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# Short-term Culture of Epithelial Cells from Urine of Adults (40509)

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Many techniques have been proposed for obtaining cultures of human epithelial cells (1). However, most of them are laborious, requiring an inordinate amount of manipulation of the tissues of origin and/or of the cultured cells. In addition, even with the most elaborate procedures there is no guarantee that the resulting cultures will not be an admixture of fibroblastic and epithelial cells. To overcome some of these difficulties, human milk (2, 3), amniotic fluid (4) and semen (1) have recently been used as sources of cultured epithelial cells. However, it is self-evident that these sources place limits regarding the sex, physiological status and age of the sample donors. Thus, the availability of a common, noninvasive, easily accessible source of epithelial cell populations capable of multiplying in vitro would be advantageous. From several reports (5–9) and the present results it appears that human urine fulfills these requirements. Because of our interest in cultured cells derived from bladder tumors (10, 11), this study addresses itself to the initiation of cultures with the urinary sediments of adult individuals.

*Materials and methods.* Midstream urinary specimens were collected from normal subjects and from patients with urological conditions. Specimens were also obtained from patients with an indwelling Foley catheter. In the case of spontaneously voided urines, collection was done in sterile 50 ml centrifuge tubes or 250 ml Erlenmeryer flasks. Urines obtained through catheter were collected in centrifuge tubes. No preservatives were used. The specimens were centrifuged at 250*g* for 10 min and the supernatant was discarded. The sedimented cells were suspended in 12 ml of Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 20% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and amphotericin B (0.25 μg/ml); combined into one tube (if necessary) and centrifuged again. After centrifugation the supernatant medium was removed and the cells were resuspended in fresh culture medium. The amount of medium used at this step depended on the size of the culture containers to be employed. Thus, 2 ml were used to inoculate 30 ml T flasks (Falcon Plastics, Oxnard, CA), 1 ml when inoculated into 35 mm Falcon Petri dishes, into culture/chamber slides (LabTek Products, Naperville, IL) or into coverslip-containing Leighton tubes (Costar, Cambridge, MA). The presence of blood cells among the sedimented cells did not interfere with the initiation of growth.

The cultures were incubated at 37° in a humidified atmosphere of 5% $CO_2$ in air and left undisturbed for the initial 24 hr. Thereafter they were examined daily for cellular attachment and outgrowth. The presence of large amounts of erythrocytes did not hinder the initial microscopic examination of specimens from patients with hematuria. Cultures contaminated by bacteria and/or fungi were discarded as soon as noticed. The initial culture medium was replaced with fresh medium as soon as cellular attachment was evident or 7–10 days after culture initiation when no attachment was observable. Following the determination that cell multiplication was taking place, the medium was changed twice or thrice a week. Cultures showing no growth were kept in the incubator without subsequent medium change for up to 4–6 weeks. Pictures of living cells were taken under phase contrast using an inverted Leitz microscope equipped with a Polaroid attachment.

*Results.* Primary outgrowth of epithelial-like cells from urinary sediments of normal adult males was discernible within 5 to 20 days. Colony formation was seen with the specimens of 32 out of 47 individuals (69%). The proliferating cells were flat, spread and formed relatively densely packed colonies. Frequently round (mitotic?) cells were observed. Occasionally colonies of smaller cells



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forming dense whorls were also noticed. In no instance was proliferation of typical fibroblast cell types seen. Representative examples of the outgrowth of cells derived from normal male urines are shown on Figs. 1–3. As can be seen, by 7 days after culture initiation with the sediment of a 35-year-old male, there were relatively few cells attached to the growth surface (Fig. 1A), whereas by day 11 the same area of the culture flask showed a considerable increase in the number of cells (Fig. 1B). In some instances, pronounced proliferation was evident within 6 days (Fig. 2A), followed by the formation of a monolayer by the 10th day (Fig. 2B). With other specimens, relatively moderate growth was noticed within one week (Fig. 3A), but 3 days later colonies of loosely packed cells were evident (Fig. 3B). Cellular outgrowth has been obtained from the sediment of as little as 25 ml of normal urine. Less than 10% of the cultures from the urines of normal males had to be discarded because of bacterial and/or fungal contamination. By contrast, the proportion of contaminated cultures from normal females was close to 90%. In an effort to overcome this problem, the concentration of the antibiotic–antimycotic mixture was increased five-fold (this concentration did not affect cellular outgrowth from male urines), but no significant improvement was obtained. As a rule the urinary specimens were processed immediately, but in some instances they were kept experimentally at room temperature for up to 6 hours. Outgrowth of cells from such samples was not different than that obtained with their control counterparts.

Colony formation of epithelial-like cells has also been obtained with urines from patients with an indwelling Foley catheter. Figure 4 shows the cells cultured from a 5 ml specimen of a catheterized patient with benign nodular overgrowth of the prostate. Cell growth was vigorous by the eighth day (Fig. 4A) and by the 12th day an almost complete monolayer and numerous round (mitotic?) cells were observed (Fig. 4B). Outgrowth of urinary cells from patients with bladder tumors has also been observed and Fig. 5 documents such a case. Cell growth occurred with five out of eight specimens obtained from a patient with papillary urothelial carcinoma (grade III) and poorly differentiated adenocarcinoma of the bladder. Three cultures had to be discarded because of contamination. Cell attachment to the growth surface was evident within 3 days (Fig. 5) and cytological preparations of cells grown for 20 days revealed the presence of malignant cells.

Because around 30% of the cultures initiated with the urines of normal males did not grow, the suitability of several other media (M-199; RPMI-1640; McCoy's 5A; Waymouth's 752/1) and sera (30% fetal calf serum, human serum) were tested. However, in no instance was an improvement on the culture outcome noted. Attempts to subculture the proliferating cells have not been very successful. Although growth has been observed occasionally with cells transferred twice or thrice, it would appear that growth potential of the exfoliated urinary cells from adult individuals is limited once they have reached maximal proliferation in primary culture.

*Discussion.* Few attempts on using human urine for the initiation of epithelial cell cultures have been reported. In one study the outgrowth of cells from the urines of four fetuses between the 12th and 16th week of gestation was demonstrated, suggesting that fetal urine may contribute to the population of viable cells present in amniotic fluids (5). In another investigation it was shown that cell growth occurred with seven out of eight specimens obtained from neonates of less than 2 days of age (6) and that the proliferating cells could be used for chromosomal analysis (12). In another series cell growth was seen with 60% of urinary samples from newborns and 25% of adult specimens (7). In a study dealing only with adults, colony formation was seen with 17 of 24 urine samples from two normal individuals and with two specimens from a patient with congestive heart failure, whereas single samples from another such patient and from an individual with barbiturate intoxication grew poorly (9). No growth was seen with urines whose specific gravity was below 1.008. Outgrowth of fibroblasts was not seen in any of these studies.

From these reports and the present results it is evident that some epithelial cells recovered from human urine are capable of in vitro proliferation. However, because the exact site(s) of origin of epithelial cells present in normal urinary sediments has not been

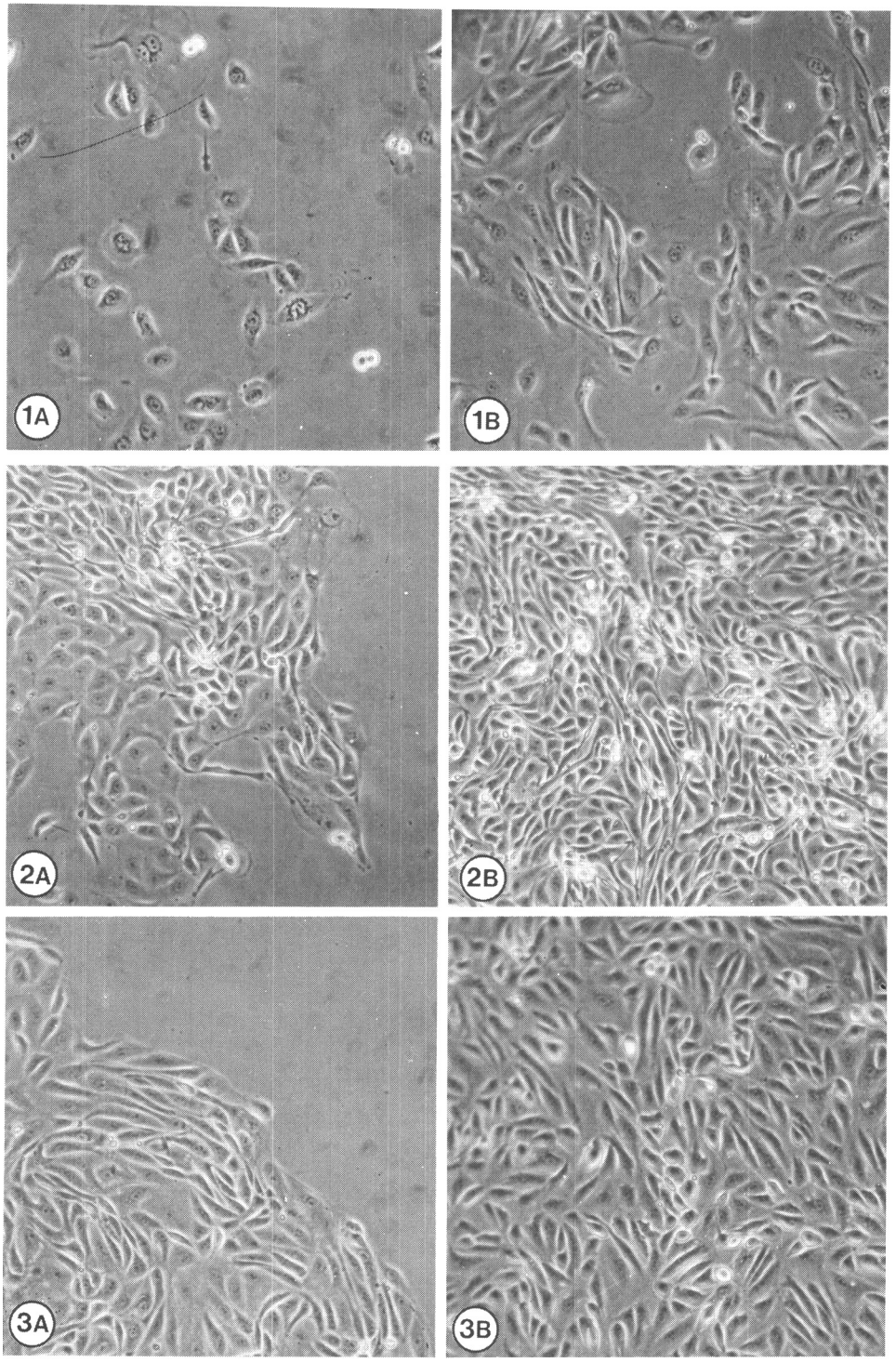


FIG. 1. Cultured urine cells from a 35-year-old normal male: A) Seven days after culture initiation; B) The same area 4 days later. Phase contrast of living cells (97×).

FIG. 2. Cells from a 30-year-old normal male: A) Six days; B) 10 days after culture initiation (97×).

FIG. 3. Cells from a 43-year-old normal male: A) Seven days; B) 10 days after initiation of culture (97×).

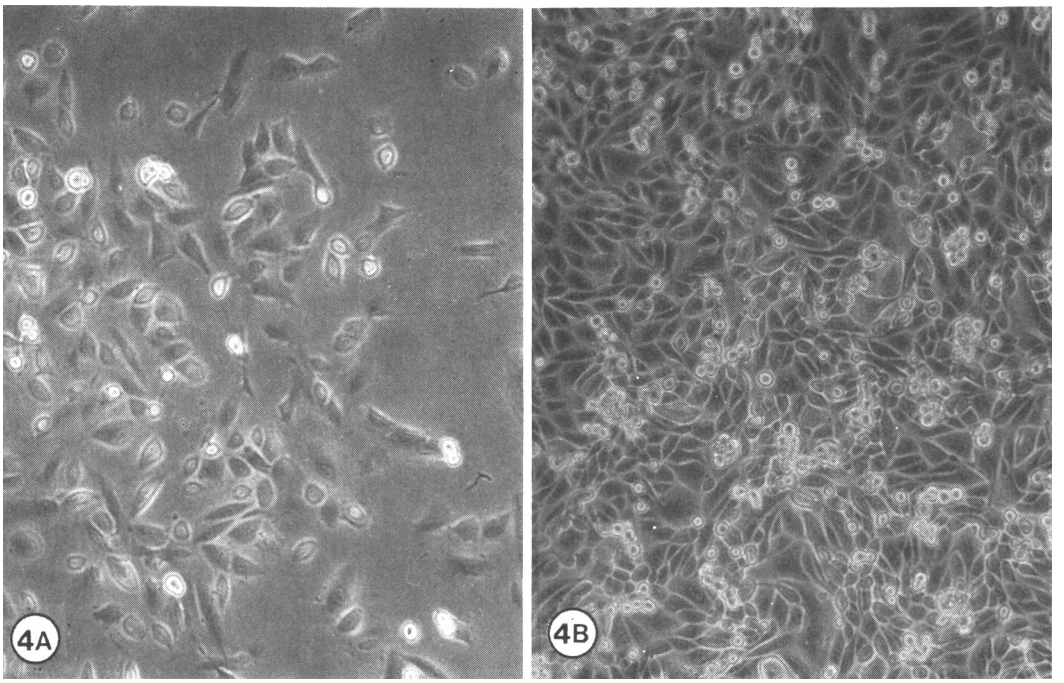


FIG. 4. Cultured urine cells from a catheterized 66-year-old patient with benign nodular overgrowth of the prostate: A) Eight days, B) 12 days. Many rounded (mitotic?) cells can be seen (97×).

established (8, 9, 13), it is difficult to indicate the anatomical source of the cells which grow in culture. Normal urine usually contains cells desquamated from the lining epithelium and they could be renal–tubular cells, transitional cells from the renal pelvis, the ureters, the bladder and the urethra. In males the urine may also contain prostatic cells and in females there may be an admixture of cells, including those of vaginal origin. The number of cells in the urine increases considerably in inflammatory disorders, lithiasis, malignancies or after catheterization (14). Attempts made to answer some questions about the origin of the proliferating cells have been only partially successful: The present observations that urines collected from the bladder through an indwelling catheter contains cells capable of multiplying *in vitro* would indicate that in these instances they may come from the bladder or above. As another approach, repeated urine cultures were initiated from a male renal transplant patient who had received a kidney from a female donor. Although in no instance was cell growth ob-

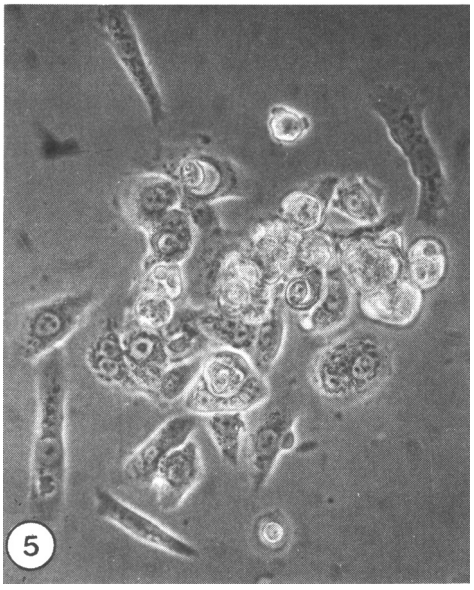


FIG. 5. Cultured urine cells from a 60-year-old female with papillary urothelial carcinoma (grade III) and poorly differentiated adenocarcinoma of the bladder; 3 days after culture initiation.

served, the idea was to carry out karyological analysis on the proliferating cells.

The urine cell culture system appears to be a promising tool for studying epithelial cells. However, many problems remain to be solved. These include: (a) Conditions favoring proliferation of cells from all urinary specimens; (b) a systematic study of the contaminating flora in female specimens and their antibiotic sensitivity; (c) conditions favoring subculture of the primary cultures; (d) determination of the anatomical site(s) of origin of the proliferating cells. The system may lend itself for diagnostic purposes, especially in those instances where the proportion of cells of interest in the urinary sediments is minimal and the cultured cells retain distinguishing diagnostic characteristics. It might be possible to use the proliferating normal epithelial cells as targets for viruses and chemicals, thus providing an *in vitro* model of human origin to study the events occurring during malignant transformation of the urothelium.

*Summary.* Outgrowth of cells occurs when sediments from voided or catheterized urines are placed in culture. Proliferation of epithelial-like cells was obtained with 69% of the urines from normal adult males and from patients with an indwelling catheter. Similar observations were made with malignant cells derived from urines of patients with urothelial carcinoma. In no instance was the outgrowth of fibroblast-like cells seen.

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