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Epithelial-Mesenchymal Interactions in Prostatic Development. I. Morphological Observations of Prostatic Induction by Urogenital Sinus Mesenchyme in Epithelium of the Adult Rodent Urinary Bladder

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ABSTRACT Tissue recombinants of embryonic urogenital sinus mesenchyme (UGM) and epithelium of the urinary bladder (urothelium, BLE) of adult rats and mice were grown for 3–30 d in male syngeneic hosts. Short-term in vivo growth indicated that prostatic morphogenesis is initiated as focal outgrowths from the basal aspect of the adult urothelium. The solid epithelial buds elongate, branch, and subsequently canalize, forming prostatic acini. After 30 d of growth in the male hosts, prostatic acini exhibit secretory activity. The marked changes in urothelial morphology induced by the UGM are accompanied by the expression of fine-structural features indicative of secretory function (rough endoplasmic reticulum, Golgi apparatus, and secretory granules). During this process, urothelial cells express prostatic histochemical markers (alkaline phosphatase, nonspecific esterase, glycosaminoglycans) and prostate-specific antigens. The expression within BLE of prostatic characteristics is associated with the loss of urothelial characteristics. These data indicate that adult urothelial cells retain a responsiveness to embryonic mesenchymal inductors. Furthermore, mesenchyme-induced changes in urothelial cytodifferentiation appear to be coupled to changes in functional activity.

Classical studies of tissue interactions in embryonic development support the idea that epithelial developmental potential is gradually restricted as a consequence of influences from inductive tissues and other environmental factors (53, 54, 56). In amphibian development, presumptive epidermis from the early gastrula is capable of differentiating into a variety of cell types (51, 54, 59). During embryogenesis this developmental plasticity is gradually restricted until the developmental fate is determined. In mammalian development, a similar temporal restriction of developmental potential has been reported for embryonic and neonatal epithelia (13, 34). The commitment to a particular epithelial differentiation is thought to be irreversible, since after epithelial determination recombination of epithelium with heterotypic mesenchyme does not modify epithelial cytodifferentiation (13, 15, 34, 50, 52). In adulthood, the stability of epithelial differentiation may be an ongoing manifestation of the irreversibility of the process of differentiation.

Recently, we have reported an exception to this concept by demonstrating that epithelium from the urinary bladder (urothelium, BLE) of adult mice can be induced by mesenchyme of the urogenital sinus (UGM, a prostatic inductor) to form glandular structures resembling prostate (18, 19). In many of these recombinants, the induced prostatic acini were filled with secretory product. This report presents additional observations that demonstrate that epithelium from the adult urinary bladder can be induced to express prostatic differentiation.

MATERIALS AND METHODS

Tissues were obtained from inbred BALB/c mice and Fisher 344 rats (Charles River Breeding Laboratories, Inc., Wilmington, MA). Urogenital sinuses were

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dissected from 15- to 16-d-old embryonic mice and 17- to 18-d-old embryonic rats (vaginal plug equals day 0). Urinary bladders were excised from 20- to 30-g mice and 140- to 200-g rats. Urogenital sinuses and adult bladders were derived from both males and females. These specimens were separated into their epithelial and mesenchymal elements after incubation in 1–2% trypsin (Difco Laboratories, Detroit, MI, 1/250) in Tyrode's solution for 2–3 h at 4°C (11). Separated tissues were stored briefly in 50% fetal calf serum (Gibco Laboratories, Grand Island, NY) in Tyrode's fluid to inactivate the trypsin. The cleanness of separation of the components was monitored by the following criteria: (*a*) inspection of separated components with a dissecting microscope and (*b*) analysis of serial sections of the separated components. These procedures are highly effective, but may not permit detection of a single epithelial cell within a mass of mesenchyme. To ascertain the possibility of such a low level of tissue contamination, epithelium and mesenchyme from trypsinized urogenital sinuses were grafted separately to subcapsular renal sites, grown in vivo for 4 wk, and then analyzed histologically in serial section.

Recombinants were prepared by placing a small fragment (1–2 mm^2) of epithelium upon a piece of mesenchyme. The recombinants were cultured overnight on a solidified agar medium (Eagle's basal medium containing 0.4% Bacto-agar (Difco Laboratories), 10% fetal calf serum, 1% L-glutamine, and 50 U each/ml of penicillin-streptomycin [Microbiological Associates, Walkersville, MD] (11) and then grafted beneath the renal capsule of syngeneic adult male hosts. Rapid vascularization of the grafts exposes them to physiologic levels of androgens and other synergistic hormones. After 3–5 wk of in vivo growth, the recombinants were harvested for analysis.

To elucidate the developmental sequence of prostatic morphogenesis in recombinants composed of UGM and adult urothelium (UGM plus BLE), and to demonstrate that the adult urothelium is the actual source of the induced prostatic acini, UGM plus BLE recombinants were grown for 3–12 d in vivo and then harvested for light microscopic analysis. Specimens destined for histological examination were fixed in Bouin's fluid, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

For histochemical analyses, specimens from mice were used. Alkaline phosphatase was assessed by the azo-dye method of Pearse (45), using sodium alpha naphthyl phosphate as the substrate and Fast Red TR as the coupling dye. This enzymatic test was performed on rehydrated paraffin sections that were fixed in 90% ethanol. Nonspecific esterase was examined in unfixed frozen sections according to the procedures of Pearse (45), using alpha naphthyl acetate as the substrate and Fast Blue B as the coupling dye. Glycosaminoglycans were demonstrated by Alcian blue staining (0.3 M $MgCl_2$, pH 5.8) on rehydrated paraffin sections of specimens fixed in Carnoy's fluid (1).

Specimens for ultrastructural analysis were fixed in Karnovsky's (32) phosphate-buffered fixative at pH 7.4 for 4–6 h at room temperature, and postfixed in osmium tetroxide in phosphate buffer for 2 h. After en bloc staining overnight in 0.25% uranyl acetate, the specimens were dehydrated in a graded series of alcohols, cleared in propylene oxide, and embedded in Araldite 506. Thin sections were cut with a diamond knife on a Porter-Blum ultramicrotome, stained with lead citrate, and examined in a Philips EM 200 electron microscope.

Preparation of a prostate-specific antiserum was accomplished by intradermal injection of rabbits with a homogenate of six mouse prostates in Freund's complete adjuvant. The rabbits received booster injections at 2-wk intervals until the antibody titer was determined by immunodiffusion to be at least 1:32 (22). Nonspecific antibodies were removed from the antiserum by extensive absorption with mouse erythrocytes and tissue powders from kidney, liver, urinary bladder, seminal vesicle, spleen, and intestine. Tissue powders were prepared by homogenizing tissues in 5 vol of acetone with a Brinkman Polytron. The homogenate was centrifuged at 5,000 *g* for 10 min, and the pellet was resuspended and washed several times with acetone until the supernatant was clear. The pellet was then evaporated to dryness with a stream of air. The antiserum was absorbed with packed erythrocytes (1:10 vol to vol) for 30 min at 4°C. The mixture was centrifuged for 10 min at 5,000 *g*, and the supernatant in turn absorbed for 48 h at 4°C with mouse tissue powder (1:1 vol to vol).

4- to 8-μm unfixed frozen sections of bladder, prostate, and the UGM plus BLE recombinants were incubated with the fully absorbed, prostate-specific antiserum or normal rabbit serum at dilutions of 1:100–1:500 for 1 h at room temperature. Sections were washed for 30 min in three changes of phosphate-buffered saline (PBS) and incubated for 30 min in goat-anti-rabbit serum at dilutions of 1:50–1:100. After rinsing, peroxidase-antiperoxidase serum (1:50 dilution) (Miles Laboratories, Inc., Elkhart, IN) was layered over the sections and incubated for 30 min at room temperature. Slides were then rinsed thoroughly in PBS, and the peroxidase was developed by the histochemical method using diaminobenzidine and H_2O_2 (57).

RESULTS

Control preparations used to assess the cleanness of the tryptic separation indicate that both epithelium and stroma were free of contaminating cells (Fig. 1, *a–d*). Grafts of UGM grown in vivo for periods of up to 1 mo consist of masses of fibromuscular cells, which were rarely (1:53) contaminated with prostatic epithelium (Fig. 1 *c*). Urogenital sinus epithelium grown for 4 wk in male hosts is maintained as an undifferentiated stratified cuboidal epithelium (Fig. 1 *d*). Since it is difficult to obtain stroma from adult urinary bladder that is free of small pieces of adherent epithelium, this stroma was used only to prepare homotypic recombinants of bladder epithelium and stroma. These recombinants exhibited a low percentage of survival (Table I), but those surviving invariably form a normal transitional epithelium (Fig. 2). Growth in male hosts of homotypic recombinations of epithelium and mesenchyme of the urogenital sinus (UGM plus UGE) consistently results in the development of prostatic acini, which after 1 mo contain secretory product within the acinar lumina (Fig. 3). In addition to prostatic acini, UGM plus UGE recombinants contain some urethral epithelium and periurethral glands. Heterotypic recombinants prepared with UGM plus BLE from adult mice or rats formed prostatic acini that frequently contained secretory product (Fig. 4 *a* and *b*). Urethral glands and urethral epithelium were present in small amounts as observed above for UGM plus UGE recombinants. The sex of the inducing or responding tissues did not affect the type or magnitude of the response (Table I).

The time course of prostatic morphogenesis from adult bladder epithelium was studied by preparing UGM plus BLE recombinants and harvesting them after 3–12 d of in vivo growth. Grafts harvested 3 d after implantation contained a hyperplastic epithelium of five to eight cell layers (Fig. 5 *a*). The apical cells of the adult BLE were smaller than normal, suggesting that some of the large apical cells had been sloughed during this brief growth period. Prostatic buds were never observed in recombinants grown for 3 d.

On days 4, 5, and 6 after grafting, prostatic morphogenesis proceeded rapidly in the adult urothelium. Solid unbranched epithelial buds evaginated from the basal aspect of the adult bladder epithelium in recombinants grown for 4 d (Fig. 5 *d*). At 5 d, the number of buds had increased, and branching was observed (Fig. 5 *c*). On days 5 and 6, canalization of the buds was initiated, and by 12 d of in vivo growth an elaborately branched network of prostatic ducts and acini was present, many of which were fully canalized (Fig. 5 *d*). However, the continued presence of solid epithelial buds at this time indicates that growth and morphogenesis are still proceeding in certain areas of recombinants.

Since homotypic recombinations of urogenital sinus and urinary bladder form prostate and urinary bladder (Figs. 2 and 3), respectively, ultrastructural and histochemical features of UGM plus BLE recombinants were compared with those of the prostate and bladder of the hosts. Prostate displays fine-structural features indicative of its secretory function (4). Prostatic acini consist of a single layer of epithelial cells situated upon a basal lamina. Nuclei are located basally where rough endoplasmic reticulum (RER) is abundant. The Golgi apparatus and secretory granules are prominent features of the supranuclear and apical regions of prostatic epithelial cells. In contrast, transitional epithelium of the adult urinary bladder is stratified (three or four cell layers in thickness). Basal cells are undifferentiated morphologically. However, in the intermediate and apical cell layers, membrane specializations are evident in the form of the unique asymmetric plasma membrane and the characteristic fusiform vesicles described earlier by Walker

(63), Hicks et al. (27), and Jacob et al. (30). Heterotypic UGM plus BLE recombinants contain ductal-acinar structures lined by a simple columnar epithelium that bears the distinctive fine structural features of secretory cells (Fig. 6). Nuclei and RER are located basally. The Golgi complex and secretory granules are present in the supranuclear and apical regions. Moreover, asymmetric membranes and elliptical vesicles characteristic of bladder epithelium are absent.

Histochemical analysis reveals the presence of nonspecific esterase in the epithelial cytoplasm of the prostate (Fig. 7*a*). The cytoplasm of urothelium lacks this marker (Fig. 7*b*). However, when epithelium of the adult urinary bladder is induced to form prostatic acini, the resultant glandular epithelium displays an intensely positive reaction for this enzyme (Fig. 7*c*). Expression of glycosaminoglycans as demonstrated by Alcian blue staining displays a similar pattern, being present in prostatic epithelium (Fig. 7*d*), absent or in minute levels in urothelium (Fig. 7*e*), but present in high levels in the acinar epithelium of UGM plus BLE recombinants (Fig. 7*f*). Alkaline phosphatase is absent in mouse prostatic epithelium, but is present in the layer of stromal cells immediately subadjacent to the epithelium (Fig. 7*g*). In contrast, the epithelium of the urinary bladder contains high levels of alkaline phosphatase (Fig. 7*h*). However, when epithelium of the adult urinary bladder is induced by UGM to form prostatic acini, the glan-

TABLE I

Developmental Response of Isolated and Recombined Epithelium and Mesenchyme from Embryonic Urogenital Sinuses and Adult Urinary Bladders Grown in Syngeneic Rats and Mice *

Specimen			
Mesenchyme		Epithelium	Developmental response
UGM		—	Fibromuscular tissue (52/53)
—		UGE	Undifferentiated epithelium (4/4)
UGM	+	UGE	Prostate (7/7)
BLM	+	BLE	Bladder (8/8)
UGM	+	BLE	Prostate (170/170)

* Since the developmental response of the above specimens is identical for mouse or rat tissues, the data for these species are pooled. UGE, urogenital sinus epithelium; BLM, bladder mesenchyme.

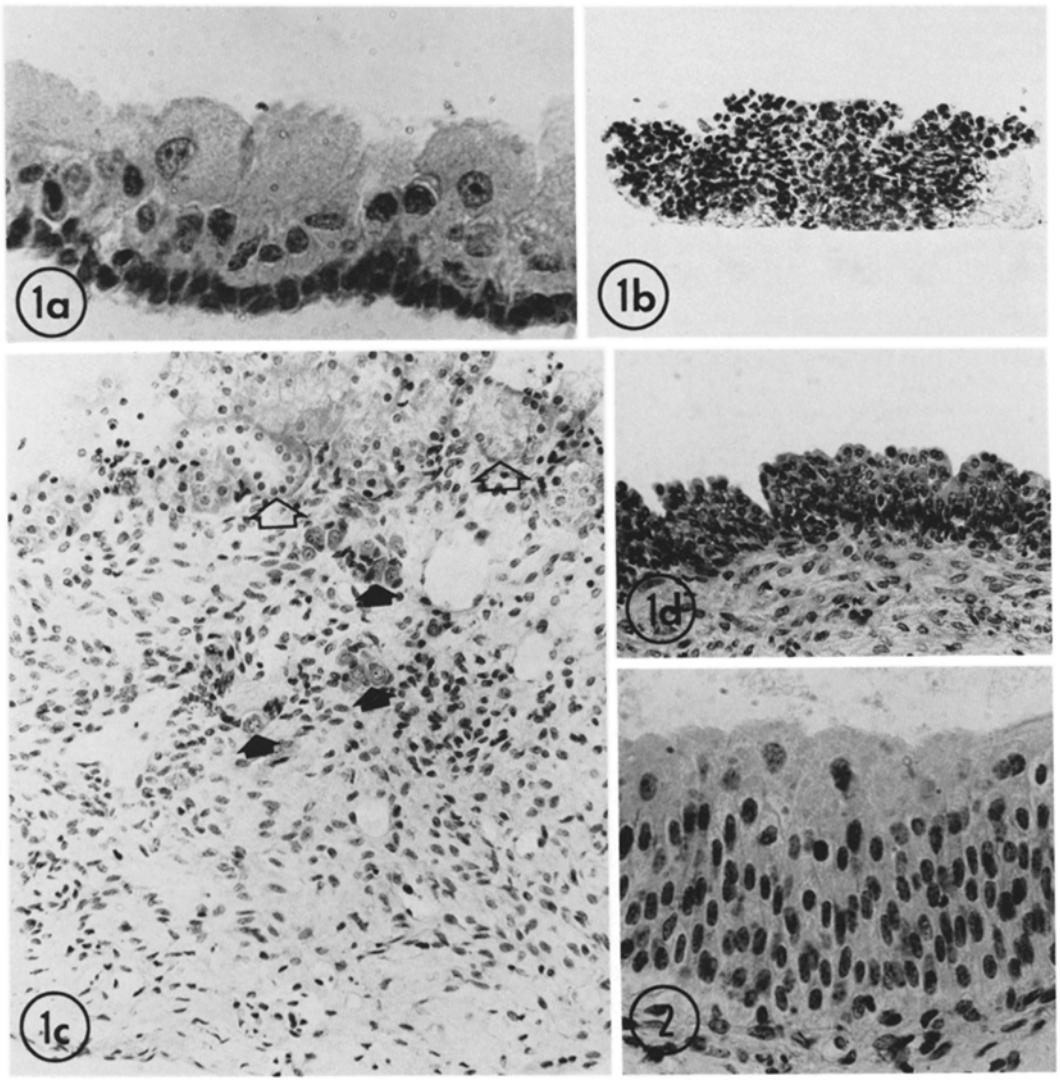


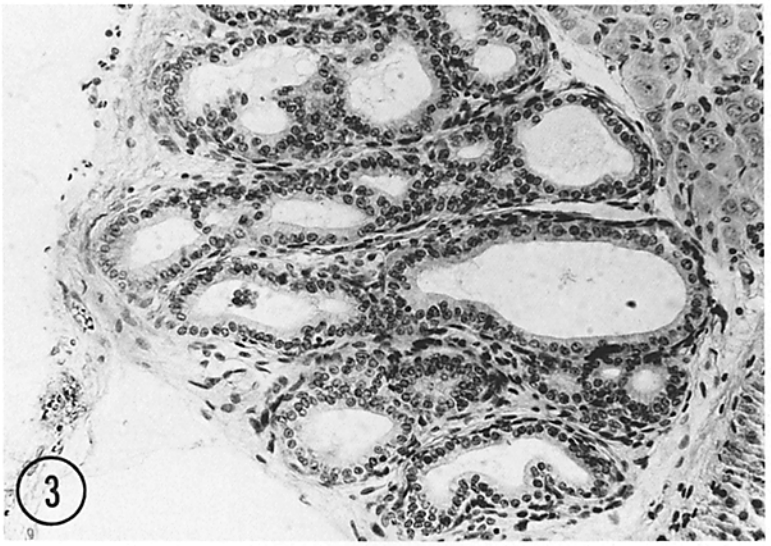
FIGURE 1 and 2 Fig. 1: (*a*) Epithelium of the adult mouse urinary bladder fixed immediately after tryptic separation. The epithelium has cleanly separated from the stroma. × 1,000. (*b*) Mesenchyme of a 16-d-old embryonic mouse urogenital sinus fixed immediately after tryptic separation. Epithelial cells cannot be detected. × 400. (*c*) UGM grown for 4 wk in an adult male host. The mesenchyme has differentiated into a mass of fibromuscular cells. Note the kidney parenchyma of the host's graft site (open arrows) and ganglion cells (solid arrows) within the graft. Prostatic epithelium is rarely (1/53) found in these preparations. × 400. (*d*) UGE of a 16-d-old embryonic mouse growth for 4 wk in an adult male host. An undifferentiated stratified cuboidal epithelium has developed. Prostatic acini are never observed in these preparations. × 400. Fig. 2: A homotypic recombination of epithelium and mesenchyme of adult mouse urinary bladder. Typical urothelial differentiation is maintained in these recombinants grown for 4 wk in male hosts. × 400.

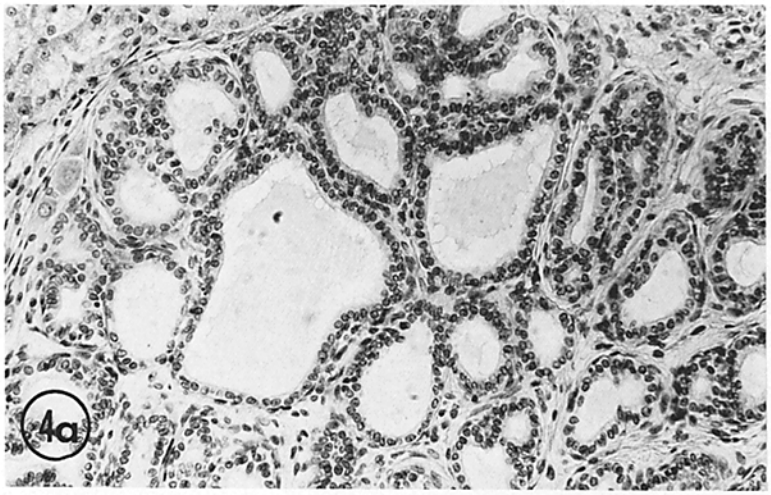
dular epithelium is completely devoid of this marker (Fig. 7*i*).

Immunocytochemical analysis using the absorbed prostate-specific antiserum demonstrates that adult urothelium is completely unreactive to this antiserum (Fig. 8*b*). By contrast, glandular epithelium and secretion of the prostate and UGM plus BLE recombinants display an intense reaction to this antiserum (Fig. 8, *c* and *d*). None of these specimens are stained when normal rabbit serum is substituted for the prostate-specific antiserum (Fig. 8*a*).

DISCUSSION

The validity of tissue recombination experiments is based upon the demonstration that the separated epithelium and mesenchyme are free of contaminating cells. Examination of serial sections of 59 specimens of isolated epithelium and mesenchyme fixed immediately after trypsinization indicated that all specimens were free of contaminating cells. Moreover, growth of trypsin-separated epithelium and stroma in adult male hosts





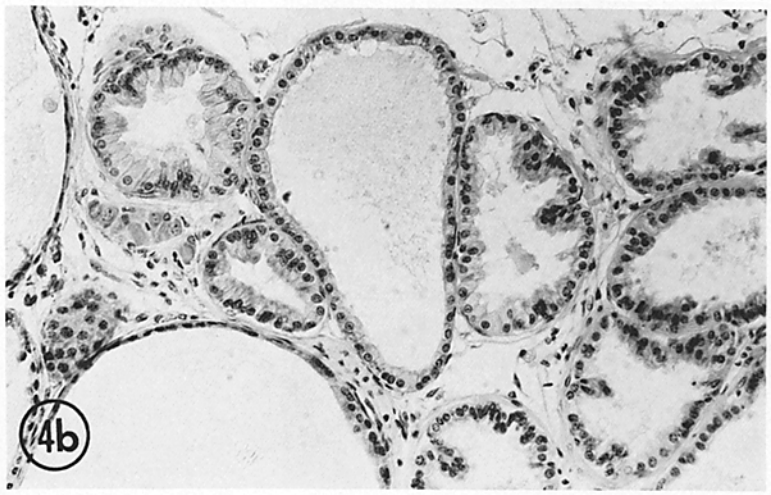


FIGURE 3 and 4 Fig. 3: A homotypic recombinant of a 16-d-old embryonic mouse urogenital sinus grown for 4 wk in a male host. Prostatic acini develop when urogenital sinus epithelium is grown in association with its homotypic mesenchyme. × 640. Fig. 4: (*a*) A heterotypic recombinant (UGM plus BLE) composed of 16-d-old embryonic UGM and BLE of an adult mouse. Prostatic acini have developed. × 640. (*b*) A UGM plus BLE recombinant prepared as in *a* but with rat tissues. Prostatic acini have developed. × 640.

revealed only one case out of 53 grafts in which prostatic epithelium developed within a graft of UGM. Thus, although a few residual epithelial cells may occasionally be retained within a mesenchymal fragment, the incidence of specimens having contamination is low (~1%). Internal controls from earlier studies further substantiate a low incidence (<1%) of mesenchymal specimens contaminated with epithelial cells. For instance, epithelia of either the salivary gland, preputial gland, seminal vesicle, or epidermis, when associated with mesenchyme of the urogenital sinus, continue their normal cytodifferentiation in the presence of this heterotypic mesenchyme. In only 1/148 (0.7%) heterotypic recombinants was prostatic epithelium present in addition to the above epithelia (10, 11, 12). This observation, in conjunction with the present controls, suggests that, in >99% of the heterotypic UGS plus BLE recombinants, the development of glandular structures can be attributed to actual inductive influences of UGM, and not to the retention of contaminating epithelial cells within the mesenchyme. Moreover, time-course studies provide direct evidence that adult transitional epithelium is capable of forming prostatic acini, since prostatic buds grew directly from the basal surface of the adult urothelium (Fig. 5).

Time-course experiments further demonstrate similarities between in situ prostatic morphogenesis and that occurring within the UGM plus BLE recombinants. Solid epithelial cords were initially recognized in the UGM plus BLE recombinants after 4 d of in vivo growth in male hosts. These epithelial cords are derived from downgrowths of the basal layer of the adult urothelium. The epithelial buds elongate, branch, and subsequently develop lumina during the 5th–12th d of in vivo growth. After 30 d of in vivo development, secretory activity was observed. The 4-d lag period between the initiation of androgenic exposure (transplantation to male hosts) and the appearance of prostatic buds is comparable with that within the embryo. Prostatic buds appear in situ on 17 ½ to 18 d of gestation in mice, 4–5 d after initiation of testosterone biosynthesis by the fetal testes (47, 48, 64). It is assumed that during this lag period the basal cells of the bladder epithelium become progressively determined toward prostatic morphogenesis in response to inductive influences from urogenital sinus mesenchyme. Whether this process of determination requires one or more cell cycles has not yet been determined. However, gross and histological observations of the increase in size of the UGM plus BLE recombinants indicate that a considerable amount of replication occurs in the bladder epithelium, which normally exhibits an extremely low rate of DNA synthesis (7, 25).

In UGM plus BLE recombinants, adult urothelial cytodifferentiation was altered markedly on the basis of light and electron microscopic observations. In these tissue recombinants, fine-structural features unique to urothelium (8, 25, 26, 62) were lost, while morphological features associated with the

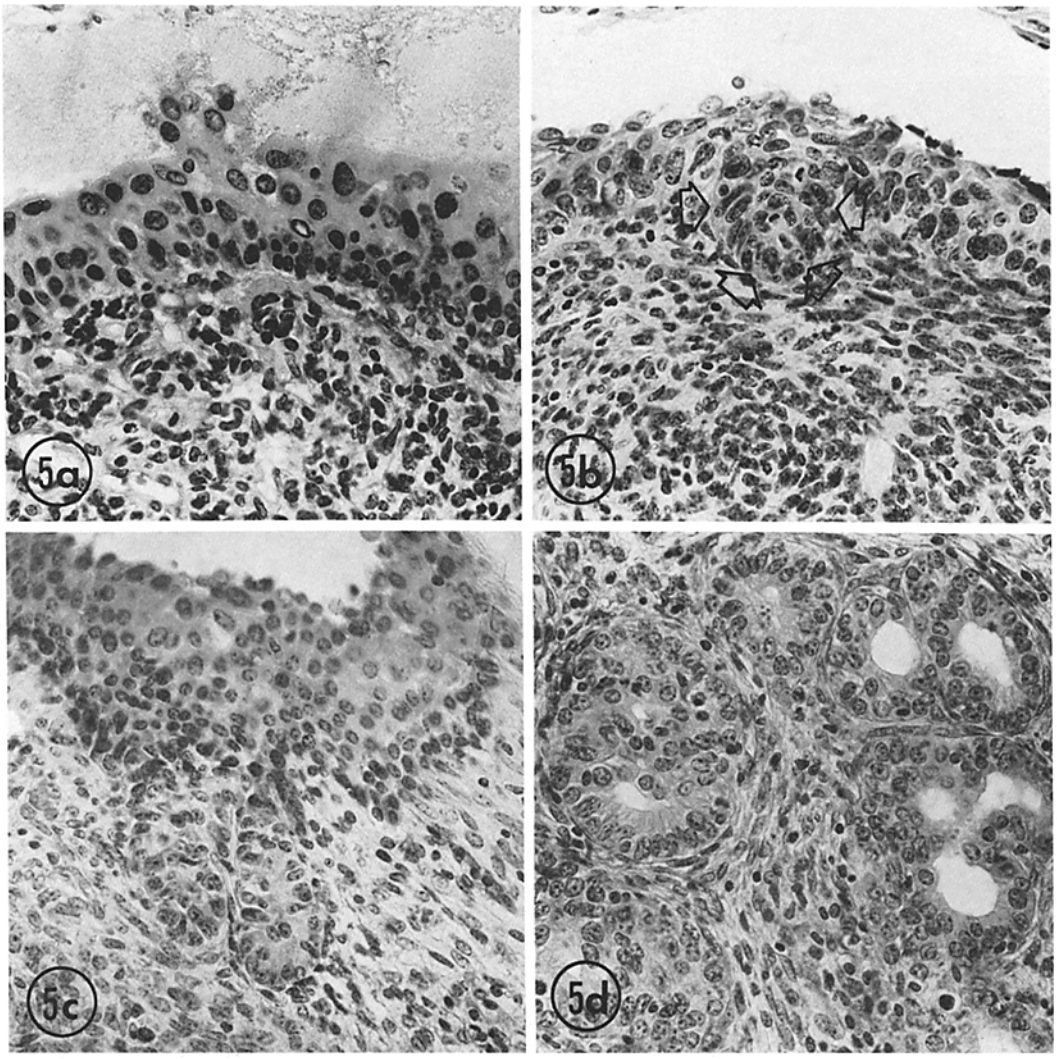


FIGURE 5 UGM plus BLE recombinants prepared with mouse tissue grown for (*a*) 3, (*b*) 4, (*c*) 5, and (*d*) 12 d. (*a*) The basal layer of epithelial cells is hypercellular, and the large apical epithelial cells appear to be sloughing (3 d in vivo growth). (*b*) Note the solid epithelial (arrows) evagination from the basal aspect of the epithelium into the underlying mesenchyme (4 d in vivo growth). (*c*) At 5 d of in vivo growth, solid epithelial buds are more numerous, longer, and in many cases are branched. (*d*) At 12 d of in vivo growth, canalization of the highly branched epithelial buds is in progress. Lumen formation is observed at many different stages. × 640.

secretory (prostatic) phenotype (4) were acquired. Similar alterations have recently been observed in tissue recombinants composed of UGM and embryonic BLE (16, 39, 40). These major morphological changes in epithelial cytodifferentiation appear to be indicative of a major alteration in functional activity of the induced bladder epithelium. This idea is supported by two additional observations: (*a*) The induced glandular epithelium of UGM plus BLE recombinants expresses prostatic histochemical markers (23) concomitant with a loss in urothelial histochemical features (28, 36). (*b*) The induced acini express prostate-specific antigens. Thus, during prostatic induction in adult urothelium, morphological changes in cytodifferentiation appear to be coupled to changes in functional or biochemical activity. This idea receives considerable support from observations in the accompanying paper (44).

Major cytological alterations have been reported earlier for

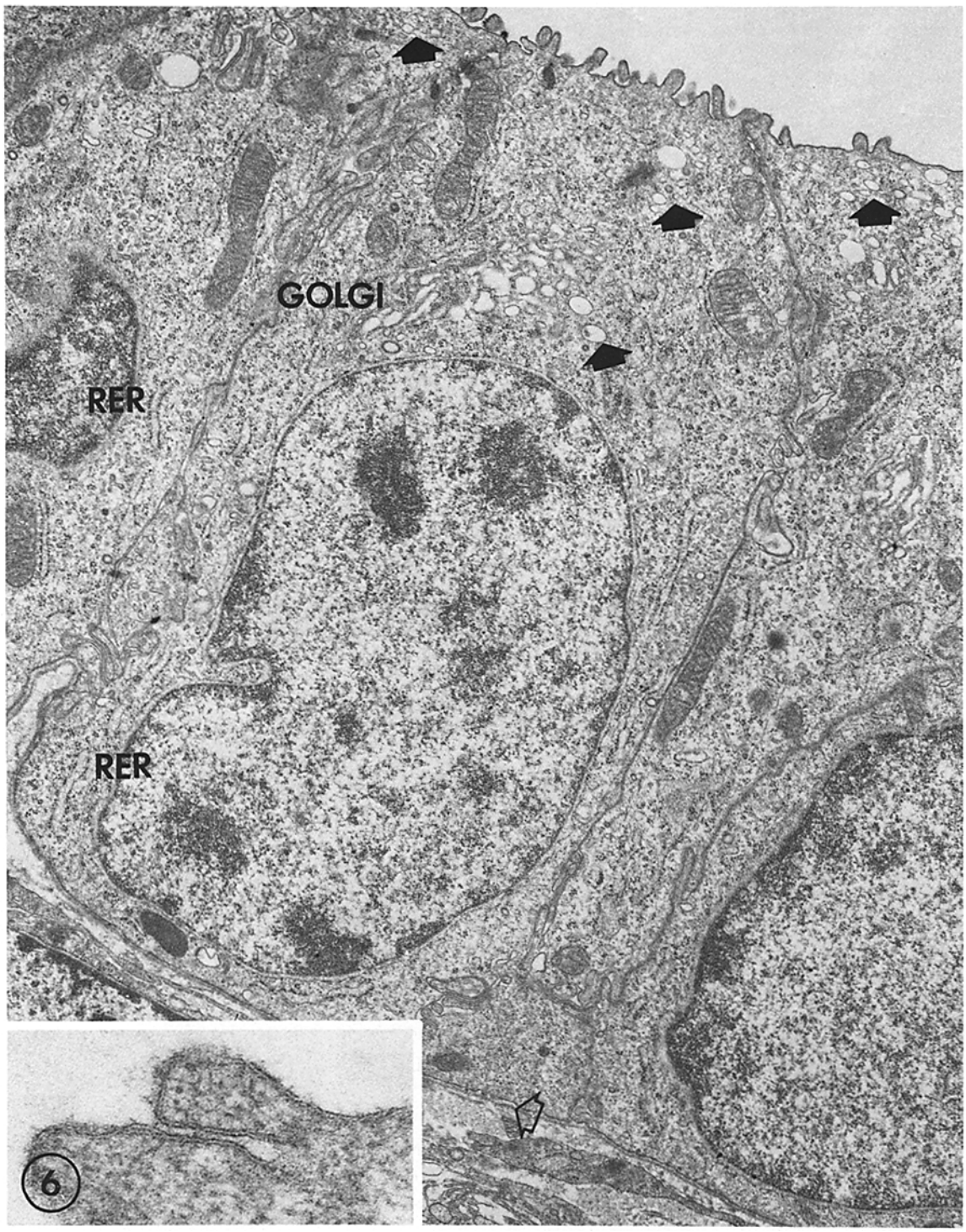


FIGURE 6 An electron micrograph of cells lining an acinus of a UGM plus BLE recombinant. The epithelium is simple columnar and rests upon a basal lamina (open arrow). RER and the nucleus are located basally. The Golgi complex is well developed in the supranuclear region. Vesicles, possibly secretory granules (closed arrows), are located apically. In the inset, note that the apical plasma membrane is symmetrical. Fusiform vesicles are absent. × 13,500; *inset*, × 150,000.

embryonic epithelial cells grown in association with heterotypic mesenchyme (14, 15, 19, 35, 41, 55, 65, 66). In most cases, the relationship of cytological alterations to changes in functional (biochemical) activity has not been explored. However, in a study of lens induction by Karkinen-Jaaskelainen (31), lens crystallins were detected immunocytochemically in lentoids formed through association of trunk ectoderm with the optic cup. In this case, induced changes in cytodifferentiation also appear to be coupled to biochemical changes. By contrast, gross morphogenetic changes in epithelial arborization (glandular branching patterns) induced by heterotypic mesenchyme do not appear to be correlated with changes in functional activity. This is illustrated by recent observations of mammary and palatal development. Sakakura et al. (50) have demonstrated that although embryonic mammary epithelium can be induced by mesenchyme of the submandibular gland to express a gross morphological branching pattern resembling that of salivary gland, the mammary epithelium retains the ability to synthesize milk proteins. Similarly, embryonic palatal epithelium can be induced by mesenchyme of the submandibular gland to form glandular structures (60). However, the ducts of the induced glands are lined with epithelium possessing histotypic characteristics indicative of palatal epithelium. Thus, it is apparent that mesenchyme-induced changes in epithelial branching patterns may not correlate with altered functional (biochemical) activity. In contrast, true changes in epithelial cytodifferentiation, i.e., when epidermal cells differentiate into lens cells or urothelial cells differentiate into a glandular epithelium, are associated with a variety of biochemical and metabolic changes (see companion paper, 44). It is apparent, therefore, the mesenchyme-induced changes in epithelial cytodifferentiation vs. changes in gross morphological organization are distinctly different processes and, therefore, should not be compared directly.

Phenotypic expression of adult mammalian epithelium, except for neoplastic transformation, is remarkably stable. Even

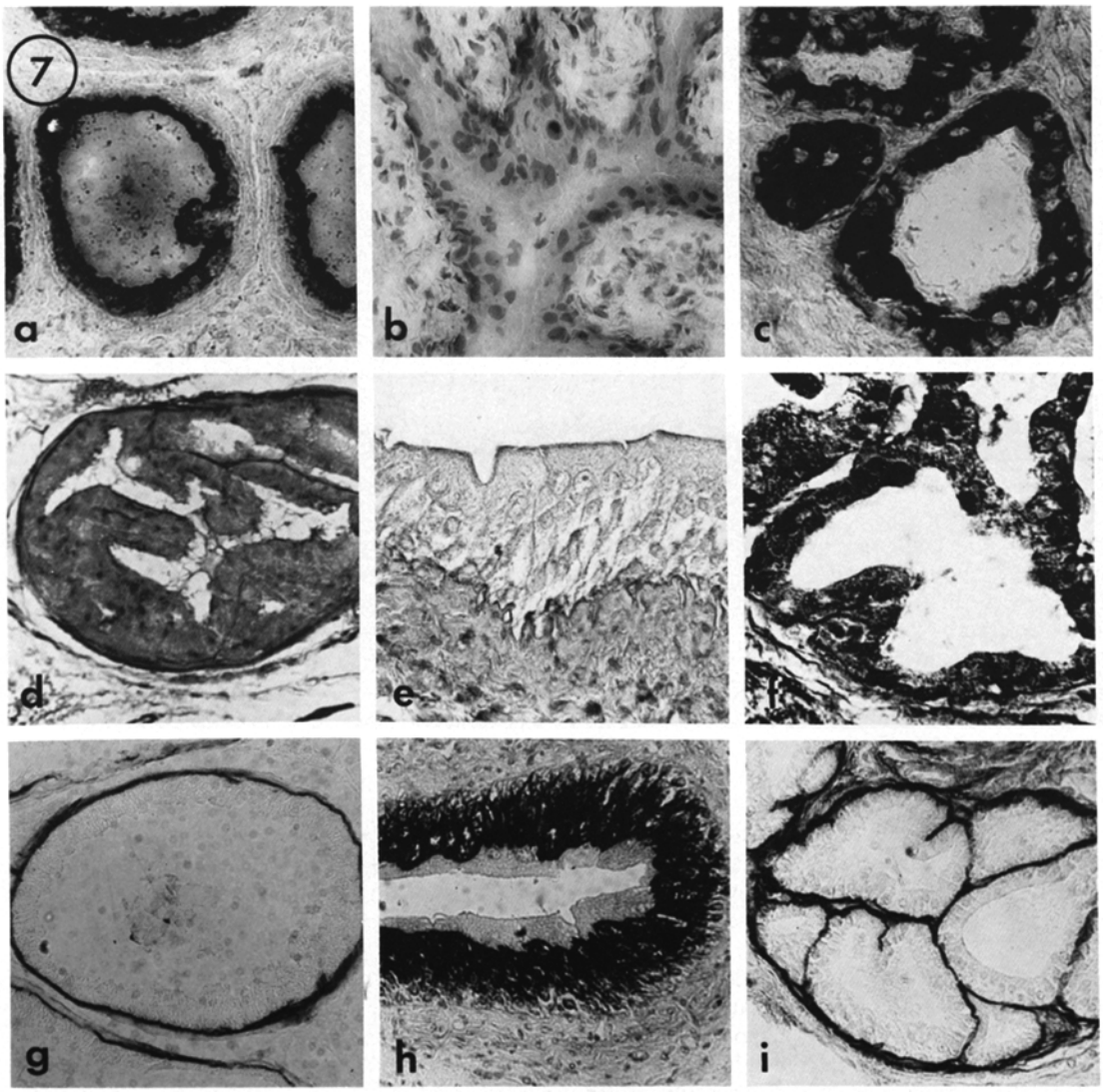


FIGURE 7 Histochemical analysis of prostate (*a, d, g*), bladder (*b, e, h*), and UGM plus BLE recombinants (*c, f, i*). Nonspecific esterase is present in prostatic epithelium (*a*), absent in bladder epithelium (*b*), and present in the acinar epithelium of UGM plus BLE recombinants (*c*). Staining of glycosaminoglycans with Alcian blue is observed in prostatic epithelium (*d*) and in the acinar epithelium of UGM plus BLE recombinants (*f*), while bladder epithelial cells (*e*) lack this marker. Alkaline phosphatase is absent in prostatic epithelium but present in the stromal cells immediately subadjacent to the epithelium (*g*). In the urinary bladder (*h*), epithelium expresses alkaline phosphatase activity. However, when BLE is induced by UGM to form prostatic acini (*i*), the glandular epithelium lacks this histochemical marker. × 400.

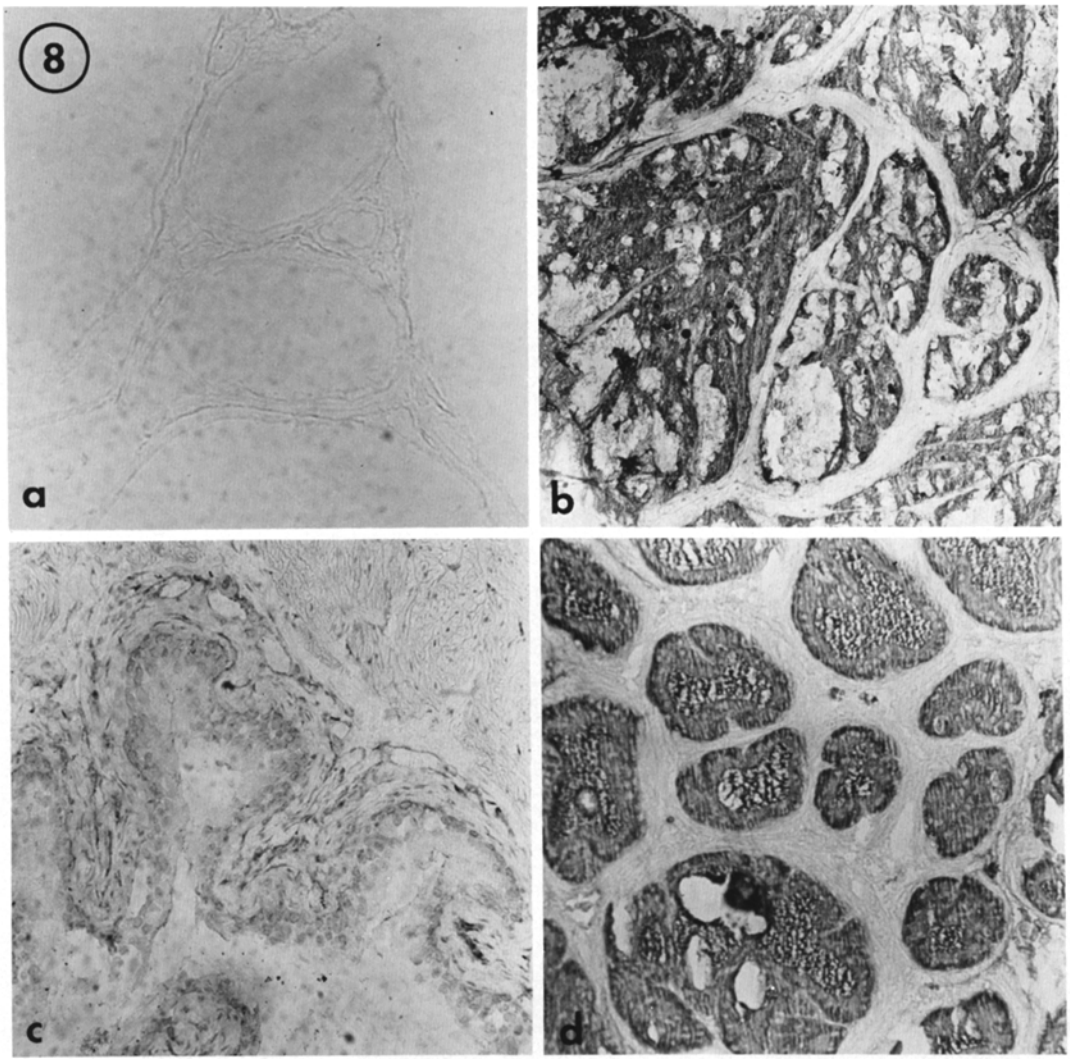


FIGURE 8 Immunocytochemical staining with a prostate-specific antiserum of mouse prostate (*b*), bladder (*c*), and UGM plus BLE recombinants (*d*) using the PAP technique of Sternberger et al. (57). In (*a*), preimmune rabbit serum was used. Staining of the prostate is negative. Using the prostate-specific antiserum, bladder epithelium (*c*) is completely unreactive. Hematoxylin counter-staining was used on this specimen so that histological detail could be discerned. In the adult prostate (*b*) and in the UGM plus BLE recombinants (*d*), the acinar epithelium and the secretion are intensely stained. × 500.

in continuously renewing adult epithelia such as skin or intestine, the progeny of mitotically-active stem cells consistently express the appropriate differentiation. This suggests that adult stem cells may not retain the wide range of developmental options characteristic of embryonic stem cells, but instead may be restricted in their developmental potential to only a few organ-specific options. For example, stem cells of the intestinal crypts only give rise to Paneth cells, enteroendocrine cells, goblet cells, and villus columnar cells (38). Thus, adult stem cells are characterized as apparently determined (46) and incapable of significant modulation into other normal cell types. Hay (24) and Lajtha (37), in fact, regard adult stem cells of renewable surface epithelia as "partially differentiated." The observation that epithelium of the adult urinary bladder can be induced by UGM to form prostatic acini demonstrates a major alteration in cytodifferentiation of adult urothelium. This demonstrates that adult urothelium contains a population of undetermined cells capable of responding to the reprogramming influence of a heterotypic mesenchymal inductor. To our knowledge, this is the first unequivocal demonstration of retention and expression of developmental plasticity in a mature adult mammalian epithelium. Such metaplasia, however, has been reported in lower vertebrates, the best example being lens regeneration in amphibians (49).

The developmental plasticity of adult BLE raises the question of whether this property is unique to the BLE or is a general property of all adult epithelia. Within the integumental system, epidermal cells may retain a limited developmental plasticity in that during wound healing cells from sebaceous glands may be able to form a keratinized surface epithelium (42, 43). Furthermore, during antler growth in deer, the neogenesis of hair follicles and sebaceous glands from the hair-bearing epidermis of the antler pedicle suggests that epidermal cells of adult deer may be capable of expressing either a cornified or sebaceous phenotype. Lastly, when corneas of rabbits are denuded of their epithelium by scraping, the surrounding conjunctival epithelium migrates over the denuded stroma and apparently undergoes metaplasia into a corneal epithelium (21). These studies suggest the retention of limited developmental plasticity within integumental epithelium. However, for each of these examples there are other possible explanations for the presumed epidermal metaplasia. Moreover, in controlled separation-recombination experiments with normal adult mammalian integumental tissues, inductive influence from dermal cells can alter epidermal organization, but in no case has heterotypic stroma been shown to induce a completely different epithelial phenotype (2, 3, 5, 6, 33, 55).

Finally, these studies of mesenchymal effects upon adult urothelial cytodifferentiation emphasize the importance in adulthood of epithelial-stromal interactions. Continued maintenance of normal urothelial characteristics is clearly subject to some form of continuing influence from bladder stroma. Im-

plicit in this idea is the concept that alteration of normal stromal-epithelial interactions may lead to abnormal epithelial expression such as benign or neoplastic growth (9, 17, 20, 29, 58, 61).

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REFERENCES

1. Bernfield, M. R., and S. D. Banerjee. 1972. Acid mucopolysaccharide (glycosaminoglycan) at the epithelial-mesenchymal interface of mouse embryo salivary glands. *J. Cell Biol.* 52:664–673.
2. Bernimoulin, J. P., and H. E. Schroeder. 1980. Changes in the differentiation pattern of oral mucosal epithelium following heterotopic connective tissue transplantation in man. *Pathol. Res. Pract.* 166:290–312.
3. Billingham, R. E., and W. K. Silvers. 1968. Dermoepidermal interactions and epithelial specificity. *In* Epithelial-Mesenchymal Interactions. R. Fleischmajer and R. E. Billingham, editors. The Williams & Wilkins, Co., Baltimore, MD. 252–266.
4. Brandes, D. 1966. The fine structure and histochemistry of prostatic glands in relation to sex hormones. *Int. Rev. Cytol.* 20:207.
5. Briggamen, R. A., and C. E. Wheeler. 1968. Epidermal-dermal interactions in adult human skin. Role of dermis in epidermal maintenance. *J. Invest. Dermatol.* 51:454–465.
6. Briggaman, R. A., and C. E. Wheeler. 1971. Epidermal-dermal interactions in adult human skin. II. The nature of the dermal influence. *J. Invest. Dermatol.* 56:18–26.
7. Clayson, D. B. 1975. Experimental studies in urothelial cancer. *In* Biology and Clinical Management of Bladder Cancer. E. H. Cooper and R. E. Williams, editors. Blackwell Scientific Publications, Oxford, United Kingdom. 1–35.
8. Cooper, E. H. 1975. Biology of human bladder cancer. *In* Biology and Clinical Management of Bladder Cancer. E. H. Cooper and R. E. Williams, editors. Blackwell Scientific Publications, Oxford, United Kingdom. 37–63.
9. Cooper, M., and H. Pinkus. 1977. Intrauterine transplantation of rat basal cell carcinoma as a model for reconversion of malignant to benign growth. *Cancer Res.* 37:2544–2552.
10. Cunha, G. R. 1972. Epithelio-mesenchymal interactions in primordial gland structures which become responsive to androgenic stimulation. *Anat. Rec.* 172:179–196.
11. Cunha, G. R. 1972. Tissue interactions between epithelium and mesenchyme of urogenital and integumental origin. *Anat. Rec.* 172:529–542.
12. Cunha, G. R. 1972. Support of normal salivary gland morphogenesis by mesenchyme derived from accessory sexual glands of embryonic mice. *Anat. Rec.* 173:205–212.
13. Cunha, G. R. 1976. Epithelial-stromal interactions in development of the urogenital tract. *Int. Rev. Cytol.* 47:137–194.
14. Cunha, G. R. 1976. Stromal induction and specification of morphogenesis and cytodifferentiation of the epithelia of the Mullerian ducts and urogenital sinus during development of the uterus and vagina in mice. *J. Exp. Zool.* 196:361–370.
15. Cunha, G. R., and B. Lung. 1979. Importance of stroma in morphogenesis and functional activities of urogenital epithelium. *In Vitro* (*Gaithersburg*). 15:50–71.
16. Cunha, G. R., and B. Lung. 1980. Experimental analysis of male accessory sex gland development. *In* Accessory Glands of the Male Reproductive Tract. E. Spring-Mills and E. S. E. Hafez, editors. Elsevier North-Holland, New York. 39–59.
17. Cunha, G. R., B. Lung, and K. Kato. 1977. Role of the epithelial-stromal interaction during the development and expression of ovary-independent vaginal hyperplasia. *Dev. Biol.* 56:52–67.
18. Cunha, G. R., B. Lung, and B. Reese. 1980. Glandular epithelial induction by embryonic mesenchyme in adult bladder epithelium of balb/c mice. *Invest. Urol.* 17:302–304.
19. Cunha, G. R., L. W. K. Chung, J. M. Shannon, and B. A. Reese. 1980. Stromal-epithelial interactions in sex differentiation. *Biol. Reprod.* 22:19–42.
20. DeCosse, J. J., C. L. Gossens, and J. F. Kuzma. 1973. Breast cancer: induction of differentiation by embryonic tissue. *Science* (*Wash. DC*). 181:1057–1058.
21. Friedenwald, J. S. 1951. Growth pressure and metaplasia of conjunctival and corneal epithelium. *Doc. Ophthalmol.* 5:184–192.
22. Garvey, J. S., N. E. Cremer, and D. H. Sussdorf. 1977. Methods in immunology. W. A. Benjamin, Inc., Reading, MA. 313–327.
23. Gyorkey, F. 1973. Some aspects of cancer of the prostate gland. *Methods Cancer Res.* 10:279–368.
24. Hay, E. D. 1968. Dedifferentiation and metaplasia in vertebrate and invertebrate regeneration. *In* The Stability of the Differentiated State. H. Ursprung, editor. Springer-Verlag, New York. 85–108.
25. Hicks, R. M. 1975. The mammalian urinary bladder. An accommodating organ. *Biol. Rev. Camb. Philos. Soc.* 50:215–246.
26. Hicks, R. M., and J. Chowaniec. 1978. Experimental induction, histology, and ultrastructure of hyperplasia and neoplasia of the urinary bladder epithelium. *Int. Rev. Exp. Pathol.* 18:199–280.
27. Hicks, R. M., B. Ketterer, and R. C. Warren. 1974. The ultrastructure and chemistry of the luminal plasma membrane of the mammalian urinary bladder. A structure with low permeability to water and ions. *Philos. Trans. Roy. Soc. Lond. Ser. B* 268:23–38.
28. Highman, B., C. H. Frith, and N. A. Littlefield. 1975. Alkaline phosphatase activity in hyperplastic and neoplastic urinary bladder epithelium of mice fed 2-acetylaminofluorene. *J. Natl. Cancer Inst.* 54:257–261.
29. Hodges, G. M., R. M. Hicks, and C. D. Spasey. 1977. Epithelial-stromal interactions in normal and chemical carcinogen treated adult bladder. *Cancer Res.* 37:3720–3730.
30. Jacob, J., C. N. Ludgate, J. Forde, and W. S. Tullock. 1978. Recent observations on the ultrastructure of human urothelium. I. Normal bladder of elderly subjects. *Cell Tissue Res.* 193:543–560.
31. Karkinen-Jaaskelainen, M. 1978. Permissive and directive interactions in lens induction. *J. Embryol. Exp. Morphol.* 44:167–179.
32. Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* 27(2, Pt 2):137*a*. (Abstr.)
33. Karring, T., N. P. Lang, and H. Loe. 1975. The role of gingival connective tissue in determining epithelial differentiation. *J. Periodontal Res.* 10:1–11.
34. Kollar, E. J. 1972. The development of the integument: spatial, temporal and phylogenetic factors. *Am. Zool.* 12:125–135.
35. Kollar, E. J., and G. R. Baird. 1970. Tissue interactions in embryonic mouse tooth germs. II. The inductive role of the dental papilla. *J. Embryol. Exp. Morphol.* 24:173–186.
36. Kunze, F., A. Schauer, and G. Krusmann. 1975. Focal loss of alkaline phosphatase and increase of proliferation in preneoplastic areas of the rat urothelium after administration of *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine and *N*-(4-(5-nitro-2-furyl)-2 thiazoly)form-amide. *Z. Krebsforsch.* 84:143–160.
37. Lajtha, L. G. 1979. Stem cell concepts. *Differentiation.* 14:23–34.
38. Leblond, C. P., and H. Cheng. 1976. Identification of stem cells in the small intestine of the mouse. *In* Stem Cells of Renewing Cell Population. A. B. Cairnie, P. K. Lala, and D. G. Osmond, editors. Academic Press, Inc., New York. 7–31.
39. Lung, B., G. R. Cunha, and B. A. Reese. 1979. Prostatic induction of mouse urinary bladder epithelium by urogenital sinus mesenchyme. *Anat. Rec.* 193:607. (Abstr.)
40. Lung, B., G. R. Cunha, and V. E. Frank. 1979. Ultrastructural analysis of prostatic induction of embryonic mouse urinary bladder. *J. Cell Biol.* 83(2, Pt 2):16*a*. (Abstr.)
41. McLoughlin, C. B. 1963. Mesenchymal influences on epithelial differentiation. *Symp. Soc. Exp. Biol.* 17:359–388.
42. Montagna, W. 1956. The structure and function of skin. Academic Press, Inc., New York. First edition. 327.
43. Montagna, W. 1962. The structure and function of skin. Academic Press, Inc., New York. Second edition. 427.
44. Neubauer, B. L., L. W. K. Chung, K. A. McCormick, O. Taguchi, T. C. Thompson, and G. R. Cunha. 1983. Epithelial-mesenchymal interactions in prostatic development. II. Biochemical observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult rodent urinary bladder. *J. Cell Biol.* 96:1671–1680.
45. Pearse, A. G. E. 1972. Histochemistry: Theoretical and Applied. The Williams & Wilkins Co., Baltimore, MD). 576–759.
46. Pierce, G. B., R. Shikes, and L. M. Fink. 1978. Cancer: A Problem of Developmental Biology. Prentice-Hall, Inc., Englewood Cliffs, NJ. 1–242.
47. Pointis, G., M. T. Latreille, T. M. Mignot, Y. Janssens, and L. Cedard. 1979. Regulation of testosterone synthesis in the fetal mouse testis. *J. Steroid Biochem.* 11:1609–1612.
48. Price, D., and E. Ortiz. 1965. The role of fetal androgens in sex differentiation in mammals. *In* Organogenesis. R. L. DeHaan and H. Ursprung, editors. Holt, Rinehart & Winston, New York. 629–652.
49. Reyer, R. W. 1954. Regeneration of the lens in the amphibian eye. *Q. Rev. Biol.* 29:1–46.
50. Sakakura, T., Y. Nishizuka, and C. J. Dawe. 1976. Mesenchyme-dependent morphogenesis and epithelium-specific cytodifferentiation in mouse mammary gland. *Science* (*Wash. DC*). 194:1439–1441.
51. Saxen, L. 1977. Morphogenetic tissue interactions: an introduction. *In* Cell Interactions in Differentiation. M. Karkinen-Jaaskelainen and L. Saxen, editors. Academic Press, Inc., New York. 145–152.
52. Saxen, L. 1977. Directive versus permissive induction: a working hypothesis. *In* Cell and Tissue Interactions. J. W. Lash and M. M. Burger, editors. Raven Press, New York. 1–10.
53. Saxen, L., M. Karkinen-Jaaskelainen, E. Lehtonen, S. Nordling, and J. Wartiovaara. 1976. Inductive tissue interactions. *In* The Cell Surface in Animal Embryogenesis and Development. G. Poste and G. L. Nicolson, editors. Elsevier North-Holland, New York. 331–408.
54. Saxen, L., and S. Toivonen. 1962. Primary embryonic induction. Prentice-Hall, Inc., Englewood Cliffs, NJ. 271.
55. Spearmen, R. I. C. 1974. Alteration of keratinization in mouse ear epidermis in recombinant grafts with tail dermis. *Acta. Anat.* 89:195–202.
56. Spemann, H. 1939. Embryonic development and induction. Yale University Press, New Haven, CT. 289.
57. Sternberger, L. A., P. H. Hardy, J. J. Cuculis, and H. G. Meyer. 1970. The unlabelled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. *J. Histochem. Cytochem.* 18:315–333.
58. Tarin, D. 1972. Tissue interactions and the maintenance of histological structure in adults. *In* Tissue Interactions in Carcinogenesis. D. Tarin, editor. Academic Press, Inc., New York. 81–94.
59. Tiedemann, H. 1976. Pattern formation in early developmental stages of amphibian embryos. *J. Embryol. Exp. Morphol.* 3:437–444.
60. Tyler, M. S., and W. E. Koch. 1977. In vitro development of palatal tissues from embryonic mice. III. Interactions between palatal epithelium and heterotypic oral mesenchyme. *J. Embryol. Exp. Morphol.* 38:37–48.
61. VanScott, E. J., and R. P. Reinertson. 1961. The modulating influence of stromal environment on epithelial cells: studies in human autotransplants. *J. Invest. Dermatol.* 36:109–131.
62. Walker, B. E. 1958. Polyploidy and differentiation in the transitional epithelium of mouse urinary bladder. *Chromosoma.* 9:105–118.
63. Walker, B. E. 1960. Electron microscopic observations on transitional epithelium of the mouse urinary bladder. *J. Ultrastruct. Res.* 3:345–361.
64. Weniger, J. P., and A. Zeis. 1972. Sur la secretion precoce de testosterone par le testicule embryonnaire de souris. *C. R. Acad. Seances Paris* 275:1431–1433.
65. Wolff, E. 1968. Specific interactions between tissues during organogenesis. *Curr. Top. Dev. Biol.* 3:65–94.
66. Yasugi, S., and T. Mizuno. 1978. Differentiation of the digestive tract epithelium under the influence of the heterologous mesenchyme of the digestive tract in the bird embryos. *Dev. Growth Differ.* 20:261–267.