Stromal-Epithelial Interactions in Sex Differentiation

GERALD R. CUNHA,[1,2] LELAND W. K. CHUNG,[3]
JOHN M. SHANNON[1] and BETH A. REESE[1]

Department of Anatomy[1]
University of Colorado School of Medicine
Denver, Colorado 80262
and
Pharmacology/School of Pharmacy,[3]
University of Colorado,
Boulder, Colorado 80303

INTRODUCTION

In the last 10 years much has been learned about the roles of sex steroids in embryonic development, morphogenesis and expression of functional activity of mammalian sex organs. In male embryos, androgens (produced by the fetal testes) are required for masculine morphogenesis of certain mesonephric tubules, the Wolffian ducts, urogenital sinus, external genitalia and mammary glands (Table 1) (Price et al., 1971; Jost et al., 1977). Regression of the Mullerian ducts is triggered in male fetuses by the Mullerian inhibiting substance, which is also produced by the fetal testes (Josso, 1977; Donohoe et al., 1977). Female urogenital development occurs in the absence of these testicular secretions and, at least initially, estrogens are not required for normal development of female accessory sex organs (Price and Ortiz, 1965; Jost, 1965; Jost et al., 1977). Developing reproductive tracts of both sexes, however, are sensitive to exogenous estrogens which may elicit a variety of teratogenic and possibly carcinogenic effects (McLachlan and Dixon, 1977; Takasugi, 1976; Bern et al., 1976; Kohrman, 1978). Growth, morphogenesis, differentiation and expression of functional activity of urogenital organs are controlled postnatally by estrogens, androgens and/or progestins (Liao, 1975, 1977; Mainwaring, 1978; Schrader and O'Malley, 1978; Clark et al., 1978). In most instances, hormonal effects have been shown to be mediated through high affinity, low capacity cytoplasmic receptors that translocate with bound hormone to nuclear acceptor sites on the chromatin. This ultimately triggers synthesis of specific proteins that are required for growth, morphogenesis, differentiation and expression of functional activity. These studies have focused almost exclusively upon hormonal effects on epithelial tissues. Indeed, dramatic morphological and biochemical effects are elicited in epithelial tissues in response to hormonal stimulation. Epithelium is responsible for secretory activity and many other functions of urogenital organs. By contrast, stroma has been regarded as an inactive matrix that is involved in maintaining histotypic organization of the epithelium. Morphological as well as biochemical effects of hormones upon stromal elements are subtle and as yet difficult to interpret; consequently, the role(s) of stroma in growth and differentiation has not been explored in depth.

Biochemical studies of hormone target organs have in most cases utilized homogenates containing both epithelial and stromal elements. This precludes the possibility of defining the respective roles of epithelium and stroma in the function of urogenital organs. Definitive studies necessary to clarify the individual roles of epithelium and stroma require clean separation of the two elements prior to analysis. Technical difficulties in separating complex glandular organs into pure populations of epithelial and stromal cells have precluded this type of investigation. For these reasons, the function(s) of stroma in hormonal response has received little attention.

All epithelial and stromal tissues are potentially separable. Their line of union and also their line of demarcation is a distinct morphological entity, the basal lamina, an ultrastructurally defined, highly specialized layer of fine fibrillar collagen and glycoprotein (Kefa-

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[2] To whom reprint requests should be addressed.

TABLE 1. Effects of hormonal conditions on development of the mammalian genital tract, external genitalia and mammary glands.

Structure	Hormonal effects	
	Androgens present	Androgens absent
Mesonephric tubules	Maintenance and differentiation into efferent ducts	Degeneration
Wolffian duct	Maintenance and differentiation into: a) Epididymis b) Ductus deferens c) Seminal vesicle d) Ejaculatory duct	Degeneration
Urogenital sinus	Differentiation into: a) Prostate b) Bulbourethral gland c) Urethra	Differentiation into: a) Vagina b) Urethra
External genitalia	Development of masculine phenotype	Development of female phenotype
Mammary glands	Arrested development or degeneration of the epithelial rudiment	Development of glandular elements

lides, 1973). Digestion of the basal lamina can be easily accomplished by a variety of proteolytic enzymes (Carlson et al., 1976). Following dissolution of the basal lamina, stromal tissue (primarily fibroblasts and smooth muscle cells) either remains as a cohesive mass of cells embedded in a meshwork of collagen fibers, or under the influence of collagenolytic enzymes may be dissociated into a suspension of single cells. By contrast, epithelium may be enzymatically loosened from the stroma by limited proteolytic digestion as intact sheets whose integrity is maintained by a variety of intercellular junctions.

Unfortunately, digestion of the basal lamina or extracellular matrix in adult glandular organs does not usually lead to clean separation of these tissues because of the exquisite complexity of interdigitation of the epithelium and stroma. However, through utilization of organs or organ rudiments of simplified histotypic architecture, clean separation of the component tissues can be obtained. Separated epithelium may be reassociated with its native stroma (homotypic recombinations) or with stroma derived from other organs (heterotypic recombinations) (Fig. 1). The recombinants can be grown under a variety of in vitro or in vivo conditions, with or without hormonal stimuli. The role of local diffusible factors or cell contact may be studied by interposing Millipore or Nucleopore filters between epithelium and stroma (Saxen et al., 1977). Hormonal influences can be evaluated through light and electron microscopic analysis of morphological change, histochemical estimation of enzymatic activity, autoradiography, immunocytochemical localization of tissue-specific antigens, or biochemical analyses.

By use of these versatile techniques, the role of mesenchyme (stroma) has been partly defined for embryonic development and, in some cases, for differentiation of the mature epithelial phenotype. Influence of stroma upon epithelium has been investigated not only in terms of morphology, but also in terms of functional activity, including the expression of androgen receptor activity and androgen-induced stimulation of DNA synthesis. The possible role(s) of stroma in the etiology of pathologic epithelial growth has also been investigated. The most significant results derived from application of tissue separation and recombination techniques are summarized below.

Role of Mesenchyme in Sex-Steroid-Induced Morphogenesis

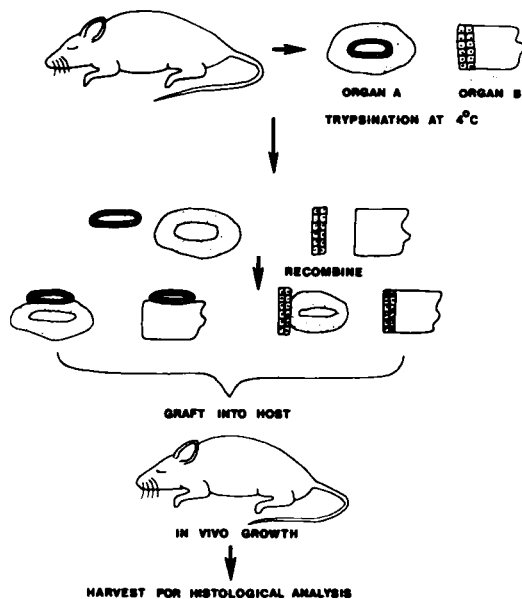*Embryonic morphogenesis of sex glands.* Morphogenesis of the male or female pheno-

FIG. 1. Summary of the methodology of tissue separation and recombination. Organs are dissected from the animal, trimmed to size and incubated for 2—3 h in 1—2% trypsin in Tyrode's solution at 4°C. Following trypsinization the organs are separated into their epithelial and mesenchymal (stromal) components, recombined either homo- or heterotypically and grown in vivo in male or female hosts.

type of urogenital anlage is directed by hormonal signals; that is, the presence or absence of androgen produced by the fetal testes. For instance, in the presence of androgen, the urogenital sinus develops the elaborately branched ductal network of the prostate, while in its absence a stratified vaginal epithelium forms. Development of either of these organs requires an interaction between epithelium and mesenchyme. Isolated embryonic epithelium usually dies when grown in vitro or in vivo, with or without hormonal stimuli. In contrast, mesenchyme survives under these same conditions but merely forms a mass of fibroblasts and smooth muscle cells. Conversely, if trypsin-separated epithelium and mesenchyme of the urogenital sinus are reassociated (homotypic recombination), harmonious morphogenesis of both components proceeds and during 4 weeks of growth in an adult male host prostatic morphogenesis occurs; in many cases functional (secretory) activity may be expressed. Homotypic recombinants grown in this fashion are virtually indistinguishable histologically from the host's prostate (Cunha, 1976a; Cunha and

Lung, 1979).

To explore the possible role of mesenchyme in transmitting morphogenetic influences of androgens to epithelium, heterotypic recombinants were prepared by associating epithelium of the urogenital sinus with mesenchyme from the embryonic integument. Under these conditions, androgenic stimulation had no apparent effect; the epithelium of the urogenital sinus differentiated into a stratified squamous epithelium (Cunha, 1972a). This response may represent vaginal differentiation, since formation of skin appendages (hair or glands) was never elicited by the integumental inductors (Kollar, 1972; Sakakura et al., 1976; Tyler and Koch, 1977). In fact, the morphogenetic response to androgen (prostatic organogenesis) only occurred when epithelium of the urogenital sinus was combined with homo- or heterotypic mesenchyme of *urogenital* origin (Cunha, 1972a,b,c; Cunha and Lung, 1978). Likewise, epithelium of the embryonic seminal vesicle only expressed androgen-induced morphogenesis when combined with mesenchyme of either the urogenital sinus or seminal vesicle and not with nontarget integumental mesenchyme (Fig. 2). We have interpreted these data as indicating that masculine morphogenesis, induced by androgen, depends upon relatively specific mesenchymal conditions. The morphogenetic effects of androgen upon urogenital epithelium are apparently mediated through unique properties of urogenital mesenchyme that are not present in mesenchyme from other organ systems such as the integument (Cunha, 1972a,b,c, 1976a; Cunha and Lung, 1979). Similar studies of prostatic development in rats have led Lasnitzki and Mizuno (1979) to the same conclusion.

The importance of mesenchyme as a mediator of androgenic effects during prostatic morphogenesis is further emphasized by studies utilizing tissues from Tfm mice (testicular feminization syndrome). Masculine morphogenesis of internal and external genitalia and mammary glands is aborted in Tfm/Y males because their target tissues are insensitive to androgens even though these hormones are secreted in significant amounts by the fetal testes (Bardin and Bullock, 1974; Bardin et al., 1978). Androgen receptors are apparently absent or nonfunctional in all cells of Tfm/Y males. However, if Tfm/Y urogenital sinus epithelium is heterotypically recombined with mesenchyme of the urogenital sinus from a
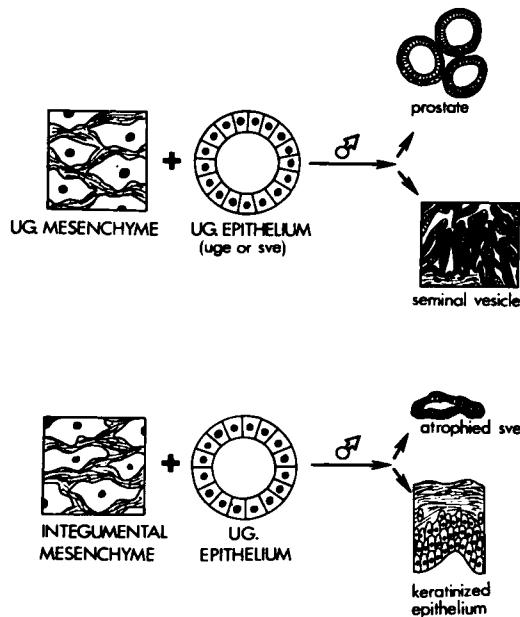
FIG. 2. When epithelium of the urogenital sinus (UGE) is associated with integumental mesenchyme and grown in intact adult male hosts, the UGE differentiates as a stratified squamous vagina-like epithelium, instead of expressing androgen-induced prostatic morphogenesis. Likewise, epithelium of the seminal vesicle (SVE) forms narrow tubules of atrophied epithelium when grown in association with integumental mesenchyme. Conversely, if these urogenital epithelia (UGE or SVE) are associated with urogenital mesenchyme and grown under identical conditions, typical androgen-induced glandular morphogenesis proceeds and after 4 weeks of growth, secretory activity is expressed.
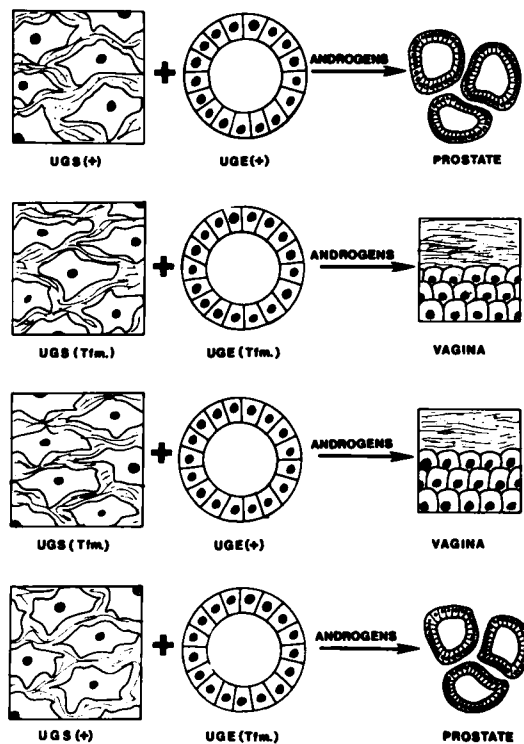

FIG. 3. A summary of recombination experiments between urogenital sinus components from Tfm/Y and wild-type embryos. A positive androgenic response (prostatic morphogenesis) occurs when wild-type mesenchyme is grown in association with either wild-type or Tfm/Y epithelium. Conversely, vagina-like differentiation occurs when either wild-type or Tfm/Y epithelium is grown in association with Tfm/Y mesenchyme in male hosts.

wild-type embryo, the Tfm/Y epithelium participates in androgen-induced prostatic organogenesis by forming prostatic acini (Fig. 3). By contrast, the reciprocal recombinant composed of wild-type epithelium and Tfm/Y urogenital sinus mesenchyme, when grown under the same conditions, differentiates as a stratified vaginal mucosa (Cunha and Lung, 1978). These observations, when considered in conjunction with the results of recombination experiments employing urogenital and integumental tissues, demonstrate that mesenchyme, specifically androgen-sensitive wild-type mesenchyme, is an essential mediator of the morphogenetic effects of androgens upon epithelial organogenesis. During *embryonic* development, mesenchyme appears to be the actual target of androgen in the urogenital tract. Finally, these data demonstrate that prostatic induction by

mesenchyme of the embryonic urogenital sinus is dependent upon the presence within the mesenchyme of the wild-type allele at the Tfm locus (Cunha and Lung, 1978, 1979). Additional studies of mice carrying both the Tfm and sex reversed mutations have led Ohno (1979) and Drews and Dietrich (1978) to similar conclusions.

Studies on androgen-induced regression of embryonic mammary epithelium are in complete agreement with our studies of prostatic organogenesis. In Tfm/Y male fetuses (in contrast to wild-type fetuses) androgen does not trigger regression of mammary epithelium (Kratochwil and Schwartz, 1976). However, when Tfm/Y mammary epithelium is combined with wild-type mammary mesenchyme, epithelial regression occurs in the presence of androgens (Fig. 4). Androgen-induced epithelial
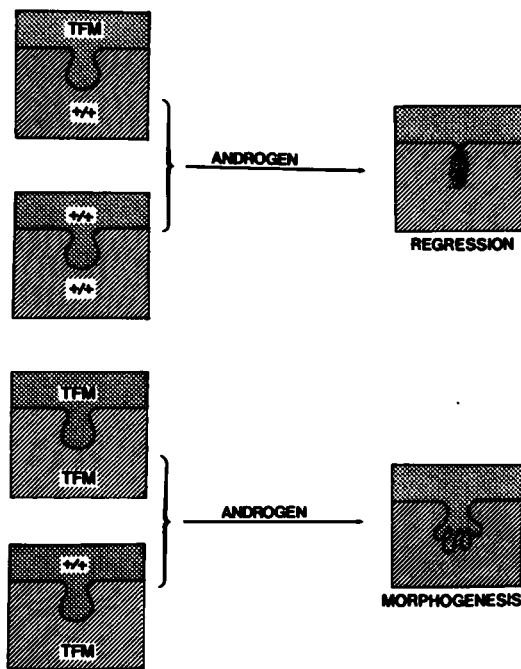
FIG. 4. A summary of experiments of androgen-induced regression of mammary rudiments composed of Tfm/Y and wild-type tissues. The positive androgenic response (regression of the epithelial rudiment) occurs when either wild-type or Tfm/Y epithelium is associated with wild-type mammary mesenchyme. In contrast, both wild-type and Tfm/Y mammary epithelium fail to exhibit androgen-induced regression when cultured in association with mesenchyme from Tfm/Y embryos.

regression does not occur when Tfm/Y mammary mesenchyme is associated with wild-type epithelium (Kratochwil and Schwartz, 1976; Drews and Drews, 1977; Dürnberger et al., 1978). Therefore, evidence in the mammary gland also suggests that mesenchyme is the actual target for testosterone and that morphogenetic effects of androgens upon epithelial development are mediated by the mesenchyme.

A possible hint at the mechanism that accounts for mesenchymal primacy during development of accessory sexual structures comes from autoradiographic studies of estrogen-binding sites in the urogenital tract of chick embryos (Gasc et al., 1978). Morphogenesis of the cloacal-anal region of the embryonic chick is under hormonal control. In contrast to the situation in mammals, castration of the chick produces masculine development, while exposure to exogenous estrogen produces the female pattern of morphogenesis. Steroid

autoradiography shows that nuclear estrogen binding sites are found *only* in the mesenchyme; estrogen binding sites are absent in the adjacent epithelial cells of this region. Therefore, the primacy of mesenchyme as a target and mediator of morphogenetic hormonal effects within the developing urogenital tract may be due to the fact that it is the mesenchyme alone which possesses hormone receptor activity during *embryonic* periods.

Thus far, this discussion has dealt with permissive inductive influences of mesenchyme; that is, the ability of urogenital mesenchyme to permit or mediate normal hormone-induced morphogenesis in urogenital epithelia. Urogenital mesenchyme may also express inductive influences which are instructive or directive; that is, the ability of mesenchyme to reprogram or change the developmental fate of an epithelium. Examples of this type of inductive influence are presented in Table 2. In most cases these heterotypic recombinants have been analyzed solely by light microscopy. Some of these morphological observations have raised the possibility that the observed mesenchyme-induced phenotypic alterations in the responding epithelium involve both morphological and functional (biochemical) alteration of phenotypic expression (see below).

Neonatal morphogenesis of accessory sexual organs. Morphogenetic processes initiated during fetal stages continue into neonatal and prepubertal periods and are completed only shortly before the onset of sexual maturity. At birth most urogenital organs, particularly those derived from the Mullerian ducts, are in a rudimentary state. In mice, Mullerian epithelium gives rise to the oviducts, uterus, cervix and the upper portion of the vagina (Forsberg, 1973; Cunha, 1975a). Because of the common Mullerian origin of the epithelium of these structures, a problem arises in explaining the development of the marked regional differences in morphology and function of Mullerian epithelium. Recombination experiments have strongly implicated the stroma as the regional determinant of epithelial differentiation (Cunha, 1976b). Stromata of the uterus, cervix and vagina of neonatal mice have been shown to induce and specify the morphological differentiation of heterotypic urogenital epithelium (Fig. 5). Thus, stroma is the regional determinant of morphogenesis of Mullerian epithelium during female urogenital development.

Closer examination of certain tissue recom-

TABLE 2. Mesenchyme (stroma)-induced alteration of epithelial differentiation in accessory sexual structures.

Mesenchymal inductor	Responding epithelium	Epithelial response	Reference
Uterus (neonatal)	Vagina (neonatal)	Uterine	Cunha (1976b)
Vagina (neonatal and adult)	Uterus (neonatal)	Vaginal	Cunha (1976b)
Cervix (neonatal and adult)	Uterus (neonatal)	Cervical	Cunha and Lung (1979)
Seminal vesicle (embryonic)	Epidermis (embryonic)	Glandular	Cunha (1972a)
Seminal vesicle (neonatal)	Bladder (neonatal and adult)	Glandular	Fujii and Cunha (unpublished)
Urogenital sinus (embryonic)	Bladder (neonatal and adult)	Prostatic	Cunha and Lung (1978) Cunha et al. (1980)
Bulbourethral gland (neonatal)	Bladder (neonatal)	Bulbourethral gland	Fujii and Cunha (unpublished)
Urogenital sinus (embryonic)	Vagina (neonatal and adult)	Prostatic	Cunha (1975b)
Urogenital sinus (embryonic)	Skin (embryonic)	Glandular	Cunha (1972a)
Mammary (embryonic and adult)	Skin, salivary gland, lung (embryonic)	Glandular	Kratochwil (1969) Propper and Gomot (1977) Sakakura et al. (1979a)
Chick oviduct (embryonic)	Chick skin (embryonic)	Glandular	Moscona (1961)

binants suggests that stroma may also elicit the expression of a new functional epithelial phenotype. For example, vaginal and cervical epithelia in the mouse are stratified epithelia whose differentiation fluctuates during the estrous cycle from the cornified to the mucified state in response to changing levels of estrogens and progestins. Furthermore, in intact (cycling) female hosts, epithelial differentiation also cycles in grafts of vaginal or cervical mucosa, as well as in homo- or heterotypic recombinants that express the vaginal or cervical phenotype. Such grafts form cysts in which sloughed, cornified and mucified cells accumulate as concentric layers. In recombinants prepared with vaginal or cervical stroma associated with uterine epithelium, the normally simple columnar, glandular epithelium of the uterus is induced to form a stratified epithelium whose differentiation varies from the mucified to the cornified state in exact concordance with the differentiation of the host's vaginal or cervical epithelium (Fig. 6). Thus, both morphological organization and certain functional characteristics (cyclical alteration of differentiation) of Mullerian epithelium appear to be induced and specified by stroma during neonatal morphogenesis (Cunha, 1976a,b; Cunha and Lung, 1979).

During the neonatal and prepubertal periods, stroma also plays a role in restricting the morphogenetic responsiveness of the female urogenital sinus to androgens. Urogenital sinuses of male and female fetuses have two primary developmental options, prostatic or vaginal morphogenesis. Under the influence of exogenous androgens, prostatic morphogenesis is elicited in the female urogenital sinus during embryonic and early neonatal periods, but by 5 days postpartum this morphogenetic sensitivity to androgens is lost (Turner, 1939; Cunha, 1975b). Thereafter, the sinus vagina (that portion of the vagina derived from the urogenital sinus) responds to androgens by undergoing mucification but not by forming prostate. To determine which tissue (epithelium or stroma) is responsible for this age-dependent loss in morphogenetic responsiveness to androgens, tissue components from the sinus vagina of 1–20-day-old mice were associated with epithelium or mesenchyme from the embryonic urogenital sinus as depicted in Fig. 7. After 4–5 weeks of in vivo growth within intact adult male hosts, the developmental response of the recombinants was analyzed morphologically.

Epithelium from mice of all ages (1–20 days postpartum) was found to be able to form prostate-like acini when combined with mesenchyme of the urogenital sinus (Cunha, 1975b). Recent experiments have, in fact, shown that vaginal epithelium from adult mice can differentiate as prostate-like glandular cells when associated with mesenchyme of the urogenital sinus and grown under androgenic conditions (Cunha, G. R., Reese, B. A. and Shannon, J. M., unpublished). Conversely, when epithelium of the embryonic urogenital sinus is combined with vaginal stroma from 1–20-day-old mice, there is a progressive, age-dependent decrease in the incidence of prostatic morphogenesis. In fact, at 20 days postpartum vaginal stroma appears incapable of participating in prostatic morphogenesis with epithelium of the embryonic urogenital sinus. These observations suggest that maturational changes within the stromal component of the sinus vagina are responsible for the age-dependent loss of the ability of this organ to express prostatic morphogenesis in response to androgenic stimulation (Cunha, 1975b). The molecular basis of this alteration has not been investigated, but may be related to a change within vaginal stroma in the levels of androgen-receptor activity or the capacity to metabolize androgen.

Mesenchyme-induced alteration of functional differentiation of embryonic epithelium. Morphological observations (see above) have suggested that certain directive inductions may involve alteration of both morphological and functional (biochemical) aspects of epithelial differentiation. In this regard, one of the most well characterized models of mesenchyme-induced alteration of epithelial differentiation is the heterotypic recombination composed of mesenchyme of the urogenital sinus and epithelium of the urinary bladder of embryonic mice (UGM + BLE). These recombinants grown in adult male hosts develop prostate-like acini lined by a simple columnar epithelium (Cunha and Lung, 1978). After 4 weeks of growth in male hosts, many of the acini contain putative secretory product (Fig. 8). Four types of control procedures involving analysis of hundreds of specimens demonstrate that the formation of prostatic acini within the UGM + BLE recombinants is attributed to inductive influences of UGM upon the embryonic BLE and not to the presence of residual epithelial cells contaminating the mesenchyme of the urogenital sinus.
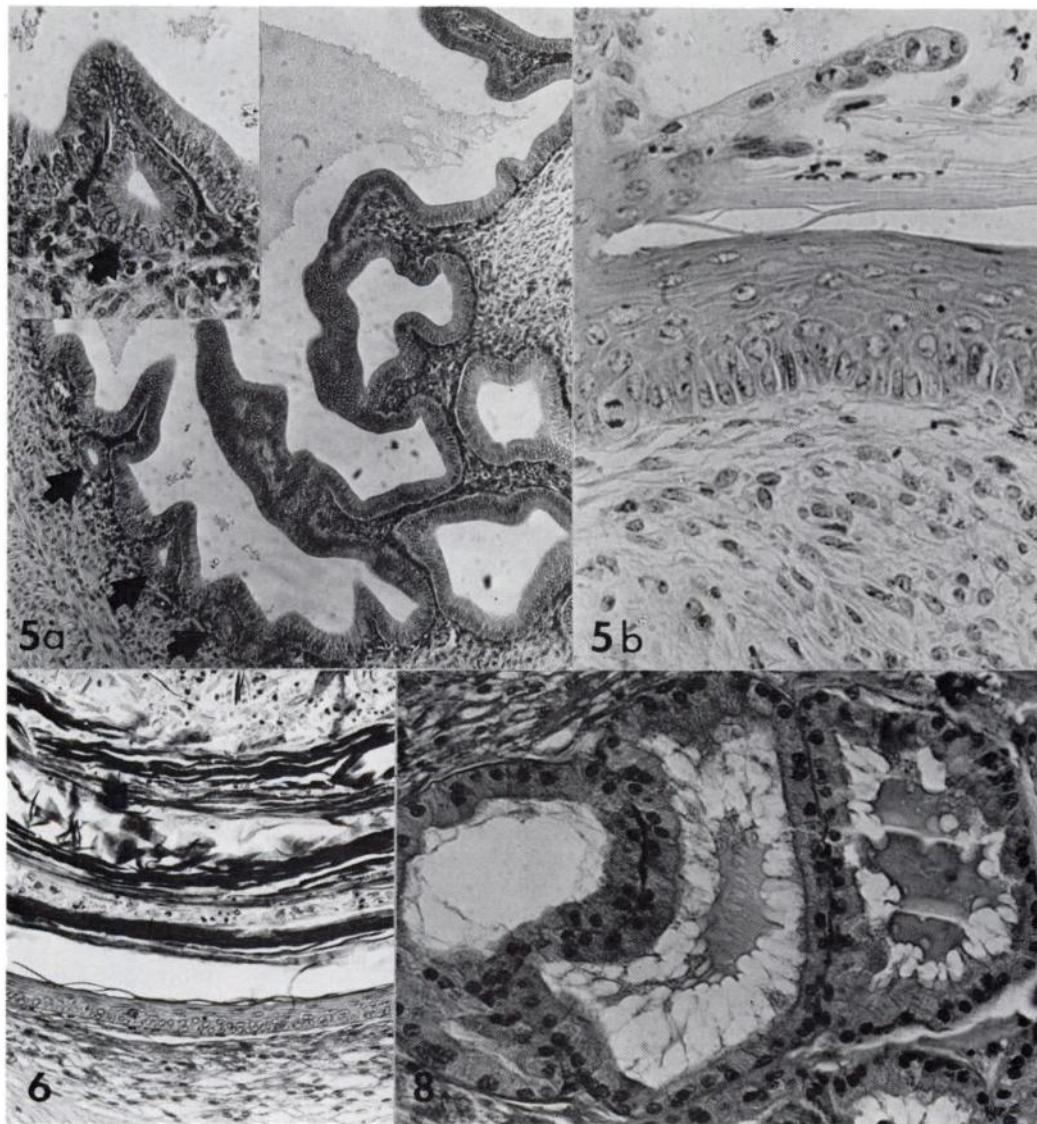
FIG. 5a. A recombinant prepared by associating uterine stroma with vaginal epithelium from 3-day-old mice. After 1 month's growth in a female host, the normally stratified vaginal epithelium has differentiated as uterus. Note the simple columnar epithelium which has formed several uterine glands (arrows). ×200. Inset ×500 (from Cunha, 1976b).

FIG. 5b. Vaginal differentiation occurs when responsive uterine epithelium (1 day) is associated with inductive vaginal stroma from either neonatal or adult mice. In this recombinant, the vaginal stroma is derived from an adult mouse. The recombinant was grown for 6 weeks in a normal, cycling female host which was sacrificed during the estrous phase of the cycle. For this reason the induced uterine epithelium is thickened and cornified, indicative of vaginal epithelium during estrus. ×800 (from Cunha, 1976b).

FIG. 6. A recombinant composed of cervical stroma and uterine epithelium from 5-day-old mice. After 5 weeks' growth in a cycling female host, the normally simple columnar uterine epithelium has differentiated as a stratified squamous epithelium. Note the alternate layers of cornified and mucified cells which are evidence of cycling of the graft during the host's estrous cycles. ×320 (from Cunha and Lung, 1979).

FIG. 8. A recombinant composed of mesenchyme of the urogenital sinus and epithelium from the urinary bladder of 16-day-old embryonic mice. The growth period was 4 weeks in an adult male host. The mesenchyme of the urogenital sinus has induced the bladder epithelium to form prostate-like acini. Note the presence of putative secretion within the acinar lumina. ×500.
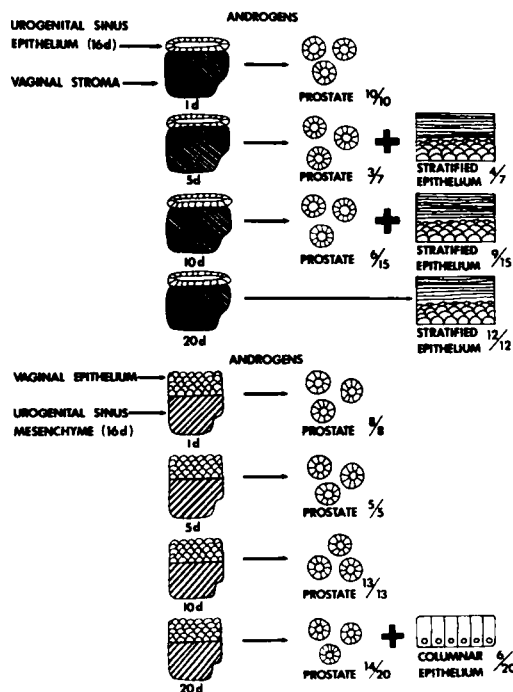
FIG. 7. To determine which tissue accounts for the age-dependent loss of morphogenetic responsiveness of the sinus vagina, recombinants were prepared with components from urogenital sinuses from 16-day-old fetuses associated with vaginal tissues from 1—20-day-old mice. After 4—5 weeks of growth in male hosts, the grafts were harvested for histological analysis. Vaginal epithelium from all ages (1 to 20 days old) is able to participate in prostatic morphogenesis. By contrast, when vaginal stroma from 1—20-day-old mice is associated with epithelium of the embryonic urogenital sinus, there is a progressive, age-dependent fall-off in the ability of vaginal stroma to participate in prostatic morphogenesis. These data suggest that maturational changes within vaginal stroma account for the loss of morphogenetic sensitivity of this organ to androgens.

histochemical patterns indicative of prostate during this induction (Table 3). Finally, the epithelial response to exogenous estradiol of UGM + BLE recombinants is similar to that of the host's prostate in that the epithelia in both cases become hyperplastic and may exhibit squamous metaplasia (Fig. 10). By contrast, the urotehlium of the bladder of the host is not affected by the exogenous estradiol (Lung et al., 1979a,b).

More direct evidence for alteration of biochemical phenotype is based upon autoradiographic studies of [^{3}H]-dihydrotestosterone (^{3}H]-DHT) binding sites. Male host mice bearing UGM + BLE recombinants were injected with [^{3}H]-DHT and autoradiograms prepared according to the thaw-mount method of Stumpf and Sar (1975). As expected, the number of silver grains was low (Fig. 11a) within the urothelium of the hosts's bladder (a nontarget organ) and their distribution random without preferential localization over cell nuclei (Cunha, G. R., Reese, B. A. and Shannon, J. M., unpublished). In contrast, epithelial cells of the host's prostate and the UGM + BLE recombinants were heavily labeled, with the majority of silver grains being localized over epithelial nuclei (Fig. 11b,c). Since there appears to be a relationship between nuclear uptake of hormone, which is mediated by specific hormone receptors (Liao, 1977), and hormone action, it is highly probable that nuclear concentration of [^{3}H]-labeled steroids as measured autoradiographically is related to the quantity of available receptor molecules (Stumpf and Sar, 1976). Our observations, therefore, are suggestive of the idea that the bladder epithelium is actually expressing androgen receptor activity.

Ultrastructural examination of UGM + BLE recombinants indicates that a fundamental change in epithelial cytodifferentiation has occurred in the urothelium (Fig. 9). The highly specialized ultrastructural features of urothelium [the asymmetric, apical plasma membrane and fusiform vesicles (Hicks, 1975; Hicks and Chowaniec, 1978)] are absent in these induced prostatic acini, which instead resemble a secretory epithelium containing arrays of rough endoplasmic reticulum, Golgi and secretory granules (Lung et al., 1979a). Furthermore, on the basis of 3 histochemical tests (Lung et al., 1979b), enzymatic markers indicative of urothelium disappear or change to

Role of Stroma in Alteration and Maintenance of Adult Epithelial Structure and Function

Stromal (mesenchymal) influence on epithelial morphology and functional activity during adulthood. The importance of stroma or mesenchyme during morphogenetic processes in the fetus and neonate is well established. Following the morphogenetic period, stromal influence appears to be involved in maintenance or continued expression of normal epithelial organization and function. Data supporting this concept is fragmentary and in many cases circumstantial. In the urogenital

tract the existence of inductive influences in adult stromal cells is based upon the observation that adult vaginal (Fig. 5b) or cervical stromas are able to induce vaginal or cervical morphogenesis (and possibly functional differentiation) from competent (responsive) uterine epithelium (Cunha, 1976b; Cunha and Lung, 1979). Gland-free mammary fat pads from adult mice are also able to support morphogenesis of embryonic mammary epithelium (Sakakura et al., 1979a). A similar permissive induction has been observed in the prostate, suggesting that stroma of the adult prostate can induce glandular morphogenesis from epithelium of the urogenital sinus when androgens are present (Cunha, G. R., Reese, B. A. and Shannon, J. M., unpublished). Finally, several other observations in the prostate, mammary gland, uterus and vagina suggest that maintenance of adult epithelial morphology and function may be dependent upon intimate association with stromal cells. For instance, Franks and Barton (1960) have demonstrated that in explant cultures of mouse prostate grown in the presence of testosterone, the retention of differentiated features as judged by electron microscopy only occurred when the epithelium was in intimate contact with the stroma. Epithelial cells in the outgrowth exhibited gross disorganization of internal structure and failed to respond morphologically to testosterone. Similarly, during short term incubation of mechanically isolated epithelium from adult human prostate, incorporation of tritiated thymidine or amino acids into macromolecules was greatly reduced as compared to explants of intact prostate (Franks et al., 1970). These observations suggest the possibility that epithelial proliferation may be influenced by connective tissue factors (Muntzing et al., 1979). Similar conclusions can be drawn from the studies of Flaxman et al. (1973) for cultured adult vaginal epithelium, Lasfargues (1957a,b), Feldman (1971) and Ceriani (1976) for mammary gland and Kirk et al. (1978) for uterus. However, interpretation of these studies is complicated by the fact that the loss of functional organization within the epithelium may also result from adverse culture conditions.

Mesenchyme-induced alteration of phenotypic expression in adult epithelium. Phenotypic expression of adult mammalian epithelium, with the exception of neoplastic transformation, is remarkably stable. Consequently, even in continuously renewing adult epithelia, the progeny of mitotically-active stem cells faithfully express the correct phenotype(s). In fact, adult epithelial stem cells have been regarded by Pierce et al. (1978) and Hay (1968) as determined and partially differentiated. Attempts to alter phenotypic expression of adult epithelium through association with heterotypic stromal inductors have largely been unsuccessful, although such inductors may alter certain morphological characteristics such as epithelial thickness (Billingham and Silvers, 1968).

The only unequivocal report of mesenchyme-induced alteration of adult epithelial cytodifferentiation in mammals is the induction by urogenital sinus mesenchyme of prostate-like morphogenesis in epithelium of the adult urinary bladder (UGM + adult BLE) (Cunha et al., 1979, 1980). The influence of this prostatic inductor upon adult urothelium appears to be identical to that observed in similar recombinants prepared with embryonic urinary bladder epithelium (see above). When UGM + adult BLE recombinants are grown in male hosts, the stratified urothelium becomes organized into glandular acini lined by a simple columnar

FIG. 9. In the course of glandular induction from embryonic bladder epithelium, a fundamental change in epithelial cytodifferentiation occurs. Normal differentiation of epithelium of the urinary bladder results in expression of the transitional cell phenotype depicted in Fig. 9a. This electron micrograph of adult bladder epithelium demonstrates the stratified nature of urothelium (usually 3 or 4 cell layers). Basal cells resting upon the basal lamina (solid arrows) are relatively undifferentiated. Maturation of urothelial cells occurs progressively in intermediate and apical layers resulting in the production of highly specialized apical cells. Fully mature apical cells contain an abundance of the characteristic fusiform vesicles and the unique asymmetric, apical plasma membrane whose border is typically scalloped. X9500. By contrast, prostatic epithelial cells in Fig. 9b are secretory and contain organelles indicative of this function [rough endoplasmic reticulum (RER), Golgi and secretory granules (solid arrows)]. X13,500. In recombinants composed of UGM + BLE, shown in Fig. 9c, the epithelial cells are organized into acini lined by a simple columnar epithelium whose ultrastructural features closely resemble glandular, secretory cells. The cells in this field resemble immature secretory cells; RER is sparse, the Golgi complex is present in the supranuclear region and a few secretory granules are present apically (arrows). X13,500.

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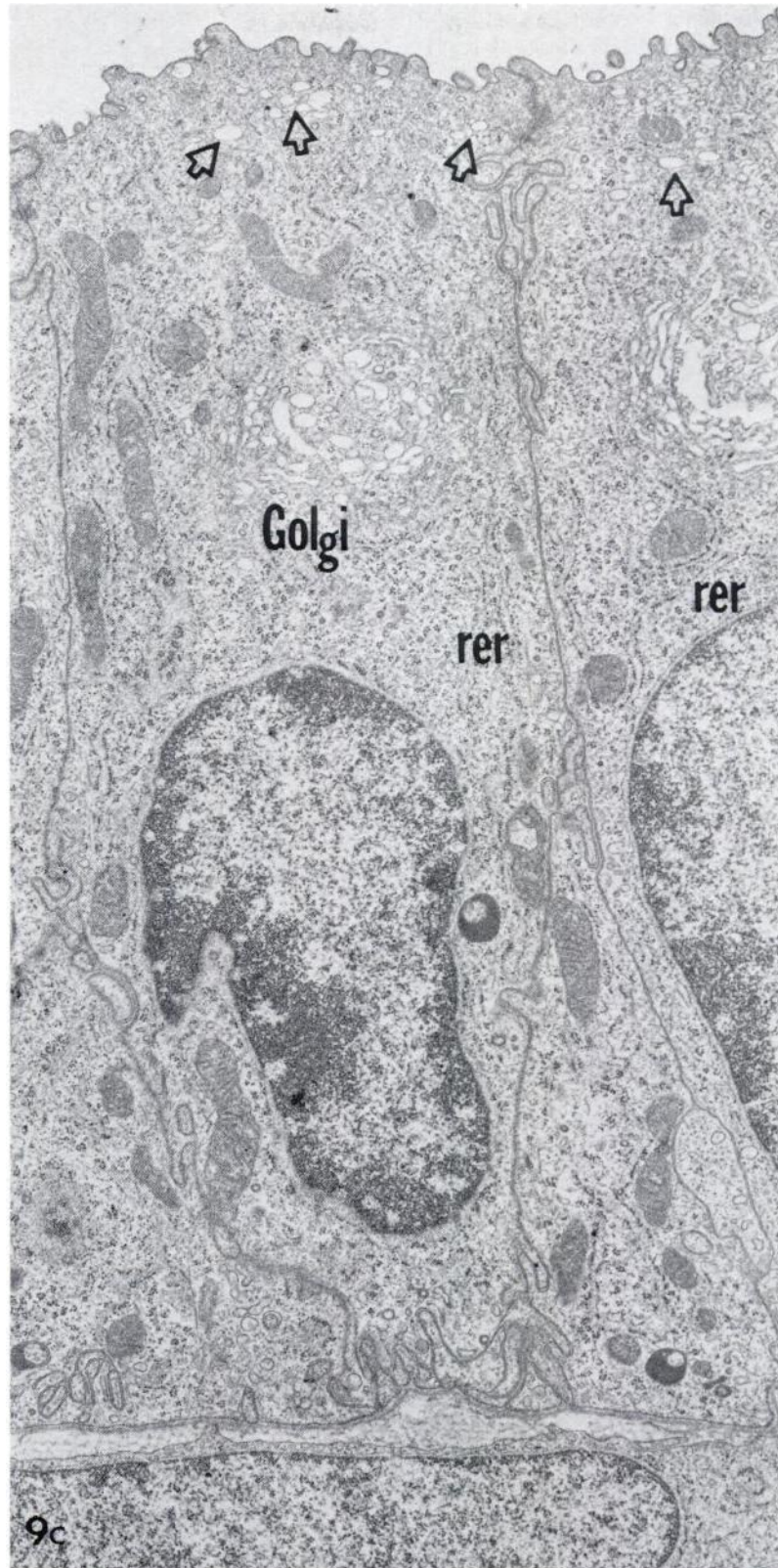
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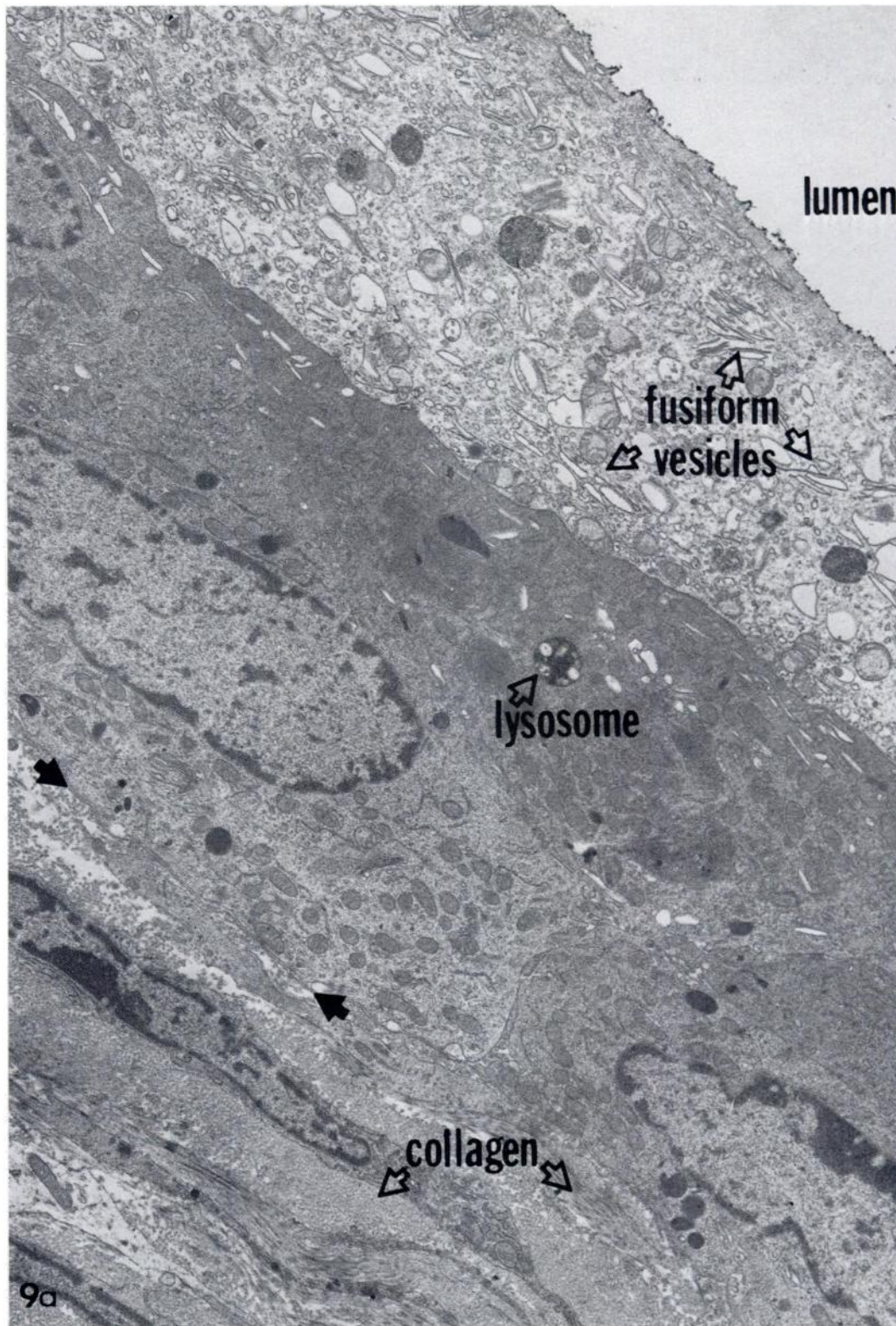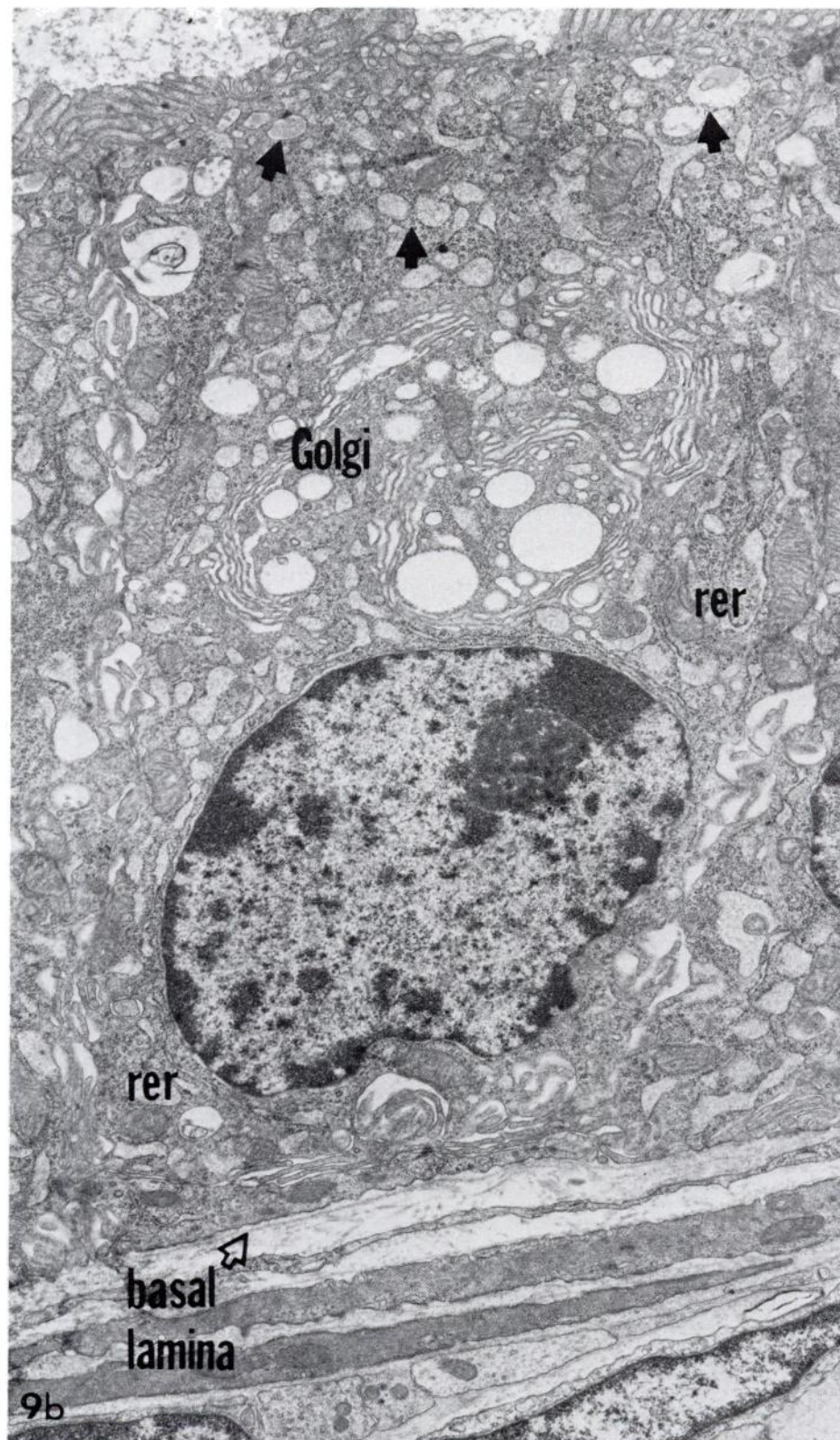
TABLE 3. Histochemical analysis of prostate, bladder and tissue recombinants prepared with urogenital sinus mesenchyme (UGM) and epithelia from urinary bladder from embryonic mice.

| Specimen	Epithelial morphology	Histochemical characteristics of epithelia		
		Alcian blue	Nonspecific esterase	Alkaline phosphatase
Adult prostate	Glandular	+	+	−
Adult urinary bladder	Transitional	−	− or ±	+
UGM + embryonic bladder epithelium	Glandular	+	+	−

epithelium (Fig. 12). Ultrastructurally, the induced bladder epithelium appears to be specialized for secretory activity by virtue of the presence of arrays of rough endoplasmic reticulum, Golgi and apparent secretory granules (Fig. 13). In the course of this induction, ultrastructural characteristics of urothelium (the unique apical, asymmetric plasma membrane and fusiform vesicles) disappear. Alkaline phosphatase, an enzyme usually present in urothelium, becomes undetectable within the epithelium of the induced glands which, therefore, further emphasize their resemblance to prostatic acini. By contrast, a prostate-associated epithelial marker, nonspecific esterase, is acquired by the induced urothelium, which is usually devoid of this enzyme (Cunha et al., 1979). These morphological and histochemical observations suggest that *adult* urothelium may be altered both morphologically and functionally.

Biochemical evidence supporting this contention has recently become available from analysis of androgen-induced stimulation of incorporation of [³H]-thymidine into DNA (Chung, L.W.K., Cunha, G. R. and Reese, B. A., unpublished). For this experiment, host males bearing UGM + adult BLE recombinants were castrated 4—6 weeks after grafting. Ten days postcastration, the hosts were injected daily for 1—5 days with testosterone propionate and incorporation of [³H]-thymidine into DNA was measured in vitro in minces of the hosts' prostates, bladders and the UGM + adult BLE recombinants. In the urinary bladder, incorporation of [³H]-thymidine into DNA was low and not influenced by injection of testosterone propionate (Fig. 14). By contrast, in the prostate and the recombinant, androgen had a distinct stimulatory effect upon incorporation of precursor into DNA, which peaked 72 h following initiation of androgen treatment (Chung, L.W.K., Cunha, G. R. and Reese, B. A., unpublished). Therefore on the basis of this single androgen-induced marker of biochemical function, the UGM + adult BLE recombinants behaved distinctly like prostate and not like

FIG. 10. In this experiment, recombinants composed of mesenchyme of the embryonic urogenital sinus and epithelium of the urinary bladder (UGM + BLE) of 16-day-old embryos were grown in male hosts for 1 month during which prostate-like glands were induced. The recombinants were then regrafted to a castrated male host which received a s.c. implant of estradiol-17β (20 mg E). After an additional 4 weeks of growth in the castrated, E treated host, the grafts and the bladder and prostate of the host were removed and processed histologically. a) Prostate from the castrated, E treated host. Note the hyperplastic epithelium of this prostatic acinus. X 400. b) UGM + BLE recombinant from the castrated, E treated host. The prostate-like acini are lined with a hyperplastic epithelium which also exhibits squamous metaplasia. X 400. Host bladder epithelium (not illustrated) is normal.

FIG. 11. Steroid autoradiograms prepared by the thaw-mount method of Stumpf and Sar (1975) utilizing [³H]-dihydrotestosterone. a) Host urinary bladder. The distribution of silver grains is random and without preferential localization over the nuclei. X 1000. b) A portion of the prostate (the coagulating gland) of the host. Note the preferential localization of silver grains over epithelial nuclei of this androgen target organ. X 1600. c) Recombinant composed of urogenital sinus mesenchyme and epithelium from the urinary bladder of embryonic mice. Note the preferential nuclear uptake of the [³H]-DHT. These epithelial androgen-binding sites may be indicative of mesenchyme-induced expression of androgen receptor activity within the nontarget epithelium. X 1000.
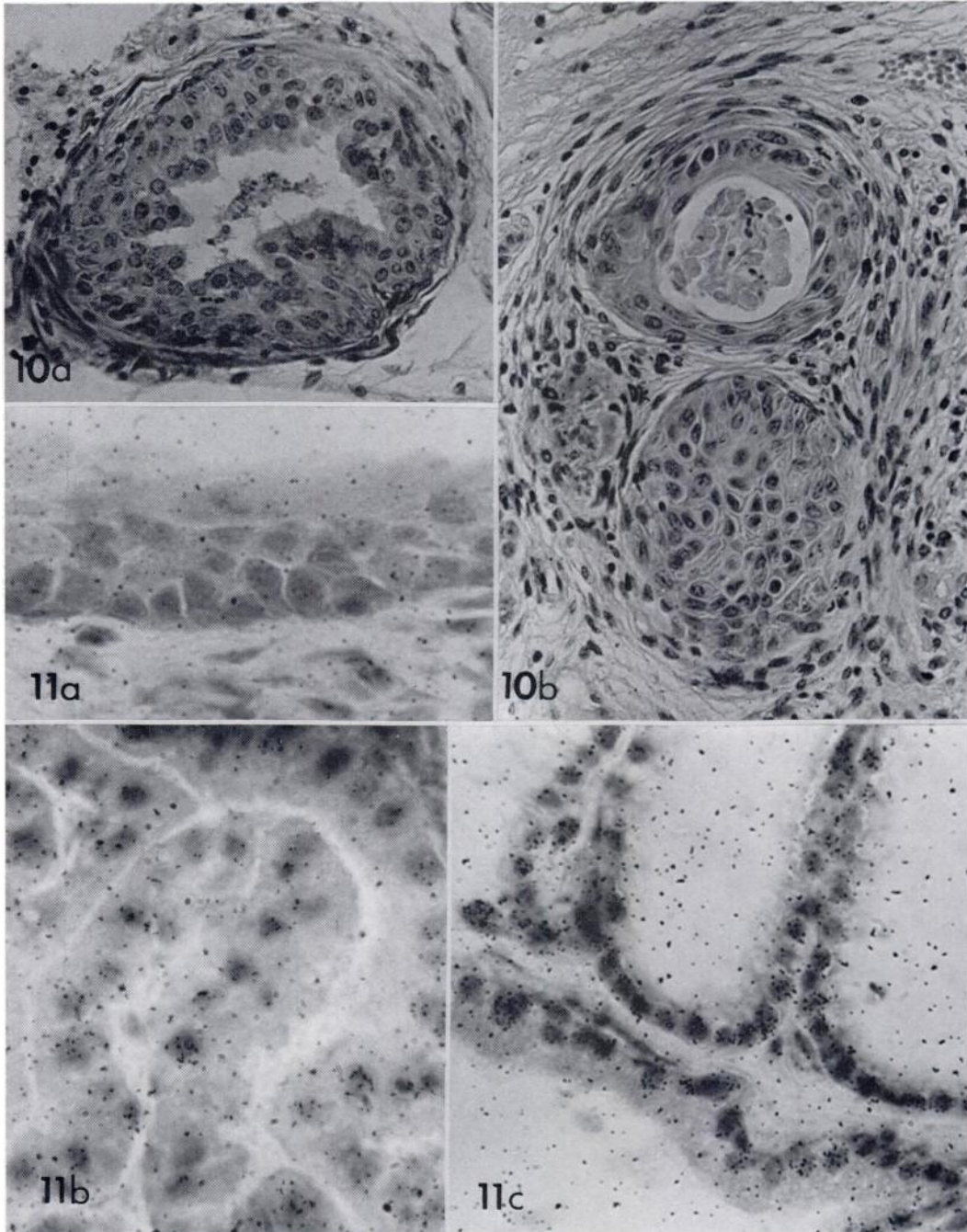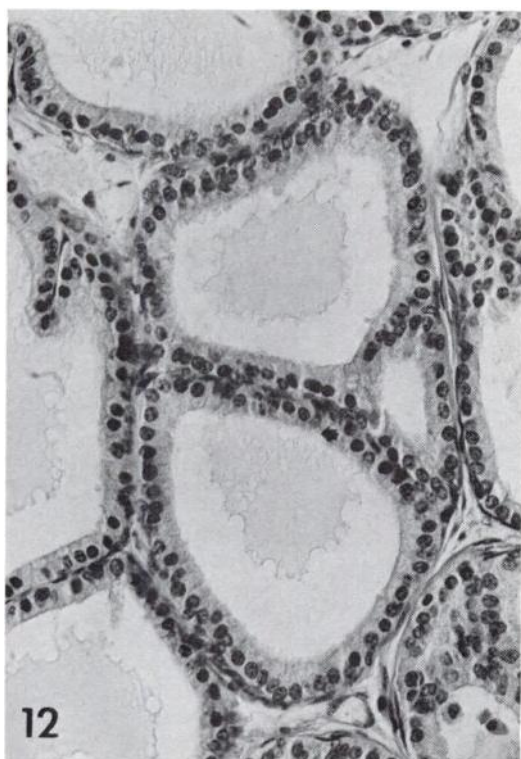
FIG. 12. Recombinant composed of mesenchyme of the 16-day-old embryonic urogenital sinus combined with epithelium of the urinary bladder of a 25 g adult female mouse. After 4 weeks growth in a male host, the epithelium is organized into prostate-like acini which ar pear to have elaborated secretory product. X 350.

urinary bladder. These observations raise the possibility that other prostate-specific functional properties may also be expressed by adult urothelium when associated with mesenchyme of the urogenital sinus.

In this regard, we (Cunha, G. R., Reese, B. A. and Shannon, J. M., unpublished) have recently demonstrated that urothelium of adult Tfm/Y mice is also induced to form prostate-like acini when recombined with wild-type urogenital sinus mesenchyme and grown in male hosts. After 4—5 weeks of growth under these conditions, many of the induced acini appear to be engaged in secretory activity; i.e., precipitated material is observed in many acini (Fig. 15). These morphological data raise the possibility that the androgen-insensitive Tfm/Y urothelium is capable of participating in androgen-induced prostatic morphogenesis and secretion. Although final interpretation of this observation awaits additional studies now in progress, biochemical data of androgenic stimulation of DNA synthesis support the concept that the Tfm/Y urothelium may actually express androgen receptor activity in the course of this inductive interaction (Cunha, G. R., Reese, B. A. and Shannon, J. M., unpublished). In this experiment, recombinants composed of wild-type urogenital sinus mesenchyme and epithelium from urinary bladders of Tfm/Y mice (UGM$^{+/+}$ + adult BLE$^{Tfm/Y\rightarrow}$ Prostate) were grown for 4 weeks in intact male nude mice which were then castrated. After 7 days to allow for regression of the hosts' prostates (as well as the recombinants), the hosts were injected with testosterone propionate alone or in combination with a 20-fold excess of the antiandrogen, cyproterone acetate (see legend Fig. 16 for experimental details). Seventy-two hours after the initiation of hormonal treatments, the recombinants and the hosts' prostates and bladders were excised and incubated for 30 min in Krebs Ringer phosphate buffer containing [^{3}H]-thymidine, and the incorporation of label into DNA was analyzed. As expected, incorporation of label into bladder tissue was not influenced by the hormonal treatments (Fig. 16). By contrast, testosterone propionate stimulated incorporation of [^{3}H]-thymidine into DNA in both the hosts' prostate and the UGM$^{+/+}$ + adult BLE$^{Tfm/Y}$ recombinants. Significantly, the testosterone-induced stimulation of DNA synthesis in the hosts' prostate and UGM$^{+/+}$ + adult BLE$^{Tfm/Y}$ recombinants was depressed $\sim$4-fold, whereas DNA synthesis in the hosts' bladder was not affected at all when cyproterone acetate and testosterone propionate were administered simultaneously. Since the antiandrogenic effect of cyproterone acetate involves a competition at the site of the androgen receptor (Fang and Liao, 1969), these data provide evidence suggestive of the existence of androgen receptor activity within the Tfm/Y epithelium and therefore suggest that the androgen-insensitive state may be reversible. If this proves to be correct, the reversibility of the androgen-insensitive state may ultimately be of therapeutic or at least heuristic value in relation to hormone-insensitive tumors of the prostate or other hormone-target organs.

Stromal Influence in Abnormal Epithelial Differentiation

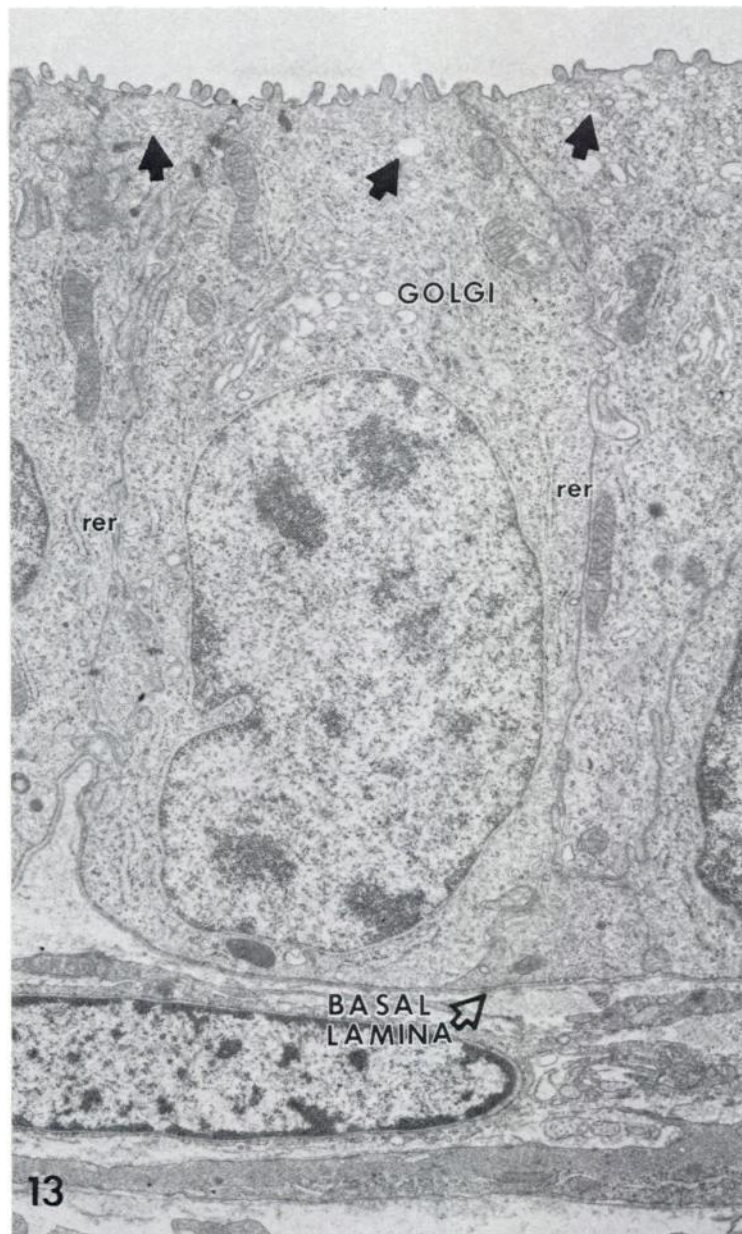Implicit in the concept that stroma induces,

FIG. 13. Electron micrograph of cells of an acinus of a UGM + adult BLE recombinant. The normally strati-fied, highly specialized urothelium (cf. Fig. 9a) has differentiated as a simple columnar secretory epithelium containing arrays of rough endoplasmic reticulum (RER), Golgi and a modest number of secretory granules (solid arrows). ×9500.

specifies and maintains epithelial morpho-genesis and functional activity is the idea that disturbance of stromal-epithelial interactions, either occurring through the natural aging process or through the action of carcinogens or other toxins, may lead to the pathogenesis of disorders involving growth or differentiation, such as various forms of nonmalignant or malignant growth (Tarin, 1972). If this concept is correct, then it should be possible to induce abnormal changes within a normal epithelium by associating it with "abnormal" stroma. Conversely, reversion to a normal phenotype may be possible by associating abnormal
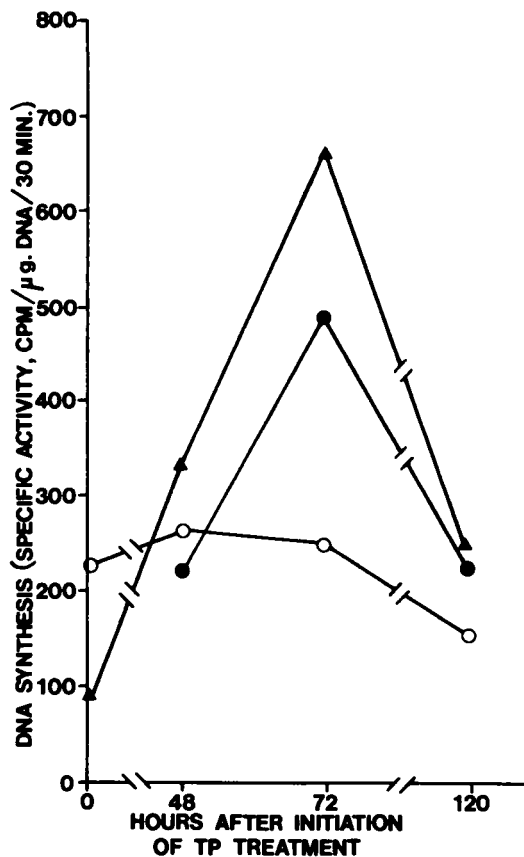
FIG. 14. Time course of DNA synthesis in host prostate, host bladder and recombinants composed of UGM + adult BLE grown in adult male mice. Host males bearing UGM + BLE recombinants were castrated for 10 days and treated with testosterone propionate (TP, 0.2 mg/day) for 24—120 h. Minces of host prostate (▲), bladder (○), and UGM and adult BLE recombinants (●) were incubated in Krebs Ringer phosphate buffer (pH 7.4) containing [^{3}H]-thymidine (400 mCi/mmole, 1 X 10^{-5} M) for 30 min at 37°C. The rate of DNA synthesis was determined according to the methods of Chung and Coffey (1971). Bladder is unresponsive to TP treatment, as expected for a nontarget organ. By contrast, in the host's prostate and the UGM + adult BLE recombinant, the rate of DNA synthesis was stimulated to comparable levels by TP.
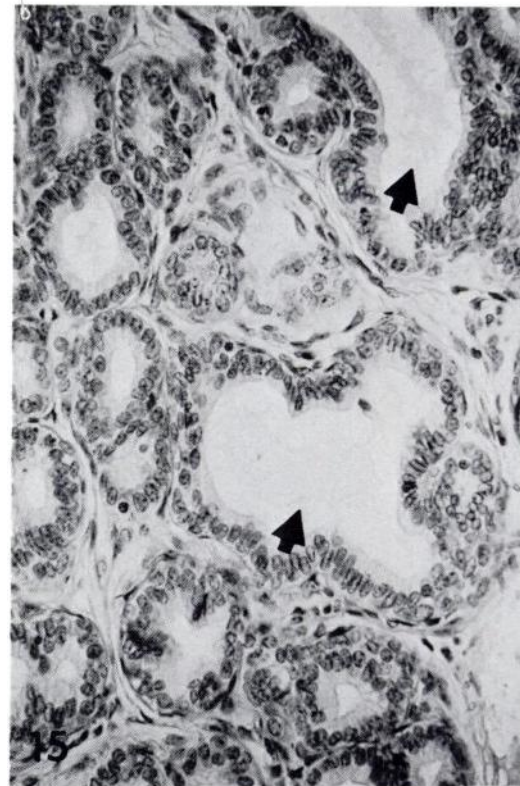

FIG. 15. A recombinant composed of wild-type mesenchyme of a 16-day-old embryonic urogenital sinus combined with epithelium of the urinary bladder of an adult male Tfm/Y mouse. The growth period was 4 weeks in a male athymic nude mouse. Prostate-like acini have developed, many of which appear to contain secretory product (arrows). X 350.

epithelium with normal stroma. As an example of the latter possibility, Van Scott and Reinertson (1961) and Cooper and Pinkus (1977) have observed normal epidermal differentiation and loss of neoplastic characteristics when epithelium of basal cell carcinoma is associated with normal stroma. Similarly, De Cossa et al. (1973) have demonstrated that association of mammary carcinoma cells with normal embry-

onic mammary mesenchyme produces a more orderly epithelial arthitecture and reduces the proliferative rate of the tumor cells. Although these authors have suggested that stromal cells may be involved in expression and possible reversal of epithelial neoplasia, interpretation of these data must be guarded because it is known that many tumors are composed of both normal and neoplastic cells (Pierce, 1974). Therefore, it is unclear as to which cells (normal or neoplastic) actually accounted for the apparent change in growth characteristics.

The role of stroma or mesenchyme in viral tumorigenesis has been investigated by Dawe et al. (1976) on tumors of the submandibular salivary gland induced by polyoma virus. Dawe's studies have demonstrated that mesenchyme (specifically homotypic salivary mesenchyme) facilitates viral transformation and tumori-
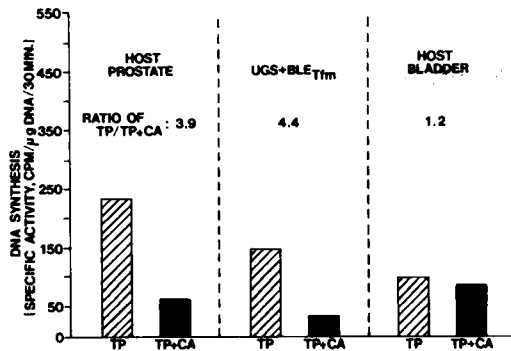
FIG. 16. Effects of the antiandrogen, cyproterone acetate (CA), on testosterone propionate (TP)-induced DNA synthesis in host prostate, host bladder and recombinants composed of UGS$^{+/+}$ + adult BLE$^{Tfm/Y}$. Host males bearing these recombinants for 4 weeks were then castrated and given hormonal treatment 7 days later. The hosts ($\sim$25 g BW) were injected with either TP (0.2 mg/day) or TP + CA (0.2 mg TP + 4.0 mg CA/day) daily for 3 days. The above tissues were minced and incubated in Krebs Ringer phosphate buffer (pH 7.4) containing [^{3}H]-thymidine (400 mCi/mmole, 1 X 10^{-5} M) for 30 min at 37°C. The rate of DNA synthesis was determined according to the methods described in Fig. 14.

genesis within the epithelium and, furthermore, influences the histotypic and biologic character of the carcinomas which emerge. Similarly, in studies on carcinogen-induced neoplasia of the urinary bladder, Hodges et al. (1977) have demonstrated that certain tumor-associated cell surface markers can be experimentally induced within normal (untreated) urothelium when it is combined with stroma previously treated with carcinogen.

Evidence also supports the concept that expression of abnormal epithelial features within the urogenital tract may also be linked to altered stromal characteristics. The generation of new ductal-acinar architecture in the pathogenesis of human benign prostatic hyperplasia (BPH) has been suggested by McNeal (1978) to be related to a re-expression of embryonic induction in adult prostatic stroma. McNeal's hypothesis is based upon the assumptions that 1) adult epithelium of urogenital sinus origin (specifically adult prostatic ductal epithelium) retains a morphogenetic responsiveness to stromal inductors; and 2) the stroma of hyperplastic prostate (BPH) is inductive. Although neither of these assumptions has been subjected to direct experimental test, the finding that adult urothelium, which shares with prostatic epithelium a common develop-

mental origin from the primitive urogenital sinus (Hutch, 1975; Felix, 1912), retains a morphogenetic responsiveness to an embryonic prostatic inductor raises the distinct possibility that adult prostatic ductal epithelium may also express a similar morphogenetic responsiveness (Cunha et al., 1980).

Tissue interactions and stromal influences also appear to be involved in abnormal vaginal cytodifferentiation. Estrogen is normally required for proliferation and cornification of vaginal epithelium. However, vaginal epithelial proliferation and cornification persist in adult mice which have been neonatally treated with exogenous estrogen, even following a variety of endocrine ablations (ovariectomy, adrenalectomy and hypophysectomy). This suggests that under these conditions vaginal cornification and hyperplasia (designated ovary-independent persistent vaginal cornification) are in fact occurring in the absence of estrogen (Takasugi, 1976). Our observations (Cunha et al., 1977) indicate that the interaction between vaginal epithelium and stroma during the period of exposure to the exogenous estrogen is necessary for maximal expression of this condition. Furthermore, once ovary-independent persistent vaginal hyperplasia is induced, the continued expression of this condition appears to be due to permanent alterations in the developmental characteristics of both the epithelium and stroma of neonatally estrogenized mice (Cunha et al., 1977).

Finally, the elegant studies of Sakakura et al. (1979b) also suggest a role of mesenchyme in mammary tumorigenesis. These investigators implanted fragments of embryonic mammary mesenchyme into the fourth mammary gland of adult mice. This mesenchyme, when associated in this fashion with adult mammary epithelium, influences both the morphogenesis and growth of the mammary epithelium, resulting in the production of abnormal epithelial configurations termed ductal-alveolar nodules. Of interest is the finding that the latency of tumorigenesis within these nodules is significantly reduced in comparison to untreated or sham-operated mammary glands of the same host. Thus, although alteration of genetic material appears in many cases to be the primary mechanism of action of carcinogenic agents upon epithelial tissue, expression of certain neoplastic characteristics of carcinomas (epithelial tumors) may be controlled at least in part by interactions with stromal cells. Indeed,

for several tumors alteration of the basal lamina (the interface between epithelium and stroma) is one of the earliest morphological features of incipient carcinogenesis (see Tarin, 1972, for reviews). In summary, the concept that stromal-epithelial interactions play a role in abnormal epithelial behavior, including carcinogenesis, is supported by studies from a variety of systems dealing with tumors induced by chemicals, viruses, or hormones (Tarin, 1972; Hodges et al., 1977; Dawe et al., 1976; Nandi, 1978). The elegant studies of Mintz (1978) and her collaborators, which demonstrate that cell-cell interactions may cause normal differentiation of malignant embryonal carcinoma cells, strengthen the concept that stromal-epithelial interactions may also regulate certain aspects of abnormal epithelial behavior, including carcinogenesis, its induction, development and biological characteristics.

DISCUSSION

At present, evidence of stromal (mesenchymal) influence during development and function of accessory sexual glands is primarily descriptive. During development, urogenital mesenchyme is involved in mediating hormonal effects upon epithelium and inducing and specifying epithelial morphogenesis and differentiation. Biochemical function, including the possible expression of epithelial androgen receptor activity, also appears to be controlled in large part via stromal (mesenchymal) influences. Furthermore, the maturational state (age) of vaginal stroma determines the morphogenetic responsiveness of the sinus vagina to androgenic influences. In adulthood, stromal influence is involved in maintaining epithelial architecture and functional activity; evidence suggests that certain adult pathological conditions may be related to disturbance of stromal-epithelial interactions.

Although the importance of stroma during epithelial development and function of urogenital organs has recently become appreciated, the mechanism(s) of these tissue interactions is unknown. Developmental studies on the role of cell-cell contact in kidney and tooth morphogenesis (Saxen et al., 1977; Thesleff et al., 1977), on the role of cell-extracellular matrix interactions during corneal development and cellular growth (Meier and Hay, 1975; Gospodarowicz et al., 1978) and studies on the role of basal laminar glycosaminoglycans in salivary

gland morphogenesis (Bernfield et al., 1973) may provide models for pursuing mechanistic studies within the urogenital system. Our limited studies in this area have demonstrated that the spatial distribution of glycosaminoglycans at the epithelial-stromal interface (basement membrane) in seminal vesicles is responsive to hormonal conditions that are permissive (androgens) or inhibitory (castration or estrogens) for morphogenesis (Cunha, 1976a; Cunha and Lung, 1977). The significance of these observations, which are similar to Bernfield's findings, are as yet uncertain. Clues to the mechanism of stromal-epithelial interactions await critical analysis of the molecular biology of fibroblasts and smooth muscle cells of urogenital origin. Particular emphasis is required in the area of how these cells alter, condition, or contribute to the extracellular matrix, which in part stabilizes epithelial architecture.

Remodelling of the extracellular matrix clearly plays an important role in certain normal and abnormal developmental processes in the urogenital tract. Extensive degradation of collagen occurs within the adult rat uterus 1–3 days postpartum (Gross, 1976). Production of collagenase, which accounts for this degradative process, appears to be triggered in part by falling levels of progestins during the early postpartum period (Koob and Jeffrey, 1974; Jeffrey et al., 1975). Remodelling of the stromal matrix must also occur during normal growth and probably is also involved in a variety of normal and abnormal invasive processes. Penetration of epithelial ducts into prostatic or mammary stroma during morphogenesis and growth of these organs is likely to involve collagenolytic enzymes for remodelling of the extracellular matrix. Since the invasive behavior of tumors in many cases is associated with the production of collagenase (Gross, 1976), benign and neoplastic growth such as the formation of new ductal-acinar architecture during the pathogenesis of human benign prostatic hyperplasia or the metastasis of prostatic adenocarcinoma may also involve collagenolytic enzymes. It will be of interest to determine which tissue (epithelium or stroma) is responsible for the production of collagenase during normal and abnormal developmental processes.

Progress in the area of stromal vs epithelial function in adult urogenital organs will be dependent upon development of procedures for

cleanly separating epithelial and stromal cells. Those engaged in this area of investigation will have to demonstrate rigorously the accuracy of their tissue separations or at least the degree of enhancement of cell purity. Utilizing Ficoll gradients, Helms et al. (1975) in their purest fractions have produced suspensions which are 81.0 ± 12.2% prostatic epithelial cells. Cowan et al. (1977) have purported to have separated prostate into epithelial and stromal fractions. The preponderance of 5α-reductase was found in the stromal fraction. This intriguing finding suggests that prostatic stroma may enzymatically convert testosterone to dihydrotestosterone, which then stimulates epithelial function. The validity of this concept depends upon establishing the purity of the cell fractions. Unfortunately, this aspect cannot be evaluated from Cowan's data. Validation of the purity of cell fractions will lead to rapid biochemical advances in elucidating the respective functional roles of epithelium and stroma in urogenital organs. Quantitative biochemical studies, in conjunction with morphometric (Bartsch and Rohr, 1977; DeKlerk and Coffey, 1978), autoradiographic, histochemical and morphological studies, will lead to a more comprehensive view of accessory sexual glands, a view based upon the respective roles of the functional activity of both organ components, the epithelium and the stroma.

ACKNOWLEDGMENTS

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