characteristic ridged appearance. Scanning electron micrograph. × 2612.

Fig. 12.—Higher magnification of cells in the fornix. This field shows large, flat surface cells with characteristic microplicae and a single, underlying less mature cell covered by short, bleb-like microvilli. Scanning electron micrograph. × 3900.
mouths, the surface cells are regular in size, dome-shaped and bulge out into the lumen. Their borders are not particularly prominent and their free surfaces are covered by variable numbers of short, bleb-like microvilli (Figs 5, 6). Some of the cells possess a single cilium which may be straight or curved (Fig. 5) while others, which bulge out into the lumen as if about to desquamate, have a deeply wrinkled or plicated surface between which small bleb-like microvilli can frequently be seen (Figs 5, 6). The surface cells of Zone B of the area cribrosa away from the duct mouths differ from Zone A primarily by their flatter, more pavement-like aspect. They are more polygonal in shape and vary somewhat in size (Fig. 7). Their free surfaces, like the cells in Zone A, are covered with variable numbers of short bleb-like microvilli (Fig. 8) but in most specimens a few cells with longer microvilli and an occasional ciliated cell were also observed (Fig. 9). Examination of thin sections of the area cribrosa in the transmission electron microscope revealed that in general the surface membrane of the cells, including that limiting the bleb-like microvilli, was coated with a fine glycocalyx (Fig. 10).

At the fornix, the epithelium changes and develops the appearance of a slightly immature urothelium. The surface is composed of a pavement of variably sized cells with prominent raised boundaries, and the surface of these cells shows a transition from the bleb-like microvilli found over the renal papilla to a microridge pattern more characteristic of maturing intermediate or superficial urothelial cells (Fig. 11). As in the urothelium, the microridges on the surface cells develop from the bleb-like projections of the underlying intermediate cells which are occasionally revealed when a surface cell desquamates (Fig. 12). Transmission electron microscopy of these transitional epithelial cells in the fornix confirms them to be similar to maturing intermediate urothelial cells and to be devoid of a glycocalyx.

Calyces, pelvis and ureter

The epithelium lining these 3 regions, including the full length of the ureter with its intramural portion, is a transitional epithelium, or urothelium, morphologically comparable to that lining the bladder. Histologically, it appears to be 3–5 cells thick with an orderly pattern of differentiation from basal to large flat surface cells (Fig. 13). The luminal surface has a flat, pavement-like appearance and is composed of large polygonal-shaped cells with randomly distributed surface ridges or microplicae (Fig. 14). These cells are delineated by prominent cell borders which are further emphasized by bleb-like microvilli which are aligned parallel with these cell borders. The number of ridges varies greatly from cell to cell and, in some specimens, where the muscle of the ureter was contracted, the cell surfaces are folded to form deep indentations. Occasional smaller, more immature cells covered with short bleb-like microvilli were found between the larger, mature superficial cells (Fig. 15). Transmission electron microscopy of thin sections through the urothelium lining the calyces, pelvis and ureter showed the mature, ridged surface cells to have the same characteristic surface membrane and fusiform vacuoles as found in mature superficial cells in the bladder.

Bladder, excluding the urethro-vesical junction

Samples were taken from the different regions of the bladder so that the epithelium covering the trigone, posterior wall, dome, anterior wall and the 2 lateral walls could be examined separately and compared. The apparent thickness of the urothelium varied according to the degree of distension of the bladder, so that histologically it appeared to be 2–3 cells thick in the dilated bladder and up to 5 cells thick in the contracted bladder. The appearance of the luminal cells also reflected the degree of contraction of the bladder wall, appearing as large flat
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Fig. 13.—Section through the transitional epithelium lining the ureter. The urothelium here is 3–5 cells thick and is limited at the luminal face by large, flat superficial cells. Wax-embedded section. H. & E. × 1200.

Fig. 14.—Surface view of the large, flat, polygonal superficial cells in the ureter. The cell boundaries are prominent and there is a characteristic random arrangement of microvilli on the luminal surface. Scanning electron micrograph. × 2100.

Fig. 15.—Between the fully differentiated superficial cells an occasional smaller, less mature, intermediate cell can sometimes be seen at the surface. Characteristically these cells are covered by short, bleb-like microvilli. This specimen shows some sign of postmortem deterioration with separation of the cells along their lateral borders. Scanning electron micrograph. × 4050.

Fig. 16.—Section through the transitional epithelium lining a dilated bladder. The urothelium is 2–3 cells thick and the cytoplasm of the large surface cells covers a number of underlying intermediate cells. The nuclei of the surface cells are arranged parallel with the surface of the urothelium. Epon-embedded section. Toluidine blue. × 1600.

Fig. 17.—Section through the transitional epithelium of a contracted bladder. The urothelium now appears to be 3–4 cells thick and the nuclei are crowded together. The surface cells are cuboidal in shape and their surface area is considerably smaller than in the dilated bladder. Epon-embedded section. Toluidine blue. × 1400.
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“umbrella” cells in the dilated bladder but as relatively cuboidal cells with a smaller surface area in the contracted bladder (Figs 16, 17). The urothelium covering the trigone area mostly appeared to be in a stretched or dilated state because of the underlying base plate which limits the degree of contraction of this part of the bladder wall relative to the rest of the bladder.

In the dilated bladder, the superficial cells have a large surface area with few folds or deep indentations and variable numbers of randomly distributed microridges or microplicae (Fig. 18). By contrast, in the contracted bladder the surface of these cells may be deeply folded and ruffled (Fig. 19). As previously reported, the luminal face of the superficial cells of the normal human urothelium is limited by a thickened, angular membrane which in the contracted bladder is invaginated into the apical cytoplasm (Fig. 20) to form fusiform vacuoles. In postmortem specimens, unless great care is taken, the large mature surface cells are lost into the urine revealing smaller underlying, less mature intermediate cells as shown in Fig. 21. The majority of vesical specimens prepared for histology from cadavers more than 12 h post mortem retain only the basal and intermediate cell layers and the sloughed superficial cells are discarded in the fixative or dehydrating fluids. The surfaces of these deeper cells revealed by postmortem desquamation are not limited by the specialized membrane found on the superficial cell and do not show a microridge pattern in the scanning electron microscope. Instead they have variable numbers of individual and/or chains of bleb-like microvilli on their surface (Figs 22, 23).

The urethra, from the urethro-vesical junction to the external urethral meatus

In view of the anatomical and morphological differences between the female and male urethra, they will be described separately.

Female urethra.—At the urethro-vesical junction normal urothelium, which is in continuity with and identical to that of the bladder, meets stratified cuboidal or columnar epithelium which extends down the urethra. The surface features of the urothelial cells are the same as those illustrated for the bladder and include randomly distributed microridges and microplicae on mature cells and isolated or chains of bleb-like microvilli on more immature cells. The columnar cells are smaller and more rounded or oval than the transitional cells and they bulge slightly into the lumen. Their cell junctions therefore tend to be less obvious than those between the urothelial cells, and a sharp junction between the transitional cells

Fig. 18.—Surface appearance of the large superficial cells in a dilated bladder. The cells have prominent boundaries and the surface varies between being relatively smooth and covered by numerous microplicae. Scanning electron micrograph. × 1330.

Fig. 19.—Surface appearance of the transitional cells in a contracted bladder. The surface area of the cells is reduced by deep infoldings plus invagination of the surface membranes which leaves the cell with a strongly ruffled appearance. Scanning electron micrograph. × 1829.

Fig. 20.—Part of a thin section through the apical surface of a normal superficial cell in the bladder urothelium. The cell is limited on its luminal face by a thickened membrane which is invaginated in the contracted bladder to form fusiform vacuoles (arrows). Transmission electron micrograph. × 71,250.

Fig. 21.—In this field, a number of the large superficial cells have been lost as a result of postmortem deterioration of the specimen. They reveal smaller rounded intermediate cells which do not have the normal mature surface pattern characteristic of the superficial cells. Scanning electron micrograph. × 1330.

Fig. 22.—High magnification of underlying intermediate cells revealed by desquamation of the superficial cells. The superficial face of these cells is covered by a variable number of blebs which may appear singly or arranged in chains. Scanning electron micrograph. × 5700.

Fig. 23.—Higher magnification to illustrate the arrangement of bleb-like microvilli on a maturing intermediate urothelial cell. The blebs appear singly at first, then aligned in chains which eventually fuse to form the microplicae of fully differentiated superficial cells. Scanning electron micrograph. × 16,450.
and the columnar cells can sometimes be found (Fig. 24). The luminal face of the columnar cells is covered by numerous short, randomly arranged microvilli with no sign of organization into a microridge pattern. In 1 patient only, occasional isolated small cells with longer and more pleomorphic surface microvilli were seen (Fig. 25), but since they were not found in the other 2 women it is not clear whether they are a normal cell type or a pathological aberration.

The upper, mid and lower regions of the urethra are lined with stratified columnar epithelium (Fig. 26). The entire mucosa is thrown up into longitudinal folds covered at their surface by luminal cells of fairly uniform size, rounded to polygonal in shape, bulging into the lumen and thus obscuring the cell junctions (Fig. 27). The free surfaces of these cells are covered by variable numbers of short, stubby microvilli (Fig. 28). Examination of thin sections of the epithelium showed the surface cells to be mucous-secreting and to contain large numbers of mucoid vesicles in their apical cytoplasm (Fig. 30). In common with other mucous-secreting epithelium, the secretion product adheres to the luminal face of the cells as an external surface coat (Fig. 30, inset). At the internal face of the urethral meatus this mucous-secreting columnar epithelium is transformed into a non-keratinizing, stratified, squamous epithelium (Fig. 29). This extends to the external face of the meatus where the stratified squamous epithelium closely resembles that of the vagina and is composed of layers of rather bloated-looking, glycogen-rich, non-keratinizing cells (Fig. 31). The surface cells of this stratified squamous epithelium are covered with a prominent pattern of microridges which resemble a thumb print (Fig. 32) and which appear to be an exaggeration of the more random microridge pattern seen on the surface of normal superficial cells in the urothelium. A few bleb-like microvilli were also seen on the surface of the squamous cells aligned with the microridges.

**Male urethra.**—At the urethro-vesical junction there is a change from urothelium to stratified columnar epithelium (Fig. 33). As in the female, 2 cells types may be found in this region, namely both the large flat polygonal cells with surface features characteristic of the urothelium, and also smaller, rounded cells which bulge into the lumen and are covered by short stubby microvilli (Fig. 34). This stratified columnar epithelium continues into the prostatic urethra where it is associated with the submucosal glands of Littré (Fig. 35).

The micro-anatomy of the male urethra is complex. On the posterior wall is a raised hillock known as the colliculus seminalis or veru montanum, and immediately lateral to this the wall is thrown up into folds and has a haphazard rugose

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**Fig. 24.**—This field illustrates the urethro-vesical junction between the normal urothelium lining the bladder and the columnar epithelium of the urethra. The flatter, polygonal superficial cells of the transitional epithelium in the lower half of the field give way to the smaller rounder cells of the urethra in the upper half of the field. The transitional cell surfaces are characteristically covered with microvilli while the cuboidal cells of the urethra are covered by numerous, short, randomly-arranged microvilli. Scanning electron micrograph. × 1550.

**Fig. 25.**—In a single patient, a number of small cells covered with bizarre microvilli were found in the area of the urethro-vesical junction (arrow). Scanning electron micrograph. × 4000.

**Fig. 26.**—Section through the stratified epithelium which lines the female urethra. The cell shape varies from cuboidal to columnar. Epon-embedded section. Toluidine blue. × 1350.

**Fig. 27.**—The entire mucosa of the female urethra is folded into longitudinal ridges and valleys. The surface is covered by uniform-sized, polygonal to rounded cells which bulge into the lumen, thus obscuring the cell boundaries. Scanning electron micrograph. × 900.

**Fig. 28.**—The surface cells of the stratified epithelium lining the female urethra are covered by short, uniform, stubby microvilli seen here at a higher magnification. Scanning electron micrograph. × 3850.

**Fig. 29.**—As the urethra approaches the meatus, the stratified epithelium becomes more squamous in appearance. This section through the internal aspect of the urethral meatus shows it to be covered by a non-keratinizing, stratified squamous epithelium. Wax-embedded section. H. & E. × 430.
Fig. 30.—Thin section through the most superficial layer of cells of the stratified epithelium lining the female urethra. The apical cytoplasm of these cells is full of mucous secretion granules, and the surface of the cell is covered by short, uniform microvilli. The inset shows the cell surface at higher magnification; the free surface is covered by an adherent mucoid coat. Transmission electron micrograph. × 13,000; inset × 55,000.

Fig. 31.—The external surface of the female urethral meatus is covered by non-keratinizing, stratified, squamous epithelium shown here in section. The surface cells appear bloated and are packed with glycogen. Wax-embedded section. H. & E. × 460.

Fig. 32.—The surface appearance of the cells covering the external face of the female urethral meatus. The cells are large, flat, squamous cells with prominent boundaries, and their free surface is covered by microplicae which give the appearance of a thumb print. Scanning electron micrograph. × 3440.
appearance when viewed in the scanning electron microscope (Fig. 36). In this area the prostatic ducts open onto the surface (Fig. 37). The veru montanum is covered with rounded cells which vary slightly in size and are convex, thus producing a cobblestone appearance. They are profusely covered by variable numbers of short, uniform microvilli (Fig. 38) and, when viewed en face, the cell junctions may be prominent and accentuated by rows of microvilli. In 1 patient ciliated cells were observed, but this does not seem to be a general occurrence. Examination of thin sections of the prostatic urethra showed that, as in the female urethra, these cells are mucus-secreting and their luminal face is covered with a thick coat of adherent mucous (Fig. 39 and inset). Distal to the veru montanum are the regions of the membranous and spongy urethra which are similar in appearance and are lined by a stratified columnar epithelium (Fig. 40). The walls of the urethra in these regions are thrown up into longitudinal folds, interrupted by the scattered lacunae and openings of the tubulo-alveolar glands of Littré. These open on to the surface of the crests, valleys, and even on to the side walls of the folds (Fig. 41). The epithelial cells have a rounded or oval, slightly convex face giving a cobblestone appearance to the luminal face of the urethra, and their free surfaces are covered by variable numbers of uniform microvilli (Fig. 42). Occasional cells appear to be ruptured, probably following discharge of mucous secretions into the lumen (Fig. 42). In a few patients, an occasional ciliated cell was also seen (Fig. 43).

As the urethra approaches the meatus, the epithelium of the fossa navicularis gradually changes in type from stratified, columnar, through non-keratinizing to keratinizing, stratified, squamous epithelium on the outer surface of the urethral meatus, ie covering the glans penis (Fig. 44). As in the female, the surfaces of the squamous cells were covered with thumb-print whorls of microridges, and the cell junctions were prominent (Fig. 45). Transmission electron microscopy of the glans penis showed the surface cells to have the characteristic sub-cellular features of keratinocytes. The usual cytoplasmic organelles had disappeared and were replaced by accumulated masses of tonofibrils (alpha keratin filaments) typical of epidermal keratinocytes, but forming a thinner layer than in normal skin.

**DISCUSSION**

The cell population in voided urine reflects the surfaces over which it has passed and the dynamics of urine flow. Urine passively enters the calyx from the collecting ducts in the pyramid of the kidney and is massaged by peristaltic waves down the ureters into the bladder where it remains till ejected at micturition. During micturition, the stream of urine in effect scours the mucosal surface of the urethra, and in the male is added to by the secretory products of the prostatic and urethral glands. While the bulk of cells found in urine sediments is clearly derived from the mucosal surface of the bladder, it is not surprising that a few cells are often observed by scanning electron microscopy the surface features of which differ from those of transitional epithelial cells.

The aim of this study was to record the in situ surface features of all cell types in the lower urinary tract which could contribute to the urine sediment. In general, postmortem material was found to be satisfactory for this purpose, and surface features as observed by scanning electron microscopy were well preserved. However, some regions of the lower urinary tract resisted postmortem change better than others; thus the subcellular structure of the cells lining the urethra and ureters remained intact longer than that of the bladder or renal pyramids as judged by examination of thin sections in the transmission electron microscope. This may reflect both variation in cytological damage due to postmortem contact with the urine and variations in the intrinsic susceptibility of different cell types to anoxia.

It is also possible to cause additional
mechanical damage to and displacement of the urothelium from the bladder wall during the initial chemical fixation. These factors have been monitored by histology and transmission electron microscopy in the selection of areas for scanning electron microscopy in this study.

Most published investigations of the epithelial lining the urinary tract have concentrated on the urothelium of the bladder, and by contrast other areas have received little attention. The surface features of the collecting tubules as far as the opening of the papillary ducts have been described in man and rhesus monkey (Andrews, 1975), as have the surface features of the fornix in a patient with pyelonephritis (Hücker et al., 1975), and of the ducts of Bellini in the rat (Burke, 1976) and of the rat renal papilla (Carroll et al., 1974). The substructure of the rat pyramid and fornices are also described by Khorshid and Moffat (1974) and by Silverblatt (1974). The description presented here of human pyramid and fornix shows them to be essentially similar to those of the rat, but in addition we have observed occasional clusters of multiciliated cells. The change-over from columnar to transitional epithelium (urothelium) which occurs at the fornix has been the subject of much discussion and is regarded as being of importance in the maintenance of the counter-current system in the medulla and of efficient urea excretion by the kidney (Silverblatt, 1974; Khorshid and Moffat, 1974). The surface features of the cells change abruptly at this junction; over the renal papilla the cells are covered with bleb-like microvilli coated with a glyocalyx, while in the fornix there is a change to large, squamous cells with the ridged surface structure typical of normal urothelium.

A few descriptions of the ultrastructure and surface features of the urothelium lining the renal pelvis and ureter have been published (Hicks, 1965; Burke, 1976; Hanna et al., 1976; Hayakawa, 1978). The surface features described here confirm that the urothelium lining the ureters is essentially the same as that lining the bladder. The occasional small cell with bleb-like projections, observed both by Hayakawa (1978) and ourselves are immature, intermediate cells which have not yet developed the typical surface characteristics of mature superficial cells; on some cells the blebs are fused to form short chains which are the forerunners of the ridges of fully differentiated surface cells. This maturation process is seen also in the bladder and has been described previously both for human (Knowles et al., 1980) and for rat urothelium (Hodges et al., 1976; Hodges, Hicks and Spacey, 1977).

The presence or absence of deep indentations in the surface of these cells is related to changes in luminal surface area which accompany the peristaltic contractions of the ureter.

The characteristic features of the uro-

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**Fig. 33.**—In the male lower urinary tract the transitional epithelium lining the bladder gives way to the stratified columnar epithelium of the urethra at the urothro-vesical junction. This section shows the junction between the two epithelial types (arrow), with the urothelium to the right of the field and the columnar epithelium of the urethra to the left. Wax-embedded section. H. & E. × 712.

**Fig. 34.**—The junction between the urothelium and the epithelium lining the urethra is clearly seen by the change in surface characteristics of the cells. Here, at the male urethro-vesical junction, the large superficial cells of the urothelium occupy the lower half of the field and the smaller cuboidal cells of the urethra the upper half. Scanning electron micrograph. × 1577.

**Fig. 35.**—The prostatic urethra in the male is lined by a stratified columnar epithelium which is associated with the mucous-secreting glands of Littre located in the submucosa. Epon-embedded section, Toluidine blue. × 617.

**Fig. 36.**—This shows the luminal face of the prostatic urethra at low magnification. At the top of the field is the colliculus seminalis or veru montanum and, below, part of the lateral wall is thrown up into folds with a haphazard rugose appearance. Scanning electron micrograph. × 53.

**Fig. 37.**—This field shows some of the numerous openings of the prostatic ducts into the prostatic urethra. Scanning electron micrograph. × 300.

**Fig. 38.**—The surface cells covering the veru montanum are rounded, slightly convex and profusely covered by variable numbers of short, stubby microvilli. Their cell boundaries are prominent and are accentuated by parallel rows of microvilli. Scanning electron micrograph. × 2612.
The urothelium lining the normal mammalian bladder have been well documented (Hicks, 1966a, b, 1968, 1975; Hicks and Ketterer, 1969, 1970; Koss, 1969; Fulker et al., 1971; Lloyd-Davies and Hinman, 1971; Firth and Hicks, 1972, 1973; Warren and Hicks, 1973; Kumagai, 1975; Noack et al., 1975; Burke, 1976; Hodges et al., 1976; Kjaer et al., 1976; Knutton and Robertson, 1976; Hodges et al., 1977; Newman and Hicks 1977; Wong and Martin, 1977; Hicks and Chowaniec, 1978; Tannenbaum et al., 1978; Nelson et al., 1979; Severs and Hicks, 1977, 1979; Knowles et al., 1980). This study confirms previous reports that the superficial cells of the normal human dilated bladder, like those of other mammals, have a flat or microridged surface pattern and present a flattened, pavement-like surface to the urine. When the bladder contracts, the urothelium folds and the surface area of the superficial cells is reduced by a concertina-like folding of the cell membrane with invagination of the cell membrane to form fusiform vesicles (Hicks, 1965, 1975; Koss, 1969), leaving a characteristically folded or ruffled surface as seen by scanning electron microscopy.

Some investigators have reported differences between the appearance of the surface cells from different areas of the bladder (Lloyd-Davies, et al., 1971; Kjaer et al., 1976; Nelson et al., 1979). There is also a persistent belief that the urothelium in the trigone area differs from that of the rest of the bladder. In fact, there are no differences in cell structure, and variations in surface appearance are accountable only to variations of folding of the urothe-
lium. When the bladder empties, the dome and body contact down towards the more rigid, flat base plate underlying the trigone area (Hutch, 1972), and the degree of folding of the mucosal surface is consequently minimal in the trigone. Thus, in the contracted bladder the surfaces of cells in the trigone area may appear flatter than those of the highly folded cells lining the body and dome. The cell boundaries of superficial cells are particularly prominent in the urothelium throughout the urinary tract, especially in partially contracted ureters or bladders, and the junctional complex frequently projects as a ridge between adjacent cells. This has been noted in the rat (Burke, 1976) and in the guinea pig (Wong and Martin, 1977), and in man the ridge is further accentuated by a parallel row of short uniform microvilli. As in the ureter, occasional immature cells derived from the intermediate layer are found at the surface of the bladder urothelium, with characteristic bleb-like projections which are progressively arranged in chains, then ridges, according to the maturation of the individual cell. This maturation process has been observed previously in normal human urothelium maintained in vitro in organ culture (Knowles et al., 1980). As reported previously (Newman and Hicks, 1977; Noack et al., 1975), this study confirms that true microvillous cells are not to be found in normal adult human bladder.

The female urethra is lined by stratified columnar cells, apart from the urethrovaginal junction, into which the transitional epithelium extends from the bladder, and the urethral meatus which is covered on its external surface by non-keratinizing squamous epithelium. Urethral glands have been reported in female rodents (Shehata, 1974), but relatively few were seen in the present study on human material. However, the bulk of the cells lining the human urethra, both male and female, are mucus-secreting cells and are very regular in appearance; their surface is covered by short uniform stubby microvilli, similar in appearance to the surface of immature basal and intermediate urothelial cells, but they are coated with the mucus they secrete. These have previously been described in the rat by Burke (1976), and were illustrated in the human bladder neck by Tannenbaum et al. (1978), who refer to them as non-pleomorphic microvilli. The overall appearance of the male urethra differs from the female only by the additional openings and lacunae of the glands of Littré and of the prostatic glands. The mouths of these glands are also limited by mucus-secreting cells covered with short stubby microvilli as previously reported by Hakky (1979). The thin squamous epithelium covering the glans penis is a keratinizing epithelium, unlike that of the external surface of the female urethra, which is non-keratinizing.

In the lower urinary tract epithelium, excluding the papillary ducts, there are thus 4 predominant cell types classified by their surface patterns. These are: first, the squamous epithelium of the urethral meatus; second, cells covered with short, mucus-coated stubby microvilli, including those lining the urethra and renal papilla; third, the immature urothelial cells with chains and ridges of bleb-like processes; and, fourth, mature urothelial cells of the bladder, ureter, calyx and pelvis which have characteristic patterns of microridges and ruffles on their free surfaces. In addition, a few more bizarre cell types are located over the renal papilla and in the male urethra where the occasional ciliated cell has also been observed. Any of these may appear from time to time in normal voided urine.

As has been demonstrated elsewhere, neoplastic disease of the urothelium lining the bladder and ureters is commonly associated with the presence in the urine of cells which are profusely covered with pleomorphic microvilli (Jacobs et al., 1977; Hayakawa, 1978; Hicks, et al., 1978; Tannenbaum et al., 1978; Croft, et al., 1979.) However, like any other single characteristic claimed to be a “marker for malignant change”, the presence of pleomorphic microvilli on the surface of a few cells in voided urine is not an infallible indicator.
of neoplasia in the urinary tract. With experience, there is little risk of confusing the regular bleb-like projections on the surface of normal urothelial cells or the stubby microvilli of the urethral cells with the pleomorphic microvilli of true neoplastic cells. (It is perhaps unfortunate that any projection on a cell surface, from a small discrete bleb to a long bizarre branched projection, is referred to as a microvillus; this frequently leads to confusion and errors in communication.) Urine cytology is only one approach to the detection of urothelial malignancies and its usefulness depends on an intelligent assessment of the entire clinical picture. The number of cells in a sediment and the presence or absence of other cell types such as inflammatory cells or erythrocytes must also be taken into consideration. If the sediment is predominantly composed of immature but otherwise normal cells, this may indicate rapid cell turnover during a phase of reparative hyperplasia in a previously traumatized urothelium, and the presence of an occasional microvillus cell in these circumstances may well be irrelevant and could have originated in the renal papilla or in the urethra. If, however, significant numbers of cells with bizarre pleomorphic microvilli on their surface are persistently found in repeat specimens from the same patient, the present study indicates that their presence is unlikely to be accounted for by the normal turnover of cells originating anywhere within the normal adult male or female lower urinary tracts. In these circumstances, microvilli are indicative of a persistent and possibly neoplastic change in cell morphology somewhere in the urinary tract which clearly merits further investigation.

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REFERENCES


Fig. 40.—Section through the stratified columnar epithelium which lines the spongy urethra. Mucoid secretion products can be seen in the apical cytoplasm of the most superficial cells. Epon-embedded section. Toluidine blue. × 1330.

Fig. 41.—The luminal surface of the spongy urethra has a cobblestone appearance and is covered by small cells which bulge into the lumen. Some of the numerous gland mouths are shown in this field. Scanning electron micrograph. × 342.

Fig. 42.—The surface cells of the spongy urethra are covered with short, uniform microvilli. One cell shown in this field appears to have ruptured, presumably following discharge of its mucoid contents into the lumen. Scanning electron micrograph. × 3800.

Fig. 43.—Although the majority of cells in the spongy urethra are similar to those shown in Fig. 42, between them an occasional ciliated cell is regularly found (arrow). The cells with deeply folded surfaces which project into the lumen may well be exhausted mucous cells which are about to desquamate. Scanning electron micrograph. × 3040.

Fig. 44.—At the urethral meatus the mucosa gradually changes to a stratified squamous epithelium which becomes keratinizing on the external surface, i.e. over the glans penis. This is shown here in an epon-embedded Toluidine-blue-stained section. × 456.

Fig. 45.—The surface of the stratified squamous epithelium at the urethral meatus is formed by large squamous cells with prominent boundaries. Their free surface is covered with microvilli arranged in a thumb-print pattern. A few bacteria are present in the upper left hand corner. Scanning electron micrograph. × 2375.


Replicas of Rat Bladder Luminal Membrane.
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