Reports

Nature of the Hormonal Influence in Mouse Mammary Cancer

Abstract. New findings indicate that hormones may play a permissive role, rather than an inductive one, in mammary tumorigenesis. Hormones supporting normal gland development may result in the emergence of precancerous lesions; hormones maintaining such hyperplastic lesions may result in the emergence of tumors. The "inherited hormonal influence" may rest in genetically determined sensitivity of mammary tissue to somatotropin.

The relations of hormones to neoplastic disease continue to attract considerable attention. In hormone-regulated tissues, it is often considered axiomatic that hormones capable of inducing normal growth and function, or of stimulating excess growth and hyperactivity, are potential carcinogens by virtue of such capabilities. Among experimental materials, the hormone-controlled mammary gland of mice has been the most intensively investigated. From the pioneer studies of Leo Loeb and on through present-day researches, it has been accepted that estrogen is the prime hormonal inducer of mouse mammary cancer, although the importance of pituitary and adrenocortical factors is becoming increasingly recognized *(1–3)*. Kirschbaum *(3)* has described estrogen as a carcinogen in the development of mouse mammary cancer, just as methylcholanthrene is considered to be a carcinogen.

The present discussion is based primarily on the work in this laboratory, where my co-workers and I have been engaged in an extended effort to determine the precise role of hormones in mouse mammary tumorigenesis. I do not intend to present our experimental data as such [these data were recently reviewed in part by DeOme *et al.* *(4)*], but rather to point out some conceptions of the well-established "hormonal influence" that may be pertinent to the experimental analysis of other instances of hormone-influenced tumorigenesis. Most of the issues considered herein have been raised as "problems" in this area by Clifton *(2)* in a recent useful review.

Where the existence of a definite precancerous state can be established, one can visualize hormonal participation in accelerating or delaying tumor formation as occurring on several possible levels: in the induction of the precancerous state, in the maintenance of the precancerous state, in the induction of the neoplastic state, and in the maintenance of the neoplastic state. In mouse mammary cancer, most tumors appear to be relatively independent of hormonal control ("autonomous"), although the growth rate of the tumor, once formed, may be influenced by hormones (see *5*). The hormonal combination necessary for the induction of a growth state need not be the same as that required for the maintenance of that state, and this distinction between *induction* and *maintenance* applies to normal, as well as abnormal, histogenesis.

The hormonal requirements for normal mammary gland development in one strain of mice (C3H/Crgl) have been well delineated by Nandi *(6)*. Inasmuch as a tumor cannot arise in a nonexistent organ, the hormones required for normal mammary gland development can be considered as playing an essential role in eventual tumorigenesis. The gland must be sufficiently developed to allow the emergence of hyperplastic alveolar nodules *(7)*; we consider it established that these are precancerous lesions *(8)*. More than estrogen alone is involved, since estrogen induces neither normal development of any kind nor precancerous lesions in the absence of the hypophysis or hypophyseal factors. At present it appears that, in addition to estrogen, a hypophyseal factor (somatotropic or mammotropic) and a second steroid (luteoid or corticoid) are minimum requirements for the development of a normal inactive gland. It can be stated that, in a genetically tumor-susceptible, virus-infected mouse, the maintenance of this limited degree of mammary-gland development will result in the appearance of precancerous lesions *(9)*. The specific hormonal milieu for noduligenesis then reduces to that necessary for development and maintenance of the normal gland. The actual inducer of the precancerous state may be elsewhere than in the hormonal background. A concentration of virus-like particles occurs in the precancerous nodules we have studied *(10)*, and these particles are certainly a candidate for the role of definitive inducer of nodule formation.

In addition to the induction of nodules, another related hormonal effect lies in the ability to increase or decrease the incidence (rate of appearance) of such nodules and thereby to affect ultimate tumorigenesis. Data indicate that the hormonal milieux associated with pregnancy and lactation *(1, 11)*, as well as administration of specific hormones, do indeed alter the incidence of nodules. Some hormonal treatments can increase the incidence of nodules even in a strain of mice wherein tumor incidence in virgins is normally high *(12)*.

Hyperplastic alveolar nodules, once formed, can be maintained partially, maintained at a "normal" level, stimulated to secrete, or stimulated to lactate fully by various hormonal combinations *(9, 13)*. Pituitary factors in the absence of ovary and adrenal have some nodule-maintaining ability *(9, 14)*, and normal and hyperactive secretory patterns can be induced by combinations (a hypophyseal factor plus a corticoid) that are ineffective in the induction of nodules *(13)*. Tumors generally arise in virus-infected mice where the mammary glands contain maintained nodules. Thus, the specific hormonal milieu for tumorigenesis may reduce to that necessary for maintenance of the hyperplastic nodules at a "proper" level, and again one may have to look elsewhere than at the hormonal factor for any specific inducer of the neoplastic transformation. The hormonal influence here, as in noduligenesis, may be a "permissive" or supportive one (see *15*), essential but not directly causative. Tumor development may conceivably be a secondary consequence of nodule maintenance—of sustained hyperplasia wherein the opportunity for new cell populations to develop and to survive is greater than normal. If one conceives of the tumor-cell population as occurring consequent to somatic mutation, certainly the opportunity for such changes is greater in a hyperplastic area. Survival of the altered cells could be facilitated by humoral factors from the proliferating cell population (see *16*).

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

As to the definitive role of estrogen in mammary tumor formation, it should be noted that in the development of the precancerous lesion, estrogen can no more be termed *the* noduligenic factor than can the pituitary hormone or the C-21 steroid. Synergism among the thee factors appears to be essential, and the specificity of the hypophyseal and of the adrenocortical factor is evidently relatively broad. Once nodules have been induced, estrogen is not essential for nodule maintenance (*9*); furthermore, tumors have arisen from nodules in the absence of ovary and pituitary when only somatotropin and deoxycorticosterone are provided (*17, 18*). The central role classically ascribed to estrogen in mouse mammary tumorigenesis is thus open to serious question. Several different hormonal milieux for successful nodule maintenance and for the origin of tumors from such nodules are being delineated experimentally (*18*).

On the basis of the hormone dosage needed to induce lactation in hyperplastic alveolar nodules, it seems that there is a spectrum of sensitivity that is shown by different nodules and even occasionally by different regions of the same nodule. The nature of the correlation between hormone sensitivity and predisposition to neoplasia is presently being investigated, and the problem raises some interesting considerations. Is there a progression of sensitivity states in a nodule that leads from an almost normal requirement of hormones to a point where no hormones at all are required (presumably the state possessed by the tumor)? If this be true, there is an abrupt qualitative change from a point where a minute amount of hormone or hormones will result in a response of, and is required by, the hyperplastic cells to a point where no amount of hormone will result in response of the neoplastic cells—that is, where "autonomy" has been attained. Or is there a progressive loss of reactivity to hormones to a point reached by the tumor? If the latter assumption is correct, there should be an inverse correlation between the hormone sensitivity of nodules and their neoplastic potential.

Experiments have established the ability of bovine somatotropin to substitute for ovine mammotropin in inducing mammary differentiation and function, including lactation, in the C3H/Crgl mouse strain (*19*), and also in maintaining and stimulating hyperplastic nodules (*13*). The endogenous hormonal requirements for nodule formation and tumorous transformation appear to differ in different strains (*20*). Thus, in virus-bearing C3H/Crgl mice, nodule and tumor incidence is almost as great in virgins as it is in breeders. However, in the three virus-bearing A lines used in our laboratory, nodule and tumor incidence is almost zero in virgins, in contrast to a high incidence in breeders. In virgin C3H mice, the minimum combination, for nodule and tumor formation, of estrogen, corticoid, and somatotropin would normally be present. In virgin A mice, this minimum combination is presumably also present, but the mammary gland appears to be unresponsive. Further studies with various strains and sublines indicate that the A sublines are similar to other strains in their ability to develop mammary lobules and to lactate when properly treated with estrogen, progesterone, and mammotropin and subsequently with cortisol and mammotropin. However, the A sublines show no such response when the mammotropin is replaced by somatotropin, and herein may lie the fundamental genetic difference in the endocrine make-up between the A strain and a strain such as the C3H: the long-debated "inherited hormonal influence" appears to involve the genetically determined sensitivity of the target organ.

The material presented above may provide somewhat new perspectives in regard to the intervention of hormones in tumorigenesis in hormone-regulated tissues. The following general possibilities emerge from recent studies of mouse mammary cancer: (i) hormonal factors involved in the evolution of a definite precancerous state may be no more than those factors involved in normal tissue development; (ii) the tumorigenic role of the hormonal milieu may be no more than the continued maintenance of a degree of hyperplasia (the precancerous state); (iii) the so-called tumorigenic hormone may be but one component of an essential milieu wherein no one hormone can be considered more essential than any other; (iv) in general, the hormonal influence may be a "permissive" one, essential for tumor appearance but not itself inductive (*21*).

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References and Notes

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Immunological Differentiation of Epididymal and Seminal Spermatozoa of the Rabbit

Abstract. Rabbit spermatozoa from the epididymis lack the antigenic material present on seminal spermatozoa, which these latter cells have in common with the seminal plasma. This observation provides further support for the indirect evidence, obtained previously, that antigenic material is taken up by the spermatozoa from the seminal plasma.

Mammalian seminal plasma is of highly complex composition (*1*). But little is known about the physiological significance of many of its components. Recently, immunological analysis has provided information suggesting that spermatozoa take up and firmly attach to themselves material from the secretions of the adnexal glands that constitute the seminal plasma. The seminal plasma and seminal spermatozoa of man and several mammals have immunologically specific components in common (*2–5*). Similar antigens are found in aqueous extracts of prostate and seminal vesicle, but not in extracts of testis and epididymis (*2, 4*). This, and the fact that azoospermic ejaculates of man contain the full complement of antigen (*2*), suggested that this material originates in the adnexal glands of the genital tract rather than in the testes. The following data provide direct evidence that this is indeed the case.

Rabbit semen was collected by means