Altered Growth Patterns in Vitro *of Human Papillary Transitional Carcinoma Cells*

CATHERINE A. REZNIKOFF, PhD,
KENNEDY W. GILCHRIST, MD,
DIANE H. NORBACK, MD, PhD,
KENNETH B. CUMMINGS, MD,
ERDOĞAN ERTÜRK, DVM, PhD,
and GEORGE T. BRYAN, MD, PhD

From the Departments of Human Oncology, Pathology, and Surgery, University of Wisconsin Medical School, Madison, Wisconsin

***In vitro* growth patterns and morphologic characteristics of five low-grade human papillary transitional cell carcinomas (TCCs) were compared and contrasted with those of normal human urothelial cells in culture. Biopsies of TCC were performed by transurethral resection. Specimens of normal human ureters were obtained surgically. Singly dispersed TCC cells grew in 0.3% agarose semisolid medium with a cloning efficiency ranging from 0.02% to 0.71%. Singly dispersed normal ureteral urothelial cells under the same conditions did not form colonies in 0.3% agarose. Neither singly dispersed TCC nor normal urothelial cells formed colonies when plated on collagen-gel substrates. In primary explant culture, normal human urothelial cells grew rapidly, to form tightly adherent flat sheets of apparently nonmotile cells. Autoradiographic labeling with ^{3}H-thymidine of growing cultures of normal urothelial cells showed cell division primarily in the zones of growth near the explant. Outgrowth of TCC from primary explants was loosely adherent. One TCC explant culture gave rise to a continuous suspension culture. Numerous multilayered cellular formations of fronds, nodules, and "walls" were seen around the periphery of TCC explant colonies. Autoradiography showed that these multilayered areas of TCC growth contained actively dividing cells. The altered ability of papillary TCC to form superficial multilayered formations *in vitro* distinguishes them from normal human urothelium and reflects the morphologic characteristic of this tumor type *in vivo*.**
(Am J Pathol 1983, 111:263–272)

TECHNIQUES for reproducibly growing and characterizing normal human urothelial cells in primary culture have not yet been reported,[1,2] although there are reports of the successful cultivation of rodent urothelium.[1,2] In contrast, there are many reports of successful techniques for establishing primary culture of human bladder carcinoma cells.[3–5] A major emphasis in many of the latter studies has been put on the "success" rate achieved for line formation from these primary cultures (usually <10%), rather than characterizations of the cells in primary culture. As a result of these studies, there are many human bladder cancer cell lines available.[6] These continuous cell lines have been characterized with respect to ultrastructure, morphologic characteristics, karyotype, growth in agar, and tumorigenicity in nude mice.[7–9] Extrapolation of findings obtained with cell lines to characteristics of the primary tumors of origin is subject to the criticism that selective and unknown cellular changes may have occurred *in vitro* during the many cellular generations required for establishment of a cell line. For the same reason, it is unclear why information obtained from cell lines would be useful in the management of an individual patient's disease.[10]

The above considerations suggest that the study of the characteristics of human bladder cancers in pri-

Supported in part by Public Health Service Research Grant CA 29525 from the National Bladder Cancer Project and Grant CA 14520.

This work has been approved by the Human Subjects Committee (Protocol 81-480-076) and the Biological Safety Committee at the University of Wisconsin, Madison (SC-81-100).

Accepted for publication January 3, 1983.

Address reprint requests to Catherine A. Reznikoff, PhD, Wisconsin Clinical Cancer Center, Room K4/550, 600 Highland Ave., Madison, WI 53792.

0002-9440/83/0607-0263$1.30

mary culture may have more pertinent clinical applications, despite the limitations inherent in using short-term cultures. Recent advances in defining the *in vitro* substrate[11] and nutritional requirements[12] of mammalian epithelial cells have resulted in reports of improved attachment, growth, and differentiation of a variety of human epithelial cell types, including human mammary epithelial cells,[13-15] human colonic epithelial cells,[16,17] human bronchial epithelial cells,[18] and human hepatocarcinoma and sarcoma.[19] In this regard, Leighton and his associates[10] have used collagen-coated sponge matrix cultures of primary tumor biopsy specimens to study three-dimensional histotypic and morphogenetic attributes of bladder cancer tissues *in vitro*. The purpose of this study was to compare the *in vitro* growth characteristics of primary cultures of normal human urothelium with those of low-grade papillary transitional-cell carcinoma (TCC).

Materials and Methods

Tissue Procurement

The 11 human ureters used in this study were from patients 1–78 years of age. Ureter tissues showed no abnormalities, as determined by light-microscopic evaluation of fixed tissues. The 5 TCC specimens were obtained by transurethral resection and were placed immediately into sterile culture medium. Representative subsamples were selected for histopathologic evaluation and tissue culture studies by dissection and separation with the use of a stereoscopic dissecting microscope.

The 5 TCC specimens used in this study were obtained from patients with multifocal Grade I/III or I-II/III papillary TCC.[20] Four patients had recurrent TCC after prior treatment with intravesical thiotepa. At the time of biopsy, these patients had been off thiotepa for a minimum of 6 months. The case of Patient 4 was newly diagnosed. Four of the TCCs were noninvasive. Patient 5 had a separate focus of nonpapillary Grade II/III TCC, which invaded the lamina propria.

Tissue Culture Conditions

The culture media used was Ham's F12 (GIBCO, Grand Island, NY) supplemented with 5 μg/ml insulin (Eli Lilly and Co., Indianapolis, Ind), 5 μg/ml transferrin (Sigma, St. Louis, Mo), 1 μg/ml hydrocortisone (Merck, Sharpe, and Dohme, West Point, Pa), 0.1 mM nonessential amino acids (Microbiological Associates, Wakersville, Md), and 2.7 g/ml dextrose (Amend Drug and Chemical Co., Irvington, NJ). Penicillin (100 units/ml; Pfizer, Inc., New York, NY) and streptomycin (100 μg/ml; Pfizer) were added to the medium. Fetal bovine serum (7%; Sterile Systems, Inc., Logan, Utah) was used. Cultures were grown at 37 C in humidified incubators in 95% air and 5% CO_2. Culture dishes used were 60 mm (P60) plastic Petri dishes from Lux (Miles Laboratory, Inc., Naperville, Ill).

Collagen Gel Substrates

Collagen gel substrates were prepared by use of the method first described by Ehrmann and Gey.[21] Briefly, collagen was extracted from rat tail tendons in 0.5 M sterile acetic acid for 48 hours at 4 C. The solution was then centrifuged at 14,000*g* for 60 minutes at 4 C. The supernatant was poured off and used for coating plastic Petri dishes (0.6 ml/P60). Reconstruction of collagen as a gel was accomplished by exposure to ammonia vapors.

Explant Cultures

Explant cultures of normal urothelium and cancer biopsy specimens were prepared as follows. TCC biopsy tissue was placed in a 100-mm glass Petri dish in culture medium. Dissection of tissue was done under a stereoscopic microscope with the use of microdissection instruments and No. 11 Bard Parker stainless steel blades. TCC tissue was separated from the lamina propria and dissected into 1-sq mm explants for tissue culture. Normal urothelium was mechanically stripped from the ureter and cut into 1-sq mm explants.

We placed explant pieces of urothelium on collagen substrates in 0.6 ml/P60 medium (sufficient to keep the explants moist) and placed them in a humidified incubator for 40–60 minutes to permit attachment. After attachment, a 2-ml volume of medium/P60 was added. The medium (2–3 ml/P60) was changed every third day. The sizes of the explants and the outgrowth of cells from the periphery of the explants were measured with the use of a calibrated micrometer disk (0.5 mm in 0.1-mm intervals) placed in an ocular of a Nikon phase-contrast microscope. The appearance of each cellular outgrowth from every explant culture was examined daily.

Cultures of Single-Cell Suspensions

Single-cell suspensions for growth on collagen substrates and for growth in agarose (see below) were prepared as follows. Fragments of normal or cancer

tissues (approximately 1 sq mm) were prepared as for explant culture. These were then put into glass Petri dishes containing 10 ml of an enzyme solution (EDTA-T-C) consisting of 0.05% trypsin and 0.02% EDTA (GIBCO) and 0.1% collagenase Type II (Sigma) and minced to smaller fragments with the use of crossed 11 Bard Parker scalpels. The cell solution was then transferred to a magnetic stir plate (warmed to 37 C) and vigorously stirred for 20 minutes in a total volume of 25 ml of enzyme solution. We placed the cell suspension into two conical centrifuge tubes for 10 minutes to allow sedimentation by gravity of fragments. We removed, pooled, and pipetted the supernatant gently to further disperse cells and dissociate clumps. An aliquot of the cell suspension was taken for light-microscopic examination. If a single cell suspension was obtained (less than 1 clump of 3 or more cells/1000 cells), the cell suspension was centrifuged for 7 minutes at 700 rpm in a clinical centrifuge. The pellet was resuspended into medium. If a single cell suspension was not obtained, the pellet was resuspended in EDTA-T-C enzyme solution, and the above procedure was repeated. Cell counts of the final solution of cells were done with the use of hemocytometers, and viability was determined with 0.1% trypan blue stain (GIBCO).

We seeded single suspensions onto collagen substrates at a concentration of 10^4 cells/P60 to determine colony-forming ability on this solid substrate.

To determine the colony-forming ability of single cell suspensions of urothelial cells in semisolid medium, we used a modification of the procedures of Hamburger and Salmon.[22] Briefly, a 3-ml underlayer of 0.5% agarose (Marine Colloids Division, Rockland, Me) in enriched medium described above was placed in a 60-mm plastic Petri dish. Cells to be tested for colony formation were suspended in a plating layer of 0.3% agarose in enriched medium. We seeded cells at 5×10^4 cells/60-mm dish to eliminate difficulties in visualizing cells due to crowding.

Immediately after seeding, all plates were examined with a phase contrast microscope. The counting of colonies at 10 and 20 days after seeding was done with the use of an inverted microscope at ×40 magnification. Only colonies consisting of 50 or more cells were scored, and no colonies that derived from clumps of cells were scored.

Autoradiography

Single explants for autoradiographic studies were grown in P30 plastic Petri dishes on 22-sq mm cover glasses precoated with collagen in 1.5 ml of medium. The method used for autoradiography has been described.[23] Briefly, medium containing 1 μCi/ml of ^{3}H-thymidine (20.0 Ci/mmol; New England Nuclear, Boston, Mass) was added to cultures for 48 hours. After the exposure period, the cover glasses were transferred to Columbia jars and rinsed three times with phosphate-buffered saline. Cells were fixed in 3:1 absolute alcohol and glacial acetic acid at 5 C for 20 minutes. After air-drying, the cover glasses were mounted with Permount onto microscope slides with the cell side up. After the Permount completely dried, the slides were dipped in Ilford L-4 emulsion (Ilford Ltd., Ilford, Essex, England), exposed at 5 C for 10 days, fixed, and developed with D-19 developer (Eastman Kodak Co., Rochester, NY). Determination of the percentage of labeled cells was done microscopically with the use of calibrated micrometer disks (1 sq mm in 0.1-mm units).

Histology

Cultures were embedded in fresh 100% Epon 812, which was polymerized in a 55 C oven for 48 hours. Cells were selected for sectioning by light-microscopic evaluation of the dish. Cells of interest and the underlying plastic Petri dish were cut with a jeweler's saw. Thick sections (2 μ) were cut with glass knives, stained with toluidine blue, and photographed with a Zeiss photomicroscope.

Results

Characteristics of Normal Human Urothelium in Primary Culture

Outgrowth of normal urothelial cells from explant cultures growing on collagen substrates was routinely obtained (Table 1). Migration of cells from the primary explants was usually observed within 48 hours, and dividing cells were observed by 72 hours. The period of rapid cell growth and increase in colony size usually lasted 1–3 weeks, followed by a variable period of maintenance in culture (Table 1). During this period cells sloughed off, and mitotic figures were only occasionally observed. None of the normal urothelial cultures gave rise to a cell strain or line growing on solid substrate. There was no apparent contamination of explant cultures of normal urothelium (or TCC) by stromal cell growth in this study, as determined by microscopic observation.

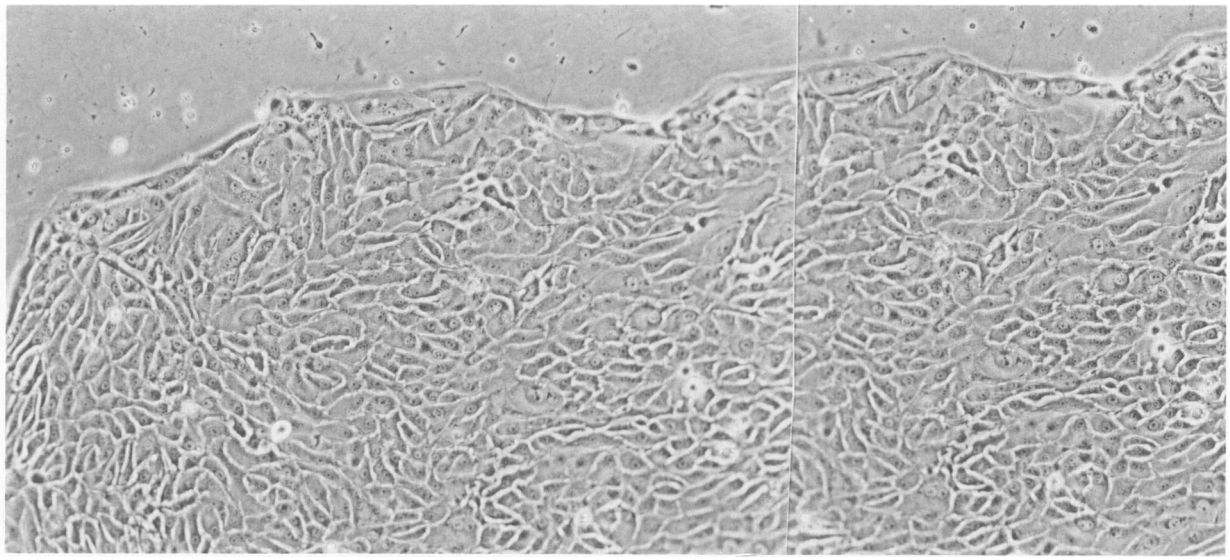
Colonies did not form when single cell suspensions of normal human urothelial cells (70% viability) were seeded at 5×10^4 viable cells/P60 in 0.3% agarose or at 10^4 viable cells/P60 on collagen gel (Table 1).

Normal human urothelial cells grew as continuous

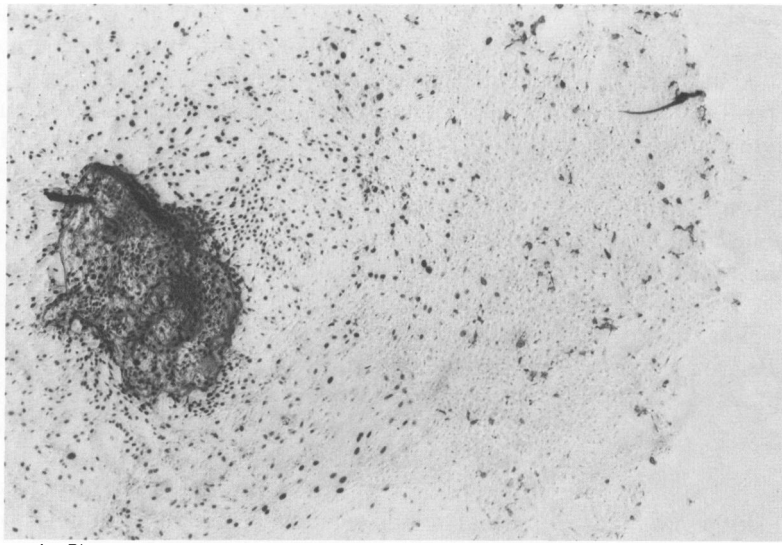
Table 1—Characteristics of *In Vitro* Growth of Human Papillary TCC

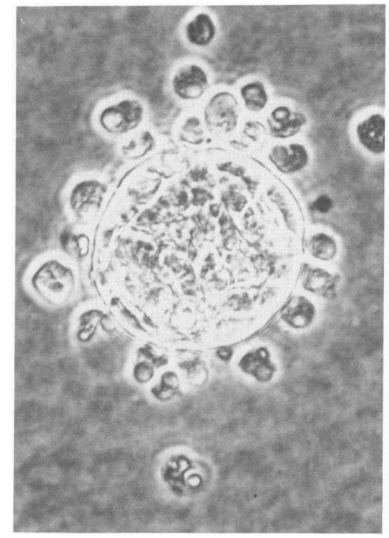
Specimen source			Colony growth in 0.3% agarose (5×10^4 cells/P60) (%)	Plating efficiency on collagen-gel (10^4 cells/P60) (%)	Explant colonies with cellular outgrowth (%)	Extent cellular outgrowth from explant colonies (mm ± SD)	Average duration of explant colonies (weeks)
TCC	Age	Sex					
1	57	M	0.02	Not done	100 (7/7)	3.6 ± 1.3	6
2	68	M	0.09	0	89 (16/18)	6.0 ± 2.9	3
3	79	F	0.01	Not done	82 (9/11)	4.5 ± 1.8	6
4	79	M	0.08	0	98 (49/50)	4.2 ± 2.1	10
5	78	F	0.71	0	100 (7/7)	5.0 ± 2.3	5
Normal ureter (11 samples)	1-78	5M and 6F	0	0	82 (397/485)	7.5 ± 1.9	14

1



2





3

Figure 1—Phase contrast photography of a sheet of living normal human ureteral urothelium growing on a collagen gel substrate for 10 days. The leading edge of growth shows tightly adherent flat cells. (×100) **Figure 2**—Autoradiography of a culture of normal human ureteral cells at 10 days showing ^{3}H-thymidine-labeled cells in the regions of growth near the explant. (×200) **Figure 3**—Phase contrast photograph of an unstained colony of TCC cells growing in 0.3% agarose. (×300)

flat sheets of tightly adherent cells (Figure 1). There was no evidence of either spontaneous separation of single cells or migration of individual cells away from the cell sheet (Figure 1). No age-related differences in growth characteristics or morphologic characteristics were observed among the explant cultures of ureters from younger patients, compared with several older patients (ages 57, 63, and 78) in this study. Autoradiographic studies showed that cell division occurred primarily in the regions near the explant and rarely in the periphery (Figure 2).

Altered Growth Characteristics of Papillary TCC

Primary explant cultures of papillary TCC showed initial outgrowth of cells similar to that observed in explants of normal urothelium. Epithelial cells appeared around the explants by 48 hours, and a period of cell division resulted in increases in the colony size. After 10 days the diameters of the TCC explant colonies were measured. There was no significant difference in colony size among the cancer explants. The largest colonies were observed in explant colonies of normal urothelium (Table 1). Although no continuous cell strains were obtained from cultures of normal urothelium, one TCC culture (5) gave rise to continuous cultures of cells growing in suspension.

Five biopsies of TCC were enzymatically dispersed, and the colony-forming ability of these single cell suspensions in 0.3% agarose (5×10^4 viable cells/P60) was compared with the colony-forming ability of the same cell suspension plated on collagen substrates (10^4 cells/P60). The viabilities of singly dispersed TCC cells were 67–91%. These low-grade papillary TCC cells had a colony-forming ability in soft agarose (Figure 3) ranging from 0.02–0.71% (Table 1). However, colonies did not form when the single cell suspensions of TCC were seeded on collagen gel substrates (Table 1).

The growth patterns of papillary TCC cells in explant culture on collagen substrate differed from those observed in cultures of normal urothelium. Rather than growing as flat, tightly adherent sheets of cells, TCC cells in culture were in general more loosely adherent than normal cells and formed multiple multilayered cellular structures, as well as altered patterns of outgrowth onto the collagen substrate (Table 2).

Explant cultures from TCCs 3, 4, and 5 formed numerous nodules around the periphery of colonies (eg, Figure 4A). Nodules typically were seen during the second week of culture and, when labeled with ^{3}H-thymidine, showed many dividing cells (Figure 4B). Cells in the nodules appeared to separate easily and could be seen floating in the medium. The morphologic characteristics of nodules from TCCs 3 and 5 (the patients had recurrent TCC after thiotepa treatment) were indistinguishable by light microscopy from the nodules seen in explants from TCC 4 (a newly diagnosed case).

Samples 1 and 5 gave rise to numerous fronds (eg, Figure 5A) during the second week of culture. These fronds appeared to grow out from the cell sheet into the liquid medium. Often they were branched. Although they were attached at the base, the upper part of the fronds moved in the medium. These fragile fronds were easily detached and usually did not persist beyond the third week of culture. Fronds consisted of actively dividing cells and showed heavy labeling with ^{3}H-thymidine (Figure 5B).

TCCs 1 and 2 showed rampartlike projections of growth from the peripheral leading edge of growth (eg, Figure 6A). This was in contrast to the even, almost symmetrical periphery seen in normal urothelial cultures (Figure 1). The outer cells in these projecting ramparts resembled basal cells. Many cells were labeled with ^{3}H-thymidine (Figure 6B), in contrast to sparse labeling of cells in the interior of the colonies.

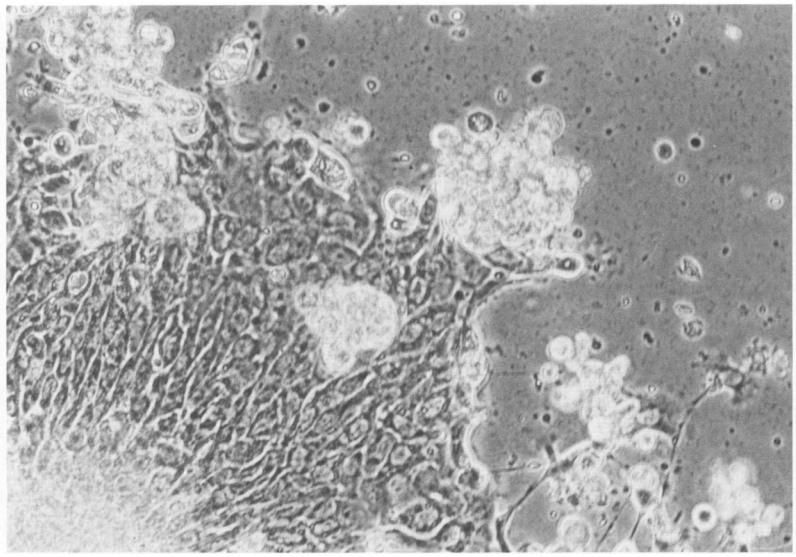
The cultures from tissue samples 1 and 5 formed multicellular multilayered "walls" around the periphery of several explant colonies (eg, Figures 7A and

Table 2—Growth Patterns of Papillary TCC in Explant Culture

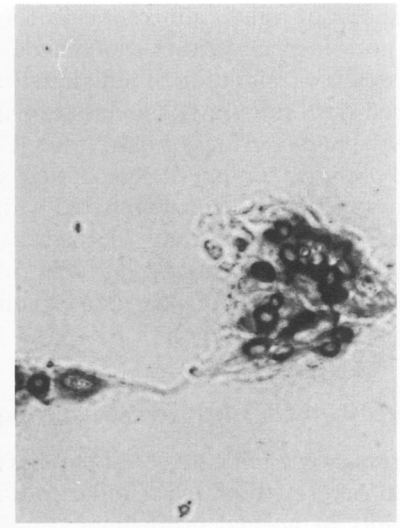
Specimen		Characteristics of multicellular formations *in vitro*			
TCC	Grade*	Nodules	Fronds	"Walls"	"Ramparts"
1	I/III	–	+	+	+
2	I/III	–	–	–	+
3	I/III	+	–	–	–
4	I-II/III	+	–	–	–
5	I-II/III†	+	+	+	–
Normal ureter	–	–	–	–	–

* World Health Organization classification.[12]

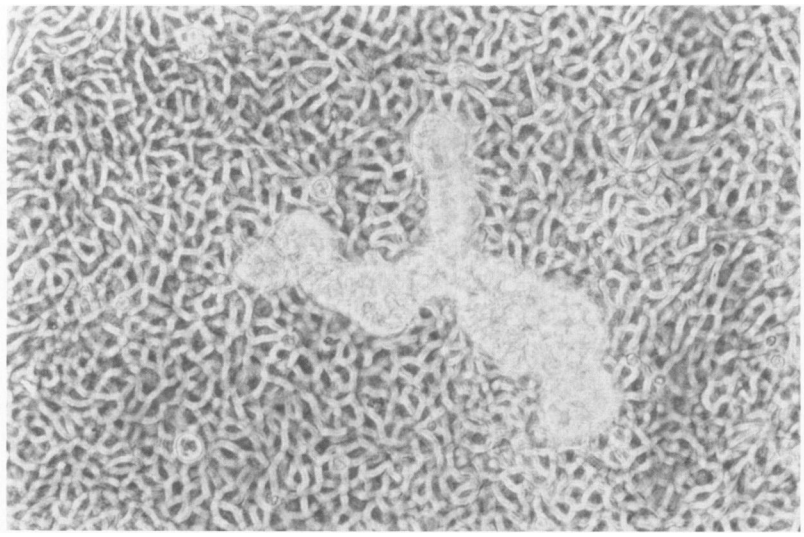
† A separate focus of nonpapillary grade II/III TCC with invasion into the lamina propria was present in this patient.



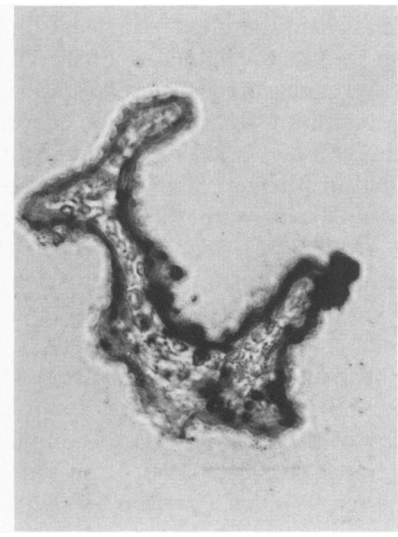
4A



4B



5A



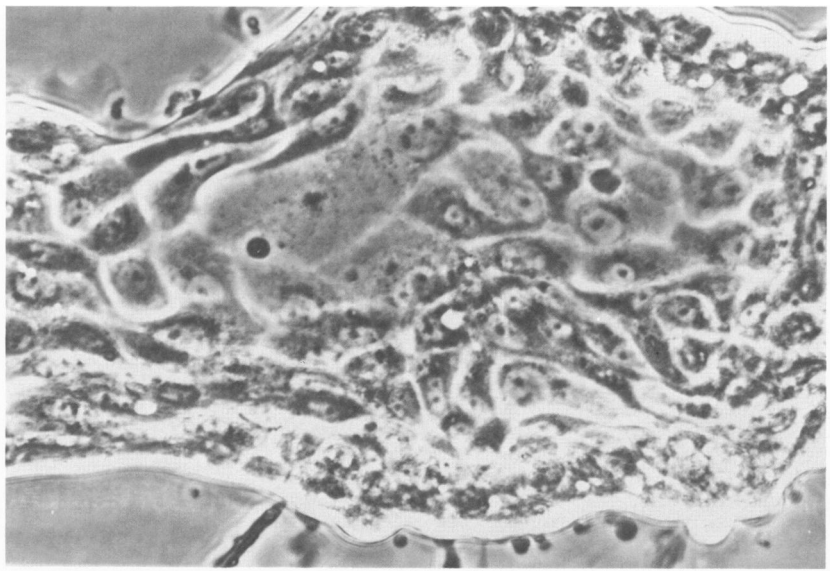
5B

Figure 4 — Explant culture of papillary TCC Biopsy Tissue 4. **A** — Nodule formation is seen around the periphery of outgrowth of cells from an explant culture. (× 100) **B** — An autoradiograph of a peripheral nodule showing many cells incorporating ^{3}H-thymidine. (× 100) **Figure 5** — Explant culture of papillary TCC Biopsy Tissue 1. **A** — Formation of a frond from the cell sheet is shown here. This frond was attached at the base and moved in the medium. The plane of focus is on the center of the frond. (× 100) **B** — An autoradiograph of a similar frond exposed for 48 hours to ^{3}H-thymidine. (× 100)

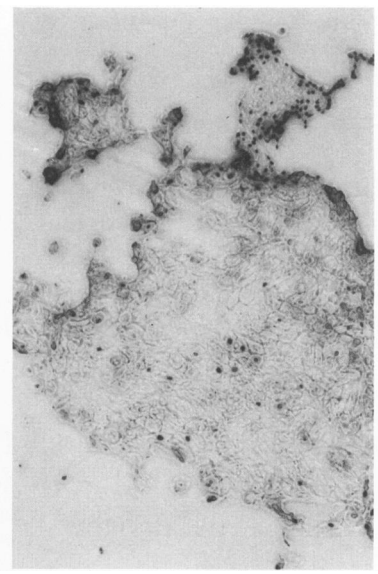
B). These "walls" also formed during the second week of culture and contained dividing cells, as determined by autoradiography.

Thick sections (2 μ) of TCC 2, which formed "ramparts" but no multilayered structures, showed that the outgrowth of cells was primarily monolayers and bilayers of cells (Figure 8), compared with TCC 1, which formed fragile multicellular "fronds" (Figure 9), and TCC 5, which formed multilayered peripheral "walls" (Figure 10). Stratification of ureter cells in sections of normal urothelium was restricted to the area adjacent to the explant (Figure 11). No nodules, fronds, or "wall" formations were seen in an extensive separate study of over 2500 explant cultures of normal human urothelium from bladder or ureter.

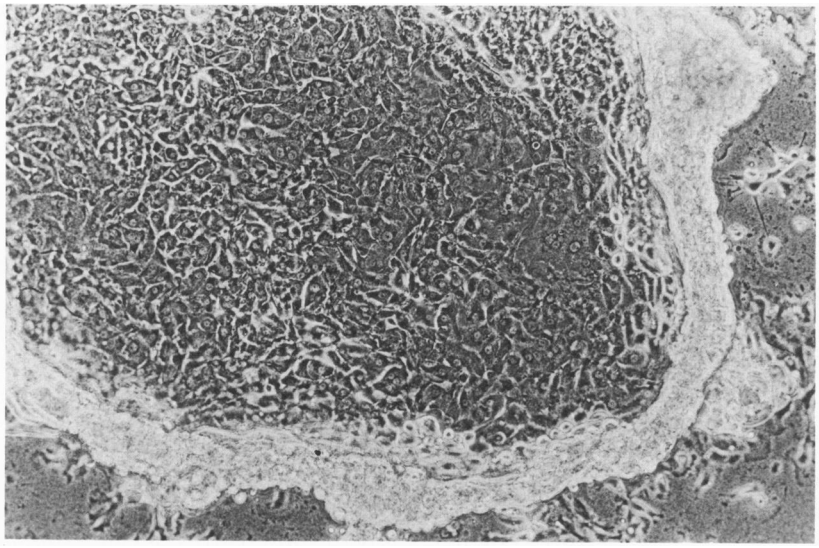
It was not practical to determine quantitatively the exact numbers of fronds, nodules, or ramparts per culture. These formations, when present, were usually multiple in each explant. Structures were usually not clearly visible until the second week in culture. Although all the TCC cultures showed altered growth patterns, all did not show the same alterations, suggesting differences between these histologically similar samples (Table 2). Normal urothelial cells, which grew as flat, tight sheets of cells, produced the largest, longest lasting explant cultures (Table 2). TCC cultures, in contrast, formed colonies with loosely



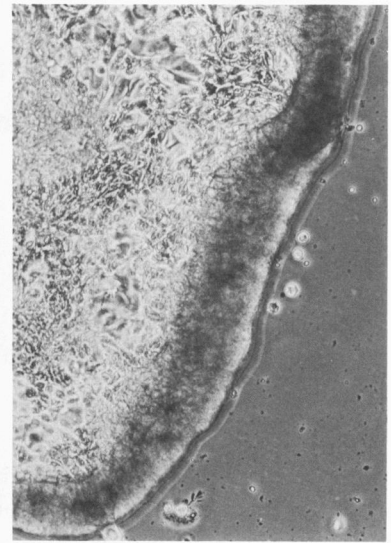
6A



6B



7A



7B

Figure 6 – Peripheral rampartlike growth from an explant culture from Biopsy Tissue 2. **A** – The outer cells have a basallike arrangement dissimilar to that seen in normal cells. **B** – Peripheral cells in the rampart formations show many dividing cells, as determined by autoradiography. (×40) **Figure 7** – Formation of peripheral wall-like structures in an explant culture from Biopsy Tissue 5. **A** – Phase-contrast photograph of a stratified wall of cells. (×200) **B** – Photograph taken out of phase at lower power to illustrate the depth of the wall. (×100)

adherent cells in fragile formations which easily separated and sloughed off into the medium.

Discussion

The *in vitro* morphologic characteristics and growth patterns of low-grade papillary bladder carcinoma cells have been studied and compared with the characteristics of normal human urothelial cells grown under similar conditions.

The finding in this study that low grade TCC cells form colonies (0.02–0.71%) in 0.3% agarose semisolid medium is consistent with the findings of others.[24,25] Singly dispersed normal ureteral urothelial cells did not grow in agarose. Since the TCC specimens were all from low-grade papillary TCC, these results suggest that the ability of TCC cells to grow in semisolid medium may be a result of their apparent loss of growth regulation, rather than an indication of an invasive or metastatic characteristic.

In contrast, neither normal urothelial cells nor TCC cells formed colonies when single cells were seeded onto collagen gel substrates. This result suggests that the requirements for growth of single cells on solid substrates may be even more rigid than growth requirements on semisolid substrates. A com-

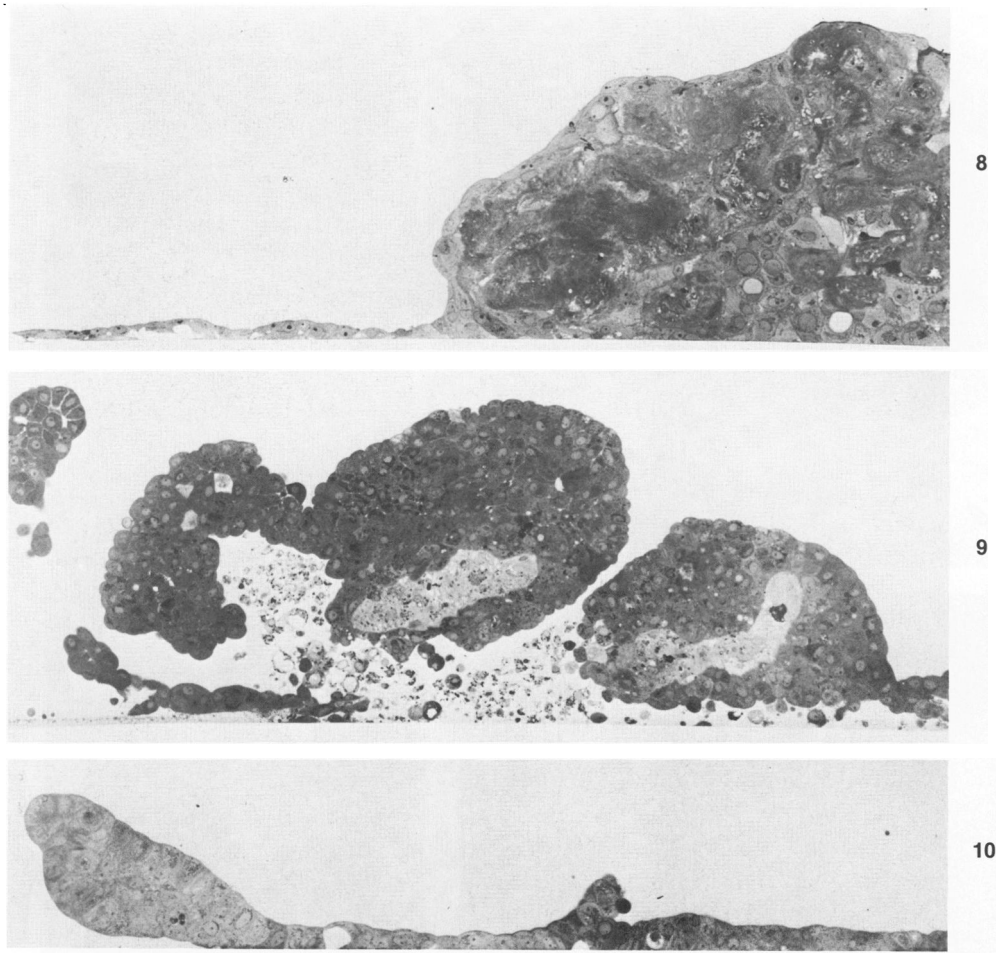


Figure 8—Thick section (2 μ) of TCC Biopsy Tissue 2. The surface epithelium of the tissue explant is continuous with the culture layer of cells. (×150) **Figure 9**—Thick section (2 μ) of TCC Biopsy Tissue 1. Epithelial cells cover the explant and extend upward in fronds. (×150) **Figure 10**—Thick section (2 μ) of TCC Biopsy Tissue 5. A multicellular mass extends upward at the leading edge of the culture layer. (×150)

parative study of the abilities of high- and low-grade bladder carcinoma cells to form colonies on solid and semisolid surfaces is in progress.

The outgrowth of cells from primary explants of normal and tumor cells growing on collagen substrates did not differ significantly, as judged by measurements of colony size at 10 days (Table 1). Tumor cells did not appear to grow more rapidly, produce larger colonies, or persist in culture longer. Papillary TCC cells in our samples were less cohesive than normal cells and easily dissociated. This characteristic may explain the relatively short duration of TCC cells in explant cultures due to sloughing off, compared with normal cells (Table 1).

The striking finding in this study was the different morphologic growth patterns of primary explant cultures on collagen substrates of TCC cells and normal urothelial cells, since only TCC cells *in vitro* formed multicellular structures that grew up into the liquid medium. These structures were reminiscent of the *in vivo* formation of papillary growths from the bladder mucosa into the lumen. Leighton and his colleagues[10] have used a collagen-coated sponge matrix tissue culture system to study the outgrowths of cells from primary TCC cultures, using minced bladder cancer biopsy specimens. These investigators also report the formation of distinct multilayered cellular structures similar to those reported here. In particular, both reports show differences in the *in vitro* growth patterns of bladder cancers of similar grade and stage. These findings suggest that papillary TCC cells *in vivo* and *in vitro* may fail to differentiate terminally. Therefore, the cells continue to divide, as do basal cells, thus forming multilayers. Evidence suggests that bladder cancer is a multistage disease.[26,27] The unregulated growth observed *in vivo* and *in vitro* may be an early change in bladder carcinogenesis. The fact that some low-grade tumors remain superficial while others become invasive is not understood. Methods for predicting which neoplasms will ultimately become invasive would be most desirable. To date, there are no reports of correlations between *in*

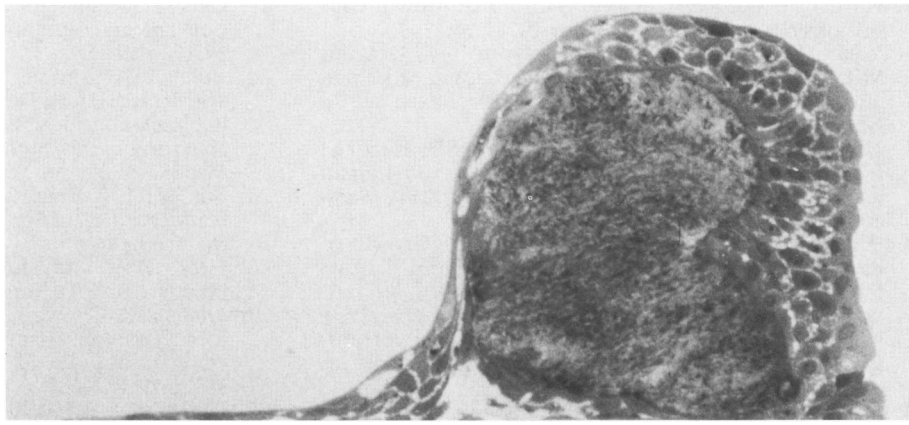


Figure 11—Thick section of explant culture of normal human urothelium showing outgrowth of transitional cells continuous with and similar to the normal stratified epithelium maintained in the explant.

vitro growth properties of individual low-grade bladder cancers and the future risks of recurrence or invasiveness.

In these studies, we have utilized culture techniques similar to those used by other investigators for better growth of human epithelial tumor cells *in vitro*.[13-19] These techniques include the use of a collagen gel substrate and culture medium supplemented with insulin hydrocortisone, and transferrin. Using these conditions, better growth *in vitro* of normal urothelial cells than previously reported[28] was obtained, and TCC cells *in vitro* formed multicellular structures reminiscent of papillary growths *in vivo*.

Normal ureter specimens (routinely obtained at the time of kidney transplantation) were used as a source of normal human transitional cells. Although it would have been ideal to use normal human bladder as control, normal human bladder specimens are not routinely available. In addition, extensive studies in this laboratory have shown no difference between the growth and morphologic characteristics of normal ureteral transitional cells and human embryonic bladder with normal mouse or rat bladder transitional cells *in vitro* grown under similar conditions. None of the alterations described for human papillary TCC cells have been observed in any of these cultures of normal mammalian urothelial cells.

Although four of the TCC samples used in this study were from patients previously treated with thiotepa, thiotepa has no known permanent morphologic effect on normal urothelium.[29] Therefore, the observations made cannot be attributed to thiotepa, because treatment was completed at least 6 months prior to biopsy. Many more samples of TCC must be studied before any significant correlations can be made between *in vitro* growth patterns and grade and stage of tumor. The system we have developed may have usefulness in the study of the sensitivity or resistance of low-grade papillary TCC to drug therapeutic manipulation. We hope to determine whether the ability of therapeutic drugs such as thiotepa to prevent altered growth patterns *in vitro* is a useful index of its biologic effectiveness *in vivo*.

References

1. Chlapowski FJ, Haynes L: The growth and differentiation of transitional epithelium *in vitro*. J Cell Biol 1979, 83:605-614
2. Pauli BU, Anderson SN, Memoli VA, Kuettner KE: The isolation and characterization *in vitro* of normal epithelial cells, endothelial cells and fibroblasts from rat urinary bladder. Tissue Cell 1980, 12:419-436
3. Elliott AY, Bronson DL, Stein N, Fraley EE: *In vitro* cultivation of epithelial cells derived from tumors of the human urinary tract. Cancer Res 1976, 36:365-369
4. Malkovský M, Bubeník J: Human urinary bladder carcinoma cell line (T24) in long-term culture: Chromosomal studies on a wild population and derived sublines. Neoplasma 1977, 24:319-326
5. Fogh J: Cultivation, characterization, and identification of human tumor cells with emphasis on kidney, testis, and bladder tumors. Natl Cancer Inst Monogr 1978, 49:5-9
6. Williams RD: Human urologic cancer cell lines. Invest Urol 1980, 17:359-363
7. Elliott AY, Bronson DL, Cervenka J, Stein N, Fraley EE: Properties of cell lines established from transitional cell cancers of the human urinary tract. Cancer Res 1977, 37:1279-1289
8. Hastings RJ, Franks LM: Chromosome pattern, growth in agar and tumorigenicity in nude mice of four human bladder carcinoma cell lines. Int J Cancer 1981, 27:15-21
9. Marshall CJ, Franks LM, Carbonell AW: Markers of neoplastic transformation in epithelial cell lines derived from human carcinomas. J Natl Cancer Inst 1977, 58:1743-1751
10. Abaza NA, Leighton J, Zajac BA: Clinical bladder cancer in sponge matrix tissue culture: Procedures for collection, cultivation, and assessment of viability. Cancer Res 1978, 42:1364-1374
11. Kleinman HK, Klebe RJ, Martin GR: Role of collagenous matrices in the adhesion and growth of cells. J Cell Biol 1981, 88:473-485

12. Hayashi I, Larner J, Sato G: Hormonal growth control of cells in culture. In Vitro 1978, 14:23-30
13. Yang J, Guzman R, Richards J, Jentoft V, DeVault MR, Wellings SR, Nandi S: Primary culture of human mammary epithelial cells embedded in collagen gels. J Natl Cancer Inst 1980, 65:337-343
14. Yang J, Elias JJ, Petrakis NL, Wellings SR, Nandi S: Effects of hormones and growth factors on human mammary epithelial cells on collagen gel culture. Cancer Res 1981, 41:1021-1027
15. Yang NS, Kube D, Park C, Furmanski P: Growth of human mammary epithelial cells on collagen gel surfaces. Cancer Res 1981, 41:4093-4100
16. van der Bosch J, Masui H, Sato G: Growth characteristics of primary tissue cultures from heterotransplanted human colorectal carcinomas in serum-free medium. Cancer Res 1981, 41:611-618
17. Friedman EA, Higgins PJ, Lipkin M, Shinya H, Gelb AM: Tissue culture of human epithelial cells from benign colonic tumors. In Vitro 1981, 17:632-644
18. Stoner GD, Katoh Y, Foidart JM, Trump BF, Steinert PM, Harris CC: Cultured human bronchial epithelial cells: Blood group antigens, keratin, collagens, and fibronectin. In Vitro 1981, 17:577-587
19. Vlodavsky I, Lui GM, Gospodarowicz D: Morphological appearance, growth behavior and migratory activity of human tumor cells maintained on extracellular matrix versus plastic. Cell 1980, 19:607-616
20. Mostofi FK: Histological Typing of Urinary Bladder Tumors, International Histological Classification of Tumors, No. 10. World Health Organization, 1973
21. Ehrmann RL, Gey GO: The growth of cells on a transparent gel of reconstituted rat-tail collagen. J Natl Cancer Inst 1956, 16:1375-1403
22. Hamburger AW, Salmon SE: Primary bioassay of human tumor stem cells. Science 1977, 197:461-463
23. Caro LG, Van Tubergen RP, Kolb JA: High-resolution autoradiography: I. Methods. J Cell Biol 1962, 15:173-188
24. Buick RN, Stanisic TH, Fry SE, Salmon SE, Trent JM, Krasovich P: Development of an agar-methyl cellulose clonogenic assay for cells in transitional cell carcinoma of the human bladder. Cancer Res 1979, 39:5051-5056
25. Stanisic TH, Buick RN: An *in vitro* clonal assay for bladder cancer: Clinical correlation with the status of the urothelium in 33 patients. J Urol 1980, 124:30-33
26. Hicks RM: Multistage carcinogenesis in the urinary bladder. Br Med Bull 1980, 36:39-46
27. Hicks RM, Chowaniec J: Experimental induction, histology, and ultrastructure of hyperplasia and neoplasia of the urinary bladder epithelium. Int Rev Exp Pathol 1978, 18:199-280
28. Knowles MA, Hicks RM, Berry RJ, Milroy E: Organ culture of normal human bladder: Choice of starting material and culture characteristics. Methods in Cell Biology. Vol 21B. New York, Academic Press, 1980, pp 257-285
29. Murphy WM, Soloway MS, Lin CJ: Morphologic effects of thio-TEPA on mouse urothelium. Acta Cytol 1977, 21:701-704

Acknowledgments

We are very grateful to Dr. David T. Uehling, Department of Surgery, Division of Urology at the University of Wisconsin Medical School and Hospital, for supporting this project by supplying the human ureter tissue used in this study. We thank Melanie Ignjatovic for excellent technical assistance and Karen Scott for her skillful typing.