Studies on Carcinogenesis of Human Prostate. II. Long-Term Explant Culture of Normal Prostate and Benign Prostatic Hyperplasia: Light Microscopy[1,2,3,4]

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ABSTRACT—Light microscopic and histochemical changes in normal human prostate and benign prostatic hyperplasia (BPH) during long-term explant culture were characterized. Normal human prostate obtained at immediate autopsy of young adults or BPH obtained at the time of surgery were maintained in explant culture as long as 24 weeks. Morphologic alterations in glandular epithelium and stroma in response to culture conditions were assessed by light microscopy and histochemistry of mucosubstances. The histologic and histochemical responses of normal prostate and BPH to in vitro conditions were essentially identical. Within 1 week, secretory epithelial cells became necrotic and sloughed into acinar lumina. Remaining epithelial cells proliferated, repopulated acinar structures, and migrated onto explant surfaces forming a new well-differentiated epithelium characterized by synthesis and secretion of neutral and acidic mucosubstances. During subsequent periods in vitro, synthesis and secretion of mucosubstances gradually diminished whereas the stroma and deep glandular structures became necrotic. Our observations suggest that cells comprising the new epithelium in cultured explants of normal human prostate and BPH are derived from prostatic basal cells.—JNCI 1982; 69:751–756.

Spontaneous adenocarcinoma of the prostate is the most common malignant neoplasm in the adult male and the third most common cause of death from cancer in the United States; only bronchogenic and colorectal carcinomas are more common causes of death. Despite numerous studies of the disease, little progress has been made in understanding the etiology, histogenesis, clinical behavior, and other features of this important neoplasm. Clearly, a suitable model of this disease is needed.

However, the validity of extrapolation from animal models of prostatic adenocarcinoma to the human neoplasm is questionable, and the need for models that are based on human tissues has been recently emphasized (*1–3*) despite inherent difficulties. For example, induction of adenocarcinoma in prostatic tissue of humans can only be done in vitro, with subsequent xenotransplantation for the assessment of tumorigenicity. However, one objection to this approach has been that human prostate tissue usually available for such studies is obtained from surgical specimens of BPH from older patients, which is *not* normal prostate (*1*). Moreover, numerous authors report maintenance of human prostate in explant culture for relatively short periods, usually less than 2 weeks (*4–10*); others report maintenance for periods of 6 weeks (*11*) to many months (*12*). However, few details are available with respect to morphologic or other changes occurring in glandular epithelium during short- or long-term culture. Establishment of these changes is vital as a base line for the interpretation of cellular alterations in response to agents such as hormones or chemical carcinogens in vitro.

In this paper, we provide this base-line information by describing histologic changes in normal human prostate of young adults throughout long-term explant culture as long as 24 weeks, with emphasis on epithelial cells and their responses to in vitro conditions. Further, we compare responses in normal human prostatic epithelium with those of BPH.

MATERIALS AND METHODS

Tissues.—Human prostate tissues were obtained from 8 patients, 16–31 years old, during IA after accidental death (*13*) or surgically from 30 patients, 55–82 years old, treated for BPH by transurethral resection or suprapubic prostatectomy.

Explant culture.—Complete details pertaining to culture techniques used in this study are presented elsewhere (*14*). Explants were cultured in CMRL-1066 medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with antibiotics, 5% heat-inactivated fetal bovine serum, hydrocortisone, and insulin. Cultures were incubated at 37°C on rocker platforms (Bellco Glass, Inc., Vineland, N.J.) in an atmosphere of 5% CO_2, 45% O_2, and 50% N_2. Culture medium and gas phase were changed three times each week.

Fixation.—During intervals of culture, from zero time to 24 weeks, explants were sampled and fixed in mixed alde-

Abbreviations used: AB=alcian blue, pH 2.5; AF=aldehyde fuchsin, pH 1.0; BPH=benign prostatic hyperplasia; H & E=hematoxylin and eosin; IA=immediate autopsy; LM=light microscopy; PAS=periodic acid–Schiff.

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hydes (4% formaldehyde–1% glutaraldehyde in 200 mos*M* phosphate buffer) (*15*) and were processed for LM and histochemistry.

LM.—Fixed tissues were dehydrated through a series of graded alcohols, embedded in paraffin, cut at a thickness of 4–5 μm, and routinely stained with H & E.

Histochemistry.—For the study of mucosubstances, serial sections were stained with PAS with and without α-amylase digestion, AB–PAS sequence, AF–AB sequence, and mucicarmine. Before being stained with PAS, tissue sections were treated with dimedone (*16*), which blocked free aldehyde groups contributed by the glutaraldehyde component of the fixative groups that would otherwise react with the Schiff reagent producing a false-positive result.

RESULTS

Explants showed good preservation of morphology in culture for as long as 24 weeks for normal prostate and as long as 20 weeks for BPH, at which times all explants had been sampled. The observations described below are characteristic of all explants of normal prostate or BPH which showed survival of epithelial cells.

Zero Time Culture

Normal glands, IA.—By LM, normal prostate consisted of numerous tubuloalveolar glands and ducts surrounded by dense eosinophilic fibromuscular stroma (fig. 1). The glandular epithelium was composed of two cell types: *a*) columnar to cuboidal secretory cells with a foamy cytoplasm and large, round nuclei and *b*) flattened basal cells which lacked foamy cytoplasm and possessed a triangular or elongated, densely staining nucleus (fig. 2). Eosinophilic corpora amylacea were occasionally present in glandular lumina (fig. 1).

Histochemically, in dimedone-blocked histologic sections, PAS-positive, α-amylase-resistant granules were seen scattered within the cytoplasm of secretory epithelial cells (fig. 3). At the cell apex, the granules appeared coarser than elsewhere. Within gland lumina, these granules appeared free or in coarse aggregates, sometimes in association with PAS-positive amorphous material. PAS-positive granules and amorphous material were negative for acidic component by the AB–PAS or AF–AB sequence. In some acini, rather large PAS-positive, α-amylase-resistant droplets of varying diameter were present, especially in basal portions of secretory cells as well as within acinar lumina.

BPH.—LM of specimens of BPH showed varying degrees of glandular and stromal hyperplasia. The acini were sometimes enlarged with numerous papillary processes projecting into the lumina. Corpora amylacea were more frequently seen in BPH than in normal prostate. Otherwise, the histology and histochemistry were essentially identical to the histology and histochemistry of normal prostate, as described above.

One Week of Culture

Normal glands, IA.—Within explants during the first 2–3 days of culture, rapid degeneration and necrosis of secretory epithelial cells were striking features, although the rate of degeneration varied from acinus to acinus (figs. 4, 5). In some acini, an occasional pyknotic nucleus was seen; in other acini, large numbers of necrotic secretory cells sloughed into the lumen. Compared to acini near the surface of explants, degeneration appeared sooner and was more extensive in acini deep within explants. Eventually, within this time, some gland lumina were filled with heterogeneous eosinophilic cellular debris (figs. 5, 6). Some acinar cells did not degenerate but remained viable and appeared to repopulate acini and ducts denuded of secretory cells. Mitotic figures were sometimes seen among these cells during and subsequent to degeneration of secretory cells.

At the same time degenerative changes were occurring within the explants, epithelial cells migrated from exposed ducts or glandular spaces over the cut surfaces of explants. By 1 week of culture, the cut surface was shown by LM to be completely covered with a sheet of migrating cells, which was continuous with the cell sheet lining the exposed ducts or glandular spaces exposed to view after sectioning had been done. In occasional explants consisting only of stromal tissue, few migrating cells were seen.

Cells repopulating acini and cut surfaces of explants formed a multilayered epithelium characterized by widened intercellular spaces. The epithelium consisted of columnar to polygonal or flattened, squamous-like cells; polygonal cells predominated (fig. 6).

By histochemistry, the surface cells of the explant as well as the cells lining the glands within explants exhibited numerous PAS-positive, α-amylase-resistant granules, particularly in the apical portion above the nucleus (fig. 7). At the cell apex, granules were dense and intensely PAS-positive and were also frequently stained with AB (fig. 8) but not with AF; these staining patterns are consistent with both neutral and sialic acid-containing mucosubstances. In the supranuclear zone within these cells, granules usually stained only with PAS. AB-positive mucosubstances also stained with mucicarmine to varying degrees. The apical surfaces of cells appeared to stain with AB, AF, and mucicarmine. Substances with similar staining properties also were present within gland lumina.

BPH.—LM and histochemical observations for BPH were essentially identical to those described above for normal prostate. Squamous metaplasia was sometimes seen in specimens of BPH (fig. 9) but was not seen in normal prostate.

Two Weeks of Culture

Normal glands, IA.—Fewer tall columnar cells were seen at this time. An occasional mitotic figure was present among acinar and surface cells of explants. Few viable cells were noted in the fibromuscular stroma.

At this time, mucosubstances were abundant in cells with markedly convex apical surfaces. This observation was especially true for columnar cells, in which the convex apical surfaces persisted. Outer surfaces of cells continued to stain with AB, AF, and mucicarmine.

BPH.—Explant surfaces were covered by a mucus-secreting multilayered epithelium (fig. 10), essentially identical to that described above for normal prostate.

Three to Twenty-Four Weeks of Culture

Normal glands, IA.—Most cultures were terminated at 12–14 weeks. However, one experiment with normal prostate was terminated at 22 weeks and another was terminated at 24 weeks. Throughout this period, the surface sheet was maintained even in 24-week explants. It differed in thickness from area to area, yet the overall organization remained virtually the same (figs. 11, 12). Intact acini were lined by polygonal, cuboidal, or squamous-like cells and were generally limited to the peripheral zones of explants. Occasional mitotic figures were seen. During this interval, mucosubstances were present but in amounts that appeared diminished when compared to amounts during earlier intervals. There were fewer apical granules present in surface or acinar cells, yet histochemical staining was qualitatively similar to staining at earlier times. The surfaces of these cells continued to show an affinity for AB and mucicarmine. Few viable cells were present within the necrotic stroma.

BPH.—Morphologic and histochemical changes occurring in explants of BPH were identical to those changes described above for normal prostate. Squamous changes persisted in some BPH explants.

DISCUSSION

The present study is the first to describe LM of long-term explant culture of normal, nonhypertrophic, nonneoplastic human prostate obtained from young adult patients, with a comparison to BPH obtained from older individuals. Previous studies of cultured human prostate have usually used BPH from older men which had been obtained surgically because of obstructive prostatic disease (*4–10, 12*). Noyes (*11*) used prostate tissue from patients 50–70 years old, which was sampled at the time of cystectomy, and concluded that it was histologically "normal." However, BPH is a common entity beyond the age of 50 and is usually unnoticed until obstructive symptoms appear. Despite the similar response of BPH and normal prostate to in vitro conditions in the present paper, Franks (*1*) has concluded that BPH is *not* normal prostate. Moreover, there is a possibility of inadvertently sampling preneoplastic or neoplastic cells in older patients, which would invalidate studies on induction of malignant transformation by carcinogenic agents (*1*).

On the basis of continuity and similarities in morphology and histochemistry of cells colonizing explant surfaces and acinar structures throughout long-term culture of normal prostate and BPH, we conclude from the present work that epithelial cells covering explant surfaces are identical to and are derived from epithelial cells of acini. In these acini, cells in a basal location appeared to remain viable during degeneration and necrosis of secretory cells. The presence of occasional mitotic figures among these basally situated cells suggests proliferation followed by repopulation of acini and migration and colonization of explant surfaces. Thus it seems likely that proliferation of basal cells accounts for repopulation of acinar structures, colonization of explant surfaces, and synthesis and secretion of mucosubstances during early periods of culture.

Previous studies of BPH or normal prostate in explant culture have reported maintenance of viability of glandular epithelium for periods of typically less than 2 weeks to periods as long as 48 months (*4–9, 11, 12*). Usually, no distinction was made in these studies between columnar secretory cells and basal cells with regard to degeneration and necrosis nor were morphologic features described in detail, as we attempted in the present study. Maintenance of secretory epithelial cells with a columnar profile has been possible for as long as 10 days (*6*), although reduction in secretory vacuoles and cell height has been reported after only a few days in vitro (*4, 10*). Squamous changes have also been seen during early periods of culture (*4, 6, 9–11*). On the basis of the present study, it is very unlikely that epithelial cells seen during long-term explant culture of human prostate in previous work are viable columnar secretory cells. Rather, we propose that cultured prostatic epithelial cells seen by previous authors were derived from basal cells we have defined ultrastructurally (*17*).

The implication for a potential role of basal cells in the genesis of adenocarcinoma of the prostate is strongly suggested by the above observations and by morphologic evidence of premalignant and malignant changes in basal cell derivatives in response to *N*-methyl-*N'*-nitro-*N*-nitosoguanidine in vitro (*18, 19*). In this regard we also find it interesting that differentiated basal cells synthesize and secrete acidic mucosubstances during explant culture, which we report in the present paper. Although acidic mucosubstances are not demonstrable histochemically in the epithelium of normal prostate, they are frequently detected in prostatic adenocarcinoma (*20, 21*) and have staining affinities (neutral and acidic components) similar to those seen in the present study. Using histochemical techniques, Noyes (*11*) also detected neutral mucosubstances in the epithelium of prostatic explants after culture for 12 days, but he did not stain for acidic components, and thereby he failed to recognize synthesis and secretion of acidic mucosubstances in vitro.

REFERENCES

(*1*) Franks LM. Tissue culture in the investigation of prostatic cancer. Urol Res 1977; 5:159–162.

(*2*) Handelsman H. The limitations of model systems in prostatic cancer. Oncology 1977; 34:96–99.

(*3*) Merchant DJ. Model systems for the study of prostatic cancer. Oncology 1977; 34:100–101.

(*4*) Schrodt GR, Foreman CD. In vitro maintenance of human hyperplastic prostate tissue. Invest Urol 1971; 9:85–94.

(*5*) Stonington OG, Hemmingsen H. Culture of cells as a monolayer derived from the epithelium of the human prostate: A new cell growth technique. J Urol 1971; 106:393–400.

(*6*) McMahon MJ, Thomas GH. Morphological changes of benign prostatic hyperplasia in culture. Br J Cancer 1973; 27:323–335.

(*7*) McRae CU, Ghanadian R, Fotherby K, Chisholm GD. The effect of testosterone on the human prostate in organ culture. Br J Urol 1973; 45:156–162.

(*8*) Harbitz TB, Falkanger B, Sander S. Benign hyperplasia of the human prostate exposed to steroid hormones in organ culture. Acta Pathol Microbiol Scand Suppl 1974; 248:89–93.

(*9*) Lasnitzki I, Whitaker RH, Withycombe JF. The effect of steroid hormones on the growth pattern and RNA synthesis in human benign prostatic hyperplasia in organ culture. Br J Cancer 1975; 32:168–178.

(*10*) Dilley WG, Birkhoff JD. Hormone response of benign hyperplastic prostate tissue in organ culture. Invest Urol 1977; 15:83–86.

(11) Noyes WF. Effect of 3-methylcholanthrene (NSC-21970) on human prostate in organ culture. Cancer Chemother Rep 1975; 59:67–71.

(12) Franks LM, Riddle PN, Carbonell AW, Gey GO. A comparative study of the ultrastructure and lack of growth capacity of adult human prostate epithelium mechanically separated from its stroma. J Pathol 1970; 100:113–119.

(13) Trump BF, Valigorsky JM, Jones RT, Mergner WJ, Garcia JH, Cowley RA. The application of electron microscopy and cellular biochemistry to the autopsy. Observations on cellular changes in human shock. Hum Pathol 1975; 6:499–516.

(14) Sanefuji H, Heatfield BM, Trump BF. Studies on carcinogenesis of human prostate. I. Technique for long-term explant culture. Tissue Cult Assoc Manual 1978; 4:855–856.

(15) McDowell EM, Trump BF. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch Pathol Lab Med 1976; 100:405–414.

(16) Pearse AG. Histochemistry: Theoretical and applied. Vol I. 3d ed. Boston: Little, Brown & Co., 1968:455–456.

(17) Heatfield BM, Sanefuji H, Trump BF. Studies on carcinogenesis of human prostate. III. Long-term explant culture of normal prostate and benign prostatic hyperplasia: Transmission and scanning electron microscopy. JNCI 1982; 69:757–766.

(18) ———. Studies on carcinogenesis of human prostate. IV. Comparison of normal and neoplastic prostate during long-term explant culture. Scan Electron Microsc 1979; 3:645–656.

(19) Sanefuji H, Heatfield BM, Trump BF. Studies on carcinogenesis of human prostate. V. Effects of the carcinogen, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), on normal prostate during long-term explant culture. Scan Electron Microsc 1979; 3:657–663.

(20) Franks LM, O'Shea JD, Thomson AE. Mucin in the prostate: A histochemical study in normal glands, latent, clinical, and colloid cancers. Cancer 1964; 17:983–991.

(21) Hukill PB, Vidone RA. Histochemistry of mucus and other polysaccharides in tumors. II. Carcinoma of the prostate. Lab Invest 1967; 16:395–406.

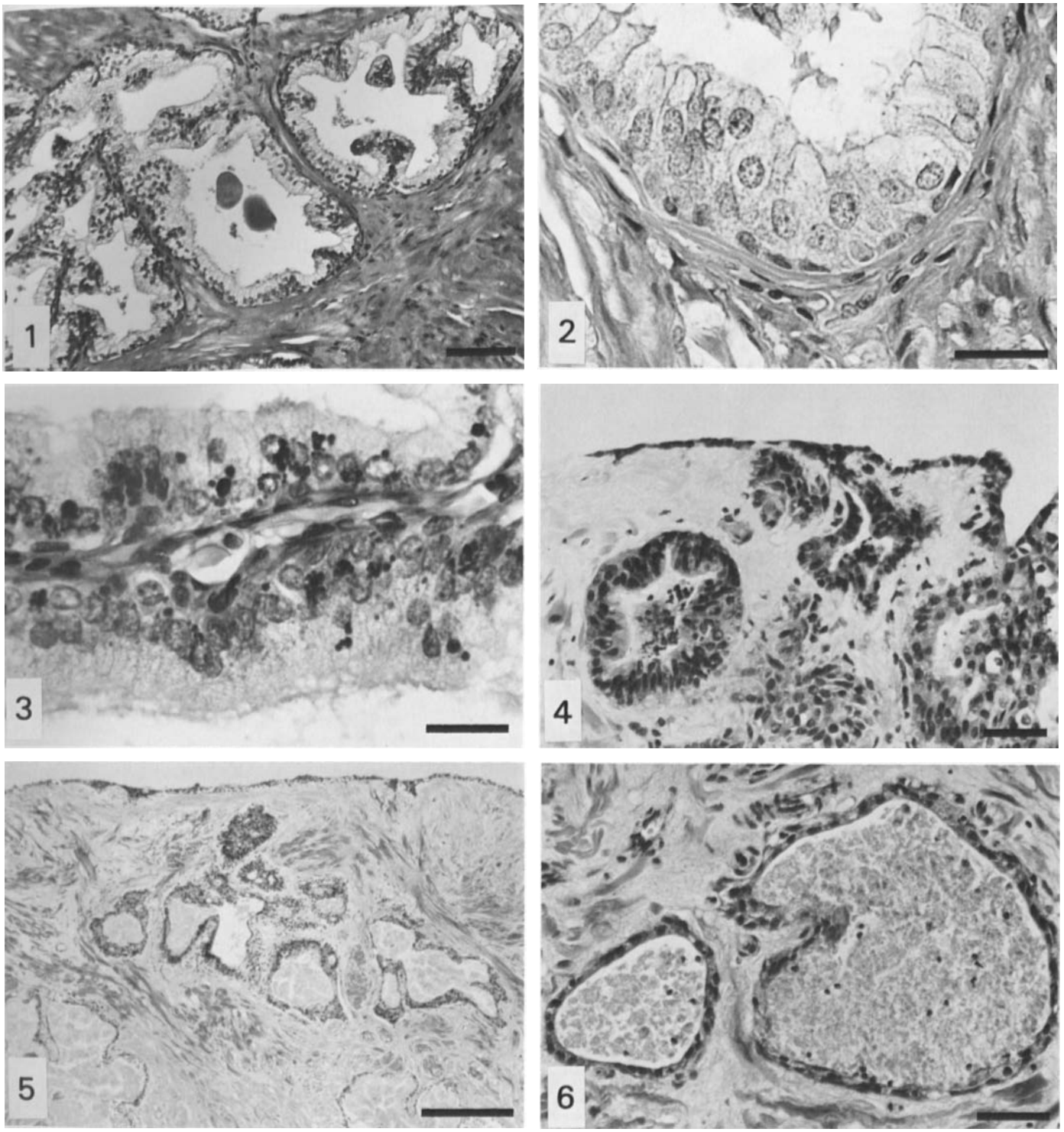

FIGURE 1.—Normal prostate, IA, zero time culture. Glandular lumina are lined by foamy-appearing epithelial cells and surrounded by fibromuscular stroma; two eosinophilic corpora amylacea appear within the central gland lumen. H & E. *Bar*=100 μm. × 120

FIGURE 2.—Higher magnification of fig. 1. Columnar secretory cells with foamy cytoplasm are oriented toward the gland lumen; basal cells beneath columnar cells are seen flattened along the basement membrane. H & E. *Bar*=25 μm. × 625

FIGURE 3.—Same tissue as in fig. 1. α-Amylase-resistant, PAS-positive fine granules are present in the cytoplasm of columnar cells. PAS after α-amylase digestion. *Bar*=25 μm. × 540

FIGURE 4.—Normal prostate, IA, 2-day culture. Epithelial cells appear on explant surfaces and adjacent glandular structures near the surface or deep within the explant. H & E. *Bar*=50 μm. × 225

FIGURE 5.—Normal prostate, IA, 1-wk culture. Viability of epithelial cells varies with distance from the explant surface. Deep within the explant, extensive necrosis and accumulation of cell debris are seen. H & E. *Bar*=250 μm. × 60

FIGURE 6.—Normal prostate, IA, 1-wk culture. Higher magnification of same explant as in fig. 5. Eosinophilic cellular debris fills glandular lumina lined by cuboidal or polygonal epithelial cells. H & E. *Bar*=50 μm. × 250

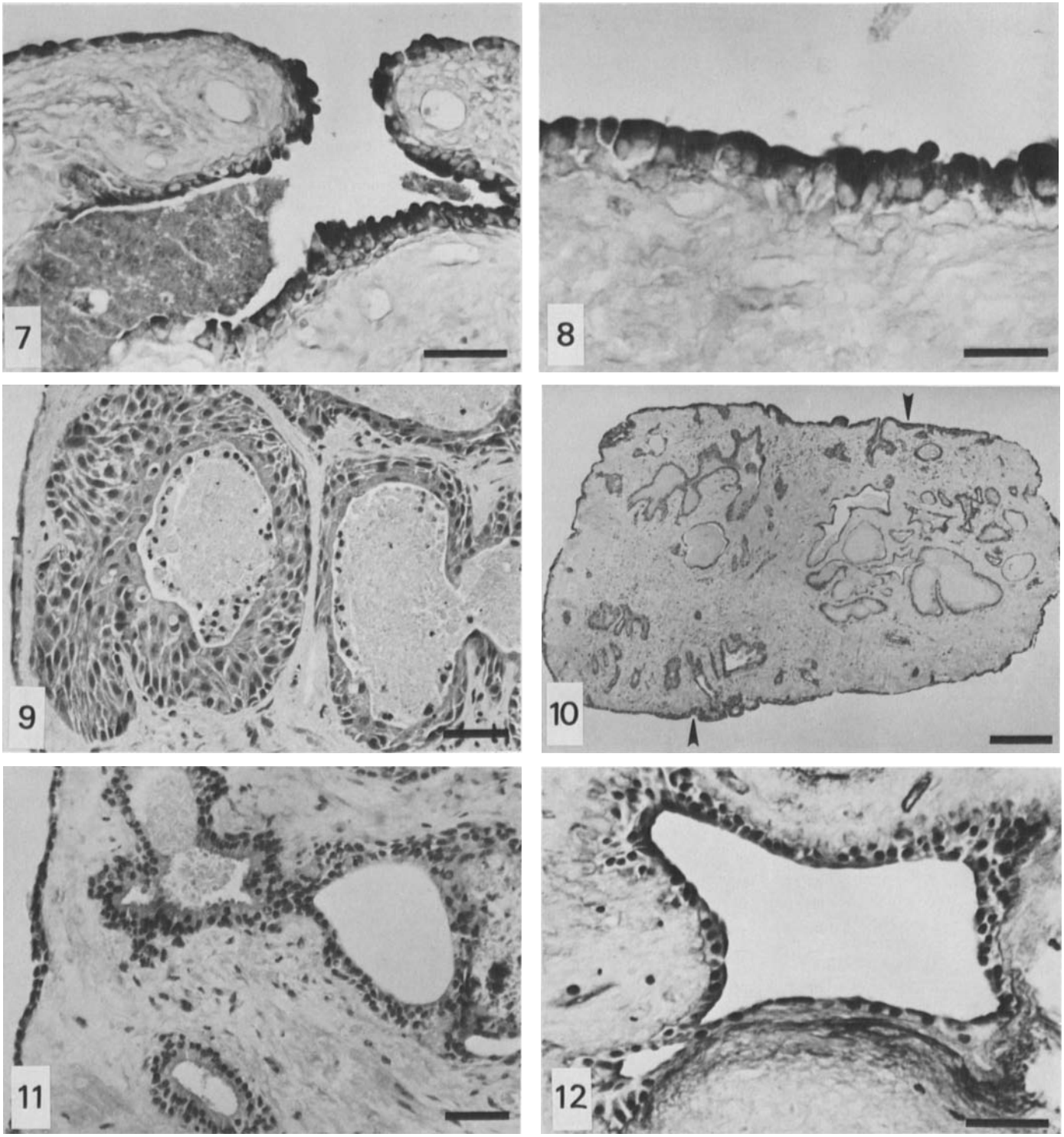

FIGURE 7.—Normal prostate, IA, 1-wk culture. PAS-positive, α-amylase-resistant material, for the most part, is restricted to the apical portion of epithelial cells. *Note* continuity of surface epithelial cells with cells of adjacent glandular structures and *note* identity of staining properties. PAS after α-amylase digestion. *Bar*=50 μm. × 280

FIGURE 8.—Normal prostate, IA, 1-wk culture. Mucosubstances are found in the apical portions of epithelial cells and at the outer cell surfaces. AB PAS. *Bar*=25 μm. × 575

FIGURE 9.—BPH, 1-wk culture. Morphologic appearances resembling squamous metaplasia are seen. H & E. *Bar*=50 μm. × 215

FIGURE 10.—BPH, 2-wk culture. A thin sheet of epithelial cells covers the cut surface (*arrowheads*) of the explant and is continuous with the epithelial cell layer lining glands and ducts within the explant. H & E. *Bar*=250 μm. × 45

FIGURE 11.—Normal prostate, IA, 5-wk culture. Well-preserved glands are lined by epithelial cells similar to those at the explant surface. H & E. *Bar*=50 μm. × 210

FIGURE 12.—Normal prostate, IA, 11-wk culture. Epithelial cells lining glandular spaces are cuboidal or flattened. H & E. *Bar*=50 μm. × 285