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ULTRASTRUCTURAL CHARACTERISTICS OF THE FETAL AND NEONATAL RAT URINARY BLADDER

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Abstract

The embryologic and neonatal development of the normal rat urinary bladder was investigated in Sprague-Dawley rats by light, transmission, and scanning electron microscopy from day 11 of gestation through 21 days of age. The epithelium at day 11 of gestation is composed of small, loosely-connected, rounded cells with occasional short microvilli on their surfaces. The large polygonal cells characteristic of the adult bladder begin to appear by day 15, but the microridges are not apparent until day 17. By day 20, the epithelium appears morphologically similar to the adult bladder. Several morphological features are observed at different times of gestation which are not seen in the normal adult bladder, but they have been found in bladder tumors. During days 12 - 15 of gestation, most of the luminal lining cells of the bladder epithelium have a single central cilium. Cilia are also occasionally seen at days 11, 16, and 17 of gestation. Occasional cells with long tentacles are present from days 13 - 16 of gestation. Cells that appear to form bridges between cells are also seen from day 14 of gestation and continue to be observed through day 11 after birth. No cells with distinctive pleomorphic microvilli, a feature of rapidly proliferating bladder epithelial cells in the hyperplastic or tumorous epithelium of the adult, were seen at any time during gestation or after birth. Small foci of superficial layer sloughing occurred at the time of birth, but were rapidly replaced by one day after birth. It is apparent from this study that the bladder epithelium is a rapidly changing, proliferating tissue *in utero* and continuing for a brief period after birth.

Key words: Bladder, Embryology, Scanning Electron Microscopy, Transmission Electron Microscopy, Sprague-Dawley rats, epithelium, bladder tumors.

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Introduction

Urinary bladder carcinogenesis evolves through several stages in humans and experimental animals. In rats, the process usually follows a sequence progressing from normal urothelium to a simple hyperplasia, continuing to nodular and papillary hyperplasia, and to the eventual development of papillomas. Within the nodular or papillary hyperplastic areas or in the papillomas, dysplastic changes occur which are indicative of non-invasive, papillary, transitional cell carcinoma. These eventually undergo invasion and occasionally metastasize. During this sequence of events, numerous morphological changes occur which have been extensively described utilizing light microscopy, transmission and scanning electron microscopy, and freeze fracture studies (Cohen, 1983; Jacobs, et al., 1983; Pauli, et al., 1983). Most notable in this process has been the disappearance of the large polygonal urothelial superficial cells with a distinctive asymmetric unit membrane. They are replaced with smaller, rounded cells with uniform microvilli and eventually pleomorphic microvilli on their surfaces.

Most of these studies have utilized administration of various compounds to animals beginning after weaning and extending for long periods of time, frequently for as long as two years of observation. However, it is becoming increasingly apparent in several animal model systems that tissues have different sensitivity to chemicals when administered either during the *in utero* or the early neonatal time period, before weaning (Koen, et al., 1983). This has been most extensively evaluated utilizing the compound, sodium saccharin (IARC Working Group, 1980; Schoenig, et al., 1985). When this compound is administered at high doses in the diet to rats beginning after the weaning period, tumors of the urinary tract rarely occur, generally in less than 1% of the test animals. In contrast, if it is administered to rats during the *in utero* period, during lactation, and after weaning for the remainder of its life, a significant incidence of bladder tumors are induced in the male.

Despite this significant toxicological problem, there is surprisingly little known about

the normal bladder during the in utero and neonatal time periods with which to make comparisons and evaluations of the toxicity of various compounds during this time period (Gyllensten, 1949; Firth and Hicks, 1970; Ayres, et al., 1985). In the previous studies in rats and mice, the late in utero time period was evaluated, generally from day 17 of gestation onward. During this late in utero time period, the bladder appears to undergo transition from an organ with a relatively poorly differentiated epithelium to the characteristic adult epithelium with large polygonal superficial cells with a characteristic asymmetric membrane. Although not specifically stated in these previous papers, there has been considerable difficulty in examining the in utero bladder in rats and mice prior to day 17 of gestation because of the technical difficulty of identifying, isolating, and processing the bladder epithelium for morphological and other evaluations. In our laboratory, we developed a variety of techniques for examining the lower urinary tract allowing for evaluation by light microscopy and transmission and scanning electron microscopy as early as day 11 of gestation (Cano, et al., 1986). The rapid and distinctive changes occurring during embryogenesis and early post-natal development of the bladder epithelium are described in this paper.

Materials and Methods

The methods used for locating and identifying the bladder were those developed by us and described previously (Cano, et al., 1986). Sprague-Dawley rats, 10 weeks of age, were obtained from Charles River Breeding Laboratories (Portage, Michigan) and housed in plastic polycarbonate cages with corncob bedding. Food (Agway Prolab 3000 rat chow) and deionized, distilled water were available ad libitum. The rats were housed in a room with a temperature of 22° ± 2° C and relative humidity of 45%. The room was lit from 2300 to 1100 hours and was dark from 1100 to 2300 hours. At 11 weeks of age, males were placed individually in the cages of singly caged females at 1300 hours and visually observed for copulation. If copulation occurred, the female was considered mated. This time was considered day 0 of gestation. If mating did not occur within 20 minutes, the male was removed from the cage and used the next day for another mating. The mating procedure was repeated daily until all females were mated. Males were sacrificed after mating was completed. Two pregnant females were sacrificed at 1300 hours on each of days 11 through 21 of gestation. The total number of fetuses for each litter were recorded, and the number of males and females indicated when possible.

Pregnant rats were anesthetized by Nembutal (Abbott Laboratories, North Chicago, IL) injection i.p., and fetuses were removed by Caesarean section and immediately immersed in 2% glutaraldehyde in 0.1 M phosphate buffer. From days 16 through 21 of gestation, the fetuses were decapitated to aid in fixation of internal organs, especially the bladder. Tissue specimens were fixed at 4° C for a minimum of 24 hrs before further processing. Location and identification of bladders at the different times of gestation were as described by Cano, et al. (1986). Specimens for light microscopy were embedded in paraffin after dehydration using an ascending series of ethanol concentrations and xylene. Sections were cut 4-5 microns thick and stained with hematoxylin and eosin. For scanning and transmission electron microscopy, tissues were washed with 0.1 M phosphate buffer, post-fixed in 1% OsO_4 and dehydrated through an ascending series of ethanol concentrations. Specimens for transmission electron microscopy were embedded in araldite and thin sections stained with uranyl acetate and lead citrate. Specimens for scanning electron microscopy were immersed in Freon 113, critical point dried with Freon 13, mounted on aluminum stubs, and sputter-coated (Polaron E5100, Polaron, Inc.) with gold. Specimens for transmission electron microscopy were examined with a Philips 201 or 300 electron microscope. For scanning electron microscopy, the specimens were examined at 20 kV in an ETEC Autoscan scanning electron microscope (Perkin-Elmer, Hayward, CA).

Results

A total of 262 fetuses and neonatal rats were examined for the time period evaluated. Fetuses from 2 litters were examined for each day of gestation from day 11 through day 21.

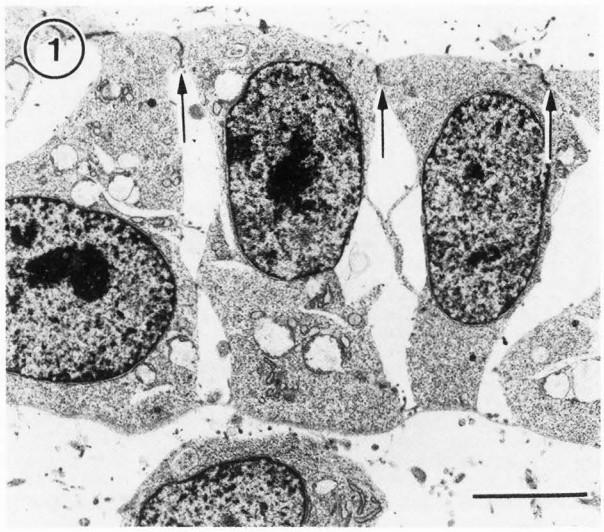
Figure 1. Cloacal epithelium from a rat fetus at 11 days of gestation. The luminal surface of the cells occasionally have microvilli. There are junctional complexes (arrows) between the cells near the luminal border, but the cells are loosely arranged. Bar = .4 µm.

Figure 2. Bladder from a rat fetus at day 15 of gestation showing immature epithelial cells, but with some differentiation toward a surface cell. Underlying stroma is loosely arranged. Bar = 8 µm.

Figure 3. Electron micrograph of the full thickness of the epithelium from the same bladder as shown in Figure 2. Epithelial cells are less loosely arranged, but again show junctional complexes (arrows) between cells near the luminal surface. Bar = 2 µm.

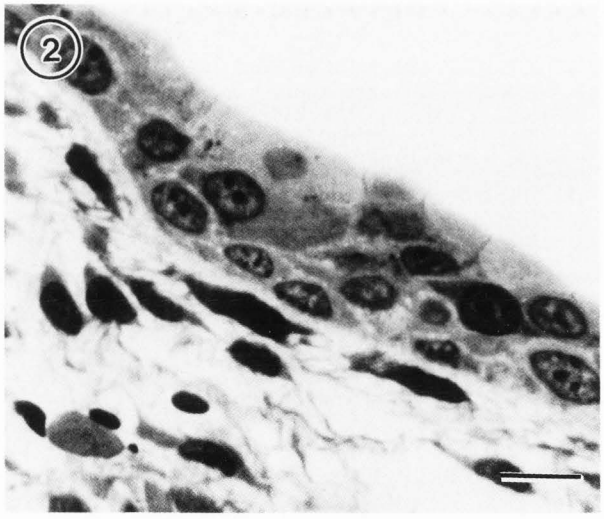
Figure 4. Epithelium from a bladder of a rat at day 17 of gestation showing the epithelium with features of differentiation to superficial cells, loosely arranged stroma beneath the epithelium, and a well-defined muscle layer present (arrows). Bar = 100 µm.

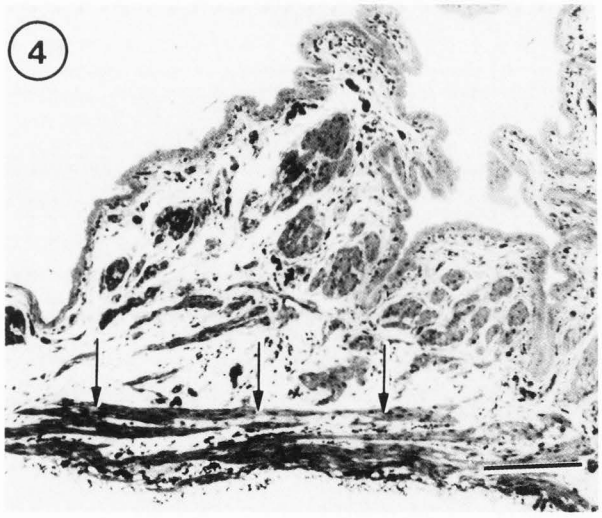
Figure 5. Fully mature epithelium from a bladder of a rat at day 19 of gestation. Mitoses are frequent (arrow) and numerous cells are labeled with grains following ^{3}H-thymidine injection 1 hr prior to sacrifice. Autoradiograph with hematoxylin and eosin staining. Bar = 25 µm.

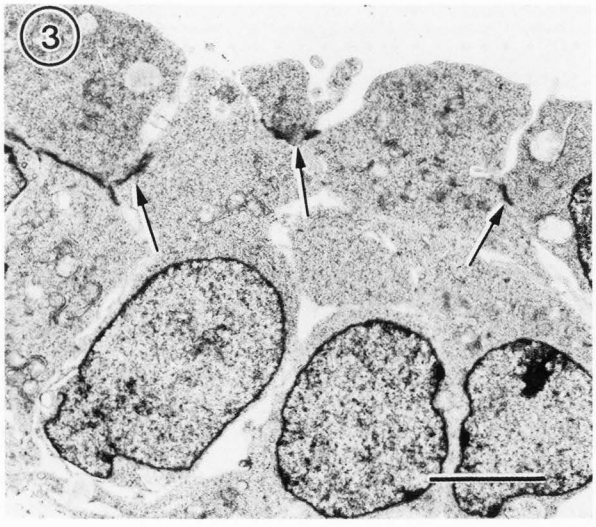


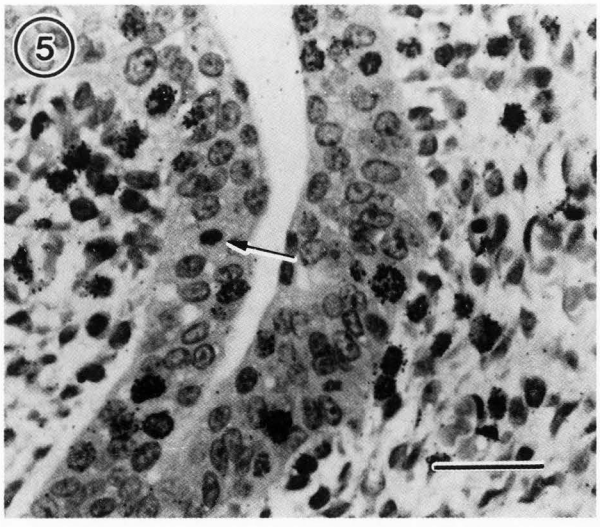
Also, the neonates of at least 2 litters were examined for each day after birth from day 0 through day 7 and also on days 11, 14, and 21 post partum. In most instances, all of the fetuses and offspring of the entire litter were examined. No differences in morphologic appearance were detectable between males and females at any given time point. At any given time of gestation or lactation there was variation between litters, and even within litters, as to the extent of development of the bladder epithelium. Thus, changes in morphologic appearance that occurred with time, occurred gradually and varied slightly from animal to animal. The variation, however, was never more than a range of two days.

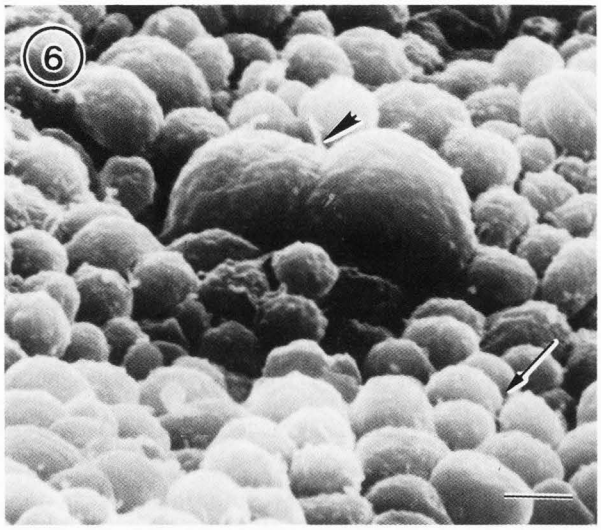
The fetal urinary bladder is a soft, gelatinous structure until about day 19 of gestation (Figs. 1-3). The first evidence of a muscle layer (distinct bundles of spindle cells in the bladder wall) is seen as early as days 15 to 18 of gestation; it is distinctively present by day 17 to 19 (Fig. 4). With the appearance of the











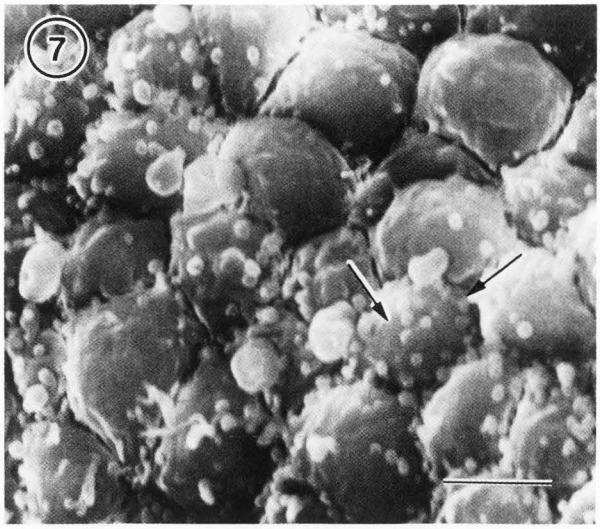


Figure 6. SEM of the surface of the cloaca at day 11 of gestation showing loosely arranged rounded cells (arrow), generally with a smooth surface. Other areas show microvilli on the surface of the cells. A single cilium is seen (arrow head). Bar = 1 μm.

Figure 7. SEM of the bladder surface from a rat at day 13 of gestation showing rounded cells covered predominantly with short uniform microvilli (arrows). Bar = 10 μm.

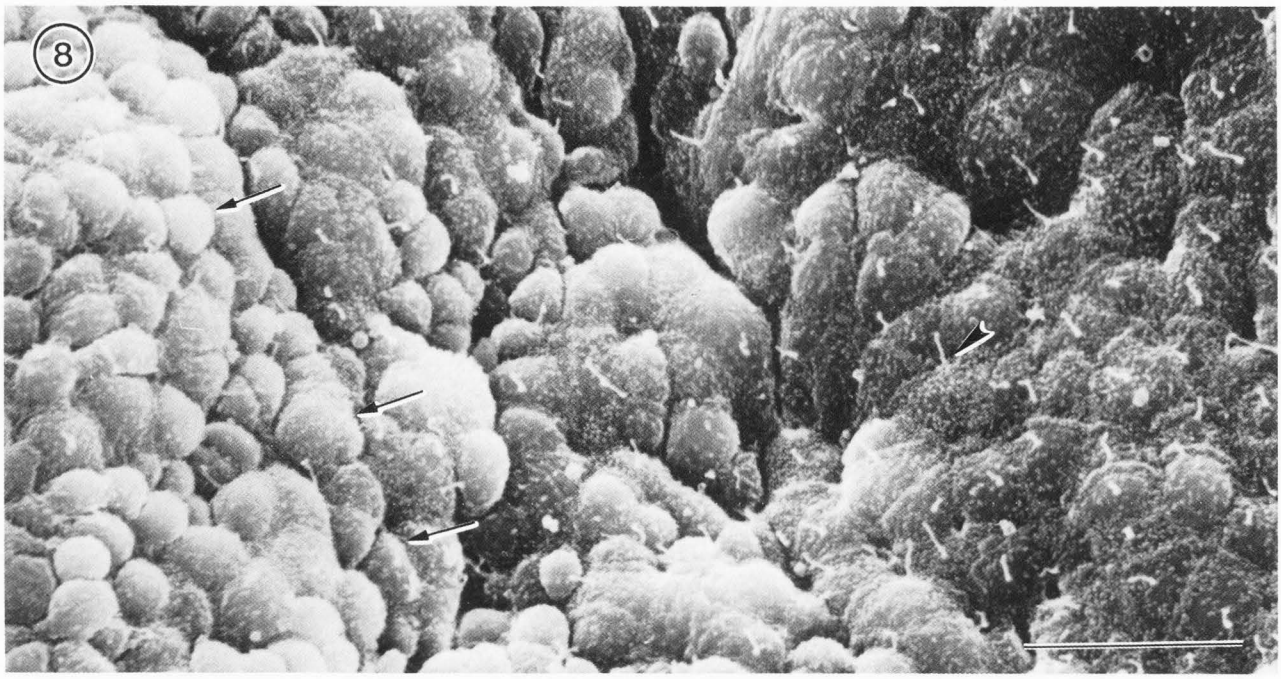
Figure 8. SEM of bladder epithelium from a rat at day 15 of gestation. The left and lower portion of the figure shows rounded epithelial cells (arrows) with microvilli or smooth surfaces gradually merging into the area at the right and upper portion of the figure with more polygonal-shaped cells having a single cilium (arrowhead) centrally located on each of the cells. Bar = 10 μm.

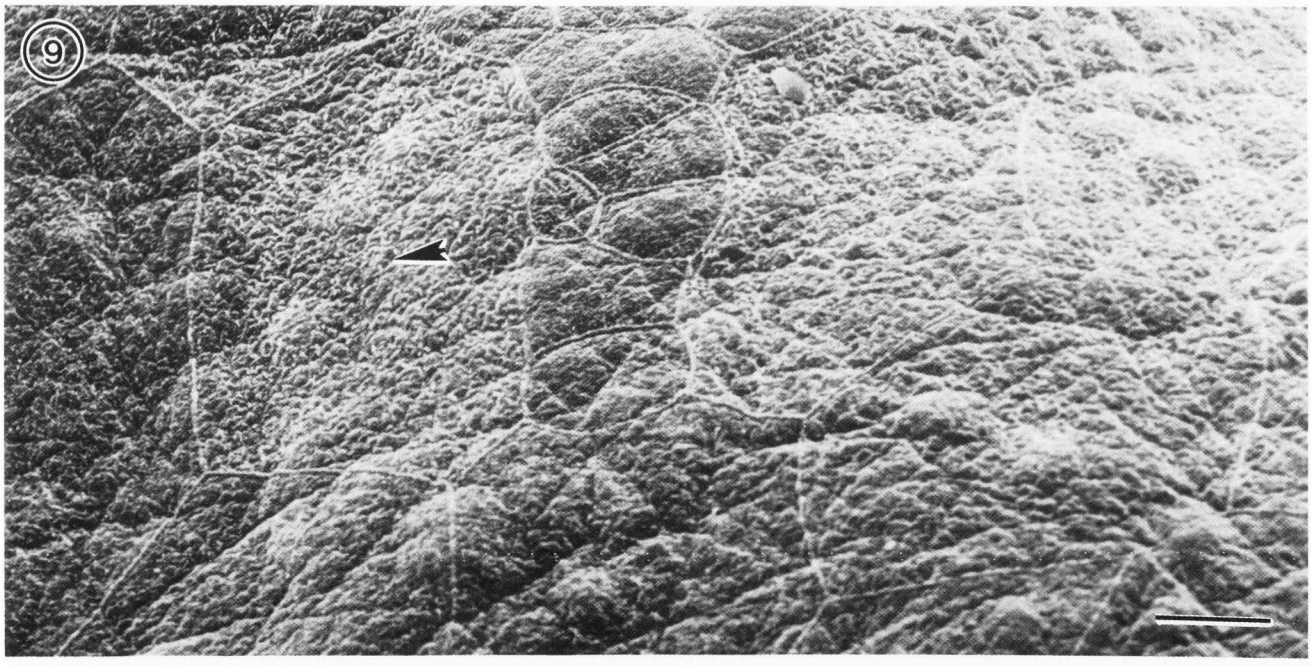
Figure 9. SEM of the bladder surface from a rat at day 18 of gestation showing the usual appearance of the mature bladder epithelium with large polygonal cells with a microridge system (arrowhead). Bar = 10 μm.

smooth muscle wall, the bladder becomes considerably firmer. The marked friability of the tissues during the in utero time period requires that the entire fetus be fixed before handling of the bladder for processing for examination.

At day 11 of gestation, the bladder (cloacal) epithelium consists of 1 - 3 layers of loosely-arranged cuboidal to columnar epithelial cells with round to oval nuclei (Fig. 1). The epithelium usually is more than one cell layer thick and rarely up to 4 - 5 cells thick. During days 12 and 13 of gestation, the bladder epithelium continues to range in thickness from 1 - 3 cell layers, but not as loosely arranged. By days 14 and 15 of gestation, the epithelium is 2 - 3 cell layers thick (Fig. 2). During these early periods of time, the epithelial cells appear undifferentiated, even those not in the basal layer. By day 15, differentiated features, such as the appearance of polygonal cells on the surface, begin to appear in the bladder epithelium (Fig. 3). The mature-appearing bladder with large superficial cells is observed from day 20 of gestation onward. Rapid turnover is present in the bladder epithelium during fetal life, as evidenced by numerous mitoses, prominent nuclei, and frequent shedding of cells into the lumen of the bladder (Fig. 5). Also, the labelling index of the bladder epithelium following a 1 hr pulse of ^{3}H-thymidine is approximately 10% or higher prior to birth (Cohen, et al., unpublished observations).

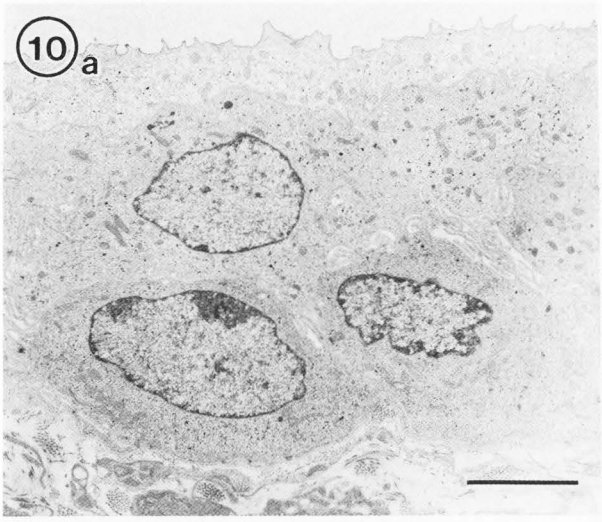
By scanning electron microscopy, the surface features of the bladder epithelium could be examined, and the findings are summarized below (Table I). At day 11 of gestation, these cells were round in appearance, relatively small, and had a predominantly smooth surface (Fig. 6), although occasionally they had short microvilli. The round nature of the cells continued through days 12 and 13 of gestation (Fig. 7), but was decreasingly observed during days 14 and 15 of gestation (Fig. 8). In contrast to the smooth surface usually seen at day 11 of gestation, uniform microvilli covered the surfaces of most of the cells beginning at day 12 of gestation and continued through day 17 of gestation. They were decreasingly observed through day 19 of gestation. The round cells covered with uniform short microvilli are similar in appearance to the intermediate and basal cells seen in the adult bladder (Jacobs, et al., 1983). Pleomorphic microvilli were not observed at any time in utero or after birth in the normal rat. The short microvilli tended to merge in the region where cells met, resulting in a thickened seam at the intercellular boundaries. This thickening became apparent during days 13 and 14 and was particularly prominent during days 15 and 16. It was less common and less prominent thereafter and not seen after 4 days after birth. Also present on the surface of cells at days 11 and 13 were numerous blebs (Fig. 7). They became less frequent as the cells became polygonal in shape, and were rarely seen in

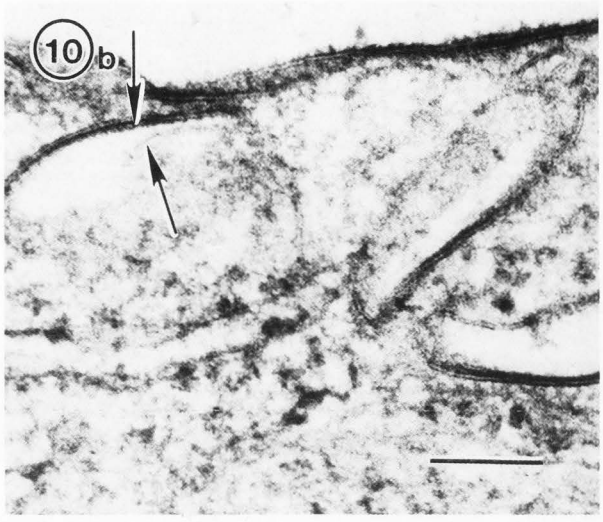


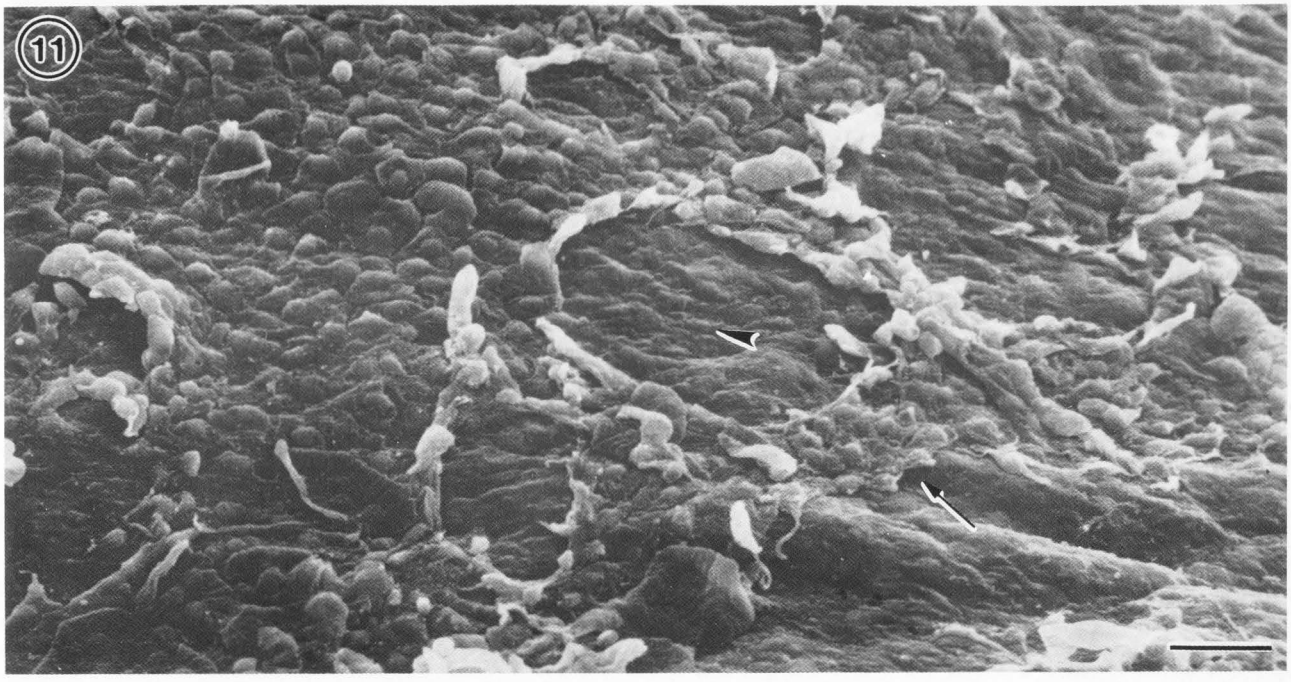


bladders from rats after birth.

Beginning at day 15 of gestation, the large polygonal superficial cells characteristic of the mature bladder epithelium appeared. These increased in frequency very rapidly so that by day 16, most of the bladder surface was covered with these large polygonal cells, and they completely covered the bladder by day 17. At first, these large polygonal cells were covered with the same uniform microvilli as seen on the round cells. However, by day 18 of gestation, these microvilli had begun to fuse, with the appearance of ropy microridges. Very rapidly, even as early as day 18 of gestation (Fig. 9), leafy microridges were present on the bladder epithelial surface as seen in the mature bladder. This type of microridge system reflected the presence of fusiform vesicles in the cytoplasm of the superficial cells with the distinctive asymmetric unit membrane present in the fusiform vesicles and on the bladder luminal surface membrane (Fig. 10). Beginning about day





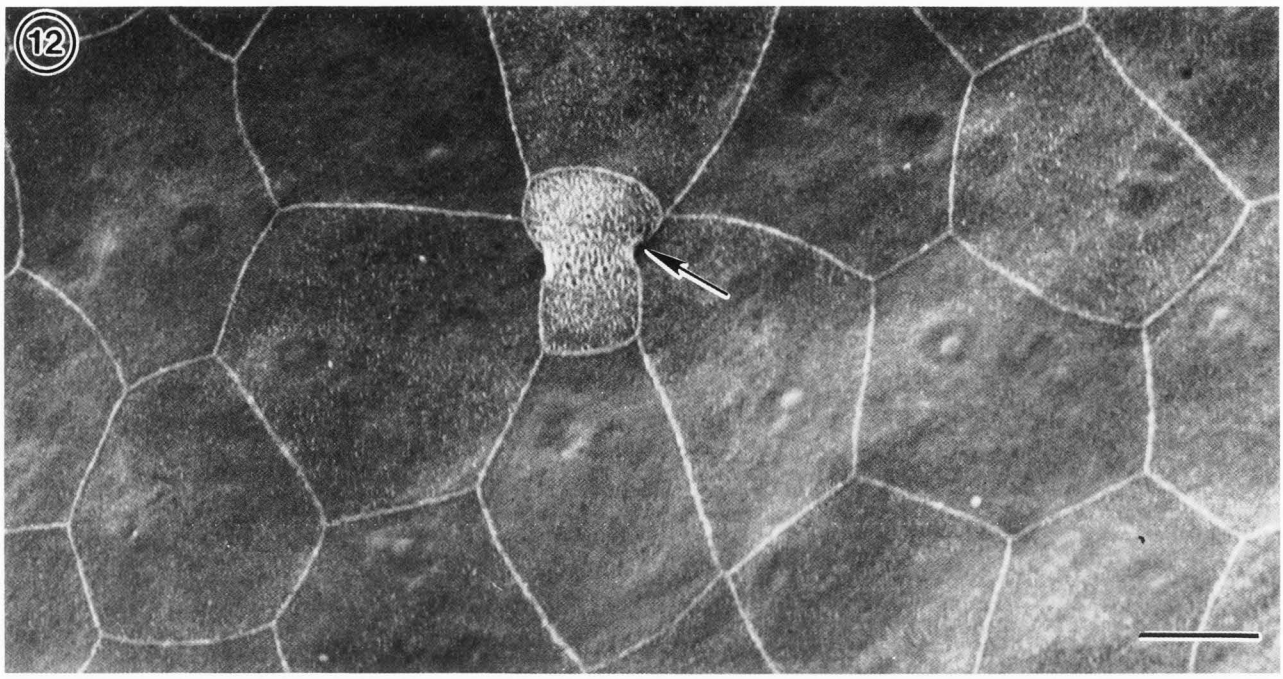


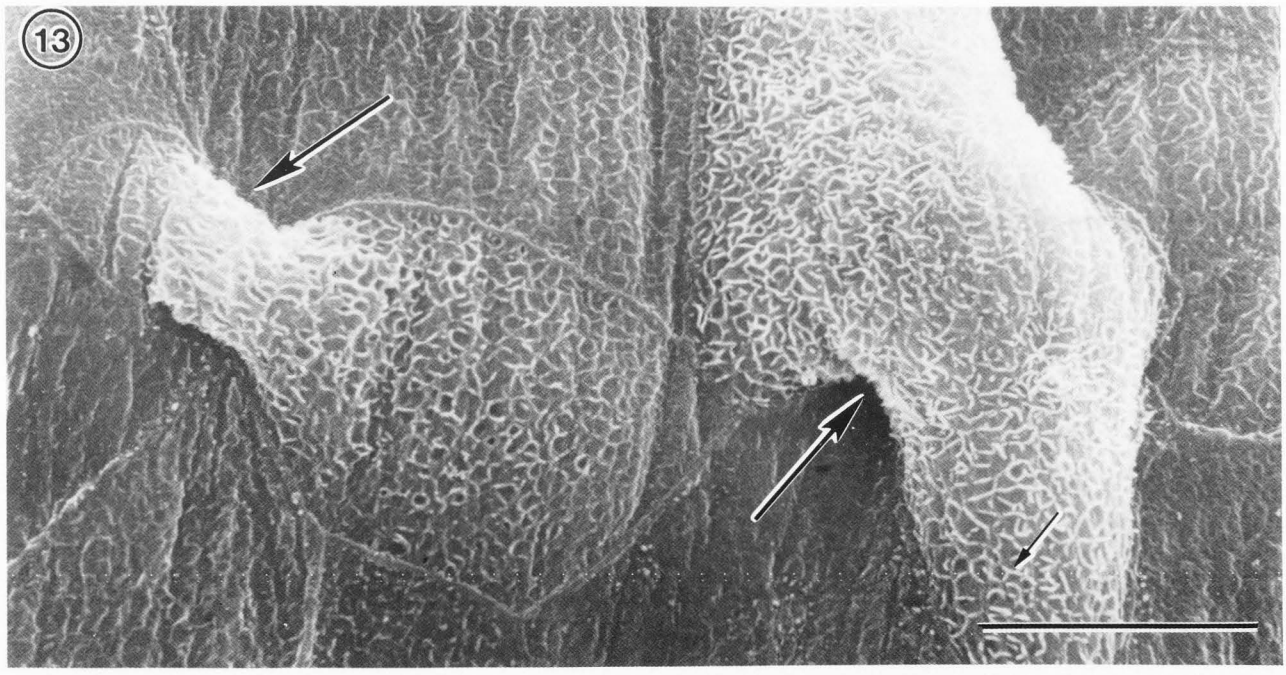
20 of gestation and continuing thereafter, the bladder had the appearance of the fully mature organ as observed by SEM (Jacobs, et al., 1983).

Just after birth, there appeared to be focal sloughing of the superficial layer, with single cells and small sheets of cells exfoliated into the lumen (Fig. 11). These foci of sloughing revealed underlying polygonal cells with microridges or rounded intermediate cells covered with uniform short microvilli as seen in the adult bladder. We did not see any areas of sloughing of the full thickness of the epithelium. The epithelium rapidly reassumed the appearance of the adult bladder within 24 hrs after birth (Fig. 12). It is unlikely that this sloughing represents artifact since it was seen

Figure 10.a) Transmission electron micrograph showing the fully mature bladder epithelium present at day 20 of gestation. Junctional complexes are evident, as well as fusiform vesicles. Bar = 3 µm.
b) The surface and vesicle membranes are predominantly composed of asymmetric unit membrane (arrows) as shown in greater detail in the adjacent figure. Bar = .1 µm.

Figure 11. SEM from the bladder of a rat immediately after birth. Small areas of exfoliated cells (arrow) can be seen, but the underlying epithelium already has the mature superficial cell surface with a microridge system (arrowhead). Bar = 20 µm.





only immediately after birth, not in bladders before birth or 1 day post partum, despite fixing and processing them the same way. Also, a single layer of cells comes off, and there is nearly complete maturation of the underlying cells.

In addition to the cells with either uniform short microvilli or microridges, other distinctive cells were observed at various times during the gestational and neonatal time periods. Cells which appeared to form a bridge were first observed at day 12 of gestation.

Figure 12. SEM of the bladder of a rat at 3 days after birth showing the typical appearance of mature bladder epithelium. A bridge cell (arrow) is present at the top of the figure. Bar = 10 μm.

Figure 13. Bladder epithelium from a rat at day 19 of gestation showing cells which appear to bridge (large arrows) across two areas of the bladder epithelium. The surface of the cells is covered with leafy and ropy microridges (small arrow). Bar = 10 μm.

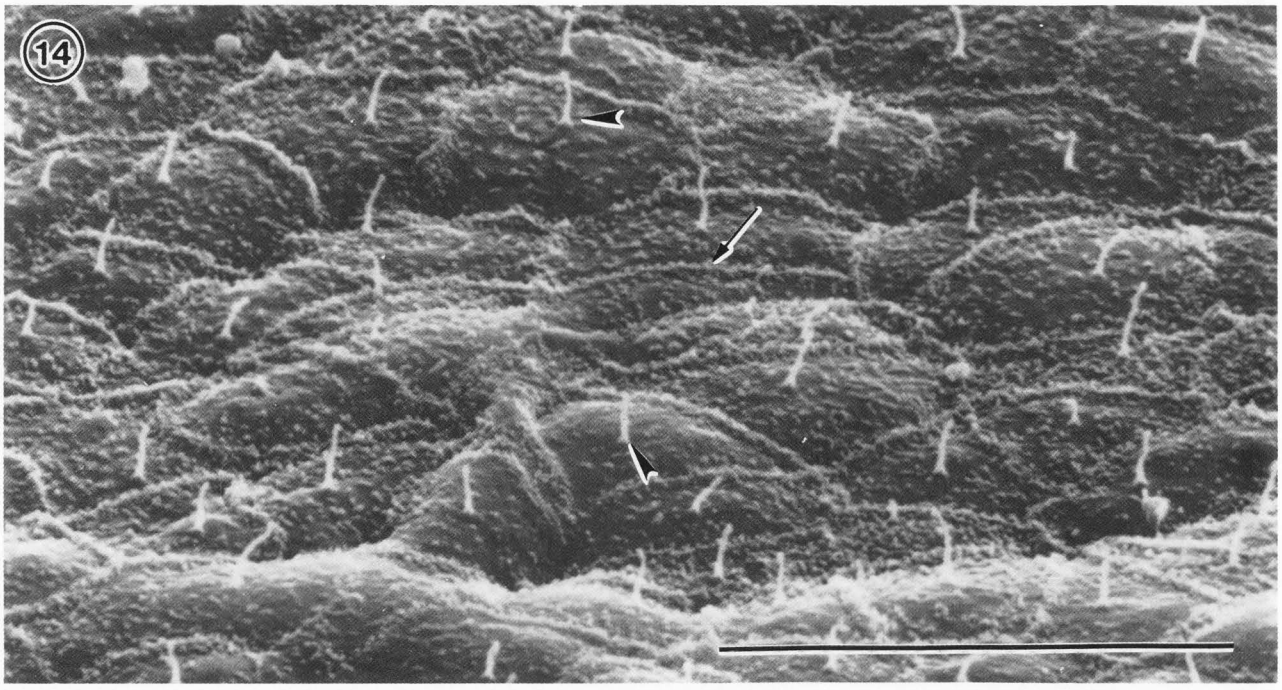


Figure 14. Bladder epithelium from a rat at day 15 of gestation showing polygonal cells covered with microvilli and a single central cilium (arrowheads). The junction between cells (arrow) is markedly thickened with prominent microvilli. Bar = 10 μm.

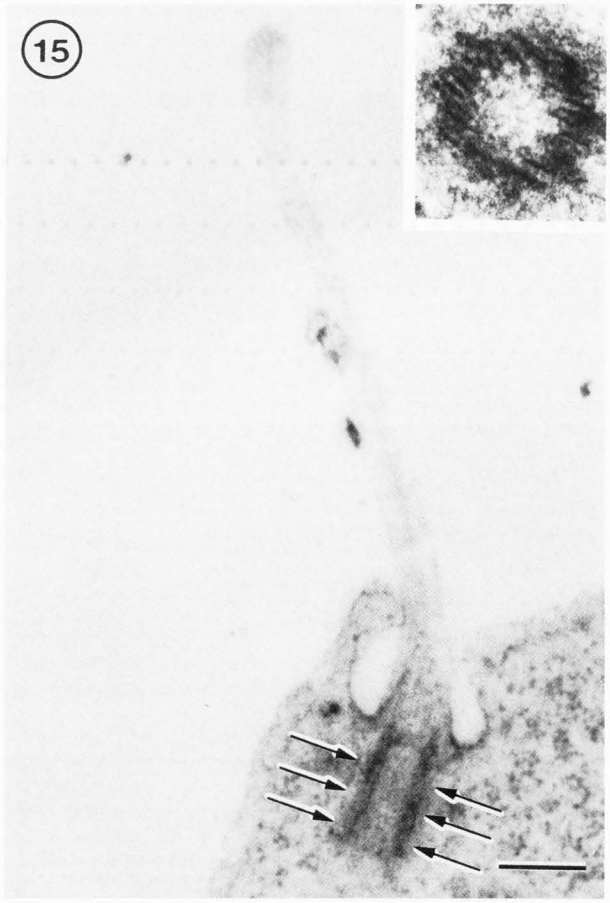


Figure 15. Transmission electron micrograph showing the fine structure of the cilium, with a cross-sectional area illustrating the 9 + 0 configuration of microtubules (inset). Bar = .3 μm.

Figure 16. Bladder epithelium from a rat at day 17 of gestation showing an "octopus"-appearing cell (arrowhead) on the surface. Bar = 1 μm.

Figure 17. Transmission electron micrograph of an "octopus"-appearing cell showing desmosomal (arrowheads) connections with adjoining urothelial cells. Bar = 1 μm.

Figure 18. Bladder epithelium from a rat at day 16 of gestation showing fine filamentous connections (arrow) between cells. Bar = 1 μm.

Figure 19. Bladder epithelium from a rat at day 17 of gestation showing the epithelium covered with numerous microvilli (arrowhead), but with large depressions (arrows) between cells. Bar = 10 μm.

They were frequently observed through day 19 of gestation (Fig. 13), then observed decreasingly often thereafter, and they were not observed after day 11 after birth. These cells would appear to be related to the rapid proliferation present in the in utero and early neonatal bladder.

Beginning at day 11 of gestation, some surface lining cells of the bladder lumen had a single cilium in the center of the cell surface.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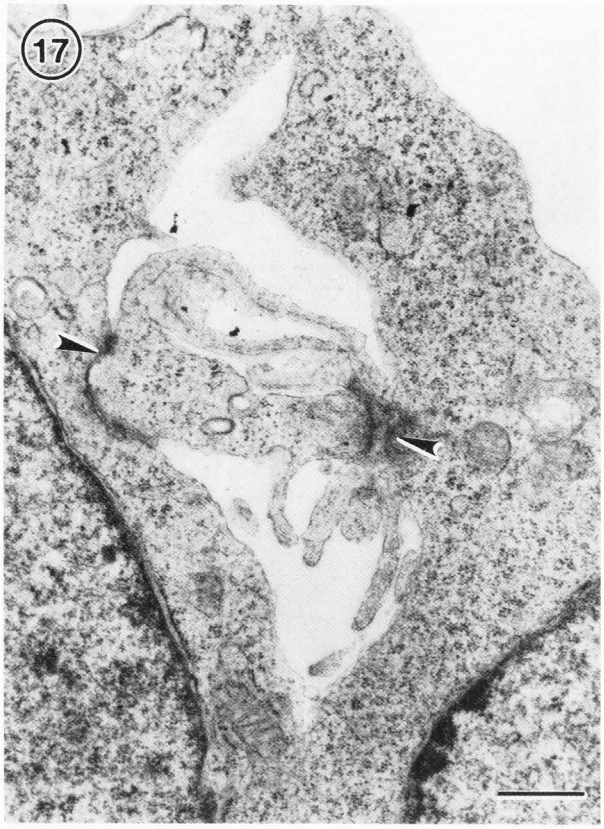
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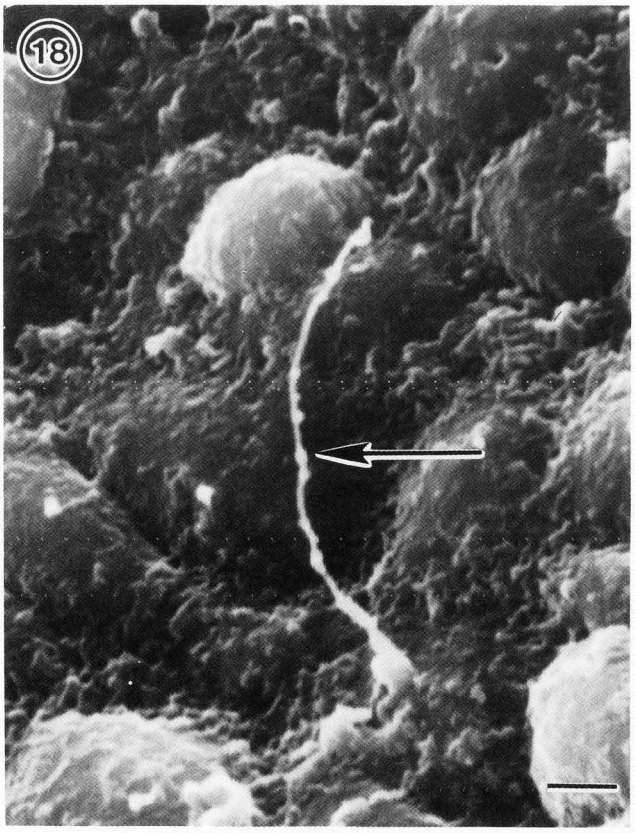
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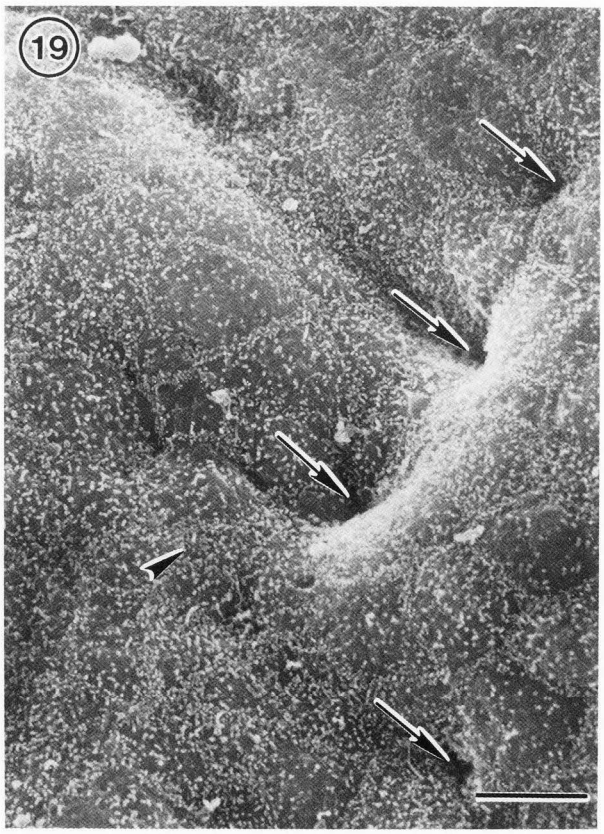
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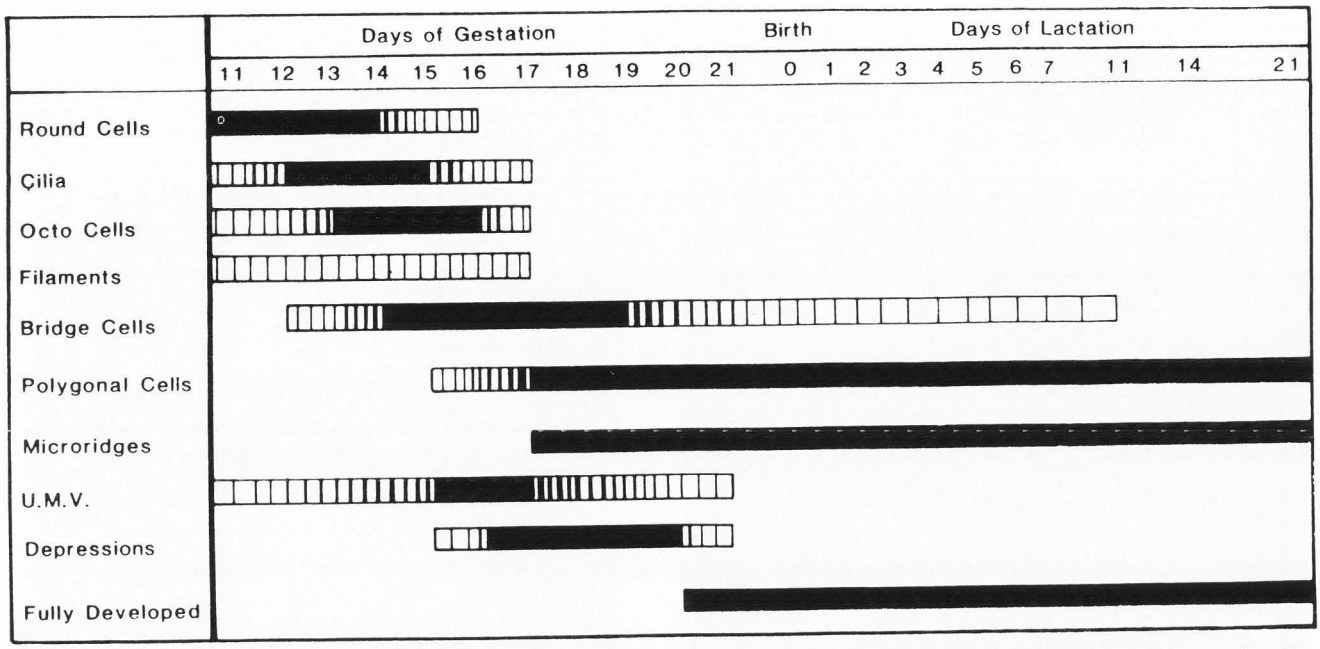

Table I. Diagramatic representation of the times of appearance of the various features during fetal and neonatal periods of the rat urinary bladder. Microridges refers to peaked, leafy microridges as seen in the fully differentiated superficial cells.

By day 13 of gestation and continuing through day 15 of gestation, most of the surface cells of the bladder epithelium had a single cilium (Figs. 8,14). During days 16 and 17 of gestation, occasional cells with a single cilium were observed, but no cells after day 17 of gestation were observed with cilia. The cilia appeared shorter than the typical motile cilia, but had the characteristic ultrastructural microtubular arrangement by transmission electron microscopy (Fig. 15). Although a definite transverse section of a cilium was not obtained in the multiple specimens examined, one section did give an appearance consistent with a 9 + 0 arrangement of microtubules. Based on the comparison with the appearance of the single cilium on cells in the renal tubules, one would anticipate that each cilium would have the 9 + 0 arrangement of the microtubules.

Beginning during days 13 - 14 of gestation, cells which had the appearance of an "octopus" were observed. These were frequently seen through day 15 of gestation and were occasionally seen during days 16 and 17 of gestation (Fig. 16). However, they were not observed after day 17 of gestation. These cells were relatively small compared to the other lining epithelial cells, and had long processes extending from the cell body. Morphologically these cells appeared quite different from the usual urothelial lining cells. Nevertheless, the epithelial nature of these cells was demonstrated by the presence of desmosome connections with the adjoining epithelial cells of the bladder (Fig. 17) as seen by transmission electron microscopy of sections prepared from the SEM specimens marked for the location of the cells. The exact function and nature of these cells is unknown at the present time, but they appear to be of epithelial type, rather than being of macrophage or mesenchymal origin.

An additional feature, filamentous connections between cells (Fig. 18), were occasionally seen during gestational ages 11 to 17 days. These have been suggested to serve as an intercellular communication function in other epithelial systems (Kelley, 1985).

In addition to the cell types observed at the different times, numerous depressions between cells were apparent from day 15 through day 20 of gestation (Fig. 19). Again, these were not observed later in the life of the rat, and were not seen with the advent of the fully-developed bladder epithelium by day 20 of gestation.

Beginning at day 20 of gestation and continuing throughout the remainder of the life of the animal, the bladder epithelium appears to be mature, light microscopically and ultrastructurally (Fig. 12), except for the occasional bridge-type cells which are seen through day 11 after birth. However, although morphologically similar to the adult bladder, the bladder epithelium at day 20 of gestation, and continuing through at least day 7 after birth, continues to be rapidly proliferating as evidenced by the numerous mitoses seen by light microscopy and as measured by labeling index procedures (Cohen, et al., unpublished observations). In a preliminary experiment, the labeling index varied considerably from litter to litter, and even within litters, but was approximately 10% during late gestation and at birth following a 1 hour pulse of ^{3}H-thymidine, and was greater than 1% at day 7 after birth. It reached levels essentially those of the adult rat bladder (<0.1%) by 21 days of age.

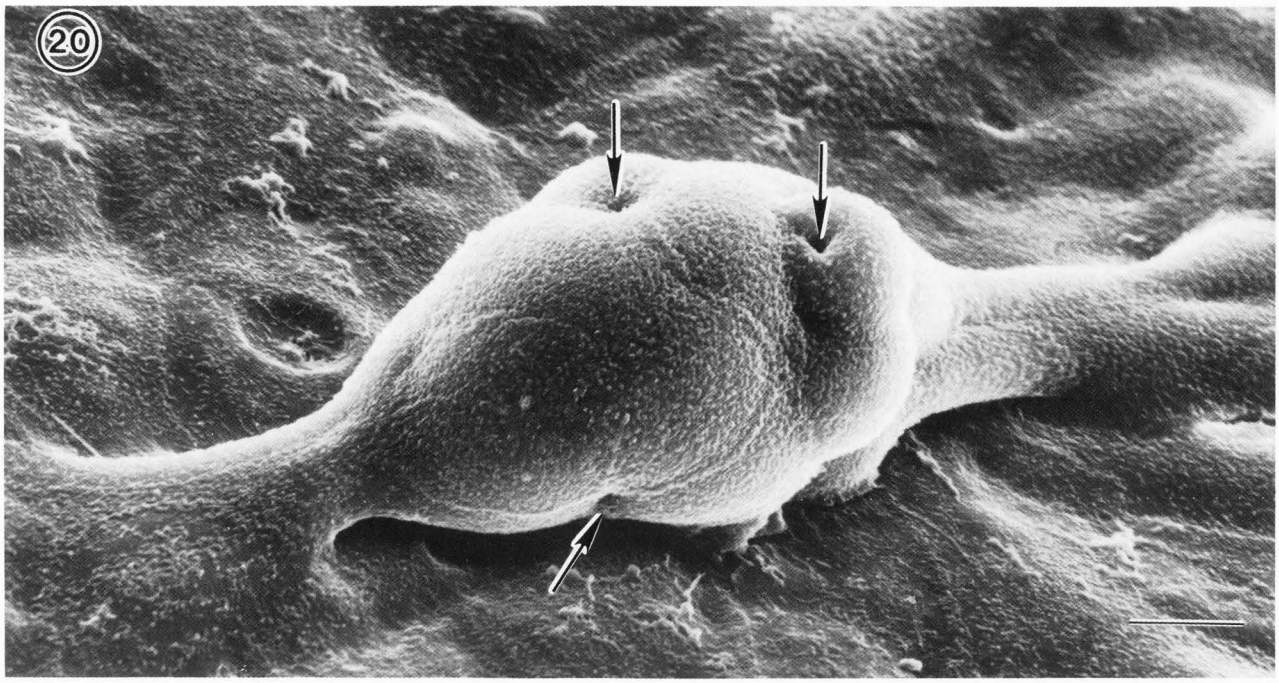

Figure 20. SEM of the surface of bladder tumor epithelial cells in tissue culture showing a bridging cluster of cells and depressions (arrows) between the cells. Bar = 10 μm.

The various cellular features seen at different times in the fetus and newborn rat are summarized in Table I.

Discussion

As detailed in this study, the bladder epithelium is a rapidly evolving tissue during gestation, reaching its adult appearance morphologically (Hodges, 1978; Jacobs, et al., 1983; Pauli, et al., 1983) by approximately day 20 of gestation, or approximately 1-2 days prior to birth. Although the morphological characteristics of a fully differentiated bladder epithelium are reached by this time point, the level of proliferation continues high through the time of birth, and it then begins to gradually decrease, reaching the mitotically quiescent levels of the adult bladder by approximately 3-4 weeks after birth (Jost, 1985; Cohen, et al., unpublished data). Thus, there is some separation between the proliferation and differentiation processes in the bladder epithelium.

Developmentally, it appears that the cloaca is not completely separated into bladder and rectum until approximately day 11 to 12 of gestation. Before that time, the luminal surface of the cells in the cloaca are similar to those extending into the allantois and the more proximal intestine. It is during days 11-12 that the characteristic features of the fetal bladder epithelium are observed, with small rounded cells covered with uniform microvilli. In addition, at days 11-12 the intestinal epithelium takes its more characteristic appearance, with somewhat rounded cells covered with long microvilli rather than the short microvilli seen in the urothelium.

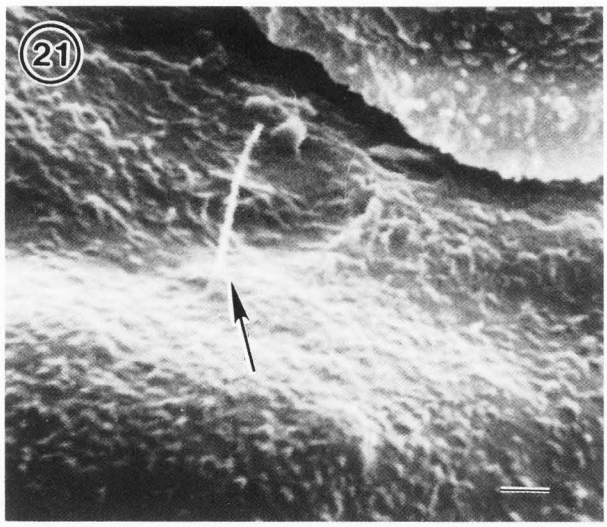

Figure 21. SEM of bladder tumor epithelial cells in tissue culture showing a single cilium (arrow). Bar = 1 μm.

During the maturation of the bladder in utero, the large superficial polygonal cells seen in the adult bladder are first visualized on day 15 of gestation. Until that time, the cells look more like the basal and intermediate cells of the adult bladder, being rounded, smaller, and covered with uniform microvilli. In addition, early in gestation a variety of cells are visualized which are not seen in the

normal adult bladder. These include the cells with the single, centrally-located cilium. These cells are similar in appearance to the tubule lining cells of the kidney which continue to be present into adult life (Bulger and Dobyan, 1982). Numerous cells which appear to bridge between two locations on the bladder are also present. They are not seen in the normal adult bladder. Furthermore, small "octopus"-appearing cells are seen during gestation, the function of which is not yet clear. However, the presence of desmosomes between these cells and adjoining urothelial cells indicate that they are part of the epithelium and are not macrophages, endothelial cells, or other types of cells which have infiltrated into the epithelium. Supporting this hypothesis has been our recent finding that these "octopus"-appearing cells are present in a urothelial tumor cell line which we have maintained in culture (Cohen, et al., 1981) for greater than 10 years. Similar features have also been visualized on the endothelial lining cells of the developing heart (Pexieder, 1981). These do not appear to be the same types of cells as lamellapodia which have been reported in a variety of tissues (Pexieder, 1981).

Some of these cellular features which we have identified in the fetal bladder epithelium have also been seen during carcinogenesis and/or rapid regenerative hyperplasia of the adult bladder, but they have not been observed in the normal adult urothelium. For example, bridge cells have been observed in a variety of circumstances involved with rapid proliferation of the adult bladder, but have been reported to occur most extensively following the intravesical instillation of tetradecanoylphorbol-13-acetate (Izumi, et al., 1984). Bridging cells have also been observed in cell culture of urothelial tumor cell lines (Fig. 20). Ciliated cells are not seen in the normal adult bladder epithelium, but they have been reported in carcinomas induced in animal models (Yalciner and Friedell, 1973), and we have also observed them in rat bladder carcinoma cell lines in tissue culture (Fig. 21). In reviewing previous photographs of this cell line, occasional cilia are seen (see Fig. 10 in Cohen, et al., 1981).

Early in the process of regenerative hyperplasia or carcinogenesis, there is a loss of the asymmetric unit membrane characteristic of the urothelium. This is replaced with cells covered with uniform microvilli and, eventually, pleomorphic microvilli (Wolf, 1966; Mooney and Hinman, 1974; Wong and Martin, 1977; Jacobs, et al., 1983; Pauli, et al., 1983; Anderström, et al., 1984). During fetal development, the bladder cells are covered with numerous uniform, short microvilli, but we did not observe pleomorphic microvilli in the fetal bladder epithelium. Thus, it appears that pleomorphic microvilli are a distinct feature of rapidly proliferating adult bladder epithelium (Jacobs, et al., 1983), which is not seen in rapidly proliferating fetal urothelium. The pleomorphic microvilli seen in tumors may be a reflection of their origin from cloacal cells. Although the urothelial epithelium evolving from the cloaca does not have pleomorphic microvilli, the intestinal epithelium which arises also from the cloaca has cells covered with the long microvilli identical to those seen in rapidly proliferating adult urothelium, whether regenerative or neoplastic. Thickened seams between cells have been observed in malignant tumors, particularly high grade tumors in humans (Jacobs, et al., 1981; 1983), and also in benign, rapidly proliferating lesions such as polypoid cystitis (Anderström, et al., 1984).

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Discussion with Reviewers

T.J. Poole: The "octopus" cells appear to resemble early urinary podocytes. Are they of similar size and are they developmentally related?
Authors: The "octopus" cells have a cell body that is approximately 3-4 μm whereas renal podocytes are generally more than twice that size. Also, the processes of podocytes are much longer with multiple branches. It is unlikely, therefore, that the "octopus" cells represent embryonic podocytes.

T.J. Poole: What is the significance of the pleomorphic microvilli seen in bladder carcinogenesis but not in the rapidly proliferating fetal urothelium?
Authors: Pleomorphic microvilli have been observed in adult urothelium in circumstances of increased proliferation, whether benign or malignant. Why fetal bladder, with an even higher proliferative rate, does not have pleomorphic microvilli is unknown.

T.J. Poole: Can you speculate on what would happen to your bladder carcinoma cell line if you introduced it into a fetal bladder? Would you expect such cells to undergo any of the developmental changes you have observed?
Authors: Whether the appropriate cellular controls in the embryonic bladder would be effective in forcing neoplastic urothelium to undergo these developmental changes is unknown. However, as demonstrated by G. Barry Pierce, placing neoplastic epithelium into the blastocyst stage of embryogenesis forces the tissue to differentiate rather than behave as a neoplasm (Cancer Res., 44: 3987-3996, 1984).

T.J. Poole: Is it possible to observe the switch from short microvilli to ropy microridges in fetal urothelium placed in tissue culture?
Authors: The progression from microvilli to ropy microridges apparently represents a step in the differentiation of the urothelium. As far as we are aware, this has not been demonstrated yet in tissue culture but is likely as better culture systems are developed for differentiating epithlia.

J.B. Reitan: Do the apparently fully matured urothelium around birth contain polyploid nuclei? Is the DNA synthesis monitored with incorporation of tritiated thymidine regarded as a proof of rapid proliferation, or can it be a sign of DNA synthesis necessary for building up of polyploidy?
Authors: Dr. Reitan raises an important point regarding proliferation and differentiation. We have not done ploidy analyses during this neonatal time period, but can provide some comments in response to the question. By "mature," we are referring to the morphological appearance of the large polygonal superficial cells with fully differentiated asymmetric membrane. Undoubtedly, Dr. Reitan is correct that the rat bladder goes through a similar process as the mouse, including change in proportion of cells with different ploidy, and that this accounts for some of the high labeling index rate. This is further supported by the finding of numerous labeled cells by autoradiography in the superficial layer as well as the basal and intermediate layers, and also by the finding of mitotic figures in all layers. Nevertheless, there

is also likely to be a high rate of proliferation of the urothelium since most of the numerous mitoses and labeled cells were present in the basal layer and because the bladder is rapidly growing in size during this period of life.

J.B. Reitan: Urothelium in fetal sheep is highly sodium permeable and reacts to antidiuretic hormone (France et al., J. Physiol., 239: 499, 1974). The observed ciliated cells resemble tubule-lining cells. Can such observations challenge the traditional description of bladder embryology indicating fetal urothelial derivation from ureteric or mesonephric tissues? Can the observation of cellular desquamation be related to loss of such cells during the adaption from fetal "aquatic" environment to life after birth?
Authors: In contrast to sheep and rabbits, the rat bladder, like that of humans, is relatively impermeable (A.G. Renwick, Fd. Chem. Toxicol., 23: 429, 1985). Although the ciliated cells bear a striking resemblance to those in the renal tubules, it is difficult to extrapolate implications about the embryologic derivation of the urothelium. The reason for the desquamation of cells at birth is unknown, but we believe that it is likely to be related to the trauma associated with birth.

A.P. Evan: Of what value will scanning electron microscopic observations play in the understanding of toxicological problems of the neonatal urinary bladder?
Authors: Scanning electron microscopic observation has proven to be a highly sensitive technique for detecting changes in the adult bladder epithelium in response to toxic stimuli. Similar sensitivity is expected for the neonatal time period.