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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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

of the artificial vagina (*6*). Seminal plasma and spermatozoa were separated by centrifugation at 12,800*g*. Epididymides of rabbits were removed from the animals immediately after exsanguination, and the spermatozoa were washed out of the dissected organs. Both kinds of spermatozoa were washed thrice in physiological saline solution, counted in a counting chamber, and suspended in physiological saline solution in the desired concentration. Complement fixation tests (Kolmer method) were made with these suspensions as antigens and the sera of guinea pigs immunized either with rabbit seminal plasma or with washed seminal spermatozoa. Controls with normal guinea pig serum and with immune serum from guinea pigs against whole rabbit serum remained entirely negative. Details on the techniques employed have been reported (*2, 4*).

The data obtained by this highly sensitive method show that epididymal spermatozoa lack the strongly antigenic material present in seminal spermatozoa and in seminal plasma (see Table 1).

These findings should not be interpreted as a denial of antigenicity of the spermatozoa as they originate in the testes. In fact, the data of Henle and his associates (*7*) and of Voisin (*8*) and Freund (*9*) show that testicular spermatozoa are not devoid of antigenic properties, and those of Pernot (*3, 5*) provide evidence that such antigenic properties can still be discerned in seminal spermatozoa. The point that concerns us here is that spermatozoa take up antigenic material from the seminal plasma before or at the time of ejaculation. It would be of interest to obtain information on the chemical nature of these antigens and their role, if any, in the physiology of reproduction (*10*).

Table 1. Fixation of complement by guinea pig immune serum (diluted 1/10) and antigen. The antiserum and antigen controls, also antigens plus normal guinea pig serum and antigens plus anti-rabbit serum immune serum, remained negative. ++++, No hemolysis; ++, moderate (about 50 percent) hemolysis; −, complete hemolysis.

Antigen dilution	Antiserum	
	Anti-spermatozoa	Anti-seminal plasma
Spermatozoa from semen (4 × 10^6 cells/ml)		
1/1	++++	++++
1/2	++++	++++
1/4	++++	++++
1/8	−	++
1/16	−	−
Spermatozoa from epididymis (4 × 10^6 cells/ml)		
1/1	−	−
1/2	−	−
1/4	−	−
1/8	−	−
1/16	−	−
Seminal plasma (diluted 1/1000)		
1/1	++++	++++
1/2	++++	++++
1/4	++++	++++
1/8	++++	++++
1/16	−	−

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Influence of pH on the Toxicity of Nitrogen Mustard

Abstract. The experiments reported here demonstrate the unexpected finding that, in mice, the *p*H of nitrogen mustard solution at the time it is injected into the animal appears to determine the toxicity of the material. At certain doses, highly acid solutions of nitrogen mustard show no toxic effects, while alkaline solutions at the same dose are invariably lethal. From further experiments on the antitumor activity of acid solutions of nitrogen mustard, it is concluded that the toxic effect is separable from the antitumor effect.

In the course of an investigation of the mechanism of toxicity of nitrogen mustard [methyl bis (β-chloroethyl)-amine·HCl; HN2] several facts have emerged: (i) toxicity can be prevented by the administration of any of several compounds before injection of the mustard (*1*); (ii) toxicity can largely be prevented in dogs by prior splenectomy and the administration of norepinephrine just before injection of HN2 (*2*); such protection is not conferred by splenectomy in mice (*3*); and (iii) intraperitoneal injections of mice with saline homogenates of spleen, mixed with nitrogen mustard, were uniformly more toxic than the same dose of nitrogen mustard alone (*4*). At the LD_{50} of nitrogen mustard (in saline), mixtures of mustard and spleen homogenates were uniformly lethal. The LD_{50} for HN2 in saline, in mice, is approximately 4.0 mg/kg, injected intraperitoneally (*5*).

In analyzing the data on the toxicity of mixtures of nitrogen mustard and spleen homogenates, it was apparent that the *p*H of the mixture was substantially higher than the *p*H of the mustard in saline alone. To control this discrepancy, Ringer's solution was substituted for the saline. When this was done, the *p*H's of the mixture of mustard and spleen homogenates and of the mustard in Ringer's solution were roughly the same, usually about 6.8. At this higher *p*H, the mustard was more toxic than it had been at the lower *p*H, and it was nearly as toxic as the mixtures of mustard and spleen homogenates. Therefore, a study of the effect of *p*H upon the toxicity of nitrogen mustard was started (*6*).

Male mice of the Swiss-Webster and C57Bl strains were used. All were healthy young animals maintained on a balanced ration with free access to food and water. Each animal was weighed just prior to injection, the dose of nitrogen mustard being based on its weight. At each *p*H, and for each dose, groups of 10 to 25 animals were used. To date, more than 3000 normal mice have been used in this toxicity study. For most of the work, intraperitoneal injections were used. For single doses 1.0 ml was given, and for fractionated daily doses the mustard was given in 0.5 ml of solution. In separate experiments the material was given intravenously in 0.2 ml of solution.

Nitrogen mustard (methyl bis (β-chloroethyl) amine·HCl; HN2], supplied commercially as Mustargen and diluted for injection (10.0 ml of saline or water per 10.0 mg of HN2) was found to have an initial *p*H of 4.7. When HN2 was diluted further for injection in mice, the *p*H of the material was usually about 5.2. To vary this *p*H, stock solutions of saline were made up with HCl or NaOH so that, upon addition to these solutions of HN2, a final *p*H of 2.0, 4.0, 6.0, 8.0, or 10.0 resulted. Several different doses of HN2 at each *p*H were used; through careful manipulation, the final *p*H's of the injected solutions within each *p*H group were quite close, in spite of tenfold differences in the concentration of HN2. All the solutions were injected within 60 seconds of mixing. An aliquot was taken, and the *p*H of each solution was measured thereafter in a Beckman model G *p*H meter.

Experiments were performed with doses of nitrogen mustard which ranged from 2.25 to 20.0 mg/kg, injected intraperitoneally as a single dose. In a few experiments the intraperitoneal dose was given as five consecutive daily injections, the total amounts ranging