Urol Res (1983) 11:131–137

Urological Research

Effect of Hyperthermic Vesical Irrigation with Bleomycin on the Ultrastructure of the Well-Differentiated Tumour and Non-tumorous Mucosa of the Human Urinary Bladder

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Accepted: March 14, 1983

Summary. Bladder tumours and non-tumorous bladder mucosa were studied by scanning and transmission electron microscopy in seven patients who had undergone hyperthermic vesical irrigation with bleomycin. The treatment induced sloughing of the outermost tumour cells, an increase of blebs and a decrease of cytoplasmic processes of the deeply located tumour cells as well as cellular degeneration. Although less severe, non-tumorous mucosa showed similar changes. Microvilli also appeared on the superficial cells of non-tumorous mucosa after the treatment. This treatment is effective by inducing cell degeneration and desquamation but not selective to the bladder tumour.

Key words: Human bladder tumour, Non-tumorous human bladder mucosa, Hyperthermic vesical irrigation, Bleomycin, Ultrastructure, Scanning electron microscopy.

Introduction

Recently, hyperthermic vesical irrigation has been utilised as a treatment for bladder tumour and reported to be effective in superficial, multiple and reccurent tumours [7,12]. Electron microscopic changes of the tumour after this modality of treatment have already been reported [8,9]. One of the authors (N. M.) observed that hyperthermic vesical irrigation of bleomycin induced cell detachment and degeneration in bladder tumours. Bleb formation was also found on the free surface of the tumour cells after the treatment [8]. However, previous studies did not include the non-tumorous mucosa of the bladder.

Cystoscopically, non-tumorous urothelium appeared congested and oedematous after the treatment. These experiences prompted us to perform this transmission (TEM) and scanning (SEM) electron microscopic study.

Materials and Methods

Seven patients suffering from well-differentiated bladder tumours received hyperthermic vesical irrigation with bleomycin. The bladder was irrigated through a two-way urethral catheter connected to an automatic irrigating apparatus. This closed irrigation system was filled with 400 ml of saline and 90 mg of bleomycin was added. Each irrigation lasted for 1h with a flow rate of 2 l/h. The inflow temperature was adjusted to 45–46 °C and the outflow temperature was 40–43 °C. The irrigation was performed every other day and ten irrigations comprised a standard course of treatment.

Of 7 cases, 4 were primary and 3 were reccurent tumours. Tumours disappeared in one case, were reduced in size in 5 and did not change in 1 after the treatment (Table 1). Specimens were obtained by a cold punch from tumorous and non-tumorous portions of the bladder before and 1 week after the treatment. For TEM study, the tissues were cut into small pieces and fixed overnight in aldehyde solution (1% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4). They were postfixed in 2% osmium tetroxide solution for 1h, dehydrated and embedded in Epon. Thin sections were cut by a glass or a diamond knife equipped on LKB 8800 Ultratome. After staining with uranyl acetate and lead citrate, they were examined in Hitachi 12-A TEM. For SEM study, specimens were similarly fixed and immersed overnight in 2% tannic acid in 0.1 M cacodylate buffer pH 7.4 for conductive staining. They were postfixed in 2% osmium tetroxide solution for 6 h, dehydrated in ascending ethanol series, then transferred in isobutyl acetate and finally dried in a critical point drying apparatus using liquid carbon dioxide. After mounting on the aluminium studs, specimens were coated with gold and examined at 15–25 KV in a Hitachi S 430 SEM.

Table 1. List of patients with some pertinent clinical data

Case	Age/Sex	TNM classification	Clinical effect
1. T. T.	52 M	rTa(m) NX MO G1	disappearance
2. N. O.	44 M	rT1(m) NX MO G1	decrease
3. T. M.	60 M	T1 NX MO G1	decrease
4. Y. N.	62 M	rT1(m) NX MO G2	decrease
5. H. F.	49 M	T1 NX MO G2	decrease
6. R. F.	63 M	T1 NX MO G1	decrease
7. S. N.	72 M	T2 NX MO G2	no change

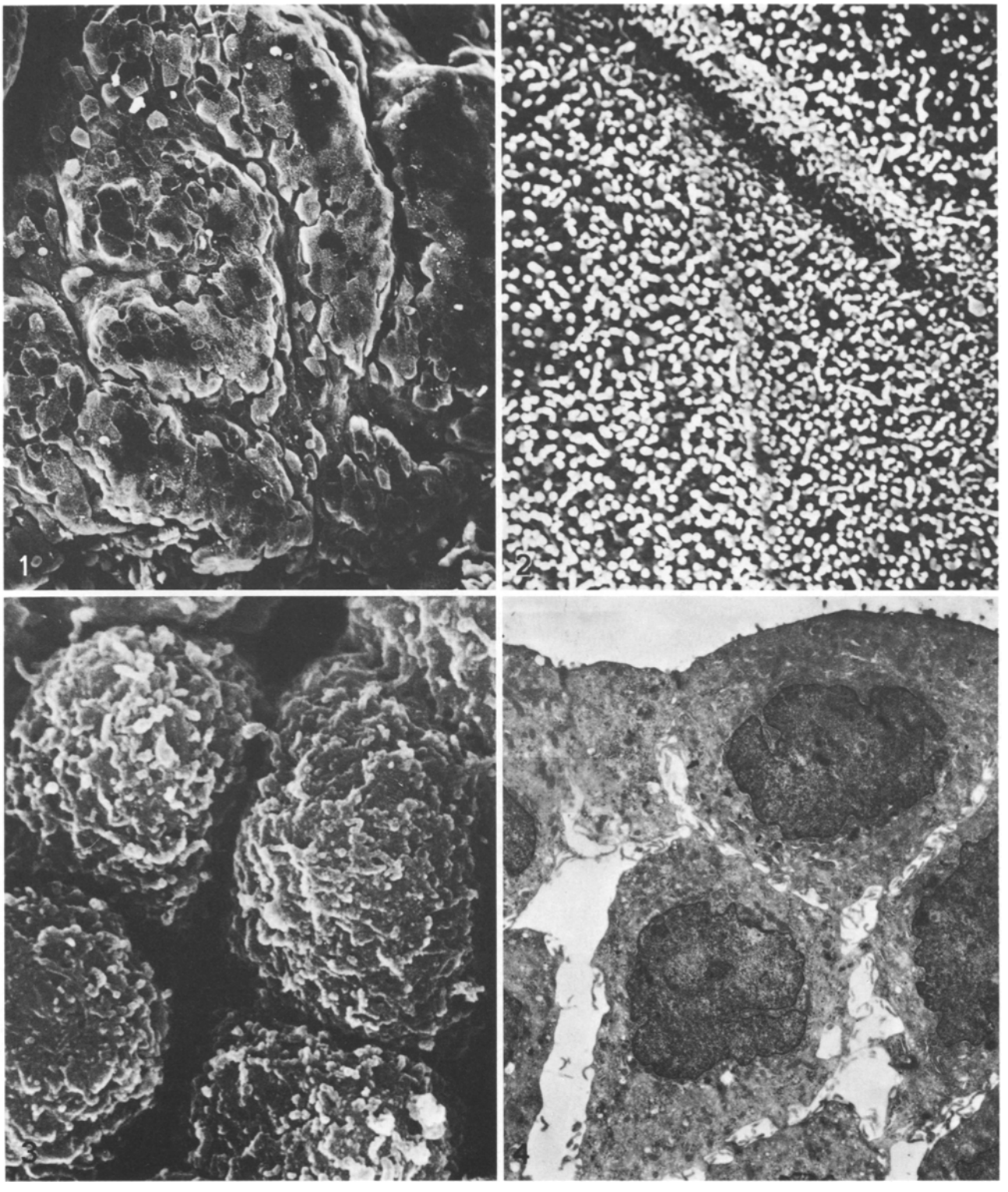

Fig. 1. A SEM picture of a tumour of Case 6 before treatment. Tumour surface is covered with polygonal outermost cells. x240

Fig. 2. A SEM picture of a tumour of Case 5 before treatment. Pleomorphic microvilli diffusely cover the surface. x6,400

Fig. 3. A TEM picture of a tumour of Case 2 before treatment. The deeper tumour cells exposed to the vesical lumen show globular and convoluted processes. x5,000

Fig. 4. A TEM picture of a tumour of Case 5 before treatment. The outermost cells possess microvilli. Nuclei are irregular in contour having deep notches. Plasma membranes of lateral outermost cells and of deeper cells show slender cytoplasmic processes. x4,800

Results

Ultrastructure of the Tumour Before the Treatment

Tumours were composed of about 10 layers of cells. The outermost cells appeared cuboidal and possessed many microvilli of various sizes and shapes. The intermediate or deeply situated tumour cells were spindle–shaped and arranged with their long axes perpendicular to the basement membrane. SEM revealed that the outermost cells of the tumour were polygonal in shape and the luminal surfaces were covered by many microvilli of various shapes and sizes. Some microvilli were short and stubby, and others were upright with varying heights. Branching of microvilli was often observed and pleomorphism was evident (Figs. 1 and 2). Deeper cells were occasionally exposed and had a cuboidal appearance with a size of 10–15 μm. Globular or convoluted cellular processes were seen on their surfaces (Fig. 3). TEM showed that the luminal membranes of the outermost cells were symmetrical. Presence of microvilli and absence of discoid vesicles were apparent. Mitochondria were scattered in the supranuclear region of the cell. The nucleus was irregular showing an ocassional deep notch. Heterochromatin granules were not conspicuous. The cellular features of the deeply situated tumour cells were similar to the outermost cells. There were many slender cytoplasmic processes and the intercellular spaces were sometimes dilated. Secondary lysosomes were seldom observed (Fig. 4).

Changes of Tumour Ultrastructure After Treatment

Sloughing of the cells was apparent at the tumour surface by SEM and TEM (Figs. 5, 7 and 8). The previous tumour site in a case of complete tumour disappearance revealed mucosal denudation (Fig. 8). The microvilli of the remaining outermost cells decreased in number in 4 cases (Fig. 6), all of which showed tumour reduction following the treatment. On the contrary, microvilli increased in number in 2 cases (cases 4 and 7) having large tumours after the treatment. Changes of the deeper cells were also remarkable. The cell surface became smooth and slender, cytoplasmic processes disappeared. Instead, small numbers of short processes resembling microvilli appeared on the surface of the newly exposed cells. An increase of large blebs was observed. Some showed shrinkage (Fig. 7). The tumours remaining after the treatment were composed of 5–6 cell layers by TEM. Detatchment of outermost cells and enlarged intercellular spaces were found. Decrease and destruction of cytoplasmic organelles and vacuolation of rough endoplasmic reticulum indicated the cellular degeneration. Nuclei became swollen and electron lucent in these cells (Fig. 8). Blebs contained amorphous cytoplasmic matrix with reduced electron density (Fig. 8 insert).

Ultrastructure of the Non-tumorous Mucosa Before Treatment

Specimes of non-tumorous portions were available in 6 cases. The mucosa was composed of 4–5 layers of cells. SEM revealed the mucosa was covered with large polygonal cells in 5 cases. Microridges with occasional microvilli were found on the luminal surfaces in these 5 cases (Fig. 9). By TEM observation, luminal surfaces of the flattened superficial cells were composed of microridges and plaques with an asymetrical unit membrane. Rows of scaphoid vesicles and mitochondria were found beneath the luminal membrane. Secondary lysosomes and microfilaments were scattered in the cytoplasm. Ovoid nuclei had scanty chromatin granules and inconspicuous nucleoli (Fig. 10). These polygonal cells were identified as the normal superficial cells. However, one of 6 cases possessed surface cells diffusely covered with microvilli by SEM. TEM revealed that these cells were smaller than normal superficial cells and that luminal plasma membranes were symmetrical. Other features of the atypical superficial cells were a cluster of mitochondria and secondary lysosomes in the supranuclear region as well as irregular nuclei.

Ultrastructural Changes of the Non-tumorous Mucosa After Treatment

SEM revealed that the superficial cells were lost in many places and the remaining surface cells possessed numerous microvilli instead of microridges (Fig. 11). Intermediate cells exposed to the vesical lumen were 15–20 μm in size. Bleb formations were also found (Fig. 11 Insert). Cell-to-cell junction was dilated in many places. TEM observations verified the SEM findings showing the detachment of the superficial cells, presence of microvilli on the surface cells and exposure of many intermediate cells to the lumen (Fig. 12). Intercellular spaces were often enlarged. These structural alterations were similar to those observed in the tumour portions after the treatment.

Discussion

There is a number of SEM studies on the surface morphology of bladder tumour and bladder mucosa [2–5, 10, 11, 14, 15]. The ultrastructural changes of human bladder tumours after intravesical bleomycin instillation or hyperthermic vesical irrigation with or without bleomycin have also been studied [8, 9]. Our previous light microscopic and TEM study showed that these treatments induced cellular degeneration and detachment of tumour cells and resulted in reduction of the tumour [8]. Nakamura [9] performed a TEM study on tumour cells after hyperthermic irrigation and observed cell degeneration. The effect of these treatments on the non-tumorous bladder mucosa, however, has never been studied. Further, SEM observation has not been done on bladder tumour after this treatment.

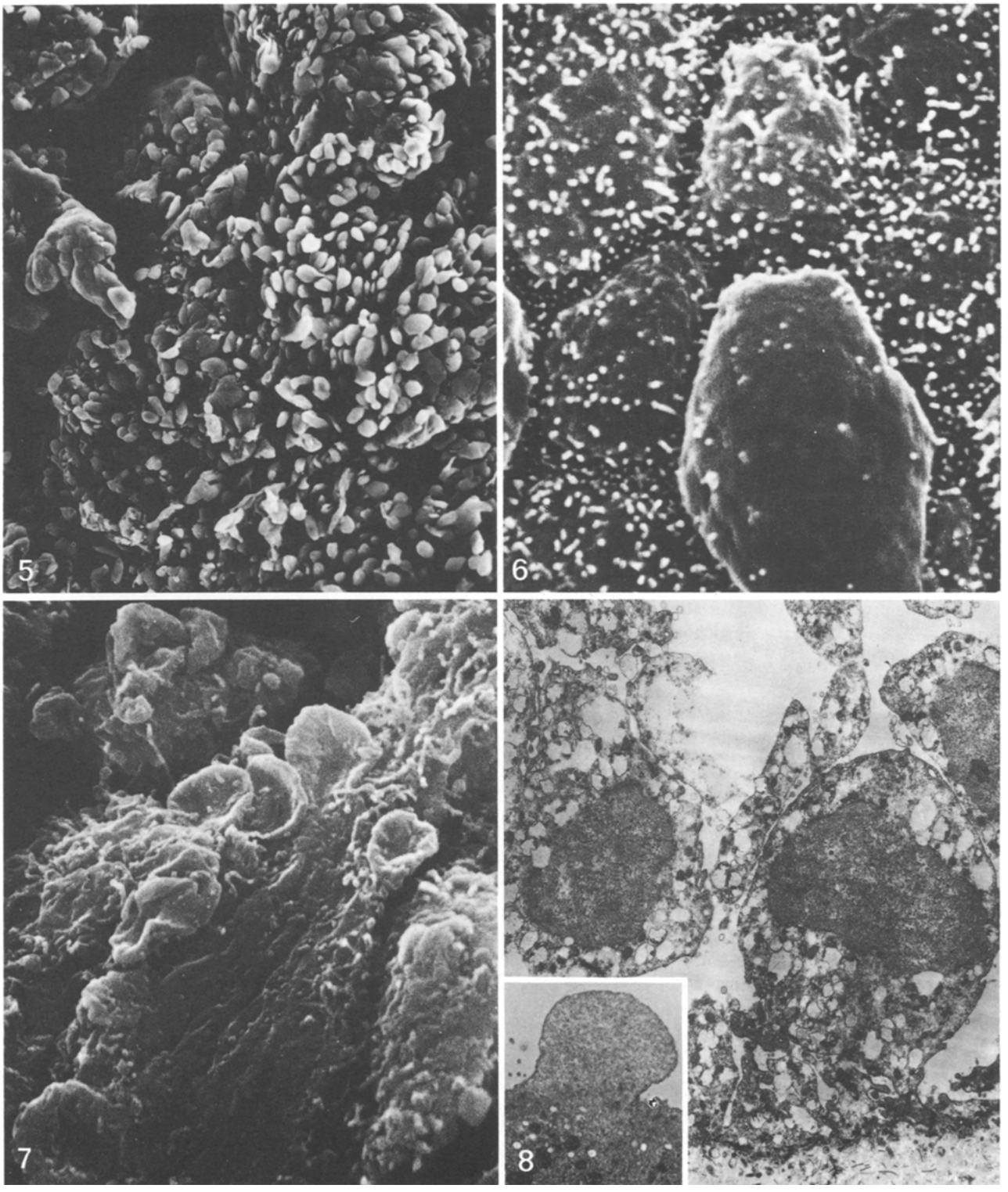

Fig. 5. A SEM picture of a tumour of Case 6 after treatment. The outermost cells are almost completely detached and the deeper cells are exposed to the lumen. x240

Fig. 6. A SEM picture of a tumour of Case 5 after treatment. The microvilli decrease in number on the residual outermost cells. x5,600

Fig. 7. A SEM picture of a tumour of Case 2 after treatment. An increase of large blebs and a decrease of cell processes are apparent in the deeper cells exposed to the lumen. x2,800

Fig. 8. A TEM picture of a tumour of Case 5 after treatment. Single layer of cells remains to attach the basal lamina. Other separated cells are accompanied by enlarged endoplasmic reticulum, condensed mitochondria, swollen and electron lucent nuclei as well as loss of cytoplasmic processes. x4,800. *Insert:* Case 4. A bleb is filled with an amorphous matrix. x7,200

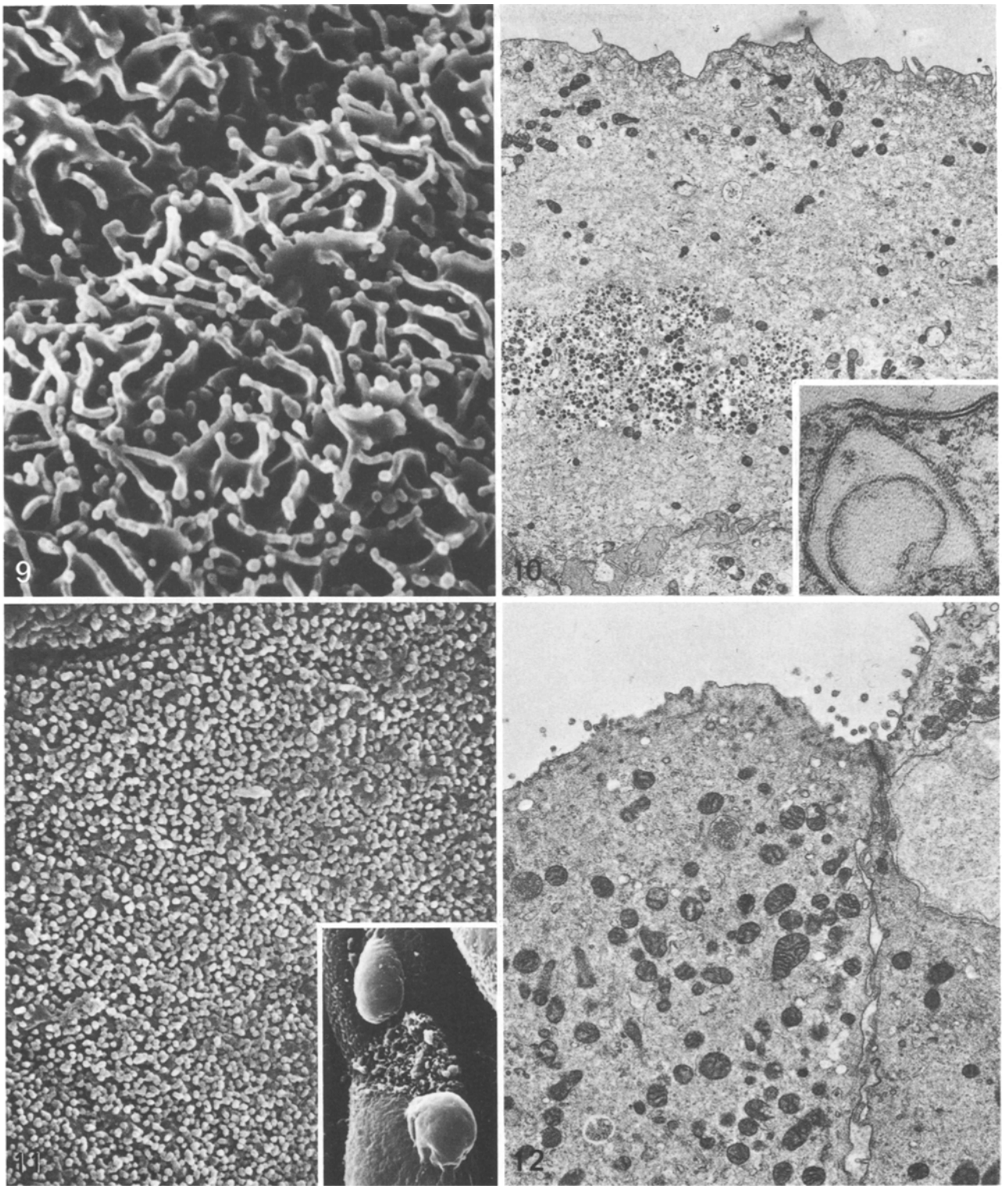

Fig. 9. A SEM picture of a non-tumorous mucosa of Case 2 before treatment. The numerous microridges with microvilli are noted on the luminal surface. x6,000

Fig. 10. A TEM picture of a non-tumorous mucosa of Case 1 before treatment. Microridges and concave plaques accompanied by occasional microvilli are found on the flat surface cells. x4,800. *Insert:* The membrane of luminal surface and discoid vesicle are obviously of asymmetrical unit membranes. x96,000

Fig. 11. A SEM picture of a non-tumorous mucosa after treatment. Surface of the ramining superficial cells is diffusely covered by microvilli (Case 2. x5,600), while that of intermediate cells appeared smooth with some blebs (*Insert:* Case 6. x2,400)

Fig. 12. A TEM picture of a non-tumorous mucosa of Case 4 after treatment. The superficial cells are cuboidal in shape. Loss of plaques and ridges as well as appearance of microvilli are apparent at the luminal plasma membranes. x9,600

The present study using both SEM and TEM revealed that the tumour cell surface was covered with many microvilli instead of plaques and microridges, being in accord with many reports [4–6, 10, 11, 14, 15]. The changes in bladder tumours after hyperthermic vesical irrigation with bleomycin were the desquamation of surface cells, a decrease of microvilli of the remaining outermost cells, a decrease of cell processes and an increase of large blebs of the deeply situated tumour cells and an enlargment of intercellular spaces. The presence of blebs in the bladder tumour cells has already been reported by Kjaergaard [5]. Trump et al. [17] observed many large blebs in Ehrich ascites tumour cells after cell injury. An increase of blebs, both in number and size, in the deeply located tumour cells would be an expression of cell degeneration. TEM observations verified the SEM findings and further demonstrated disorganized and destroyed organelles in the tumour cells. Thus, the study substantiated the morphological evidence that the treatment caused tumour cell autolysis and detachment.

The superficial cells of normal bladder mucosa are known to have microridges and plaques on their surfaces [10, 11, 14, 15]. Five of 6 non-tumorous areas were identified as being covered by normal superficial cells, while one non-tumorous mucosa had surface cells having numerous microvilli. The lesions could be a bladder dysplasia or carcinoma in situ since an increasing number of microvilli of various shape was found on the preneoplastic, dysplastic and neoplastic urothelium of animals [2, 3, 11] and humans [6, 10, 11]. Other possibilities are pleomorphic microvilli of an infected urothelium (unpublished data), or immature urothelial cells [4, 5].

This study, for the first time, showed the appearance of pleomorphic microvilli on the luminal surface of non-tumorous urothelium after anticancer treatment. Since the generation time of human bladder mucosal cells was reported to be about 6 days [16] and our standard therapy lasted about 30 days, the surface cells of non-tumorous areas were supposed to be replaced during the treatment. The presence of microvilli on these surface cells would be a result of stimulation by either one or both of hyperthermia and bleomycin. An accerelated cellular regeneration might also be a possible explanation since the microvilli were reported to appear in bladder cells after mucosal ulceration either by cyclophosphamide administration or freezing [2, 3]. Further studies are necessary to evaluate the significance of these findings.

Other cellular changes of non-tumorous mucosa were a slough of the superficial cells, enlarged intercellular spaces, bleb formation and vacuolation of intermediate and basal cells, cellular degenerations as well as reduced density of nucleoplasm. These changes were essentially similar to those of tumours after the therapy, although less severe in non-tumorous urothelium.

Biochemically, bleomycin exerts its antitumour activity through a non-enzymatical reaction with DNA and impairment of single strand scission [18]. Hyperthermia shows cytotoxic effects and inhibits cell growth of cultured human bladder tumour cells [7]. The mechanism of action has been found to be inhibition of oxygenation, increase of lysosomal activity and damage of RNA and DNA synthesis [13]. The tumour cells are more susceptible than the corresponding normal cells [1]. Combined therapy with bleomycin and hyperthermia has a synergic cytotoxic effect on tumour cells [7]. The topical application of high concentrations of bleomycin coupled with hyperthermia may cause the bladder cell death through the inhibition of DNA replication and direct toxic actions [7].

In conclusion, hyperthermic vesical irrigation with bleomycin induces cell sloughing and cell degeneration of the bladder tumour and results in tumour disappearance or reduction. The therapy, however, causes similar alterations in non-tumorous urothelium. Therefore, this treatment is effective but not specific to bladder tumours.

Acknowledgement. This work was supported in part by a grant from the Japanese Ministry of Education (No 557364) to M. Yokoyama.

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