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Culture and characterisation of human urothelium in vivo and in vitro

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Abstract The aim of this study was to culture human urothelium and generate enough cells for subsequent reconstructive surgery. Using a modification of the Rheinwald-Green method for the routine culture of keratinocytes from patients with burns, we successfully cultured 91% of 57 biopsies from the renal pelvis, ureter, bladder and urethra of paediatric patients. The cells could be split one to three up to 9 times at 7–10 day intervals, giving a surface area of 1000 cm^2 after a 2 month culture period. Primary cultures could not be initiated in defined medium MCDB153, although cells initiated using the Rheinwald-Green method could subsequently be propagated in this medium. Cytokeratin patterns in vitro were similar to those in vivo in the expression of keratins 7, 18 and 19 (characteristic of simple epithelia) and keratin 13 (characteristic of non-cornified stratified epithelia). Cultured urothelium also expressed keratin 14 (characteristic of cornified stratified epithelium) in about 25% of cells and keratin 16 (characteristic of fast-growing cells). These findings indicate that urothelial cells can be propagated in vitro for autologous grafting, and the next step is to identify substrates suitable for urothelial cell growth and differentiation and surgical manipulation.

Key words Urothelium · Primary cell culture · Defined medium · Epidermal growth factor · Cytokeratins

Urothelial cells derived from urine, bladder washings or biopsies have been maintained in vitro using a variety of tissue culture conditions [6, 10, 12, 27, 30]. The aim of this study was to identify culture conditions in which urothelial cells could be grown routinely from patients with the long-term goal of using the cultured cells for reconstructive surgery.

Three culture methods of differing levels of complexity were chosen, based on experience of the culture of keratinocytes. The simplest system was urothelial cells grown on plastic in serum-free defined medium MCDB 153 supplemented with bovine pituitary extract. This medium contains low levels of calcium, which are known to influence the degree of differentiation of keratinocytes in vitro [3]. Secondly, we used a modification of the system developed by Rheinwald and Green [29, 33] for the bulk propagation of keratinocytes from the epidermis and other stratified epithelia, which not only supports long term culture through multiple passages but also permits some features of keratinocyte differentiation such as keratin expression, stratification and the production of squames. In addition to the presence of irradiated Swiss 3T3 feeder cells, the additives were epidermal growth factor (EGF), hydrocortisone, transferrin, cholera toxin, liothyronine, adenine and foetal calf serum (FCS). Thirdly, urothelial cells were cultured on contracted collagen gels containing fibroblasts derived from various sites in the urogenital tract [2].

Urothelium is a highly specialised tissue lining the mammalian urinary tract, and is described as a transitional epithelium, sharing features of both stratified squamous and simple columnar epithelia. In the relaxed state it is 4–8 cell layers thick. The basal layer is composed of cuboidal cells, the intermediate layers are polygonal and the superficial cells are large "umbrella" cells containing fusiform vesicles, an asymmetrical "hinged" plasma membrane and a *zonula occludens* [11]. The urothelium must not only be able to expand as the bladder fills, but also protect the underlying tissue from urine.

The major structural proteins of all epithelial cells are keratins. These are separated into two biochemical families (the acidic and basic keratins) and two gene families (Type I and II), each keratin being the product of a distinct gene [8]. At least 19 keratin polypeptides have been found

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in human epithelia [8]. The combination of these proteins expressed by a cell can be characteristic of specific epithelial type or tissue specific differentiation [4]. This study reports the expression of multiple keratins in urothelial cultures derived from the kidney pelvis, bladder mucosa and ureter under various conditions, in comparison with staining patterns in the urogenital tract in vivo.

Materials and methods

Samples

For primary culture surgical specimens of kidney pelvis, ureter and bladder mucosa were collected in DMEM (Gibco, Paisley, UK) supplemented with 10% FCS (Gibco), 100 IU/ml penicillin (Sigma, Poole, UK), 100 μg/ml streptomycin (Sigma) and 2.5 μg/ml amphotericin (Gibco) and stored at 4°C. Fifty seven samples were obtained from 43 patients (aged between 6 months and 12 years) undergoing surgery for conditions including hypospadias, ureteric implant, bladder augmentation, and nephrotomy. The samples were processed within 48 h of surgery, although in most cases they were processed immediately.

Primary urothelial cell cultures

The specimens were minced into 1–2 mm^3 fragments and incubated in 10–15 ml of 0.25% trypsin (Gibco, Paisley, UK) in 0.02% ethylene diammine tetraacetic acid (EDTA) (Gibco) at 37°C. After 30 min incubation the tissue was filtered through a stainless steel mesh (approximately 1.0 × 0.5 mm pore size across the two diameters) and the filtrate collected in an equal volume of DMEM/10% FCS (Gibco) to neutralise the trypsin, centrifuged at 70 g for 5 min, and the resulting cell pellet resuspended in Rheinwald-Green medium [29, 33]. The tissue remaining on the mesh was reincubated as before and the process repeated three times to give a total of 4 separate samples.

The urothelial cells were plated in a 3:1 mixture of Ham's F12:DMEM (Gibco) supplemented with 10% FCS, 0.4 μg/ml hydrocortisone (Sigma), 4 μg/ml cholera toxin (ICN-Flow, High Wycombe, UK), 5 μg/ml transferrin (Sigma), 13 ng/ml liothyronine (Koch Light, Suffolk, UK), 24 mg/ml adenine (Sigma) and 20 ng/ml epidermal growth factor (Sigma). The modifications from the original descriptions of this medium [29, 33] were the doubling of the concentration of FCS from 5 to 10% and the exclusion of insulin. The 4 aliquots of urothelial cells obtained from each sample were plated in a T25 flask (Gibco) with 5×10^5 lethally irradiated Swiss 3T3 feeder cells.

Fibroblasts

The minced tissue left after serial trypsinisation to produce urothelial cell cultures was used as the starting material. About 10 pieces of tissue were placed on a tissue culture petri dish, left to adhere for 30 min and then covered with DMEM with 10% FCS and incubated at 37°C in 8% CO_2. The medium was changed weekly and the cultures examined for growth. When the cells became confluent they were detached by exposure to 0.05% trypsin in 0.02% EDTA for 2–5 min at room temperature, washed and plated in DMEM/10% FCS.

Feeder cells

Swiss 3T3 cells were maintained in DMEM/10% FCS. The cultures were passaged twice weekly when approximately 90% confluent at a ratio of 1 to 10 using 0.05% trypsin in 0.02% EDTA. For use as feeders the cells were trypsinised and the cell suspension exposed to 6000 rads from a ^{137}Cs source (Gammacell 100, Atomic Energy of Canada Limited) at room temperature, washed, resuspended in DMEM/10% FCS, counted, stored at 4°C and used within 24 h.

Maintenance of primary cultures and production of cell lines

Defined Medium

First passage cells grown in the modified Rheinwald-Green medium were transferred – following washing in MCDB153 to reduce calcium levels – to MCDB153 (Clonetics Corporation, San Diego, Calif.) supplemented with 60 μg/ml bovine pituitary extract (Clonetics) at 37°C in 8% CO_2. The medium was changed twice weekly and the cultures monitored for growth. The cells were passaged approximately every 3 weeks when 80–90% confluent using 0.05% trypsin in 0.02% EDTA for 10–15 min at 37°C, and split at a ratio of 1:3.

Modified Rheinwald-Green medium

The cells were kept at 37°C in 8% CO_2 and media-changed twice weekly. The cells were passaged as described above at approximately two weekly intervals and replated with fresh lethally irradiated 3T3s.

Contracted collagen gels

For the preparation of collagen, rat tails were collected and stored at −20°C. The tendons were removed, finely minced in 5 ml of 70% ethanol (Hayman, Witham, UK) and then transferred to glacial acetic acid (Sigma labgrade, Poole, UK) diluted 1:1000 in double distilled water (250 ml/tail) for 48 h at 4°C. The solution was clarified by centrifuging at 37,000 g for 1 h, the supernatant removed and neutralised with one-sixth the volume of 0.1 M NaOH (Sigma) and gently mixed on a magnetic stirrer for 90 min at 4°C. The solution was then centrifuged at 8000 g for 20 min at 4°C and the pellet resuspended in 1:1000 glacial acetic acid. The protein concentration in the collagen solution was measured using the method of Lowry [16] and adjusted by the addition of 1:1000 glacial acetic acid to 2–5 mg protein/ml.

Contracted collagen gels were produced by seeding collagen with fibroblasts prior to gelling, as described by Bell [2]. Fibroblast cultures from ureter or Swiss 3T3s were harvested using 0.05% trypsin in 0.02% EDTA and resuspended at a density of between 1–1.5 million cells/ml. Under aseptic conditions the following solutions were added to a 100 mm bacteriological petri dish (Sterilin, BDH, Poole, UK) in this order: 3 ml double strength DMEM (Gibco), 6 ml FCS (Gibco), 1 ml cell suspension, 3 ml collagen solution and 0.7 ml 0.1 M NaOH. The gel was allowed to set for 10 min before transfer to a 37°C incubator containing 8% CO_2. After 12 h the gels were washed 3 times with DMEM plus 10% FCS and seeded with an inoculum of 1.5×10^6 urothelial cells in modified Rheinwald-Green medium by placing a stainless steel annulus with an interior diameter of 5 mm on top of the gel and adding the cell suspension to the central hole. The annulus was left in place for 48 h and then removed to allow the gel to float freely in culture medium. The gels were assessed for growth every 2–3 days under the conditions described above and maintained for a maximum of 3 weeks.

Effect of mitogens on cell proliferation

Prior to measuring cell proliferation second passage cells were transferred to a minimal medium consisting of DMEM:Ham's F12 (3:1) with 2% FCS. The cells were then trypsinised, washed and viable cells estimated using trypan blue exclusion. The cells were then plated in 6 well plates at a density of 5×10^5 cells/well with 10^5 lethally irradiated Swiss 3T3 feeder cells. To each well one of the following mitogens: EGF, cholera toxin, liothyronine, hydrocortisone, trans-

ferrin or insulin, was added to give the concentration used in the Rheinwald-Green formulation. At 48 h intervals thereafter until 10 days the number of urothelial cells per well was determined by selectively removing the feeder cells by a 5 min incubation in EDTA and then the urothelial cells were released with trypsin/EDTA and clumps disaggregated by repeated passage through a 25 g needle (Gillette Surgical, Isleworth, UK). The number of cells was counted on a haemocytometer. The experiment was carried out with 3 samples per time point and the mean of the cell count plotted aginst time.

Colony forming ability

Freshly derived primary urothelial cells were counted on an haemocytometer using trypan blue (Gibco) exclusion to determine the proportion of viable cells, and seeded out on a preformed layer of lethally irradiated Swiss 3T3 fibroblasts in a tissue culture plate at densities of 10^2, 10^3 and 10^4 cells/plate. The cultures were allowed to grow for 10–14 days, fixed and differentially stained for urothelial cells using Rhodanile blue (ICN-Flow). Colonies were counted and expressed as colony forming units per 1000 cells.

Population doubling time

First passage urothelial cells were plated in six well tissue culture plates (Nunc, Gibco) at a density of 5×10^5 cells/well with 10^5 lethally irradiated Swiss 3T3 feeders. At 48 h intervals until 10 days the number of urothelial cells per well was determined by selectively removing the feeder cells with 0.02% EDTA for 5 min. The urothelial cells remained firmly attached and were then released with 0.05% trypsin in 0.02% EDTA and any clumps disaggregated by passaging approximately 5 times through a 25 g syringe needle and the cell number counted on a haemocytometer. The mean of the triplicate readings for each time point was calculated and plotted as a function of time. From the graph produced the doubling time was read directly.

Immunocytochemistry

Fresh tissue

Tissue specimens obtained within 5 min of surgery were snap frozen in liquid nitrogen and stored at −70°C. Six samples from each of the 4 sites studied (renal pelvis, ureter, bladder and urethra) were obtained from 15 patients aged between 18 months and 12 years. 6 µ sections were cut using a Reichert-Jung cryostat at −17°C and transferred to glass slides. Parallel sections were stained with HE.

Cell cultures

Passage 2 cells from 6 different samples from 3 sites (renal pelvis, ureter and bladder) were cultured on 100 mm petri dishes in both MCDB 153 with the bovine pituitary extract and under the modified Rheinwald-Green conditions. When the cultures were 60–70% confluent they were washed once in PBSA, fixed in a 1:1 mixture of methanol (BDH) and acetone (Sigma), washed 3× in PBSA and kept at −70°C until stained.

Contracted collagen gels

These were processed for immunohistochemistry after 14 days culture. The gels were washed in PBS and taken through a series of increasing concentrations of sucrose in PBSA; 30 min each in 5%, 8.75%, 12.5%, 16.25% sucrose and then overnight in 20% sucrose at 4°C. The gels were taken into tissue embedding medium (Tissutek, Lab-Tek, USA); 30 min each in 3:1 20% sucrose:Tissutek, 1:1 20% sucrose:Tissutek, 1:3 20% sucrose:Tissutek and finally 30 min in Tissutek. The gel was then frozen in a bath of isopentane (BDH) cooled by a mixture of frozen CO_2 and methanol and stored at −70° C. The gels were cut in 6 µm sections with a Reichert-Jung cryostat at −22°C on to glass slides.

Indirect immunofluorescence

The slides were air-dried for 30 min and 50 µl of a 1:200 dilution of the primary antibody [9, 13, 14, 22, 24, 26, 32] in DMEM with 5% FCS and 5% human serum applied for 1 h. The slides were washed in tap water for 5 min and 50 µl of a 1:50 dilution of FITC-conjugated mouse IgG antibody (Dako, High Wycombe) was applied for 1 h. The slides were washed for 10 min in tap water, counterstained with 1 mg/ml propidium iodide (Sigma) for 5 min and mounted in Univent (Gurr microscopy materials, BDH) and scored with a Zeiss Photomic 3 fluorescence microscope. Murine anti-vimentin PK-V antibody was used as a negative control and CAM5.2 (hybridoma provided by CA Makin, ICRF) as a positive control for simple epithelia [17, 31].

Results

Primary cultures were obtained using the modified Rheinwald-Green procedure in 52/57 (91%) of samples. These included 11/11 renal pelvis, 29/31 ureter, 11/13 bladder and 1/2 urethral biopsies. The 5 failures were due to bacterial contamination in 3 cases and for unknown reasons in 2 cases.

The primary cultures grew slowly at first, becoming confluent after 3 to 4 weeks. The cells grew either as closely packed irregular polyhedrons (Fig. 1) or more diffuse long fusiform cells, both types growing as discrete colonies. Approximately one-third of cultures contained both morphologies; the morphologies were interchangeable on passage and keratin staining patterns were identical. No differences in morphology were seen in the cultures from the different sites. These cultures could be passaged at 7–10 day intervals up to 9 times over a 2 month period, corresponding to an expansion of more than 2000 fold over at least 25 generations. Maintaining the cells without passage resulted in cultures 2–4 cell layers thick, although there was no apparent difference in morphology between the cell layers. After 7–9 passages the cells began to senesce. The cells had a more ragged appearance with an increased surface area, a higher cytoplasm to nucleus ratio, cytoplasmic vacuolation and either failed to settle or divide following passage.

Three unsuccessful attempts were made to produce primary cultures in MCDB153. However, passage 1 cells were grown in this medium from 10 cultures initiated using the modified Rheinwald-Green procedure. The cells grown in MCDB153 tended to grow either separately or were only loosely attached to each other and did not form colonies (Fig. 2). The cells grew relatively slowly as judged by time taken to reach confluence and could be passaged after 10–15 days up to 4 times.

The cells grown on contracted collagen gels were difficult to assess microscopically due to the opacity of the

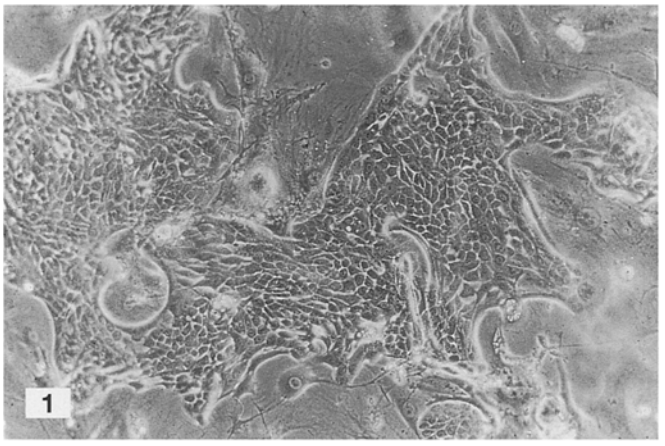

Fig. 1 Primary culture of bladder urothelium after 10 days in vitro showing a colony of tightly packed polygonal cells growing in modified Rheinwald-Green medium

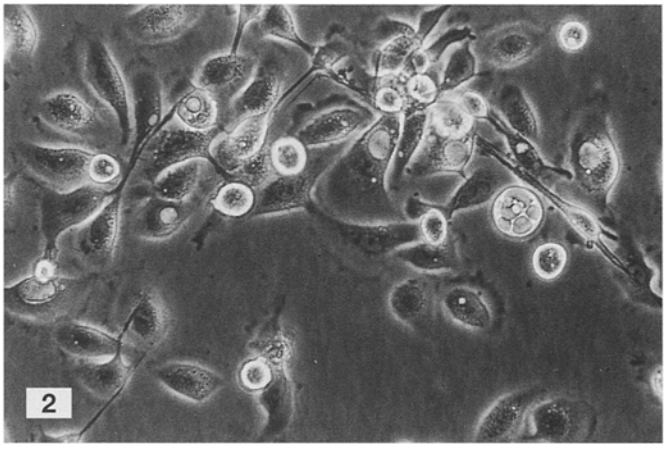

Fig. 2 Second passage kidney pelvis urothelial cells growing in MCDB153 medium supplemented with 70 µg/ml bovine pituitary extract showing individual and loosely attached cells

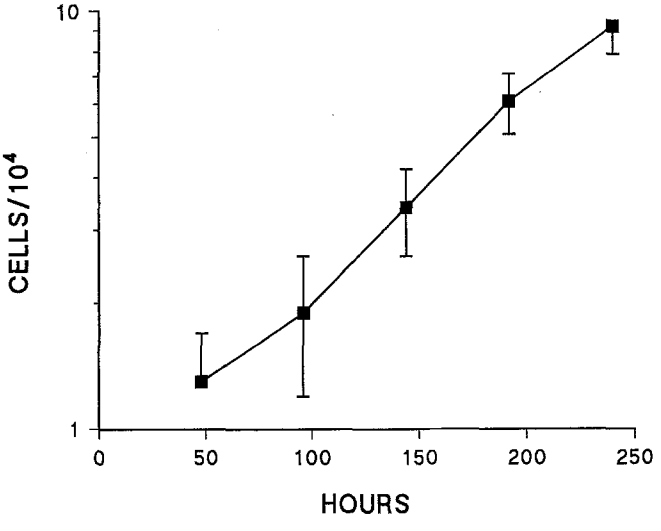

Fig. 3 Growth curve of second passage cells in Rheinwald-Green medium showing cell counts estimated using a haemocytometer at 2 day intervals. The data are derived from 3 independent experiments done in triplicate. Error bars indicate the SD

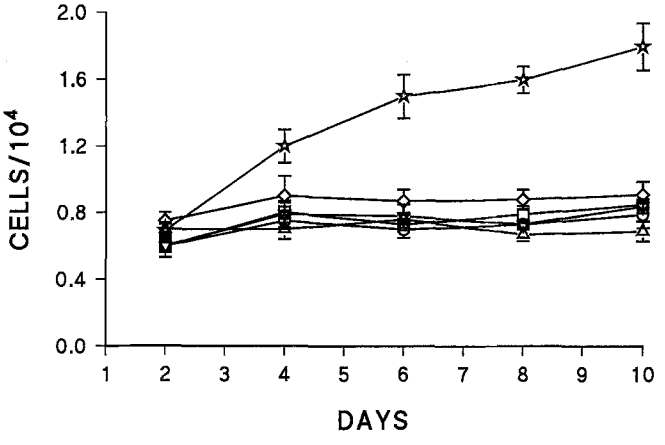

Fig. 4 Effect of mitogens on the growth of second passage urothelial cells. Cell counts were estimated on a haemocytometer at 2 day intervals. The data are derived from 1 experiment done in triplicate showing the intraexperimental error. ○, Control; □, liothyronine 13 ng/ml; ◇, hydrocortisone 0.4 µg/ml; △, transferrin 5 µg/ml; ▽, insulin 5 µg/ml; ☆, EGF 20 ng/ml

gel and the ridged surface created by the remodelling of the gel by the fibroblasts. The irregularity of the substrate favoured patchy growth. Colonies of urothelial cells could be distinguished 2–3 days after seeding and by the 10th day the cells covered most of the surface of the gel. Sections taken from the gel after 12 days culture show a disorganised trilayer of urothelial cells over part of the gel. There was no evidence of differentiation within the 3 layers, each of which had a similar appearance. The urothelial cells adhered strongly to the gel, and attempts to passage the cells resulted in fibroblast cultures as a result of cells being released from the gel. When maintained for periods of longer than 3 weeks the gels began to disintegrate. No differences were observed between the cultures grown on gels containing ureter fibroblasts and those containing Swiss 3T3s.

The urothelial cells growing in the modified Rheinwald-Green medium had a low colony forming ability (0.38 ± 0.36 SD, range 0.01–0.74 in 6 experiments). The cell doubling time was 56 h with a SD of 6.5 h (Fig. 3).

Little or no increase in cell number was seen in cells grown in Ham's F12:DMEM (1:3) with 2% FCS (Fig. 4). The addition of 10 ng/ml EGF stimulated cell growth, giving a population doubling time of approximately 5–6

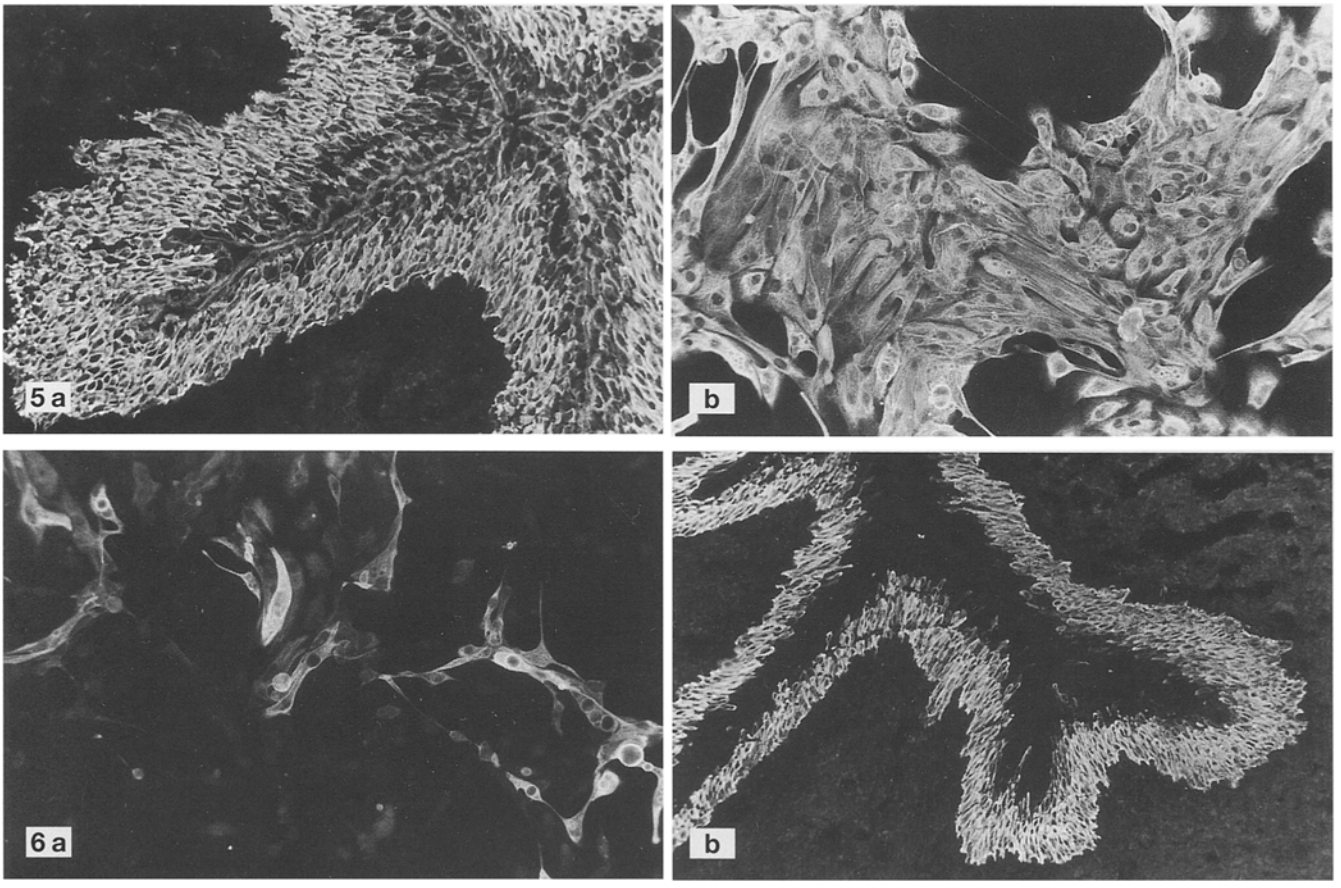

Fig. 5a Section of ureter stained for keratin 7 showing staining throughout the urothelium. **b** Second passage ureter urothelial cells growing in modified Rheinwald-Green medium and stained for keratin 7 showing staining in all cells. **Fig. 6a** Section of ureter stained for keratin 13 showing staining of the basal layers of the urothelium. **b** Second passage ureter urothelial cells growing in modified Rheinwald-Green medium and stained for keratin 13 showing staining of approximately 25% of the cells

Table 1 Monoclonal antibodies 6B10 and RCK105 (Bio Nuclear Services, Reading, UK); 1C7 (Euro Path, Cornwall, UK); LMM3 (gift from Dr Gardener); LE61 (gift from Dr Lane); LH2, LL001 and LP2K produced in IL's Department

Antibody	Keratin
6B10	4
RCK105	7
LH2	10
1C7	13
LL001	14
LMM3	16
LE61	18
LP2K	19

days. However, the other supplements in the Rheinwald-Green medium did not increase the growth rate over that of the controls (Fig. 4).

The results of monoclonal antibody (MAb) staining of cytokeratins in sections of fresh frozen biopsies are shown in Table 2. Samples of renal pelvis, ureter, bladder and urethra showed positive staining throughout the urothelium with antibodies to keratins 7 (Fig. 5a) and 18 (Fig. 7a) and 19 (Fig. 8a), with the exception of keratin 19 which was only present in the basal layers of renal pelvis urothelium. Keratin 13 (Fig. 6a) was only present in the basal layers, with the exception of bladder urothelium which stained throughout. The samples were negative for keratins 4, 10 and 14. The 6 samples from each site were read blind and all gave identical results. HE stained sections were examined and appeared histologically normal. Positive staining was seen for vimentin in the stroma, but not in the urothelium.

The staining patterns of the urothelial cultures are summarised in Table 2. All cultures of urothelial cells showed identical staining patterns regardless of the site the tissue was derived from, the media that they were cultured in or the substrate used. Universal staining of all the cells in the cultures was found with antibodies to keratins 7 (Fig. 5b), 18 (Fig. 7b) and 19 (Fig. 8b). Expression of keratins 13 and 14 was not uniform, with approximately 25% of the cells staining in each case (Fig. 6b). This keratin expression differed from that in vivo in that some of the cultured cells stained for keratin 14 and all stained for keratin 16. Expression of vimentin was not observed.

Table 2 Keratin staining patterns in vivo and in vitro

Keratin	Renal pelvis		Ureter		Bladder		Urethra
	in vivo	in vitro	in vivo	in vitro	in vivo	in vitro	in vivo
4	–	–	–	–	–	–	–
7	+	+	+	+	+	+	+
10	–	–	–	–	–	–	–
13	Basal	(+)	Basal	(+)	+	(+)	Basal
14	–	(+)	–	(+)	–	(+)	–
16	–	+	–	+	–	+	–
18	+	+	+	+	+	+	+
19	Basal	+	+	+	+	+	+

+, present in all cells; (+), present in approximately 25% of cells; *basal*, present only in basal cell layer; –, not detectable

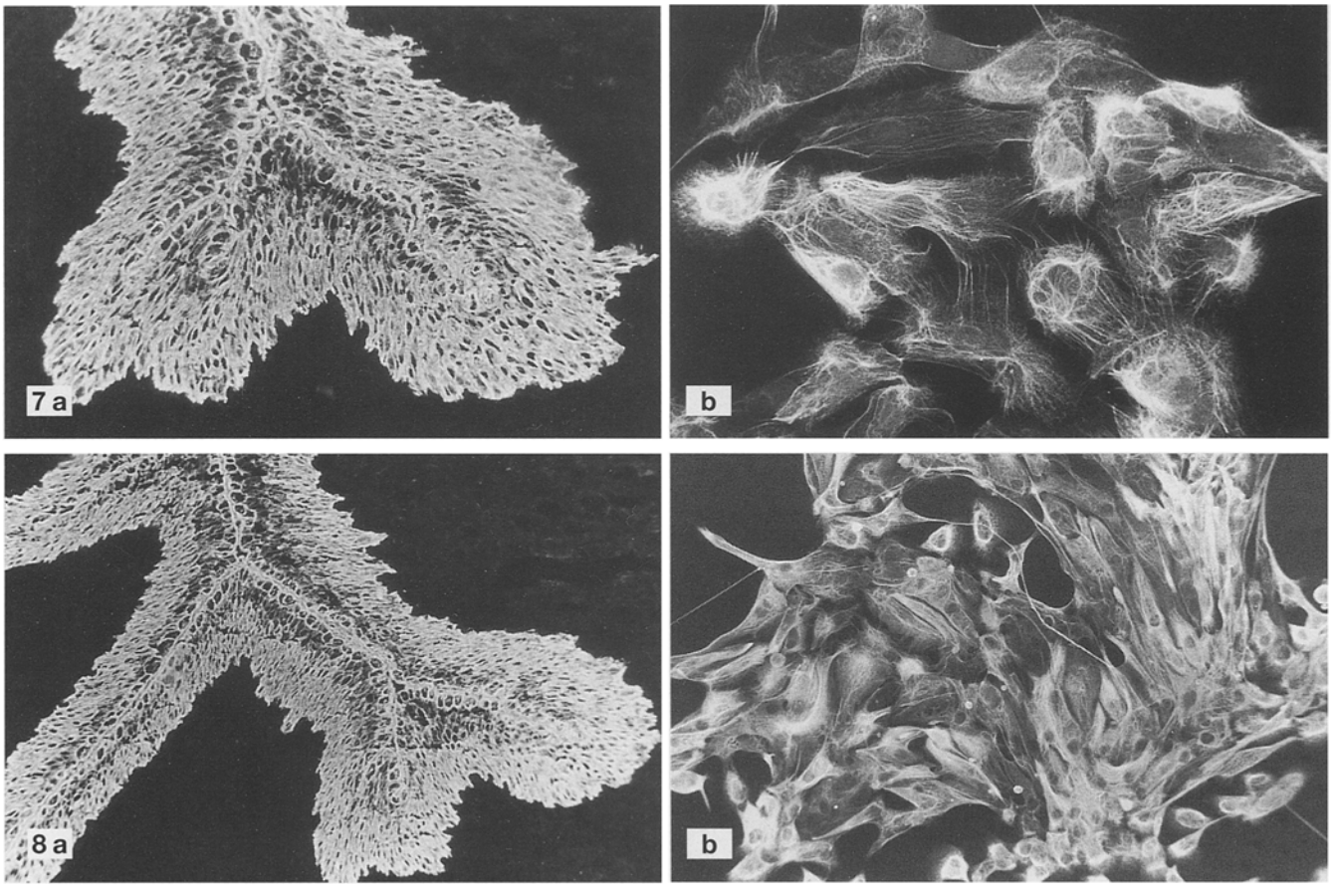

Fig. 7a Section of ureter stained for keratin 18 showing staining throughout the urothelium. **b** Second passage ureter urothelial cells growing in modified Rheinwald-Green medium and stained for keratin 18 showing staining in all cells. **Fig. 8a** Section of ureter stained for keratin 19 showing staining throughout the urothelium. **b** Second passage ureter urothelial cells growing in modified Rheinwald-Green medium and stained for keratin 19 showing staining in all cells

Discussion

Primary cultures of urothelial cells were obtained from biopsies in over 90% of cases. The cells were obtained by enzymatic dissociation and grown on a feeder layer and complex medium slightly modified from that developed for the growth of keratinocytes by Rheinwald and Green [29, 33]. The aim of these studies was to determine whether we could propagate urothelial cells in vitro for autologous grafting. Urothelial cells were propagated routinely from over 90% of cases and cultures covering an area of 1000 cm^2 could be produced within 2 months. This is equivalent to the in vitro culture of keratinocytes for the treatment of burns and ulcers, and in the urological setting would provide enough tissue, for example, for a hypospadias repair.

We could not initiate primary cultures of purified urothelial cells in serum-free medium, in contrast to

2 earlier studies using explant cultures [5, 12]. Presumably the stromal components provide the necessary conditions to supplement the serum-free medium. Cell lines derived from our primary cultures were able to grow in serum-free medium but these did not form colonies or stratify.

The morphology of the cells grown in the Rheinwald-Green system is similar to that described in earlier studies using enzymatically dissociated cells [29] or single cells grown from urine [10, 30]. The cells are small, irregular, and polygonal, growing in compact colonies similar to keratinocytes grown in these conditions, the only difference being the absence of a central pigmented area. We also observed a second cell type – a long fusiform cell with identical keratin expression that grows in swirling colonies. Rheinwald and O'Connell [30] also described a second cell type in their cultures derived from urine, although their cultures, in contrast to ours, did not form multilayered sheets.

Doubling times for human urothelial cells in vitro vary according to the culture conditions. Low serum and calcium concentrations favour proliferation over differentiation [28]. Most studies report doubling times of between 24 and 51 h [5, 12, 23, 28]. The relatively slow population doubling time of 56 h and low colony forming efficiency (CFE) of 0.2% reported in this study probably reflects the high concentration of 10% FCS. CFEs of 16.1% and 5.7% were obtained using Ham's F12 with 1% FCS and MCDB 170 containing 70 μg/ml bovine pituitary extract respectively [15].

The role of the individual mitogens in the Rheinwald-Green medium were tested by adding each individually to urothelial cells growing in 2% FCS. Only EGF stimulated cell growth. Intravesical instillation of EGF into rat bladders also resulted in an increase in the rate of DNA synthesis [19]. In serum-free medium cell growth was not stimulated by EGF [18, 28], but at high concentrations (50 ng/ml) it appeared that terminal differentiation was induced [5].

Transitional cell epithelium exhibits characteristics of simple epithelia, expressing keratins 7, 18 and 19. The basal cell layers also express keratin 13, characteristic of non-cornified stratified epithelia. The results support the idea that transitional cell epithelium is intermediate in its differentiation between simple and stratified epithelia [20]. Keratins are usually expressed in pairs, and keratin 13 is matched by keratin 4, absent in both this and previous studies [1, 7, 21]. There was little regional variation. Keratin 19 was expressed only in the basal cells in the renal pelvis, in contrast to throughout the urothelium elsewhere. Also keratin 13 was expressed throughout the bladder urothelium, in contrast to only the basal cells elsewhere.

Urothelial cells growing in vitro were uniformly positive for keratins 7, 18 and 19 and negative for 4 and 10, matching the cytokeratin patterns in vivo. Similarly, approximately 25% of the cells stained for 13, while in vivo only the basal cells stained. In contrast to the biopsies, keratin 14 was present in approximately 25% of the cells. This keratin is normally expressed in stratified cornified epithelium, so it is likely that some component of the culture system is favouring this form of differentiation. The major difference from the biopsies was the universal expression of keratin 16, characteristic of highly proliferating epithelial cell types. This presumably reflects the higher growth rate in vitro. These results are similar to those obtained by Rheinwald and O'Connell [30] in cultured urothelium from bladder, ureter and renal pelvis. Using electrophoresis they found the simple epithelial keratins 7, 8, 18 and 19 in addition to 6, 13, 17 and small amounts of 5 and 14. Keratin 6 was demonstrated (characteristic of highly proliferating epithelium) but in contrast to this study they failed to observe its partner, keratin 16. The antibody RGE53, specific for keratin 18 [31], showed selectivity for umbrella cells [25], although our data with LE61 (against cytokeratin 18) indicated uniform expression throughout the urothelium.

We have confirmed and extended previous findings for keratin expression in urothelia [1, 7, 21]. Urothelial cells can readily be expanded in vitro from biopsies to produce quantities of cells comparable to those produced from keratinocyte cultures and used for the treatment of burns. Modulation of the culture conditions with growth factors and extracellular matrix proteins (e.g. laminin, fibronectin) might be used to prevent squamous metaplasia and induce the differentiation of umbrella cells. The next goal is to find a suitable substrate which will support the growth and differentiation of urothelial cells and can be used for reconstructive surgery.

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