Alkaline Phosphatase Activity in Epithelial Metaplasia

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The etiology of epithelial metaplasia and its relation to neoplasia have been of interest for many years. Investigators have proposed (39, 41) and opposed (6) the view that metaplastic transformations in various sites may be precancerous. It seems well established that such alterations often accompany neoplastic growths (20, 25, 27, 32). The need to distinguish metaplastic from neoplastic lesions in the genital tract has received emphasis in recent pathologic studies (7, 33, 34, 35, 43).

McCullough and Dalldorf (31) have suggested that all squamous metaplasia and keratinization result from the same etiologic factor, local vitamin A deficiency, regardless of the initiating stimulus (e.g., mechanical, hormonal). Investigations on the effects of vitamin A on estrogen-induced vaginal cornification (19, 23, 47) and on epidermal differentiation (44, 45) have given some support to this suggestion. Earlier studies suggested that metaplastic lesions in the male rat genital tract caused by estrogen treatment and those accompanying avitaminosis-A are histologically identical (21). Such histologic identity, however, does not hold for all types of genital epithelial metaplasia in the male rabbit (9). In the male mouse, it has been shown that vitamin A deficiency intensifies the histologic response in the genital tract to minimal dosage with estrogen (15).

In the course of a series of studies on the relation of steroids and phosphatases in the male genital tract (11, 12), it was noted that estrogen-induced epithelial metaplasia is accompanied by alkaline phosphatase activity in the replacing epithelium. This phenomenon has been investigated further in both estrogen-induced and avitaminotic-A lesions, in addition to other instances of metaplasia; results are reported herein. Differences in the histologic picture of the metaplastic process after estrogen treatment and in vitamin A deficiency were seen in (a) the mode of growth of the lesion, (b) the alkaline phosphatase activity of the replacing epithelium, and (c) the extent of involvement of the various reproductive structures.

MATERIALS AND METHODS

Tissues with estrogen-induced metaplastic lesions were obtained from estradiol-treated male mice, rats, guinea pigs, and rabbits used in previous studies (11, 12). All sex accessory and duct tissues removed at the time of the termination of these experiments were examined for evidence of epithelial metaplasia.

A total of 21 male mice and 13 male Long-Evans rats was used in the study of avitaminotic-A lesions. Of these, 8 mice and 10 rats provided genital tissues with distinct evidences of epithelial metaplasia. Portions of submaxillary salivary glands and trachea were occasionally removed to check on the efficacy of the vitamin A-free diet. Both mice and rats were maintained on a standard vitamin A test diet (18).

Other instances of epithelial metaplasia observed in previous studies are also considered: metaplastic transformations accompanying methylcholanthrene-induced squamous-cell carcinomas of the rat ventral prostate (12) and certain spontaneous lesions of the male rabbit genital tract (9).

Alkaline phosphatase activity was demonstrated histochemically by the Barger (8) modification of the Gomori technic. Acetone-fixed tissue sections were incubated at 37° C. for 18 hours in sodium glycerophosphate at pH 9.5. Control slides were processed identically, except for poisoning of the substrate with approximately 0.01 M KCN. It is of interest that certain batches of control slides incubated for 18 hours in poisoned substrates wherein the KCN concentration was low showed some evidence of alkaline phosphatase activity in the metaplastic epithelium, although other intensely active areas were completely negative (cf. 16). Nucleic acids were demonstrated in some sections with pyronin-methyl green (Pappenheim-Saathof).
OBSERVATIONS


Estrogen-treated mice.—Estrogen-induced metaplastic changes in the male mouse, first described by Lacassagne and Villela (26) and Burrows (13), and well reviewed by Thorborg (47), have been discussed in detail previously (11, note Figures 5–10). In brief, a proliferation of alkaline phosphatase-positive basal cells has been observed in the anterior prostate, dorsal prostate, and seminal vesicle. This proliferation is most extensive in the anterior prostate and culminates therein in the transformation of many of the alveoli into small keratinized nodules (Fig. 5). Eventually, the original epithelium, which does not show alkaline phosphatase activity, is entirely sloughed, and the alveolar lumen partially or wholly obliterated by keratin. As noted by Burrows (13), the process in the dorsal prostate does not progress to keratinization. No metaplastic alterations were seen in the ventral prostate, the epithelium of which is normally alkaline phosphatase-positive. This gland becomes quite atrophic after castration, with or without estrogen treatment, and upon estrogen treatment of intact mice.

Avitaminotic-A mice.—It is ordinarily very difficult to produce the syndrome of avitaminosis-A in adult mice, inasmuch as the minimal daily requirement of vitamin A is only 1 unit (30). However, we have found that placing mice on vitamin A-free diet immediately after weaning occasionally results in the development of severe symptoms of vitamin A deficiency in the male genital tract within 2 months or less. It has been pointed out that the ability to produce the syndrome in mice is dependent upon “the stringency of prenatal and lactational treatment” (30, p. 382).

Despite the occurrence of an actively keratinizing, stratified squamous replacing epithelium, the histologic picture in sex accessories of male mice with vitamin A deficiency suggests some real differences from that found after estrogen treatment. Proliferation of alkaline phosphatase-positive basal cells was not seen in any of the avitaminotic-A accessories. The occurrence of multiple foci of basal cell proliferation, which keratinize and spread underneath the original epithelium, as described in the rat (49), was not noted. Instead, our findings agree with the general condition described previously in the mouse of “an orderly layer of keratinizing cells which seemed to begin at only one point and grow in all directions” (51, pp. 187, 189). Growth seemed to occur in part, at least, over the surface of the original epithelium, rather than underneath.

The metaplastic changes first occur in the luminal epithelium of a structure such as the seminal vesicle, the glands being initially unaffected (Fig. 1). In general, once having begun, the metaplastic growth seems largely to extend “centrifugally” (i.e., away from the lumen), instead of “centripetally” (i.e., toward the lumen) as occurs after estrogen treatment. In contrast to the situation occurring after estrogen administration, wherein only the anterior prostate is transformed into a keratinized mass, all the structures with avitaminotic-A lesions ( seminal vesicle, ductus deferens, anterior and dorsal prostates, epididymis ) show the same picture (Figs. 1–2). However, as after estrogen treatment, the anterior prostate is most readily and most noticeably affected. In their earlier study, Wolfe and Salter (51) did not find changes in the epididymis.

Intra-epithelial keratinization can occasionally be seen, and this may represent the “multiple foci” of Wolbach and Howe (49). As keratinization proceeds and involves the entire epithelium, the intra-epithelial “pearls” seem to be largely sloughed into the lumen. The cells involved are alkaline phosphatase-negative (Fig. 4). Even in the anterior prostate, where the metaplastic process eventually results in keratinized alveoli structurally indistinguishable from those resulting from estrogen treatment, such epithelial lesions are entirely alkaline phosphatase-negative (Fig. 6).

Estrogen-treated rats.—No evidence of genital tract keratinization was found in rats castrated and treated with estrogen for as long as 6 months. However, alterations interpreted as metaplastic were noted in rats so treated for 4 and 5½ months. As in the estrogen-treated mouse, these changes consisted of groups of alkaline phosphatase-positive basal cells, which were seen in the ductus deferens (Fig. 8), seminal vesicle (Figs 7 and 9), and the ducts of the dorsal prostate. These aggregations may become quite extensive and result in the transformation of the glands of the seminal vesicle into adenoma-like growths (Fig. 9). The anterior prostate, so reactive in the mouse to estrogen, was found consistently unaffected in the rat. Again, no metaplastic alterations were seen in the ventral prostate.

Avitaminotic-A rats.—The avitaminotic-A lesions occurring in the genital tract are similar to those of the mouse, except that the superficial layers (strata granulosum and corneum) often show a coloration indicative of alkaline phosphatase activity, although the more basal cell layers are always negative (Fig. 10). This “activity”—in dead and dying cells—may be an artifact. No proliferation of alkaline phosphatase-positive basal cells was seen. The original description of
the process (49) was difficult to verify, at least as
cconcerns "the multiple origin of keratinizing foci."
Although the keratinizing process may originate
as subepithelial centers which break through the
overlying original epithelium, it does not seem that
proliferation occurs consistently underneath the
original epithelium. The possibility of superficial
spreading of keratinization is certainly suggested
(Fig. 10).
Lesions were observed in the seminal vesicle,
anteor and ventral prostates, and preputial
gland. In ventral prostate tissue affected by the
deficiency in vitamin A, premetaplastic atrophy
and pluristratification were accompanied by a
loss of the epithelial alkaline phosphatase normally
characteristic of this organ (Fig. 11). Keratiniz-
ing epithelium again was alkaline phosphatase-
negative in its basal layers. The anterior prostate
was relatively unresponsive, as compared to the
extensive response of the homologous structure in
the mouse.

Estrogen-treated guinea pigs.—A distinct single
layer of basal cells is evident in the seminal vesicle
and ductus deferens of normal intact and of un-
treated castrate guinea pigs. In the normal an-
nimal, this layer is often alkaline phosphatase-
active; in the castrate, it is often inactive. In the
estrogen-treated castrate, the layer is phospha-
tase-active and stands out in distinct contrast to
the phosphatase-inactive original epithelium (Fig.
18). Metaplasia evidently begins as a result of
proliferation of this basal cell layer which breaks
through the overlying epithelium to form islands
and papilloma-like growths of alkaline phosphatase-
active and stands out in distinct contrast to
the phosphatase-inactive original epithelium (Fig.
18). The details of the process can be discerned
because of the resemblance of the replacing epi-
thelium to that normally lining the bladder.

As in other species, the metaplastic epithelium
following estrogen treatment of the rabbit is
characterized by intense alkaline phosphatase
activity. Foci of presumably premetaplastic epi-
thelium can be detected in alveolar walls. The
patches spread out and cover the original epi-
thelium in nonmetaplastic areas, in many in-
stances eventually filling the entire alveolus
(Fig. 16). The details of the process can be
observed in the seminal vesicle, the metaplastic epithelium evidently proliferates
as basal cells, which form a distinct alkaline
phosphatase-positive layer beneath the original
epithelium (Fig. 15).

Spontaneous lesions in the rabbit.—Certain
spontaneous metaplastic epithelial lesions have
been described in the prostate and vesicular gland
of the male Dutch rabbit (9). It was originally
thought that these keratinizing lesions might be
due to a local inadequacy of vitamin A. However,
administration of cod liver oil failed to result in
any evidence of their alleviation (9). In addition,
we find that this metaplastic formation is alkaline
phosphatase-positive (Fig. 8), unlike the avita-
minotic-A lesions in the mouse and rat. The
etiology of these lesions in the rabbit remains
unknown.

Methylcholanthrene-treated rats.—The loss of
specific alkaline phosphatase activity ac-
companying the neoplastic transformation in the ventral
prostate of the rat has been discussed elsewhere
(19). The parenchyma of squamous-cell carcino-
mas induced by intraprostatic injection of methyl-
cholanthrene is almost entirely negative for al-
kaline phosphatase activity, despite the activity
of the tissue of origin. In some methylchol-
lanthrene-induced abnormal growths, metaplastic
areas can be found (cf. 32) which are often alkaline
phosphatase-positive. However, apparently neo-
plastic downgrowths into the stroma arising from
these areas are alkaline phosphatase-negative
(Fig. 16).

DISCUSSION
Observations on the rat and mouse suggest
that genital epithelial metaplasia caused by estro-
Gen administration can be distinguished from that caused by avitaminosis-A on the basis of two principal criteria: (a) the consistent "centripetal" growth of replacing tissue in the former, as opposed to the secondarily "centrifugal" growth in the latter, and (b) the consistent alkaline phosphatase activity in the former. Basal cell proliferation, leading to pluristratification, occurs in both syndromes. However, the basal cells are alkaline phosphatase-active only after estrogen treatment.

A third difference may lie in the extent of involvement of the several accessories. Thus, the mouse anterior prostate is the principal accessory affected by estrogen treatment, whereas vitamin A deficiency results in cornifying metaplasia in most of the accessories and in the ductus deferens and epididymis. Moreover, in the rat, no keratinization was observed, even after 5½ months treatment with estrogen, although keratinization is extensive in vitamin A deficiency. However, it is of interest that the anterior prostate of the mouse, which is most reactive to estrogen, is also most reactive to a lack of vitamin A.

The alkaline phosphatase activity, as previously mentioned (11), serves almost as a differential stain in the detection of estrogen-induced metaplasia. The details of the process have been described in the mouse without the use of this technic (26, 29, 47); however, the technic may be of some value in distinguishing some metaplastic alterations from histologically similar neoplastic alterations (12). It would be interesting to determine if the metaplastic epithelial alterations seen in association with infarction of the human prostate and after estrogen therapy (7, 33, 35, 43) are alkaline phosphatase-positive, in possible contrast to normal and neoplastic tissue.

In a recent discussion of "epidermization" of the human uterine cervix, Motyloff (34) distinguished between the alteration of normal epithelial characteristics resulting from transformation of the original epithelium (metaplasia) and that resulting from basal cell proliferation (heteroplasia). Adami and McRae's (1) oft-quoted definition of metaplasia states that the process is not direct but involves proliferation from preliminarily dedifferentiated cells or from undifferentiated basal cells. In the male genital tract, alterations resulting from vitamin A deficiency in the rat and mouse appear to include the covering (or invasion) of the original epithelium by stratified squamous epithelium which had proliferated from relatively distant foci. If sheets of proliferating basal cells were continuous under the original epithelium, one would expect the glandular, as well as the luminal, epithelium to be undermined. This does not seem to be the case in the seminal vesicle of rats and mice with vitamin A deficiency (Figs. 1, 10). Coalescence of keratinizing areas in the surface epithelium would eventually obliterate the unaffected glands by "centrifugal" growth. It is difficult to eliminate the possibility of direct transformation of some of the original epithelium. In accordance with Motyloff's terminology (34), local transformation and keratinization would be true metaplasia. According to Adami and McRae (1), direct transformation does not occur in metaplasia, whereas estrogen-induced proliferations from basal cells would satisfy their definition. These latter changes would be termed "heteroplastic" after Motyloff (34).

In view of the evidence for the origin of metaplastic tissue from basal cells after estrogen treatment, it is difficult to reconcile some of our observations with Zuckerman's suggestion (52, 53) that estrogen may induce squamous metaplasia only in those tissues "in whose development oestrogen-sensitive [urogenital] sinus epithelium has either played a direct or indirect part" (52, p. 264). The Zuckerman hypothesis has received support in particular from the detailed studies of Raynaud (e.g., 42, pp. 53-54) and, in part, from those of Thorborg (47). It is not proposed to include a detailed discussion of this attractive hypothesis, although some re-evaluation seems warranted. A few points, however, should be made. Along with Thorborg (47), we feel that the distinction between "glandular" and "squamous" response may be invalid. The response of male rabbit sex accessories to estrogen (10, 14) is certainly not squamous regardless of embryonic origin of accessories studied; however, further studies of the embryology of the male rabbit reproductive system are needed.

In any detailed reconsideration of the hypothesis, a careful analysis of the total picture of genital epithelial changes after estrogen will be needed. Thus, our observations and those of other workers on various species of mammals would call for alterations of Thorborg's summary diagram (47, p. 169), at least as it relates to the rabbit, mouse, rat, and guinea pig. In addition, the process of true metaplasia must be defined. Is basal cell proliferation after estrogen treatment (Motyloff's heteroplasia) to be considered as a metaplastic response, regardless of whether or not it ever leads to total epithelial replacement and keratinization?

One point with real bearing on the Zuckerman hypothesis concerns the origin of the basal cells in estrogen-induced metaplasia. Recent work
The basal cell proliferation seen in the estrogen-phosphatase in fibrous protein (including keratin) is not clear. The absence of such activity associated with estrogen-induced metaplasia is not clear. The absence of such activity in many neoplastic growths, even when the tissue of origin shows considerable activity in many neoplastic growths, even when the tissue of origin shows considerable activity (5, 12, 24). Distinctive pyronin-positive ribonucleic acid concentrations were not seen in the cells showing high phosphatase activity. Differentiation of basal cells, so nicely accomplished with the Barger-Gomori (8) technic, was not observed with pyronin-methyl green. In Lacassagne and Villela’s original description (26) of estrogen-induced metaplasia in the mouse anterior prostate, a silver stain was employed. The cytoplasmic fibrils seen with this stain paralleled our observations with the Barger-Gomori technic (11) and allowed a differentiation of the early basal cells. Alkaline phosphatase activity associated with fibril production would be expected if Jeener’s suggestion (22) in regard to fibrillar protein synthesis were universally applicable.

The validity of the histochemical technic for alkaline phosphatase demonstration has been questioned, particularly with reference to accurate localization of the enzyme (e.g., 38). It is pertinent that with biochemical methods (17) rapidly proliferating (metaplastic?) epithelial growths in the guinea pig uterus after estrogen treatment show high alkaline phosphatase activity. Regardless of what the cytochemical technic used herein may actually demonstrate, and there is evidence that it is alkaline phosphatase which is demonstrated (12), it does allow distinction of metaplastic from original epithelium after estrogen treatment.

In 1938, Wolbach and Howe pointed out that “careful cytological studies [of epithelial metaplasia in vitamin A deficiency] as the deficiency progresses and during the recovery phenomenon may yield interesting correlations between morphology and function” (49, p. 524). One can add to this statement today the desirability of continued biochemical studies involving the possible relationship between vitamin A and estrogen in the processes of metaplasia and keratin formation. On the basis of work reported herein, it seems that metaplastic processes in the male genital tract in vitamin A deficiency and after estrogen administration are not completely identical. Further studies of the development of and recovery from alterations occurring after simultaneous estrogen treatment and vitamin A deficiency seem to be warranted.

SUMMARY AND CONCLUSIONS
1. A study has been made of epithelial transformations in the male genital tract of estrogen-treated mice, rats, guinea pigs, and rabbits; of avitaminotic-A mice and rats; of methylcholanthrene-treated rats; and of rabbits with spontaneous metaplastic lesions.

2. Comparison of metaplastic alterations in mice and rats after estrogen treatment and with vitamin A deficiency reveals three possible histologic distinctions: (a) consistently “centripetal”...
growth of replacing epithelium in the former compared with secondarily "centrifugal" growth in the latter; (b) the occurrence of proliferation of alkaline phosphatase-positive basal cells in the former; and (c) the considerably more extensive occurrence of keratinizing stratified squamous epithelium in the latter. Histologic similarity of the metaplastic transformations in the two syndromes may be only superficial.

3. The possible utility of the Barger-Gomori histochemical technic for alkaline phosphatase in distinguishing estrogen-induced metaplasia from neoplastic transformations is suggested.

4. The role of alkaline phosphatase activity in estrogen-induced metaplasia is considered. Possibly it is an indicator of cytoplasmic metabolic activity accompanying non-neoplastic cell proliferation. A constant relation between such activity and keratin synthesis is not supported.

5. The mechanisms of metaplastic transformations require further investigation, with emphasis on the possible relation of vitamin A and estrogen in such changes, and on the origin of replacing epithelium. The Zuckerman hypothesis offering an embryologic explanation for changes observed after estrogen treatment and definitions of metaplasia are briefly considered.

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REFERENCES


All photomicrographs are of 6-μ sections of acetone-fixed tissues, treated by the Barger-Gomori technic and lightly stained with hematoxylin. Dense extranuclear coloration is evidence of alkaline phosphatase activity.

Fig. 1.—Seminal vesicle from 7½-month-old male mouse, on vitamin A-free diet for 6½ months. Squamous metaplasia and keratinization of luminal epithelium are unaccompanied by alkaline phosphatase activity. Note relatively unaffected glands, absence of alkaline phosphatase-positive basal cell proliferation, and normal enzyme distribution in fibromuscular wall. ×80.

Fig. 2.—Epididymis of 7½-month-old mouse, on vitamin A-free diet for 6½ months. Portions of duct have undergone squamous metaplasia and keratinization, unaccompanied by alkaline phosphatase activity. Note activity of intertubular stroma. ×65.

Fig. 3.—Spontaneous lesion from vesicular gland of adult intact rabbit. Note alkaline phosphatase-positive metaplastic epithelium. ×270.

Fig. 4.—Portion of anterior prostate alveolus from 5-month-old mouse on vitamin A-free diet for 4 months. Note intra-epithelial “pearl” formation, unaccompanied by alkaline phosphatase activity, positive reaction in circumalveolar stroma. ×300.

Fig. 5.—Anterior prostate alveolus from 6-month-old mouse, castrated and treated with estrogen for 2 months. Note alkaline phosphatase activity accompanying keratinizing squamous metaplasia. ×270.

Fig. 6.—Anterior prostate alveolus from 5-month-old mouse, on vitamin A-free diet for 4 months. Note absence of alkaline phosphatase activity in this squamous metaplastic lesion; stroma retains some activity (cf. Fig. 5). ×300.

Fig. 7.—Seminal vesicle of 7-month-old rat, castrated and treated with estrogen for 4 months. Note proliferation of alkaline phosphatase-active basal cells, inactive original epithelium. ×165.

Fig. 8.—Ductus deferens of 7-month-old rat, castrated and treated with estrogen for 4 months. Note proliferation of alkaline phosphatase-active basal cells, relatively inactive original epithelium (stereocilia show some coloration). ×165.
FIG. 9.—Seminal vesicle of 12-month-old rat, castrated and treated with estrogen for 4½ months. Note transformation of glands into alkaline phosphatase-active adenoma-like structures, a continuation of the metaplastic process seen in Figure 7. × 125.

Fig. 10.—Seminal vesicle of 3½-month-old rat, on vitamin A-free diet for 2 months. Note keratinizing squamous metaplasia of luminal epithelium, coloration only in some sloughing keratin and in stroma. Glands are relatively unaffected (cf. Figs. 1, 7, and 9). ×300.

Fig. 11.—Ventral prostate alveoli of 3½-month-old rat, on vitamin A-free diet for 7 weeks. Note pluristratification preceding metaplasia with partial loss of normal epithelial alkaline phosphatase activity in some alveoli. ×190.

Fig. 12.—Metaplastic region from ventral prostate of 14-month-old rat after exposure to intraprostatic methyleneblue for 7 months. Most of tissue was squamous-cell carcinoma. This region shows alkaline phosphatase-active metaplastic epithelium; cells infiltrating into stroma are inactive. ×140.

Fig. 13.—Seminal vesicle of 10-month-old guinea pig, castrated and treated with estrogen for 4½ months. Note alkaline phosphatase-active basal cell layer, evidently proliferating and breaking through inactive original epithelium as masses of polyhedral cells. ×140.

Fig. 14.—Prostatic duct from 10-month-old guineapig, castrated and treated with estrogen for 4½ months. Upper surface of duct is lined by alkaline phosphatase-active metaplastic epithelium with precornified squamous superficial layers; lower surface of duct is lined by alkaline phosphataseinactive original epithelium. ×175.

Fig. 15.—Seminal vesicle of 8-month-old rabbit, castrated and treated with estrogen for 4½ months. Note alkaline phosphatase-positive metaplastic epithelium and basal cells, negative original surface and glandular epithelium. ×290.

Fig. 16.—Prostatic alveolus of 12-month-old intact rabbit, treated with estrogen for 4½ months. Note alkaline phosphataseactive metaplastic growths occluding lumina, in contrast to inactive original epithelium. ×270.
50. ———. Epithelial Repair in Recovery from Vitamin A Deficiency. Ibid., 87:511-26, 1955.
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