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LACK OF REGIONAL SURFACE DIFFERENCES IN MOUSE BLADDER UROTHELIUM: A SCANNING ELECTRON MICROSCOPIC STUDY

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Abstract

The surface structures of mouse urinary bladder epithelium, the urothelium, were examined in SEM. The entire surface area of both a moderately and a well stretched bladder were studied. No regional differences were found in either case. The moderately stretched bladder showed deep folds, while the well stretched bladder had only small folds giving the bladder an accordion-like appearance. In both bladders the typical surface structures were microridges arranged in a honeycomb-like pattern. The present study indicates that focal or regional differences found in bladders treated with chemicals or exposed to radiation are a result of the treatment itself, and not of differences normally occurring in the urothelium.

Key words: Mouse urinary bladder, surface structures, regional differences, scanning electron microscopy

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Introduction

The luminal surface of the normal human urothelium is known to have a nearly regular arrangement of polygonal cells exhibiting well-defined microridges and tight contact margins (Hodges 1978). Newman and Hicks (1981) found that both the thickness of and the appearance of the luminal human bladder cells reflected the degree of contraction of the bladder wall. In well dilated bladders they found large flat cells with few folds and microridges while the cells in contracted bladders were cuboidal, deeply folded and ruffled and had smaller surface area. However, Nelson et al. (1979a) found slight regional variations in their studies of human bladders, differences traceable also in tumour-bearing bladders (Nelson et al. 1979b). Other investigators (Kjær et al. 1976, Lloyd-Davies et al. 1971) have also proposed regional differences in the human bladder.

The urothelia of different species have been found to have some histological differences. Even the relief pattern of cover cells has been said to differ to some extent, but seems to be fairly uniform within the species (Wolf 1966).

Few of the existing investigations have examined different regions of the bladder, and no investigations of the mouse urinary bladder have been carried out. The size of the the mouse bladder should, however, make it a good subject for such an investigation since the whole bladder can be examined by SEM without difficulty. In order to use scanning electron microscopy as a method of measuring the urothelial reactions to irradiation and other noxious agents, we have found it necessary to undertake a study of possible regional surface differences in the mouse bladder urothelium.

Materials and Methods

Female hairless (hr/hr) mice of the Oslo strain (Gamle Bomholdt Gaard,Aarhus) aged 10-12 weeks and weighing 20-25 grams were used. Prior to the investigation the mice were kept under standard conditions with free access to pelleted food and tap water. They were exposed to artificial light from 0630 to 1830 h.

For this study 2 mice were sacrified by cervical dislocation at 1400 h. The abdomens were opened immediately and the bladders were catheterized with a galactography catheter under visual inspection. The bladders were inflated with 3% glutaraldehyde in a buffer of 0.1M cacodylate and 0.1M sucrose, pH 7.4. One of the bladders was inflated to its maximal distension in approximately 2 seconds with 0.2 ml of fixative, the other with about half the volume. The bladder necks were ligated, and the bladders excised and immersed in the same fixative as above.

After fixation for three days at room temperature the bladders were rinsed in the same buffer as used for the fixative, and cut into 5 rings from top to bottom. Each ring was kept isolated and was cut into 8-11 small pieces as described by Reitan and Feren (1986). All pieces were mounted and examined by SEM (fig.1).

About half of the pieces from each ring were thoroughly washed in bidistilled water, and impregnated with silver nitrate ($AgNO_3$) as descibed by Thiebaut et al. (1984). The remaining pieces were postfixed in 1% OsO_4 in the same buffer as above. In this way we were able to compare the two methods of impregnation and their ability to reduce charging effects caused by the topography of the specimen.

The material was dehydrated through graded alcohols, critical point dried by liquid CO_2 using a Balzers Critical Point Dryer, mounted on specimen stubs, and covered with a 200Å gold layer in a Balzers SCD 230.

The bladders were examined both at low and high magnification in a Philips PSEM 500 at a tilt angle of 20 degrees. Photographs were taken using Kodak Tri-X-Pan film.

Results

Only the bladder urothelium was studied. Possible differences in surface structure between the urothelium of the trigone and the ureters has not been examined.

Both $AgNO_3$- and OsO_4- impregnated pieces were chosen for closer examination. No differences were found between the two methods of impregnation, either in the observed surface structure or in the ability to reduce charging effects.

Of the two bladders studied one was only moderately stretched and had numerous folds. This made it difficult to examine the surface of the single cells. It was, however, possible to recognise an overall pattern of the surface structure and cell borders, and to observe the same honeycomb-like appearance as found in the fully stretched bladder. Due to the folds, the cell borders seemed to be situated in grooves. From this moderately stretched bladder 2-3 typical pieces per ring were chosen for closer examination and photography (fig. 2a & b).

The second bladder was well stretched, and the whole bladder was examined by SEM before representative areas on 5-6 pieces per ring, totally 29 of 45 pieces, were photographed (fig. 2c).

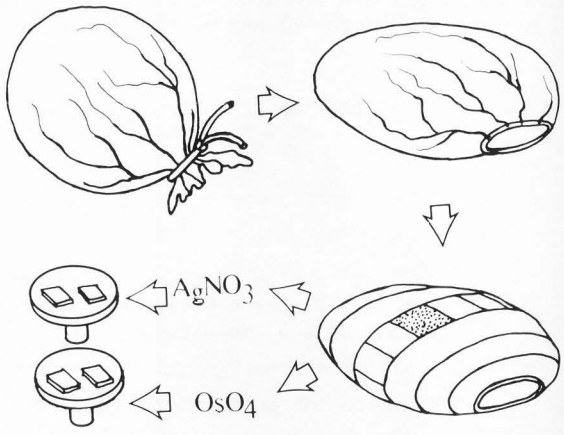


Fig.1. Schematic drawing of specimen preparation.

The examination did not reveal any regional surface differences. Everywhere the cover cells formed a continuous layer either completely stretched or slightly folded giving the bladder an accordion-like surface (fig. 2d). The typical cover cells were large, flat and polygonal with a honeycomb-like appearance and clearly raised cell borders (fig. 2e & f). Some small variations could be found between cells exhibiting more or less surface structures, mostly microridges. These variations were not pronounced and were as large within the different regions as between the regions from top to bottom. However, cells in the lowest part of the bladder were more folded than elsewhere, probably due to the ligation.

In some areas, cells with small blebs in addition to the usual surface structures were seen. Such cells are probably dying cells in the process of being exfoliated (fig. 3). In other small areas, the cover cells were missing due to either mechanical trauma during specimen preparation or physiological exfoliation. This gave a view of the deeper cell layers and lateral surfaces (fig. 4a & b).

Discussion

During embryogenesis, the bladder is formed from the vesico-urethral part of the primitive urogenital sinus. During movement of the ureteric openings the trigonum vesicae is formed mainly from mesodermal tissues, whereas the main part of the mucosa is of endodermal origin. The early connection to the allantois normally undergoes regressive changes and it is unclear whether or not the allantoic endoderm contributes to the definitive bladder dome lining. There is some intermingling of the various epithelia of the urogenital sinus. For details see Gyllensten (1949) and Hamilton et al. (1976). Thus, from an embryological point of view regional differences might be anticipated.

SEM examination of normal and neoplastic

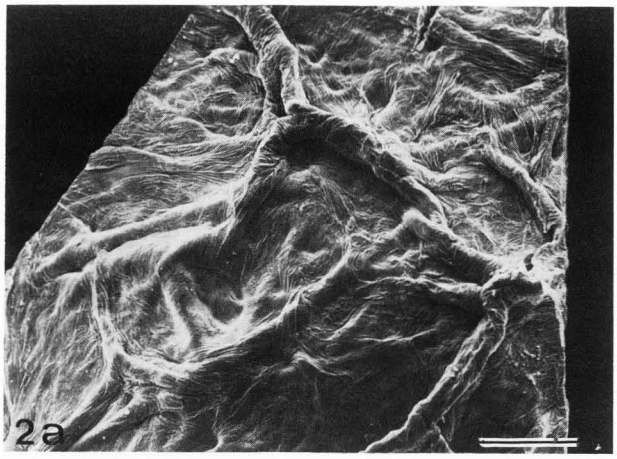
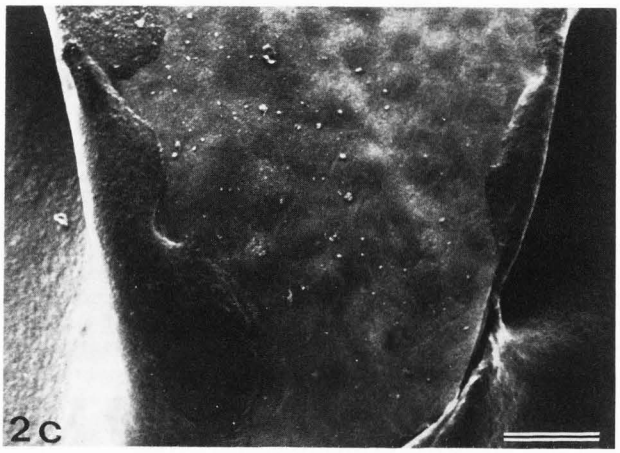
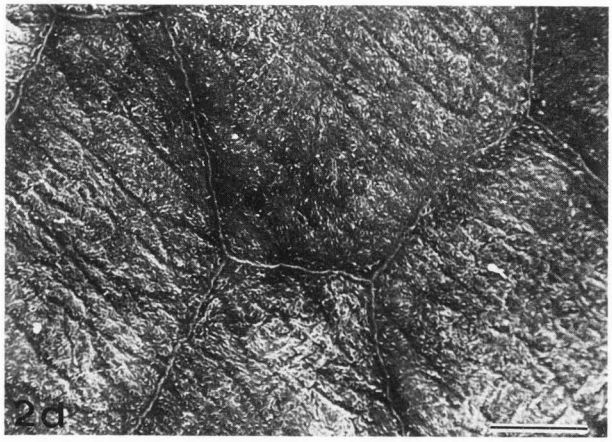


Fig.2. a) Low magnification of the moderately stretched bladder with numerous large and small foldings. Bar= 200μm

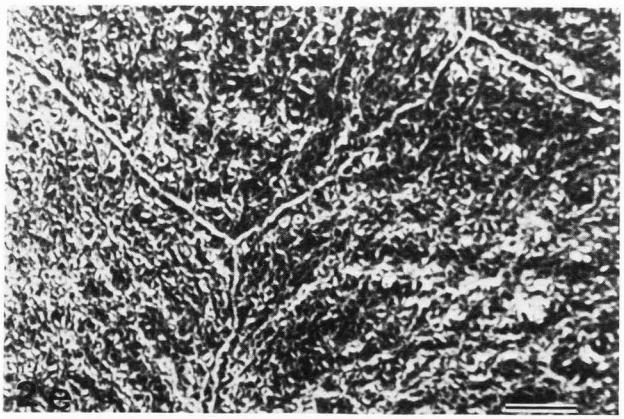
b) Higher magnification of the same bladder showing the numerous small folds raising the cell surface well above the cell borders (arrows). Bar= 10μm



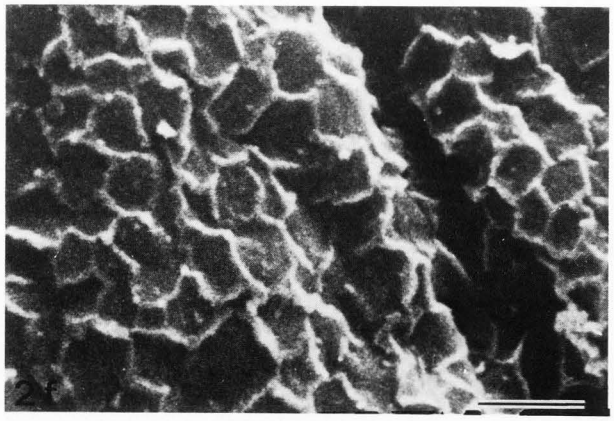
c) Overview of the surface of the well stretched bladder. Note the smooth surface view showing the cell "outlines" and the difference between this and fig. 2a. Bar= 200μm



d) The typical accordion-like surface of the well stretched bladder formed by cells with clearly raised cell borders and slight foldings. Note the difference in cell size between b and d.Bar= 20μm



e) Large, flat and polygonal cover cells of the well stretched bladder. Note the regular microridge pattern. Bar= 5μm



f) High magnification of a cover cell showing the honeycomb-like microridge pattern of the surface. Bar= 2μm

bladder urothelium has been investigated in numerous studies (Hodges et al. 1973, Noack et al. 1975, Kjærgaard et al. 1977, Wolf 1966, Lloyd-Davies et al. 1971, Tannenbaum et al. 1978, Newman and Hicks 1977, Koss et al. 1974, Nelson et al. 1979a & b). In some of these investigations regional differences were proposed. The overall findings were, however, that the bladder urothelium consists of flat polygonal cover cells in a continuous layer as we have found in our investigation. A more rounded form of cells, hiding cell contacts has also been reported. Many of these previous investigations have studied the trigonum and the urethra in addition to the urothelium, but none of them carried out scanning electron microscopic mapping of the whole bladder. In this study the trigonum was cut off and no parallel to the prominent surface differences in the human bladder between the trigonum and the rest of the bladder could be established. Nelson et al. (1979a), found that the lateral wall cells appeared to be more stretched than the rounded cells of the dome. This was attributed to a better stretch capacity of the lateral cells. However, their biopsy specimens were taken from patients with mild prostatic hyperplasia. Thus a muscular hypertrophy might have existed and the urothelium might not have been entirely normal. In our study we found that the urothelium had a slightly more folded appearance near the ligature. This is probably due to the ligature pressing the bladder together during fixation, and not to differences in the cell form.

In conclusion it can be said that no regional differences could be found in the urothelium of the mouse bladder. Focal differences found in bladders treated with chemical carcinogens, toxic substances or irradiated bladders can therefore be said to be results of treatments, and not of differences previously existing within the bladder.

Acknowledgements

We like to thank Mrs. R Puntervold for skilful technical assistance and Dr. GW Farrants for revising our English.

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Discussion with Reviewers

DB Jones: Were you able to identify a population of unusually large surface cells which might correspond to the polyploid "umbrella" cells seen in human urothelium?
Authors: Most of the surface cells were of the polyploid "umbrella" type as we have described in another study (Thiebaut et al. 1986).

CE Nelson: Of course it would have been of great interest if the vesicle trigone had also been investigated. It would have been of special interest since it has been demonstrated that the surface structures are different from the rest of the bladder in humans. Since a high percentage of malignant tumours are located in the trigone it would have been of interest to know how a normal mouse trigone appears as a basis for further examinations of the influence of toxic substances and irradiation. Are there any surface structures in the urothelium of the trigone which will predicate the existence of malignant tumours there, or will it be an effect of carcinogens here by the position of man? Will malignant tumours appear more frequently in the anterior part of the mouse bladder?
Authors: We do not know whether the trigone has surface structures predicating it for malignant tumours or whether malignant tumours appear more frequently in this part of the bladder. We fully agree, however, that there is a need for further studies including the trigone, but have found it very complicated when using the inflation fixation technique.

DR Abrahamson: As the bladder is progressively filled in vivo, would you not expect to find minor differences in the surface contours in different regions of the bladder? In contrast, these probably would not have developed with your fixation protocol.
Authors: In partly expanded bladder there may perhaps be regional differences due to various stretching of the urothelium. However, we believe such phenomena not to be of structural origin. We do not believe that the fixation procedure is a major concern in this respect.

DR Abrahamson: Have you examined different mice strains, or rats with your procedure?
Authors: No, that has not been done.

DR Abrahamson: Were exfoliating cells more numerous in stretched than in unstretched bladders?
Authors: No obvious differences with respect to exfoliating cells were found between stretched and unstretched bladders.

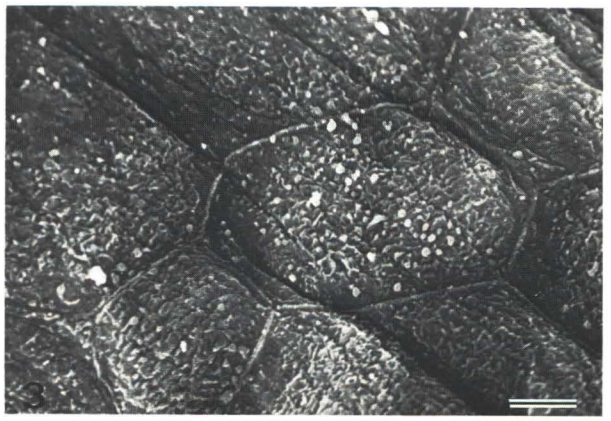

Fig. 3. Area with cells showing small blebs in addition to other surface structures. Such cells are probably on their way to be exfoliated. Bar= 5μm

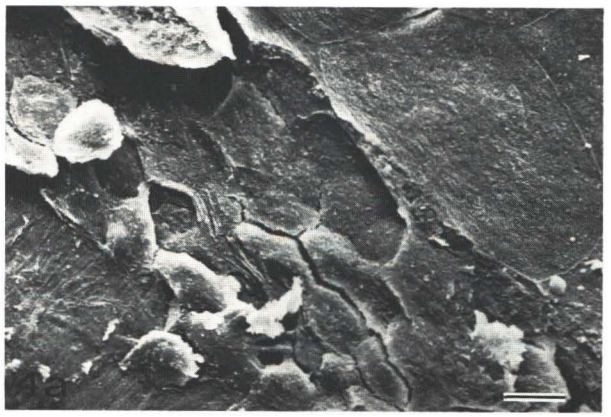

Fig. 4. a) Area of the well stretched bladder where the cover cells are missing due to mechanical trauma during preparation. Note the very large size of the cover cells compared to the underlying cells. Bar= 10μm

b) Higher magnification of an area with a missing cover cell, showing the few surface structures on deeper lying cells and the flatness of the cover cells. Bar= 5μm

S Johansson and S Cohen: We do not find it sufficient to examine only two mice and to evaluate mice only at one age. Please comment.
Authors: Since we did not find any regional differences in the two bladders examined we did not find it justified to extend the study. However, if regional differences had been found, the study would have been expanded. In another study (Reitan and Feren, 1986) we examined bladders from young (10-12 weeks) and older (62-64 weeks) animals. No age variations in surface morphology were found in that study.

S Johansson and S Cohen: We do not see the advantage of cutting the bladder into five rings, which will then be further divided into 8-11 small pieces. This will destroy a considerable amount of surface area. It is completely feasible to divide the bladder into two pieces which could then be examined, retaining a better orientation of the bladder. Please comment.

Authors: The advantage of cutting the bladder into 40-50 small pieces as we have experienced it, is to reduce the charging effect in the microscope. The method produces more scars than dividing the bladder into two parts, large surface areas are, however, not destroyed. The scars give us the opportunity to examine underlying cells where surface cells are lost. We have tried to examine bladders divided into two parts but have experienced several problems. In addition to the charging effect, the topography of such a large specimen makes it unfavourable for examination by SEM. Further, it is difficult to mount such a large specimen without drowning it in the mounting medium.

GM Hodges: Was any attempt made to examine the silver nitrate-impregnated tissue by BEI - this could have possibly provided information on underlying cells and on cell-cell relationships both within and between the different urothelial layers.
Authors: Not in this study, since the bladders were covered with gold and thus not available for BEI-examination. In another study using BEI we have described the occurrence of a structure resembling the epithelial proliferative unit of the skin in the mouse bladder epithelium (Thiebaut et al. 1986).

GM Hodges: Which locations of the bladder showed cells with small blebs? What percentage of cells within each area showed blebs? Are these cell numbers consistent with the cell numbers that would normally be sloughing off from the luminal surface of the bladder?
Authors: These cells were only seen sporadically and in no particular locations. We estimate them to cover less than 1% of the total surface area and thus be in consistence with the very low proliferative rate of the normal murine bladder (Reitan and Tverå 1985).

Additional references

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