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The 3D structure of the human urinary bladder mucosa. A scanning electron microscopy study

T. CONGIU, R. RADICE, M. RASPANTI and M. REGUZZONI

Department of Clinical and Biological Sciences, Laboratory of Human Morphology 'L. Cattaneo', University of Insubria, Varese, Italy

SUMMARY - We performed a scanning electron microscopy study on the human urinary bladder tunica mucosa. Specimens from bladder biopsies were treated with OsO_4 maceration and 1N NaOH maceration methods prior to SEM observation to disclose the three-dimensional organization of the lamina propria, basal lamina and urothelium. The lamina propria housed a well developed capillary plexus just below the basal lamina; the urothelium presented a typical three-layered organization with basal, intermediate and superficial cells. The intermediate cells appeared essentially similar to basal cells in their external features and stretched from the basal lamina up to the superficial layer. The most superficial cells appeared consistently flattened and interconnected by extensive junctional complexes. They showed a peculiar specialization, their apical plasmalemma being thickened with distinctive, stiff plaques, in contrast with the underlying globular or spindle-shaped cells whose plasmalemma was only covered by short microvillosities.

KEY WORDS *human urinary bladder - scanning electron microscopy - maceration - urothelium*

INTRODUCTION

The urinary bladder is responsible for collecting and storing urine before micturition.

The urine excreted by kidneys is remarkably different in osmolarity and chemical composition from blood. Substances such urea, ammonia and toxins, present in high concentration in urine, could cause irritation and inflammation of the bladder wall unless they were contained by the superficial layer of the urothelium, which is organized in such a way as to make and preserve the barrier between blood and urine, defending the layers below from toxic substances. This task is achieved by tight junctions between neighbouring cells as well as by a special modification of the apical plasma membrane of superficial cells (Zeidel, 1996; Negrete *et al.*, 1996). In addition, the urothelial cells from the basal to superficial layers must follow the volume changes in the bladder and the expansion of the luminal surface without breaking the seal of the superficial layer. The basal lamina and the lamina propria are also indirectly involved in the permeability barrier, respectively assuring a valid support to the urothelium during distension of the bladder, lodging a well-developed vascular plexus. Therefore, the whole tunica mucosa must be seen as a functional unit in which permeability plays a role.

Many studies on bladder structure highlight the importance of superficial cells in forming the blood/urine barrier, supported by basal and intermediate cells (Hicks, 1975; Newman and Antonakopoulos, 1989; Jost *et al.*, 1989). In the last twenty years, however, very few authors have addressed the morphological aspects of the urothelium.

In the present study, we investigated the organization of the tunica mucosa of human urinary bladder by means of high resolution scanning electron microscopy with osmic maceration (Riva *et al.*, 1998) and 1N NaOH maceration (Ohtani *et al.*, 1988) techniques. Osmic maceration is known to allow the visualization of cytoplasmic membranes and intracellular structures by removing the cytosol. Moreover, the removal of soluble proteins from the extracellular space improves the resolution of the cellular layers and basal lamina. On the other hand, to better understanding of the structure of the uppermost lamina propria responsible for metabolic exchanges, we used NaOH maceration which removes the epithelial cells and basal lamina exposing the connective stroma.

Mailing address: Dr. Terenzio Congiu, Laboratorio di Morfologia Umana 'L. Cattaneo', Dipartimento di Scienze Cliniche e Biologiche, Università degli Studi dell'Insubria, via Monte Generoso 71, 21100 Varese, Italy; e-mail: tere.c@tiscali.it

MATERIALS AND METHODS

Human urinary bladder biopsies were obtained by consent from 8 male and female patients aging from 55 to 82 years undergoing diagnostic or review cystoscopies under general anesthesia. Ethical approval for the removal of biopsies was obtained in each case. During cystoscopy the bladders were irrigated and partially filled with saline, so we can consider the bladder samples taken in a state of semi distension. The specimens were then divided into two different groups and prepared in accordance with the different protocols described as follows.

Osmic maceration

To limit the artifacts due to autolysis, the biopsies (measuring 2-3 mm^3) were immediately immersed in 0.25% glutaraldehyde and 0.25% paraformaldehyde in 0.1 M Na-cacodylate buffer (pH 7.2) for 20 min at room temperature. After an initial reduction in size of 1 mm^3, the specimens were washed in phosphate buffered saline (PBS, pH 7.2) and then postfixed in a solution of 1% osmium tetroxide and 1.25% potassium ferrocyanide for 2 h. Specimens were further reduced in slices 0.2 mm thick, followed by a second postfixation in 1% osmium tetroxide and 1.25% potassium ferrocyanide for 1 h. The slices were washed in PBS and immersed in 0.1% osmium tetroxide in PBS for 48 h (Riva *et al.*, 1998). They were then dehydrated in graded ethanol and subjected to critical point drying with CO_2. The slices mounted on aluminum stubs were coated with 10 nm of pure gold in a Emitech K250 sputter-coater.

1N NaOH maceration

The specimens were fixed for 5 days with 0.25% glutaraldehyde and 0.25% paraformaldehyde in 0.1 M Na-cacodylate buffer (pH 7.2) and then cut with a razor blade into 0.5 mm thick slices, rapidly washed in distilled water and immersed in 1N NaOH for 3 days at room temperature (Ohtani *et al.*, 1988). The slices were then washed in distilled water for 3 days at room temperature, then dehydrated in graded ethanol and subjected to critical point drying with CO_2. The slices were then mounted and coated as above.
All the specimens were observed with a Philips SEM-FEG XL-30 scanning electron microscope operated at 10 kV.

RESULTS

The urothelium consists of three types of cells arranged in three or more layers from the basal lamina to the luminal surface. Osmic maceration provides a gradual chemical dissection of the specimens, giving a good visualization of the urothelial cells' cytoarchitecture and the interfaces of the basal lamina with the epithelium and lamina propria. The polygonal shape of the superficial cells readily distinguishes them from the intermediate cells. The images of the bladder luminal surface show superficial cells, tightly fastened to each other, floating over the globular intermediate cells (Fig. 1).
The loose connective tissue of the lamina propria is occupied by a well-developed network of blood vessels. They flow very close to the abluminal surface of the urothelium and are covered by only a thin shield of collagen fibers (Fig. 2*a*,*b*).
Tough collagen bundles leave the vessel shield and bridge it to the stroma. En face images of NaOH-macerated specimens show circular apertures, well outlined by collagen bundles, about 5 µm in diameter, which correspond to a breakdown of the lamina propria covering the blood vessels (Fig. 3*a*,*b*). We also observed plentiful collagen bundles forming parallel crests in the spaces between blood vessels (Fig. 3*a*).
The basal lamina is thin and irregularly folded. It corresponds to the structures of the lamina propria already described such as crests, bridges and, particularly, the uppermost blood vessels. Sometimes recesses can be seen excavated in the lamina propria or defined by loops of blood vessels containing basal and intermediate cells and already described as Brunn's nests (Fig. 4*a*). We noted the presence of globular bodies of a uniform size of about 100 nm, but irregularly distributed on the surface (Fig. 4*b*).
Basal cells are ovoid in shape. The cellular basal membrane rests on the basal lamina and forms button-like interdigitations with the 100 nm globular bodies (Fig. 4*b*).
The intermediate cells are taller than the basal cells, and develop from a fusate body to a teardrop-like body, with a subspherical head still connected with the basal lamina by a long shaft (Fig. 5*a*). The lateral and apical plasmalemmae present short interdigitating microprojections, like the basal cells. The subspherical head contains the nucleus. The cytoplasmic organelles, observed in the opened cells and through the lacerations of the plasmalemma, consist of filiform mitochondria and rough endoplasmic reticulum, the latter made up of a network of anastomosed tubules and cisternae adjacent to the lateral plasmalemma. Multivesicular bodies were easily recognizable by the tiny vesicles within (Fig. 5*b*). Some intermediate cells elongate toward the urinary bladder lumen and wedge their apexes between superficial cells (Fig. 6).
Most of superficial cells have a wavy luminal surface (Fig. 7). This aspect is due to a distinctive apical plasmalemma, composed of relatively stiff plaques covering almost the whole surface and by globular microvillosities closer to the cell. Just below the cell border we can see an indented band which separates the apical and lateral surfaces. The lateral plasmalemma appears highly folded up in finger-like interdigitating plicae (Fig. 7).
Through the larger lacerations of apical plasmalemma organelles could be seen arranged in the apical portion. The

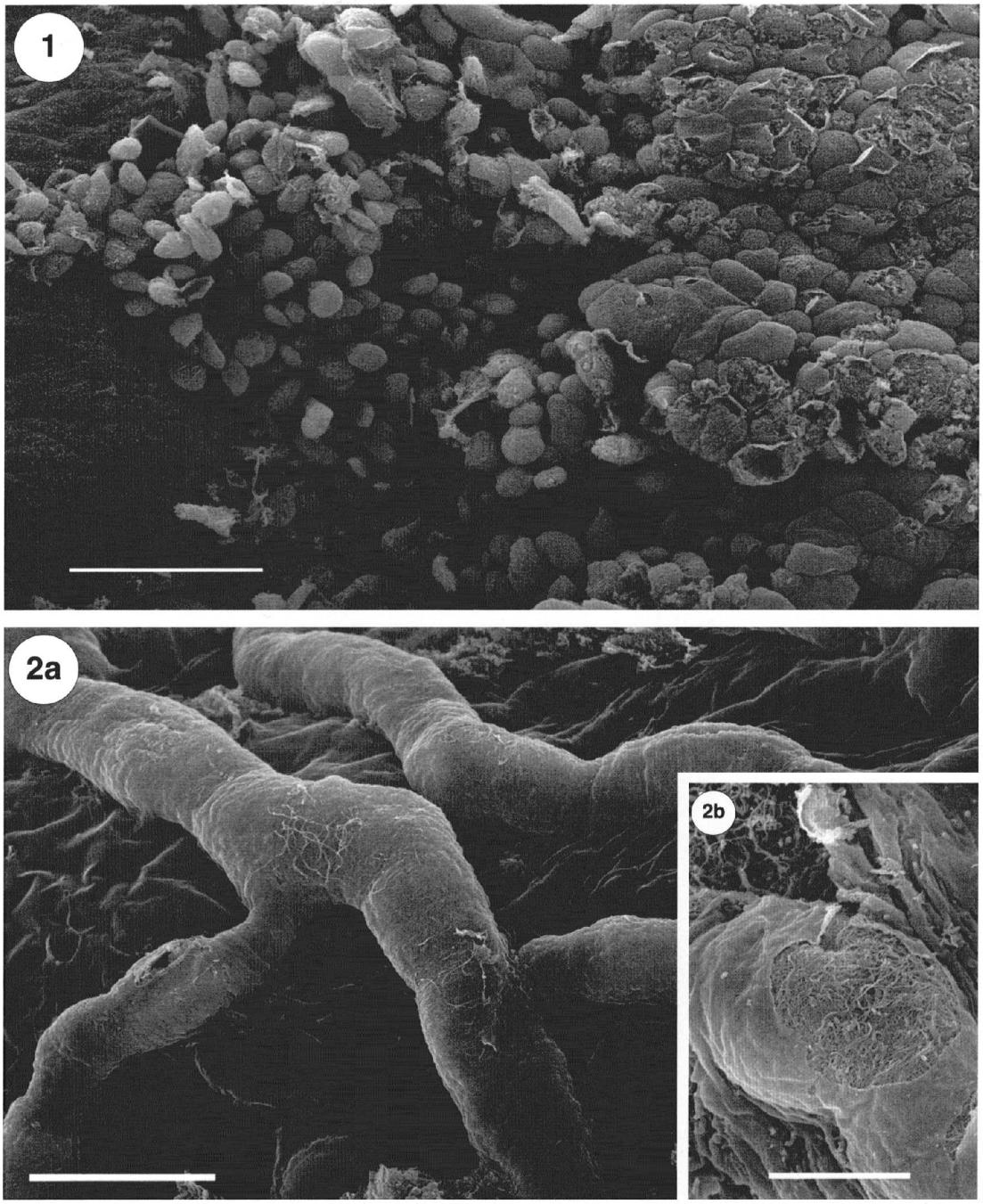


FIGURE 1 Panoramic view of urothelium and basal lamina after osmium maceration. On the right the superficial cells are visible, some of which have lost their apical membranes. The intermediate and basal cells, uncovered, show spherical apexes. Bar = 50 μm.

FIGURE 2*a,b* (*a*) The capillaries lift the basal lamina throughout their thickness forming anastomosing ridges. Bar = 10 μm. (*b*) Detail of a capillary partially free from the basal lamina of the urothelium. The different organization of collagen bundles in the shield of capillary is evident with respect to those of the lamina propria. Bar = 5 μm.

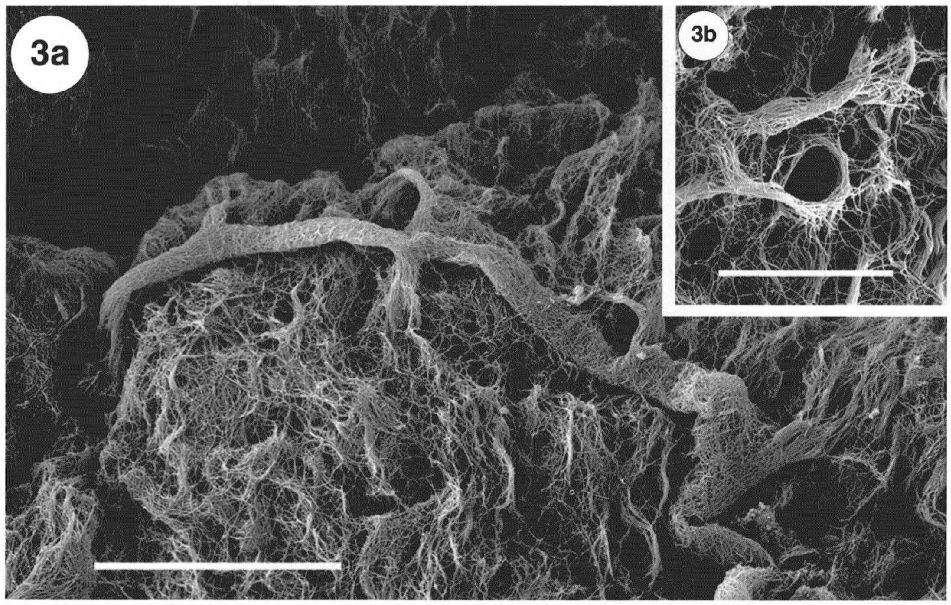


FIGURE 3*a,b* (*a*) Specimen treated with 1N NaOH viewed from the luminal side. The removal of urothelial cells, basal lamina and endothelial cells discloses the organization of connective stroma in the lamina propria, just below the basal lamina. Bar = 20 µm. (*b*) Aperture corresponding to the capillary shield detaching from the connective stroma of the lamina propria. Bar = 10 µm.

FIGURE 4*a,b* (*a*) Groups of basal and intermediate cells are contained in deep niches in a configuration typical of Brunn's nest. Bar = 50 µm. (*b*) Detail of the connection system between basal cells (bc) and basal lamina. Note the three holes (arrowhead) perfectly corresponding to the globular bodies (arrow) in the basal lamina. Bar = 5 µm.

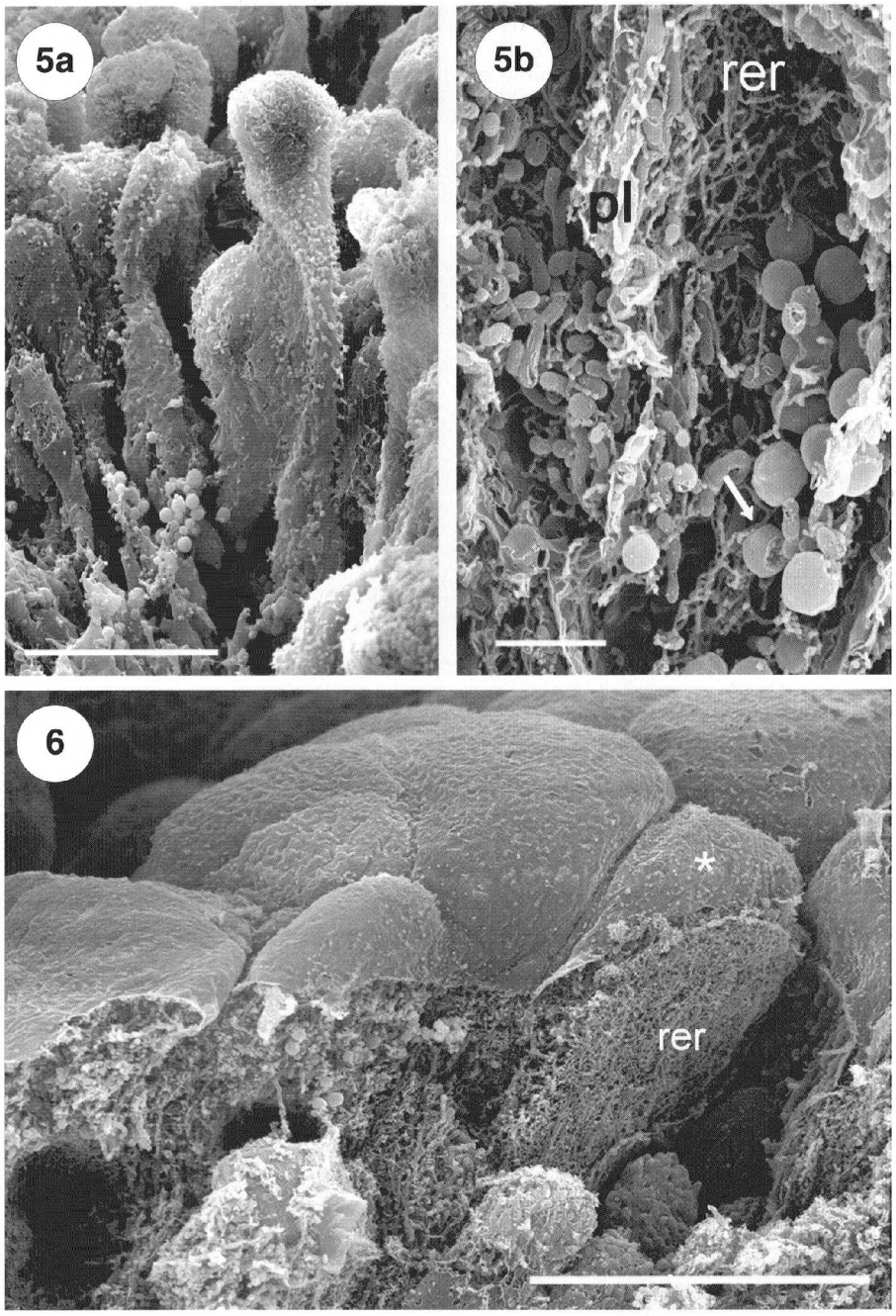
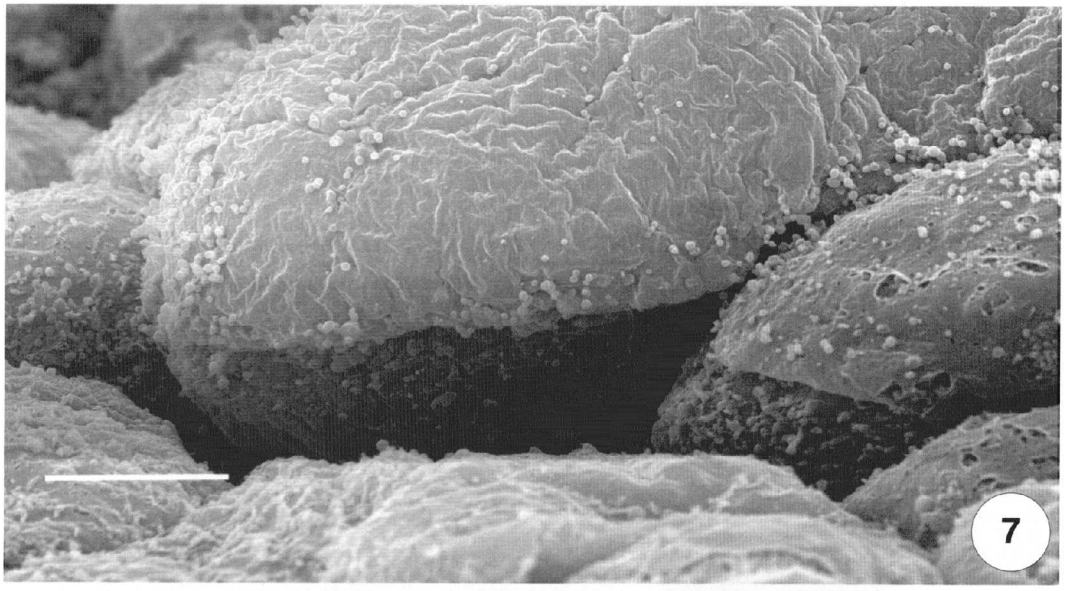


FIGURE 5*a*,*b* (*a*) Intermediate cells elongating toward the superficial layer. Apical and lateral surfaces are covered by short microvillosities. Note the subspherical apex containing the nucleus. Bar = 10 µm. (*b*) Sectioned intermediate cells showing their cytoplasmic organelles. The rough endoplasmic reticulum (rer) is placed close to the plasmalemma (pl), entrapping filamentous mitochondria. The central part of the cell is occupied by multivesicular bodies (arrow). Bar = 2 µm.

FIGURE 6 Luminal surface in oblique view. Note the superficial cell (asterisk) with a narrow luminal surface and a more elongated body, between the flat ones. Its lateral plasmalemma was stripped away, displaying a well developed rough endoplasmic reticulum (rer). Bar = 20 µm.

nucleus was surrounded by vesicles of different size: the smaller ones were also visible attached to the apical membrane. We often observed a large empty space between the organelles and the apical membrane (Fig. 8).

A few superficial cells, intermingled with more typical ones, showed a narrower luminal surface and a body more elongated toward the basal lamina. Their apical membrane was smooth and mostly covered by globular microvillosities,



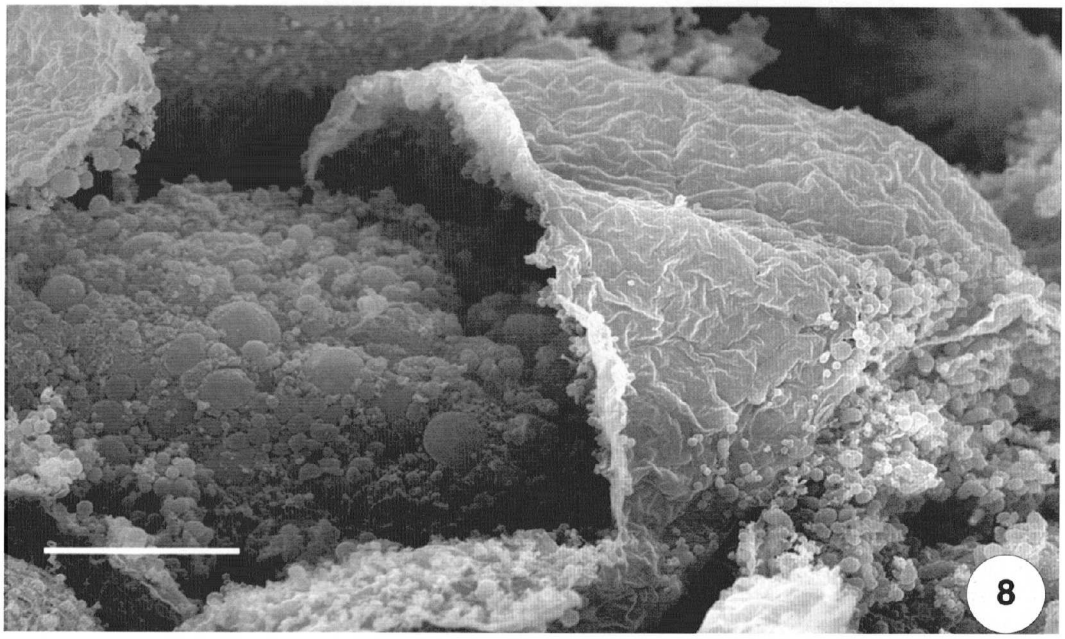
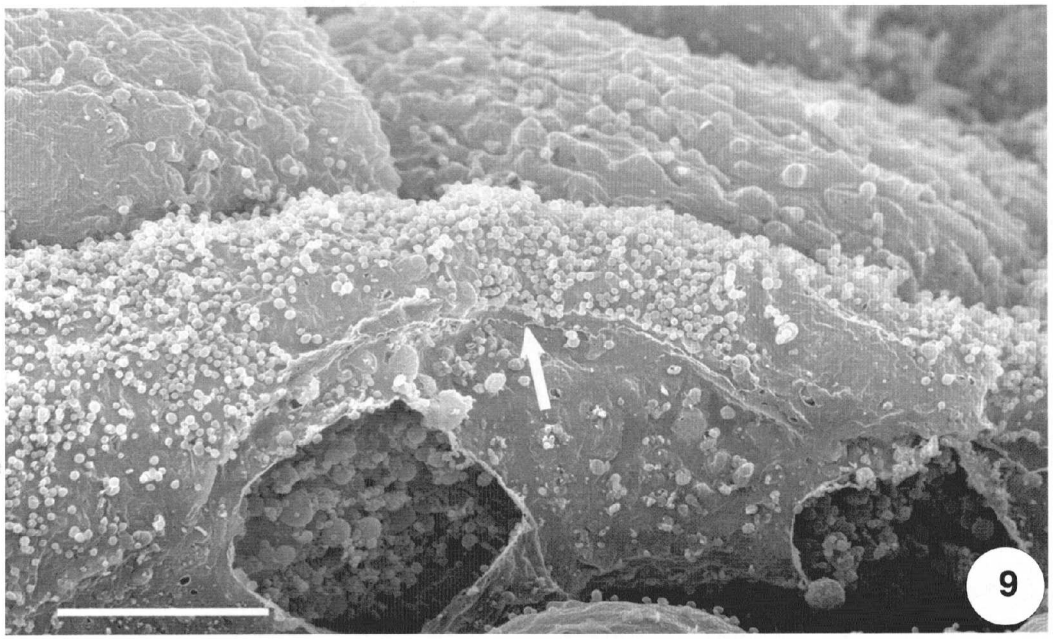


FIGURE 7 Superficial cells partially detached by osmic maceration. The apical plasmalemma is covered by plaques. Only close to the cell border do short microvillosities appear. Note the indented apicolateral border and lateral finger-like interdigitations. Bar = 10 µm.

FIGURE 8 Superficial cell partially opened near the apical membrane. Vesicles of different size appear closely packed in the cell apical region. Some tiny vesicles are visible attached to the apical membrane. Note the large space between the vesicles and the apical membrane. Bar = 5 µm.



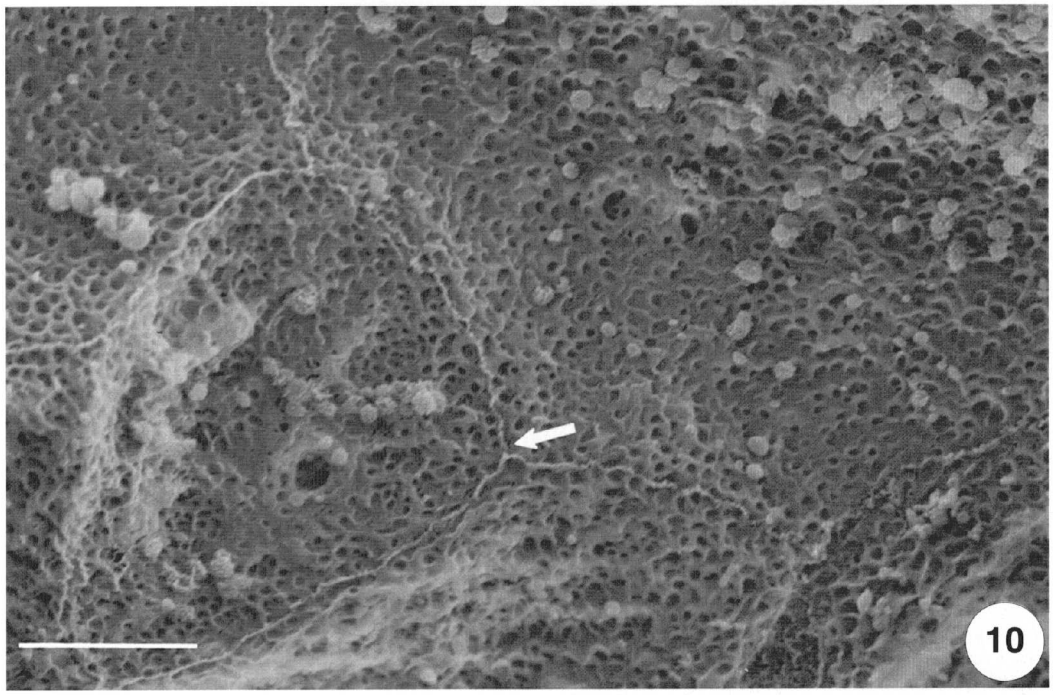


FIGURE 9 Immature superficial cell. The apical membrane is covered by short microvillosities. The horizontal ridge in the apicolateral border (arrow) denotes the presence of tight junctions. The wide concave impression on the lateral plasmalemma emphasizes the relationship with the adjacent cells removed by osmic maceration. Bar = 5 μm.

FIGURE 10 Immature superficial cell. In some cells, a flap of apical plasmalemma was stripped away from the lateral membrane and capsized. The exposed cytoplasmic side shows the cell boundary (arrow), the holes corresponding to the bases of the microvillosities and some small rounded vesicles attached. Bar = 2 μm.

while they lack typical plaques; their lateral surfaces also showed microprojections and were devoid of finger-like interdigitations. We noted an indented band among the apico-lateral border, similar to the structure described for typical superficial cells (Fig. 9).

Large portions of the apical plasmalemma of some cells were stripped away by the maceration process and capsized, but were still retained by the tight junctions. The cytoplasmic side of the apical plasmalemma revealed by this process showed a number of adherent rounded vesicles and small holes corresponding to the bases of the microvillosities (Fig. 10).

DISCUSSION

Scanning electron microscopy proved a useful approach to the three-dimensional features of the whole tunica mucosa. Thanks to the gradual dissection performed by osmic maceration, the layers of the tunica mucosa were displayed separately (Fig. 1).

The uppermost region of the lamina propria is occupied by a well-developed vascular plexus. A recent study on ischemia in mouse bladder (Korosec and Jezernik, 2000) disclosed the dependence of the barrier function on blood flow, which is of course essential in supplying the urothelium with nutrients and oxygen. The network of blood vessels, described by Inoué and Gabella (1992) as 'epithelial capillaries', accomplishes this task by running very close to the basal lamina of the urothelium, and emerging at the level of the basal cells layer. The circular apertures of 5 µm visible on the interface between the lamina propria and basal lamina are likely to correspond to the points where the blood vessels emerge toward the basal layer of the urothelium, freeing themselves from the collagen bundles of lamina propria. The presence of these epithelial capillaries makes the bed of the urothelium very irregular with anastomosing vascular ridges; sometimes the plexus meshes are so tight to enclose groups of basal and intermediate cells described as Brunn's nests by Jost *et al.* (1990). Collagen bundles start from the capillary connective shield and reach the lamina propria (Fig. 2). The role of this anchoring system is probably to fasten the capillaries position during the movements of contraction-distension of the bladder.

The urothelium consists of three distinct cell layers in different growth stages from basal lamina to superficial layer. Among them, only the superficial layer seems detached from the basal lamina according to Jost *et al.* (1989). The basal and intermediate cells present similar external features. Their rounded lateral surfaces show that basal and intermediate cells are not fastened by tight junctions (Jost *et al.*, 1989) but only by a few short interdigitations.

SEM micrographs clearly show that the intermediate cells elongate from the basal lamina, reach the superficial cells and form tight junctions with them. Occasionally, the removal of the lateral plasmalemma revealed a well-developed network of rough endoplasmic reticulum, typical of intermediate cells (Figs. 5*b* and 6). We also noted that the apical plasmalemma of these 'neo-superficial' cells is still covered by microvillosities, whereas it lacks plaques. These data confirm the studies on development of urothelium in fetal human (Newman and Antonakopoulos, 1989) and fetal mouse (Jezernik and Pipan, 1993) bladders, in which the plaques appear at a later time, after the tight junction belts have fastened the adjacent cells. Some authors (Riesco *et al.*, 1989; Wong and Martin, 1977) have already described the presence, in animal urinary bladder, of immature superficial cells characterized by short microvillosities on the apical surface, among the mature ones easily recognizable by their rough apical surface covered by plaques (Fig. 6). The lateral views reveal further differences between them. The immature superficial cells have a long body still surrounded by intermediate cells (Fig. 5*a*). Their lateral plasmalemma has only a few short microvillosities. The mature superficial cells are flat, and seem to float over the intermediate layer; their lateral surfaces are smaller, and are covered by numerous long finger-like interdigitations (Fig. 7). The large empty space between the organelles around the nucleus and apical membrane, may be due to the complete removal of a microfilaments network involved in shuffling membrane-containing endosomes into and out of the apical plasmalemma during expansions and reductions of the luminal surface, as suggested by Walton *et al.* (1982) and Chang *et al.* (1994). This empty space was never visible in the apex of immature superficial cells.

These data suggest that in coming to maturity the immature superficial cells lose their connection with the basal lamina, they reinforce their junctions with adjacent cells, and develop a distinctive superficial layer of stiff plaques protecting the blood-urine barrier during cell turnover.

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