Long-Term Organ Culture of Normal Human Bladder¹

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ABSTRACT

Normal adult human bladder obtained at cystoscopy has been maintained in long-term organ culture. Several media were tested for their ability to maintain viability and normal tissue morphology. The optimum medium was Ham’s F-12 nutrient mixture, supplemented with 10% fetal calf serum, hydrocortisone (1 µg/ml), and FeSO₄, (0.45 µg/ml). During the first 28 days in vitro, epithelial damage incurred at biopsy and during preparation of the cultures was repaired, and epithelialization of cut stromal surfaces occurred. A wave of cell proliferation was identified by [³H]thymidine autoradiography, 24-h labeling indices rising to a peak of up to 50% on the cut sides of the cultures between 7 and 21 days and falling to 0 to 5% by 21 to 28 days. The regenerating epithelium showed all the normal features of urothelial cell differentiation when examined by scanning and transmission electron microscopy. From 28 days, histology and scanning and transmission electron microscopy showed the cultured urothelium in most cultures to resemble closely that in the normal bladder in vivo, and in this mature state cultures were maintained for 100 days. Urothelium derived from certain patients, although showing normal surface maturation, developed enlarged intercellular spaces or intracellular mucin-containing acini. A study of the cytology of cells shed into the medium at different stages in culture showed that culture viability and epithelial differentiation could be monitored easily in long-term culture by this nondestructive means.

INTRODUCTION

It is well established that chemicals identified as carcinogenic in humans are generally carcinogenic in one or more species of laboratory animals but that the carcinogenic response may vary markedly between species (35). For example, for a given carcinogen, the target organs may differ; the histological type of tumor induced may differ; and metabolism, although qualitatively similar, may show considerable quantitative differences. Thus, there are many difficulties in extrapolating directly from animal experiments to humans, and it is clear that to assist interpretation of data, parallel human and animal models of carcinogenesis are required.

Cultured tissues provide appropriate model systems for such comparisons. There are several available tissue culture models for epithelial carcinogenesis in rodents, in which normal adult tissues treated with a chemical carcinogen in vitro can be seen to undergo a series of biological changes culminating in the acquisition of tumorigenicity by the epithelial cells (21, 23, 40, 42). Several adult human epithelial tissues have been maintained in organ culture for long periods (2, 3, 22, 39, 41), although to date there have been no reports of the long-term biological effects of in vitro carcinogen treatment on these tissues.

The urinary bladder is of particular interest in such comparative studies since it represents one of the few organs for which several human environmental carcinogens have been identified (for review, see Ref. 30), although these probably account for only a small proportion of tumors. The etiology of most bladder tumors is still unknown. Cancer of the bladder accounts for about 4% of all cancer deaths in the United Kingdom and United States, and of these deaths, probably less than 10% are attributed to exposure to identified bladder carcinogens. There is a significant association with cigarette smoking (1, 8) and a weak correlation with both coffee drinking (7) and consumption of artificial sweeteners (5, 19). Tissue culture models could provide appropriate systems in which to compare the effects of unknown chemicals with those of known human bladder carcinogens in parallel human and rodent tissues. An in vitro model should also provide an opportunity to study the pathology of transitional cell carcinoma in humans, the early stages of which are at present inadequately documented. In humans, bladder cancer is characteristically a multifocal disease in which recurrences may appear at disparate sites in the bladder over many years. Multiple recurrences of low-grade exophytic tumors may be controllable cystoscopically by diathermy, but undetected deep muscle invasion by a single tumor can lead to rapid death. Thus, the identification of markers of preneoplasia and/or neoplasia which could be used to aid prognosis would be of considerable clinical significance.

Rodent urinary bladder has been maintained for long periods both in organ culture (17, 36) and in tissue culture (42). In organ culture, the urothelium has been shown to maintain its normal differentiated morphology for up to 100 days (18). There have been few attempts to study human bladder in vitro and to date only one report of long-term culture (12). This showed that, when maintained in CMRL-1066 medium as rocked cultures, the urothelium reverted to an immature state in which the cells lacked the normal morphological features of membrane maturation characteristic of the bladder in vivo (32). Here, we describe a method for maintaining human bladder for up to 100 days in a morphologically normal state in organ culture. A preliminary report of this work describing short-term cultures has appeared elsewhere (24). This report represents part of a program to compare binding, metabolism, and long-term biological effects of chemical carcinogens in human and rat bladder organ cultures.

MATERIALS AND METHODS

Specimens. Normal tissue specimens were obtained from consenting patients undergoing cystoscopic investigation under general anesthesia. Only patients with no past or present neoplastic disease of the bladder and no prostatic hypertrophy or other cause of urine retention were used as donors. This choice is based on a histological assessment of over 200 biopsies, details of some of which have been published...

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(24, 33). Samples of bladder mucosa (approximately 5 sq mm) were removed as cup biopsies from an area posterolateral to the ureteric orifices. In bladders with focal inflammation, samples were taken from those areas in the bladder, away from the trigone, which were judged by the surgeon to be most "normal" by macroscopic appearance. Glycine solution (1.1%) was used in all cases for irrigation rather than distilled water. Specimens were transported to the laboratory in Waymouth’s Medium MB752/1 with 4-(hydroxyethyl)-1-piperazineethanesulfonic acid buffer ( Gibco-Biocult, Ltd., London, England) at 4° and were either cultured immediately or stored for up to 48 hr at 4°. Specimens stored at 4° shows no significant differences in long-term culture from specimens cultured immediately. Altogether, cultures have been maintained from more than 200 patients ranging in age from 18 to 85 years.

Culture. Details of the culture method are published (24). Briefly, tissue was dissected to yield explants (2 to 3 sq mm) consisting of urothelium and underlying stroma to a depth of 0.5 to 1 mm. Between 6 and 12 explants were obtained from tissue from each patient. These were placed on cellulose acetate filters (Millipore, Harrow, England) on steel grids in plastic organ culture dishes (Falcon Plastics, Oxnard, Calif.) containing approximately 3.5 ml medium.

Several media were tested for their ability to support the survival of human bladder tissue, namely, Waymouth’s Medium MB752/1, Ham’s F-10 and F-12 nutrient mixtures, CMRL 1066, Roswell Park Memorial Institute Medium 1640, Medium 199, Dulbecco’s modification of Eagle’s medium and NCI Medium (Gibco-Biocult, Ltd.). The following supplements were used alone or in combination: heat-inactivated fetal or newborn calf sera (Gibco-Biocult, Ltd.); human pooled Group AB serum; patient’s autologous serum; L-glutamine (2 mm); hydrocortisone sodium succinate (1 μg/ml); ascorbic acid (300 μg/ml); insulin (1 IU/ml); FeSO4 (0.45 μg/ml); pyruvate (1 mM); urea (0.05%); penicillin (100 units/ml); and streptomycin (100 μg/ml). The same batches of distilled water. Specimens were transported to the laboratory in Waymouth’s Medium MB752/1 with 4-(hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Gibco-Biocult, Ltd., London, England) and spun in a Shandon cytopin (Shandon, Camberley, Surrey, England) onto albumin-coated slides. All slides were stained by the Papanicolaou method.

RESULTS

Morphology of Uncultured “Normal” Human Bladder Biopsies

Histology. As described elsewhere (24, 32), the normal adult urothelium consists of 4 to 5 epithelial cell layers, showing a regular pattern of differentiation from small diploid basophilic basal cells through larger, predominantly tetraploid intermediate cells to large pale superficial cells of even higher ploidy. The basal lamina is flat, and there are no epithelial cells nests beneath the surface urothelium. Results presented here were obtained from organ-cultured tissue which showed all these normal histological features at the time of biopsy (Fig. 1).

Ultrastructure. Transmission electron microscopy confirmed previous findings with human and rat bladder (14, 31, 32). The basal cells are relatively undifferentiated and contain many free ribosomes. During maturation, there is a concomitant accumulation of cytoplasmic vacuoles and autophagic bodies, the development of an extensive Golgi apparatus, and, in human but not rat bladder, an accumulation of glycogen granules. All these features are most conspicuous in the mature superficial cells. The luminal membrane of the superficial cells is unique and is composed of thickened, rigid plaques of asymmetrical unit membrane separated by thinner "hinge" regions (Fig. 2). The apical cytoplasm contains numerous fusiform vesicles delineated by the same specialized membrane. The structure and function of this specialized membrane has been described in detail elsewhere (14, 16).

At the damaged edges of the cup biopsies and where one or more cell layers have been shed from the surface of the biopsy (Fig. 3), we have been able to study by scanning electron microscopy the surface ultrastructure of cells at different levels in the epithelium. The small basal cells have numerous small globular processes or blebs on their surfaces (Fig. 4). These processes should not be confused with true luminal microvilli, which are longer and show a central filament core in the transmission electron microscope (10). Immature intermediate cells possess larger numbers of these processes which gradually fuse to form globular chains on the surface of maturing intermediate cells (Fig. 5) and angular ridges which correspond to the hinge regions of the membrane on mature superficial cells (Fig. 6). The pattern of cell surface maturation is very similar to that described in vivo in the rat (17).

Culture Morphology

Choice of Culture Medium. The different basal media were tested initially in the presence of 10% fetal calf serum and no other supplements. Only Ham’s F-12 and Waymouth’s Medium MB752/1 gave satisfactory results. In both media, the epithelium at 28 days was immature but was as thick as or one cell layer less thick than the original epithelium. At later stages, the thickness of the epithelium tended to thin to 1 to 2 cells in these unsupplemented media.

Interpatient variation was an important factor in determining
tissue survival, such that tissue from some patients survived well in all media while that from others failed in all but the most fully supplemented. Absolute comparisons are therefore impossible, but relative effects of different supplements have been assessed by culturing from each individual patient at least one control explant in medium containing all supplements.

Glutamine, insulin, pyruvate, and urea had no significant effect on culture survival or morphology. The effects of FeSO₄, hydrocortisone, and ascorbic acid either alone or in various combinations in the presence of 10% fetal calf serum in Ham's F-12 or Waymouth's medium were studied during the first 28 days in culture. Each combination was tested on tissue from at least 8 patients. FeSO₄ (0.45 µg/ml) improved the speed and final state of epithelial maturation, but in the absence of other supplements the original thickness of the epithelium was not regained following damage at the time of biopsy, and in many cases the epithelium attenuated. In the absence of other supplements, hydrocortisone (1 µg/ml) had a striking effect on epithelial thickness, which rapidly returned to normal, and it also improved slightly the final degree of differentiation of the epithelium. Ascorbic acid inhibited maturation in both the presence and the absence of the other supplements, although it had no effect on epithelial thickness and caused a loss of normal epithelial polarity. Our preliminary findings (24) suggested that FeSO₄ and ascorbic acid inhibited the development of intraepithelial acini in some cultures. The present more detailed studies did indicate an improvement in some cultures, but the now recognized disadvantages of ascorbic acid clearly outweigh this slight advantage. No supplements had an effect on the development of enlarged intercellular spaces (see later) in some cultures. In Waymouth's Medium MB752/1 in the presence of FeSO₄, hydrocortisone, and 10% fetal calf serum, cultures were poorer than in Ham's F-12 medium similarly supplemented. Newborn calf serum, bovine serum albumin, and patients' autologous serum (at 10%) provided good alternatives to fetal calf serum up to 28 days. Bovine serum albumin and autologous serum have not been tested on long-term cultures, but newborn serum is less satisfactory than fetal calf serum. The medium of choice, therefore, is Ham's F-12 medium with FeSO₄ (0.45 µg/ml), hydrocortisone (1 µg/ml), 10% fetal calf serum, penicillin, and streptomycin.

**Histology under Optimal Culture Conditions.** During the first 2 days in culture, some degree of damage to the surface epithelium was apparent. The extent of damage varied from minor osmotic damage involving the development of extracellular spaces (Fig. 7), which was reversible and rapidly disappeared in all but the superficial cells, to more extensive damage involving the loss of one or more layers of epithelial cells. In explants with minimal damage, the superficial cells became necrotic during the first 2 days in culture. These dead cells (Fig. 8) sometimes remained attached to the upper surface of the cultures for 35 days. The majority, however, were lost rapidly. Epithelialization of the cut surfaces of the organ cultures was rapid, and by 24 hr cells of the basal layer began to migrate down the sides of the explant. By 48 hr, the cut surfaces were often completely covered by a single layer of epithelial cells. This process occurred more rapidly in small explants. In larger explants, bare areas of stroma in contact with the Millipore filter often persisted. The thickness of the epithelium on the newly epithelialized surfaces increased to 3 to 5 cells by 7 days. Sometimes, small irregularities in the base of the culture were filled by epithelium (Fig. 9), and where larger spaces existed between part of the explant and the filter an epithelium-lined lumen developed (Fig. 10). Where the epithelium was in direct contact with the filter, micropodia extended into the pores in the filter (Fig. 10). Fig. 11 shows an immature culture at 7 days. The appearance of the culture margin varied in different explants. Occasionally, a flat outgrowth one or more cells thick extended over the filter (Fig. 12). The most common appearances were a thickening of the epithelium (Fig. 13) or a piled-up rim of epithelial cells.

After 7 to 14 days, there was no further thickening of the epithelium even at the edges of the culture. A process of urothelial maturation followed, and by about 28 days the surface of the explant was covered by histologically mature superficial cells. Depending on the degree of damage sustained by the biopsies, the time taken for mature urothelium to redifferentiate varied from 14 to 30 days. The epithelium then persisted in this mature state for at least 100 days (Fig. 14).

Some variations in culture morphology appeared to be patient related. Thus, in some cultures, the intercellular spaces in the basal and lower intermediate cell layers enlarged, and some contained debris (Fig. 15). This feature, characteristic of tissue from certain patients only, occurred early in culture and remained for the duration of culture. Cultures from other patients developed rounded intraepithelial lumena which often contained a substance which stained metachromatically with toluidine blue, was Alcian blue positive, and was probably an epithelial mucin.

**Transmission Electron Microscopy.** Transmission electron microscopy showed the process of maturation to resemble closely that seen in vivo. During the first week, the undifferentiated cells on the epithelializing surfaces and in the basal layer of the surface epithelium resembled basal cells in vivo. The intermediate cells beneath the dead superficial cells on the surface showed signs of maturation characteristic of the intermediate cell in vivo including a well-developed Golgi apparatus and numerous smooth vesicles (Fig. 16). The surface epithelium was separated from the stroma by an intact basal lamina, but no basal lamina was produced at the interface between the micropodia of the lowest epithelial cells and the Millipore filter. From Day 7 onward, the epithelium showed all the features of in vivo maturation. Increased numbers of lysosomal bodies characteristic of normal urothelial cells and thought to be concerned in vivo with membrane turnover (13, 14) appeared in the upper intermediate and superficial cell layers (Fig. 17), and single particles of glycogen were also observed (Fig. 18). From 7 to 14 days onward, the most mature cells in contact with the medium gradually developed fusiform vesicles and asymmetrical membrane plaques (Figs. 17 and 18). Variations in the time scale of maturation were more readily detectable by transmission electron microscopy than by histology, and it was clear that tissue from certain patients matured much more rapidly than that from others, regardless of initial damage, and this did not appear to be related to age or sex. The rate of maturation was decreased by the retention of dead superficial cells, and in such explants mature cells were usually visible on the newly epithelialized sides of the cultures before they appeared on the upper surface. Ultimately, mature epithelium covered the top and sides of the explants, any piled-up rim around the explants, and the free surface of stroma-supported epithelium over the lumena beneath the explant. In cultures
which developed intraepithelial lumena, these were surrounded by cells with glycocalyx-covered microvilli at their free surfaces and well-developed junctional complexes at their lateral acinar junctions. The lumena contained a finely granular material (Fig. 19). Although epithelium in direct contact with stroma matured normally, those cells which formed an outgrowth over the filter or were separated from the stroma by a lumen remained immature (Fig. 20).

**Scanning Electron Microscopy.** The surface membrane changes on cells in contact with the media were analyzed in detail by scanning electron microscopy. The changes observed were directly analogous to those seen during in vivo maturation. During the first few days in culture, dead and dying superficial cells masked the upper culture surface, but when these had sloughed, small immature cells similar to basal cells in vivo could be seen (Fig. 21). As these cells matured from about 7 days onward chains of fusing globular processes characteristic of the in vivo maturing intermediate cells were seen (Fig. 22). These cells eventually resembled mature intermediate cells (Fig. 23) and/or mature superficial cells of the contracted bladder in vivo (Fig. 24). It was apparent from scanning electron microscopy studies that maturation proceeded independently in different cells on the explant so that cells at different stages of maturation could be seen on the surfaces of histologically “mature” cultures (Fig. 25). By 21 to 35 days, the mature forms predominated. Although cultures from all patients showed evidence of membrane maturation, there were often striking differences between in the numbers of globular processes and ridges produced.

**Culture Cytology**

The cytology of smear preparations from biopsies or cultures was compared with cells harvested from the culture medium at different times in vitro. Fig. 26 shows a typical smear preparation from a biopsy. These contained many superficial cells, some binucleate, with typically “foamy” cytoplasm and occasional large pale cytoplasmic inclusions. The size and nuclear characteristics of these mature cells varied in different patients. Smaller cells from the intermediate and basal cell layers were also present, and many of these showed long cytoplasmic processes suggesting attachment of cells at different levels in the epithelium to the basal lamina as described by Petry and Amon (34).

Smears prepared from early cultures showed that the superficial cells had been lost (Fig. 27) and cells of intermediate and basal-type predominated. Later, larger cells were present, and by 14 to 21 days some cultures had binucleate superficial-like cells (Fig. 28). The culture medium at all stages contained cells in various stages of degeneration. Many cultures were lost in the early stages, and small sheets of cells were often recovered from the medium (Fig. 29). If cultures were left in the same dish, many of these cells remained, even after several medium changes. By changing the dish at each medium change, it was possible to obtain some viable cells, and medium cytology proved to be a good indicator of culture viability. Absence of cells in the medium was a reliable indication of culture death. A sudden marked increase in the number of recovered cells usually indicated damage to the culture, e.g., by drying out, and was almost inevitably a precedent of culture death.

**Cell Turnover**

Tritiated thymidine labeling indices were measured over 24-hr periods up to 42 days in culture. A large interpatient variation was observed in the numbers of labeled cells per culture, but the overall pattern of labeling was similar in all cases.

On the sides and base of the cultures, no labeled cells were observed on Day 1. Labeling indices then rose to a peak (5 to 50%) between 7 and 21 days and fell to low levels once epithelialization of the cut stromal surfaces was complete. The surface epithelium followed a similar pattern of labeling during the first 21 days in culture, although the peak labeling index was lower (1 to 5%), unless there had been considerable initial damage to the tissue. Occasional stromal fibroblasts were labeled during the first 21 days.

After the initial wave of cell proliferation during repair of the epithelium and epithelialization of the cut stromal surfaces, labeling indices on the top and sides of the explants fell and remained low (0 to 5%) for the rest of the period of study. The epithelium under the base of the explants showed more widely varying indices (up to 10%) throughout the period of study.

**DISCUSSION**

The present study demonstrates that normal adult human urothelium supported by stroma can be maintained for long periods in organ culture in a morphological state which closely resembles that in vivo. We have chosen to use an organ culture rather than a cell or tissue culture system as our model for carcinogenesis studies, since normal tissue architecture is maintained, and stromal and epithelial cells remain in their normal relationships. Thus, any tissue interactions which may occur during carcinogenesis and the pathogenesis of bladder cancer in vivo can in theory be investigated in this organized tissue in vitro. These studies demonstrate that the complex process of urothelial cell differentiation and maturation in vivo is paralleled in organ cultures maintained under the conditions defined here, unlike the situation in epithelial monolayer cultures derived from the same tissue. Isolated rat bladder epithelial cells grown on plastic substrates also fail to differentiate normally, although some degree of stratification and membrane maturation has been described in long-term rat urothelial cell cultures on collagen discs (6).

The histology, transmission, and scanning electron microscopy and cytology of cells shed from the culture surface all indicate a qualitatively normal process of cell maturation in vitro. A comparison with the time course of differentiation in vivo is difficult. It has been reported by Lund (28) that regeneration of stripped urothelium in humans takes about 6 weeks, but it is not clear whether the regenerated epithelium had fully differentiated in that time or was still immature. Our labeling studies show that a wave of cell proliferation during the process of epithelialization and repair is followed by a return to a low rate of cell turnover, although our recorded labeling indices of 0 to 5% for a 24-hour labeling period are higher than those observed in vivo in rodents (9, 26, 27, 29) and probably higher than occur in vivo in humans.

3 M. A. Knowles, unpublished observations.
One of the major difficulties in interpreting results obtained with human tissue lies in the selection and assessment of the starting material. Many patients from whom bladder tissue is readily available and who therefore have been used in numerous previous published studies of normal human bladder have conditions such as prostatic disease which directly or indirectly affect the morphology of the urothelium. It is clear that absence of clinical disease in the bladder is no guarantee of a normal urothelium. On the contrary, we have shown a wide range of symptom-free histopathological conditions to exist in patients with no specific bladder disease and in whom the mucosa appears macroscopically normal at cystoscopy (24, 33). Variability in starting material derived from human bronchus and endocervix has also been described (3, 39), indicating the need for rigorous selection procedures and time zero assessment of all human tissues. A further source of bladder tissue variability arises from the damage caused by cystoscopy fluids. This may be mild (e.g., necrosis of superficial cells or transient edema) or severe (e.g., loss of more than one layer of cells) and depends both on the length of the investigation and on the irrigation fluid used at cystoscopy (24, 43). The effects of this damage may be more than merely morphological. It has been reported (25) that the intravesical instillation of saline or water into rats increased the [3H]thymidine labeling index 15- and 100-fold, respectively. Such effects, while not affecting the final state of differentiation reached by the tissue, may explain our observed variability in labeling index early in culture.

The present studies confirm the remarkable regenerative capacity of the urothelium (for review, see Ref. 14) which enables it to epithelialize cut stromal surfaces and rapidly repair cystoscopy-induced damage. The process of epithelialization resembles the process of epiboly described in epidermal organ cultures (38). The role of the various media supplements in contributing to the final attainment of normal epithelial thickness and maturity is not clear. Ascorbic acid and Fe²⁺ ions are necessary factors in collagen synthesis (20) and were included in a supplemented Waymouth's Medium MB752/1 for rat bladder organ cultures (17). We tested these supplements in our human cultures since collagen synthesis in the stroma might be involved in maintaining the normal stromal-epithelial interrelationship. FeSO₄ clearly improved the maturity of the cultures, although our earlier suggestion (24) that it inhibited the formation of intraepithelial lumena has not been confirmed. The effect of ascorbic acid in inhibiting maturation of these human cultures was surprising since the same concentration of ascorbic acid has been used in the maintenance of rat bladder organ cultures in a fully differentiated state for up to 100 days (17). However, the nutrient requirements of rat bladder in vitro clearly differ from those of human bladder. In Ham's F-12 medium in the absence of serum, rat bladder epithelium has been reported to undergo hyperplasia which can be prevented by hydrocortisone (37). By contrast, hydrocortisone is required to maintain epithelial thickness in human bladder cultures.

The epithelial abnormalities that we have observed, namely, dilated intercellular spaces persisting throughout the culture period and the development of intra- and intercellular acini containing an epithelial mucin, appear to be related to individual patients rather than to the conditions of culture. These features, although not present in the original biopsies, developed rapidly and persisted in culture. It is possible that the dilated intercellular spaces, which occurred in some cultures predominantly between basal and lowest intermediate cells, represent defective repair of tissue damage incurred at the time of biopsy. The mucin-containing acini do not resemble the classical appearances of mucous metaplasia of the urothelium as seen in vivo (15) but may represent the in vitro expression of this condition. Epithelial metaplasia has been noted in other human organ culture systems. Thus, in bronchial epithelium, nonkeratinizing and occasionally keratinizing squamous cells were reported after 3 months in vitro (3); and in endocervix, squamous metaplasia developed progressively from 4 weeks in culture (39). It seems likely that further improvements in culture conditions will delay or prevent such changes both in bladder and other tissue organ cultures.

The present studies were undertaken to provide base-line data for future in vitro carcinogenesis studies on human bladder tissue. It is clear from work with rodent epithelia in vitro that the latent period for epithelial transformation is considerably longer than for mesenchymal cell transformation (11, 23, 40, 42). It is important, therefore, that normality, as defined by as many parameters as possible, should be maintained in long-term control cultures. This is vital in experiments using human tissues where very long in vivo tumor latent periods may be reflected by correspondingly long latent periods in vitro. This study demonstrates that normal human bladder may be maintained in vitro in a normal mature state for many weeks and indeed to our knowledge for longer periods than have been described for any other human tissue to date.

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REFERENCES

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Fig. 1. Urothelium of a normal human bladder biopsy showing large pale superficial cells and smaller, more darkly staining intermediate and basal cells. Toluidine blue, × 340.

Fig. 2. Transmission electron micrograph of the urothelial surface of a normal human bladder biopsy. The scalloped profile of the luminal membrane indicates the presence of rigid asymmetrical unit membrane plaques, and the cytoplasm contains numerous multivesicular bodies and glycogen granules. Uranyl acetate-lead citrate, × 4,800. Inset, detail of asymmetrical membrane plaque. × 68,800.

Fig. 3. Scanning electron micrograph of a normal biopsy from a 31-year-old male. The large polygonal superficial cells present a flat pavement-like surface to the urine, characteristic of the normal distended bladder. Areas where superficial cells have desquamated reveal the smaller underlying intermediate cells. × 500.

Fig. 4. Basal cells in a human bladder biopsy. Small globular processes are present in various numbers on the cell surfaces, × 5,800.

Fig. 5. Maturing intermediate cells in a human bladder biopsy. Chains of globular processes and microridges cover the cell surface, × 5000.

Fig. 6. Mature superficial cells on the surface of a human bladder biopsy. The membrane is thrown into angular folds. Deep clefts between adjacent cells indicate that the urothelium was in a contracted state when fixed, × 3,000.

Fig. 7. One-day culture showing early reversible tissue injury. Superficial epithelial cells have been lost, and intercellular spaces are dilated. Toluidine blue, × 130.

Fig. 8. Urothelium on the surface of a 7-day culture showing dead, darkly staining superficial cells attached to the living cells beneath. Toluidine blue, × 130.

Fig. 9. Detail of the base of a culture showing a crevice between the cut stromal surface and the Millipore filter support which has been filled by epithelial cells. Toluidine blue, × 100.

Fig. 10. Base of a culture showing cytoplasmic processes extending from the epithelial cells into the filter support. The epithelium has formed a small lumen beneath the culture. Toluidine blue, × 130.

Fig. 11. Section of an entire, 7-day immature human bladder organ culture. Toluidine blue, × 60.

Fig. 12. Edge of a culture with a small epithelial outgrowth. Toluidine blue, × 70.

Fig. 13. Edge of a culture with a thickened rim of epithelial cells. Toluidine blue, × 70.

Fig. 14. Mature surface urothelium on a 100-day culture. Toluidine blue, × 375.

Fig. 15. Surface urothelium with enlarged intercellular spaces, some containing debris, between basal and intermediate cells in a mature 28-day culture. Toluidine blue, × 375.

Fig. 16. Transmission electron micrograph of the surface of a 14-day culture showing a dead superficial cell overlying a viable cell which has a well-developed Golgi apparatus and numerous apical smooth vesicles. Uranyl acetate-lead citrate, × 10,000.

Fig. 17. Urothelium from a mature 28-day culture. The luminal membrane shows rigid plaques, and the cells contain many lysosomes, a well-developed Golgi apparatus, and glycogen deposits, characteristic of human bladder in vivo. × 6,500.

Fig. 18. Detail of mature surface cells. The apical cytoplasm contains fusiform vesicles bounded by asymmetrical membrane, a junctional complex is present between adjacent superficial cells, and glycogen granules can be seen in the cytoplasm. × 54,500.

Fig. 19. Intraepithelial lumen. The cells have well-developed junctional complexes and stubby, glycocalyx-covered microvilli at their free surfaces. × 9,500.

Fig. 20. Epithelial cells on the Millipore filter in a 28-day culture. These cells are separated from stroma by a lumen. The cell surfaces remain immature and have scattered, stubby microvilli. × 7,100.

Fig. 21. Scanning electron micrograph of surface cells on a 14-day culture. The cell surfaces are covered by small globular processes. × 4,700.

Fig. 22. Maturing intermediate cells on a 21-day culture. A few single globular processes, some chains of fusing globules, and many microridges can be seen. × 6,200.

Fig. 23. Mature intermediate cells on a 28-day culture. Most globular processes have fused to form microridges. × 5,000.

Fig. 24. Mature superficial cells on a 28-day culture. × 2,900.

Fig. 25. Maturing urothelial surface after 21 days in vitro. The larger, more mature cells are interspersed in places with smaller less mature cells. × 1,200.

Fig. 26. Smear preparation from a normal bladder biopsy. Large superficial cells with one or 2 nuclei can be seen. Smaller, intermediate, and basal cells are also present, many showing a characteristic cytoplasmic “tail,” indicative of deep attachment to the basal lamina of cells from different layers in the urothelium. Papanicolaou stain, × 375.

Fig. 27. Smear preparation from a 4-day culture. Cells are of intermediate and basal cell size. Papanicolaou stain, × 375.

Fig. 28. Smear preparation from 14-day culture showing mature superficial-like cells. Papanicolaou stain, × 375.

Fig. 29. Cells recovered from the medium of a 2-day culture. A sheet of cell “ghosts” shed from the epithelium at the time of initiation of the culture and several small basal-type cells can be seen. Papanicolaou stain, × 375.

Fig. 30. Cells recovered from the medium of a 14-day culture. Most of the cells are of intermediate cell size and show a range of nuclear degenerative changes. Papanicolaou stain, × 375.

Fig. 31. Mature, superficial-like cells recovered from the medium of a 21-day culture. Papanicolaou stain, × 375.
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