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In Vitro Culture of Epithelial Cells Derived from Urogenital Tissues*

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Summary. A tissue culture procedure is described which yields a high percentage of successful long-term cultures of epithelial cells derived from malignant and non-malignant human urogenital tissues.

Key words: Tissue culture, prostate, bladder, kidney.

A limited number of reports have been made detailing successful long-term propagation of urogenital tissue culture cells in vitro. (1, 2, 3, 4, 11, 12, 13) The rarity of successful in vitro culture of urogenital cells probably reflects not only inherent difficulties in isolating viable cells from these tissues but also specific nutritional or hormonal requirements of the cells. An effective tissue culture system for malignant and non-malignant human tissue is crucial to analyze properly the actions of specific aetiological, pharmaceutical and hormonal agents on these tissues. In addition, the immunological and biochemical properties of malignant and non-malignant cells are determined most easily on tissue culture cells where contaminating materials are minimal and sufficient working material is available. The object of this report is to detail the procedures employed in this laboratory to establish in vitro cultures of epithelial cells from a variety of malignant and non-malignant human urogenital tissues.

Reagents. Growth Medium A: RPMI 1640 (GIBCO) supplemented with 20% heat-inactivated foetal calf serum, 0.3% tryptose phosphate broth (DIFCO), 0.0014% sodium bicarbonate, 0.005% streptomycin sulfate (Eli Lilly and Company), 62.5 units/ml penicillin G (Squibb), 0.5 µgm/ml Fungizone (Squibb), 1 µgm/ml folic acid. Growth Medium B: Eagle's MEM with Hank's salts (GIBCO) supplemented with 20% heat-inactivated foetal calf serum, 0.005% streptomycin sulfate, 62.5 units/ml penicillin G, 0.5 µgm/ml Fungizone, 0.001% sodium bicarbonate. Trypsin-EDTA solution: 0.025% trypsin - 1 mM ethylenediamine tetra-acetic acid (EDTA) in trypsin diluent (8 gm NaCl, 0.4 gm KCl, 0.06 gm Na_2HPO_4, 0.06 gm KH_2PO_4 per 1000 ml, pH 6.6). EDTA solution: 1 mM EDTA in trypsin diluent.

Cell culture. Tissues, bathed in Growth Medium A, were obtained directly from surgery. The tissues were minced into pieces of 1 mm diameter and were immobilized in multiple 60 mm petri dishes under sterile 22 x 22 mm coverslips secured to the bottom of the dish with a drop of sterile stopcock grease (9) (Dow Corning Corporation). The explant pieces and coverslips were then covered with Growth Medium A and incubated at 37 °C in a humidified 5% CO_2 atmosphere. Medium was changed at weekly intervals or earlier, if necessary, to maintain the pH near 7.0. Outgrowth of cells from the explants occurred both on the coverslip and on the petri dish over a period of one to four weeks and was monitored by phase contrast microscopy. When the cells reached a dense colony around the explant pieces (greater than 50 cells), the medium was removed and both the coverslip and dish were rinsed with 2 ml of phosphate buf-

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fered saline (PBS, pH 7.25). The PBS was replaced with trypsin-EDTA solution. The explant pieces were removed with forceps and discarded since disintegrating cells from these explant pieces might release materials toxic to the cell cultures. The dishes were returned to 37 °C for seven minutes to allow the trypsin-EDTA solution to separate the cells. The suspension of separated cells from a single dish was then added to an equal volume of Growth Medium A to inactivate the trypsin and centrifuged at 1000 RPM for 10 min in an IEC model HN-S centrifuge. The pellets were resuspended in 2 ml Growth Medium A by gentle pipetting and were dispensed in 35 mm Petri dishes. The cells were incubated at 37 °C in a 5 % CO_2 atmosphere with medium changes at weekly intervals. Rapidly dividing cultures altered the pH of the medium quickly and were fed at more frequent intervals. Cultures were passaged by trypsinization as necessary to maintain viability and to reduce population size. After the cultures were well established (4-5 transfers), Growth Medium A was replaced with Growth Medium B which has a higher concentration of pH indicator.

Alternatively, cells were passaged by EDTA dispersion in the absence of trypsin. The procedure used was similar to that described for trypsinization except the cells were washed with trypsin diluent rather than with PBS, which contains calcium salts, and were incubated with EDTA solution in trypsin diluent.

Results

Epithelial cells from malignant and non-malignant bladder and kidney tissues have been established in tissue culture (Table 1). One-third of all explants produced epithelial cells which could be maintained in vitro for at least five cell transfers. Two cultures have now been carried for more than 70 transfers and are considered established as cell lines. One kidney culture has been passaged 45 transfers with similar characteristics. Figures 1 and 2 illustrate the types of epithelial cells derived from bladder and kidney tissue respectively.

Table 1. Summary of tissue culture attempts with urogenital tissues

	Bladder	Kidney	Prostate
Epithelial cells (a) (≥ 5 transfers)	14	8	3
Discarded or lost (b) (< 5 transfers)	27	11	18
Other in culture (< 5 transfers)	9	6	6
Total	50	25	27

a) cells carried at least 5 passages in vitro over a period of several months

b) cells discarded due to outgrowth of fibroblasts or lost before being transferred 5 times

In vitro culture of prostatic epithelium is more difficult. Only one tenth of the culture attempts produced epithelial cells which could be carried for more than five transfers. Adequate outgrowth of cells from the explants of prostate tissue occurred in each instance, but most of these cultures failed to survive the initial transfer into secondary culture. Prostatic cells are apparently very sensitive to the action of trypsin and more efficient passage of viable cells has been achieved with 1 mm EDTA in trypsin diluent. Transfer of cells with EDTA in the absence of trypsin does not result in complete dispersion of the cells into single cells but in the transfer of small sheets of cells (Fig. 3). These cell sheets then grow as microexplants and spread radially across the dish. Although the degree of cell dispersion is less using EDTA alone, cell viability is increased.

Excluding the established cell lines, all of the epithelial cells exhibit slow growth kinetics in vitro. Once established in culture, the cells grow equally well on MEM, RPMI 1640 medium, Ham's F10 or F12 medium and BME medium (GIBCO). Substitution of HEPES buffer in the medium has no effect. Earle's salts and Hank's salts are equally effective for cell growth. Thus, several growth medium supplements have been ineffective in accelerating the rate of cell division in established cultures. Supplemented medium has a significant effect on cell growth only during outgrowth of cells from the explant, with outgrowth from explants more rapid in Growth Medium A compared to Growth Medium B. Medium B is a good maintenance medium after the cells have adapted to in vitro growth.

Discussion

An active tissue culture programme is essential to the proper analysis of the biology of human urogenital tissue. The procedure described in this report yields a significant percentage of long-term epithelial cell cultures from both malignant and non-malignant urogenital tissues. Approximately 30 % of all bladder and kidney culture attempts produced in vitro epithelial cell cultures which could be carried for more than five transfers over a period of several months. The infrequent contamination with other than epithelial cells is not fully explained. Attempts were made to remove supporting stroma before explantation and this may have reduced the problem of fibroblast contamination.

The explant culture procedure, as described, may not favor outgrowth of fibroblasts from the tissue pieces, thereby giving the more slowly growing epithelial cells a selective advantage in the early stages of culture.

In vitro culture of malignant bladder tissue was more successful than the culture of non-malignant adjacent tissue. No correlation has been made with the tumor stage versus ability of the tissue to be cultured in vitro. In contrast to bladder tissue, non-malignant kidney tissue could be established and propagated in vitro more easily than malignant renal tissue. Less success was achieved in establishing long-term cultures of prostatic epi-

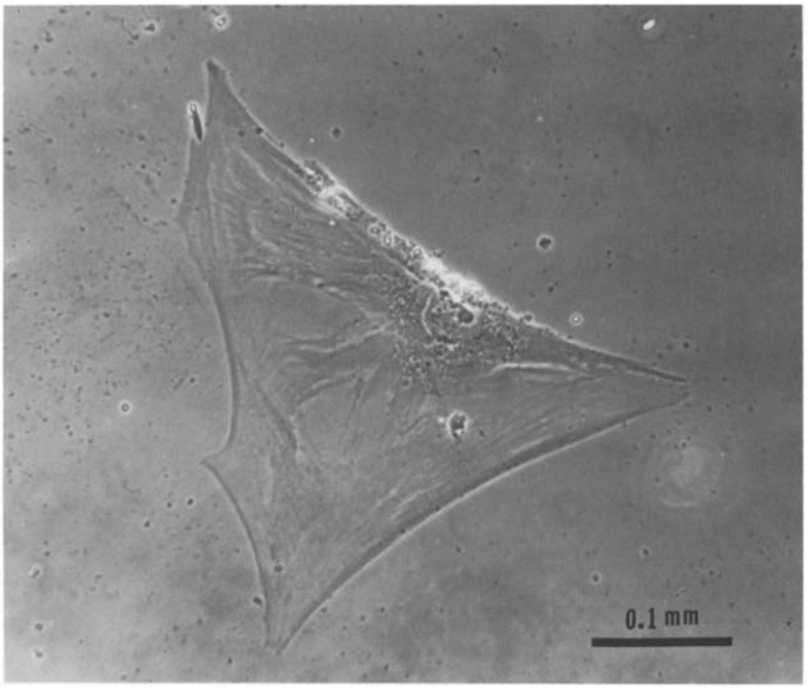

Fig. 1. Phase contrast micrograph of bladder urothelium in tissue culture

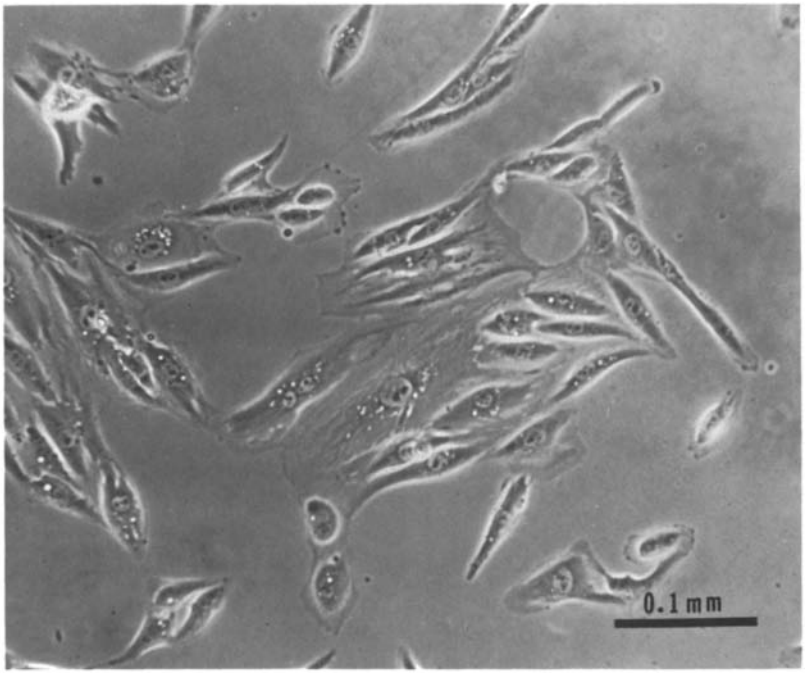

Fig. 2. Phase contrast micrograph of kidney epithelium in tissue culture

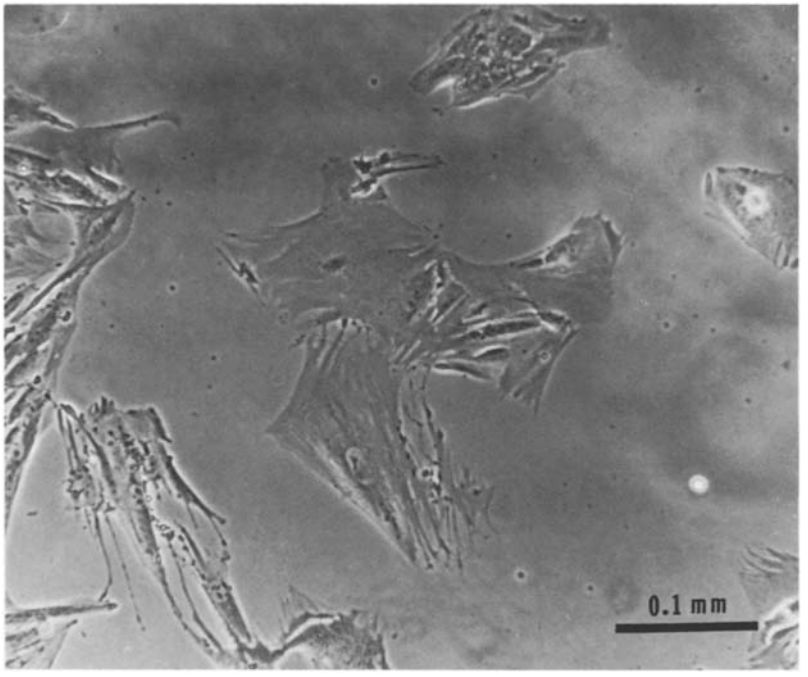

Fig. 3. Phase contrast micrograph of prostatic epithelium in tissue culture

thelium with a higher percentage of the culture attempts resulting in fibroblast outgrowth from the explants. This may reflect the lower percentage of prostatic epithelial cells compared to the other tissues examined. Also, a greater number of the culture attempts on prostate tissue failed at the first transfer from explant into secondary culture. There was adequate epithelial cell outgrowth from the explants, but the cells were more susceptible to damage during passage than were the epithelial cells of bladder and kidney. Removal of trypsin from the transfer solution improved survival during passage. Our experience indicates that prostatic glandular epithelium is easily damaged by trypsin and that use of this proteolytic enzyme is best avoided.

Ultrastructural studies on the cultured cells indicate that these cells are epithelial cells. The cells are not spindle shaped in culture like most fibroblasts and the cells exhibit no noticeable migratory properties characteristic of fibroblasts. With the exception of the cell lines, the cells are highly contact inhibited. Electron micrographs of thin sections of the cultured bladder cells indicate that they have properties similar to the intermediate urothelial cell layer of the bladder epithelial lining. (7, 8, 10)

Prolonged cell viability is best achieved if the epithelial cultures are maintained at high cell densities which supports Hosick's observation (6) that the morphology and growth rates of epithelial cells in culture are density dependent. For this reason, it is preferable to make the initial transfer of cells from explant into as small a culture dish as possible. Further subculture then should be performed when the cells are confluent and high cell densities should be maintained. This process necessitates more frequent transfer of cells, but greatly inccreases cell viability for a longer period of time. Cells in sparse culture quickly become highly vacuolated and stop dividing. It is usually impossible to rescue these cultures once cell death starts.

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