Relation of Prolactin and Estrogen to Mammary Tumorigenesis in the Rat [1,2]

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SUMMARY—The 2 most important hormones for development and growth of mammary tumors in rats are prolactin and estrogen, both under control of the pituitary gland and the brain. Estrogen stimulates prolactin secretion and acts with prolactin directly on the mammary tissues to promote tumorigenesis. Large doses of estrogen can inhibit mammary tumor growth, not by suppressing prolactin secretion but by interfering with the peripheral action of prolactin on the mammary tissues. Estrogen can neither induce nor maintain growth of mammary tumors in the absence of the pituitary, but prolactin may have a limited capacity to induce and maintain mammary tumor growth in the absence of the ovaries. Although rats show no significant change in serum-prolactin levels during growth of carcinogen-induced mammary tumors, elevations of serum prolactin produced by hypothalamic lesions, pituitary grafts, or administration of small doses of estrogen or appropriate drugs, can increase growth of spontaneous and carcinogen-induced mammary tumors. Drugs that reduce serum-prolactin levels, including ergot derivatives, iproniazid, pargyline, etc., can decrease growth of mammary tumors. When prolactin secretion is elevated before administration of a *carcinogen*, development of mammary tumors is inhibited, presumably by stimulating growth of mammary tissues and thereby rendering them refractory to the action of the carcinogen. Biogenic amines in the hypothalamus apparently serve as neurotransmitters to regulate the release of prolactin-inhibiting factor, luteinizing-hormone releasing factor, and follicle-stimulating hormone releasing factor, which in turn regulate release of prolactin, luteinizing hormone, and follicle-stimulating hormone by the pituitary. Any agent that can alter biogenic amine turnover in the hypothalamus is therefore potentially useful for controlling growth of mammary tumors.—J Nat Cancer Inst 48: 1217–1224, 1972.

[1] Presented at the Symposium on Mammary Neoplasia held by the Institute for Medical Research at Cherry Hill, N.J., November 11–13, 1971.

[2] Supported in part by Public Health Service research grant CA 10771 from the National Cancer Institute and grant AM 07484 from the National Institute of Arthritis and Metabolic Diseases.

ESTROGEN AND prolactin are the 2 most important hormones involved in development and growth of mammary tumors in rats. Administration of estrogens for prolonged periods of time (*1*) or sustained high blood levels of prolactin (*2*, *3*) can result in mammary tumorigenesis, whereas ovariectomy or hypophysectomy (*4*) can inhibit development of mammary tumors. Growth of established mammary tumors can be stimulated by appropriate doses of estrogen, estrogen and progesterone (*5*), or prolactin (*5*, *6*), whereas ovariectomy or hypophysectomy result in mammary tumor regression (*4*, *6*).

Estrogen increases prolactin (*7*) and growth hormone (*8*) secretion by the pituitary, and also acts synergistically with progesterone and prolactin to directly stimulate growth of the mammary epithelium. Estrogen has no effect on mammary tumorigenesis in the absence of the pituitary (*9*), but prolactin or prolactin and growth hormone together may promote mammary tumor development in the absence of the ovaries (*4*). Prolactin alone can maintain established mammary tumor growth for at least a limited time in the absence of the ovaries (*10*, *11*) or ovaries and adrenal glands (*6*). Whether estrogen and prolactin *per se* are potential carcinogenic agents or act only as cocarcinogens in the presence of viral or other factors has not been established, but there is no conclusive evidence that viruses are involved in mammary carcinogenesis in the rat.

EXPERIMENTS AND RESULTS

Relation of Estrogen Levels to Development and Growth of DMBA-Induced Mammary Cancers in Rats

In this experiment, the effects of different doses of estrogen on mammary cancer development and growth were investigated in ovariectomized rats given 7,12-dimethylbenz[*a*]anthracene (DMBA), according to the procedure of Huggins (*5*). Sprague-Dawley rats were ovariectomized at 45 days of age, and 7 days later (at 52 days of age) they were given a single intravenous injection of 5 mg DMBA. Beginning on the following day, the rats received subcutaneous injections every other day for 150 days (table 1). The intact (Group 1) and ovariectomized (Group 2) controls were given injections of corn oil every other day, and all other rats (Groups 3–5) were given injections of estradiol benzoate (EB) in corn oil. Mammary cancers were detected by palpation and were measured with calipers.

Table 1 shows that mammary cancers developed in all 18 intact control rats (Group 1), with an average latency period of 59 ± 3 days. No cancers developed in the ovariectomized controls (Group 2). In the 3 ovariectomized groups given estrogen, the greatest tumor incidence (in 17 of 19 rats) occurred in the animals given 2.0 μg EB (Group 4), and the average latency period for this group was 73 ± 7 days. Regression of 8% of the mammary tumors occurred in the intact controls, of 19 and 20% in the ovariectomized rats given 0.2 and 2.0 μg EB, and of 39% in the ovariectomized rats given 20 μg of the estrogen.

At the end of 150 days, serum prolactin was measured by radioimmunoassay (*12*) in the ovariectomized rats. The intact control rats (Group 1) continued to cycle normally and had an average of 27.3 ± 2.6 ng prolactin/ml during the diestrous phase and 101.8 ± 23 ng/ml on the day of estrus. This agrees with similar serum values reported in normal-cycling rats (*13*). The ovariectomized rats showed reduced levels of serum prolactin (14.7 ± 2.4). The lowest dose of EB given, 0.2 μg, produced only a small increase in serum prolactin whereas the 2 highest doses produced approximately 11.5 to 12.0-fold increases in serum prolactin over those in the ovariectomized control rats.

These results indicate that the optimal dose of the 3 doses of EB injected to produce mammary cancers in the ovariectomized DMBA-treated rats was 2.0 μg. In the rats given this dose of estrogen, the number of cancers produced were fewer and the average latency period was longer than in the intact controls, which suggests that ovarian function in the intact controls was not entirely duplicated by this dose of estrogen. The incidence of tumor regression was greater in all the ovariectomized groups given estrogen than in the intact controls, and the greatest regression occurred in the group given the highest dose of estrogen (20 μg). This is in agreement with earlier work showing that administration of estrogen promotes mammary tumor development in ovariectomized rats but inhibits growth of the tumors after they appear (*5*). The 2 largest doses of estrogen increased tumor

TABLE 1.—Effects of graded doses of EB* on mammary tumor induction and regression in DMBA-treated ovariectomized rats [from H. Nagasawa and J. Meites, unpublished data]

Group No. and treatment	Number of rats	Number of rats with tumors	Average latency period (days)	Percent and No. of completely regressed tumors	Serum prolactin concentration (ng/ml)
1. Intact controls, no EB	18	18	59 ± 3	8 (11/143)	27.3 ± 2.6(D)† 101.8 ± 23(E)‡
2. Ovax§ controls, no EB	10	0	—	—	14.7 ± 2.4
3. Ovax, 0.2 μg EB	21	12	98 ± 10	19 (4/21)	20.8 ± 1.6
4. Ovax, 2.0 μg EB	19	17	73 ± 7	20 (16/79)	169.7 ± 23
5. Ovax, 20.0 μg EB	19	10	119 ± 7	39 (9/23)	177.0 ± 21

*EB was injected every 2 days for 150 days.
†D = diestrous phase of cycle.
‡E = estrous phase of cycle.
§Ovax = ovariectomized.

regression despite marked elevations in serum-prolactin levels. This is in agreement with findings in previous work showing that large as well as small doses of estrogen increase serum-prolactin concentration in rats (*14*). These results indicate that the ability of large doses of estrogen to inhibit mammary tumor growth in rats cannot be attributed to a decrease in prolactin secretion.

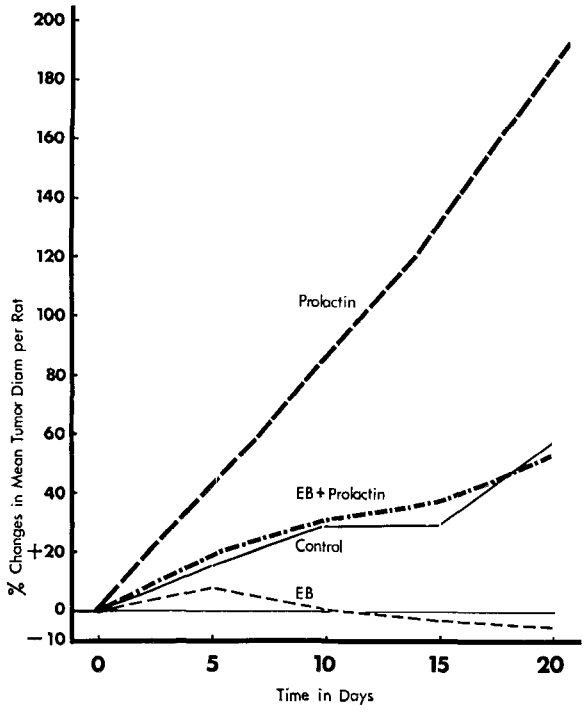
Mechanism by Which Large Doses of Estrogen Inhibit Mammary Tumor Growth in Rats

Our earlier work showed that large doses of estrogen inhibited lactation in animals by interfering with the peripheral action of prolactin on the mammary gland (*15*). Injections of relatively large doses of prolactin to such treated animals largely overcame lactation inhibition by the estrogen. It was of interest therefore to determine whether large doses of estrogen also interfered with the peripheral action of prolactin on growth of mammary tumors, and whether this could be overcome by administering additional prolactin (*16*).

Sprague-Dawley female rats were given a single intravenous injection of 5 mg DMBA at 55 days of age to induce mammary cancers. About 3 months later, when most rats had mammary tumors they were divided into 4 groups and given subcutaneous injections daily for 20 days of 1 of the following substances: 1) corn oil (controls); 2) 20 μg EB in corn oil; 3) 1 mg prolactin (NIH-P-S-8 ovine prolactin, 28 IU/mg) in 0.9% NaCl; and 4) 20 μg EB and 1 mg prolactin. Diameter and number of mammary tumors per rat were recorded every 5 days. Text-figure 1 shows that mammary tumors in the control rats increased by 50.3% in average diameter during the 20-day period, whereas growth of mammary tumors in the rats given EB was completely suppressed. Rats given EB together with prolactin showed about the same gain in average tumor diameter (49.5%) as in the controls. Rats that received injections of prolactin alone showed an average gain in mammary tumor diameter of about 190%. The effects of the treatments on total number of tumors per rat were similar to those on tumor diameter.

These results are believed to demonstrate that a large dose of estrogen can prevent prolactin from stimulating mammary tumor growth. Prolactin alone increased mammary tumor growth about 190%, whereas a combination of EB and the same dose of prolactin resulted in about a 49.5% increase in growth or about the same growth as in control rats not given the hormones. Furthermore, EB alone completely suppressed growth of the tumors, whereas prolactin given together with EB restored mammary growth to the control level.

In the previous study it was shown that the dose of EB used in the present experiment, 20 μg, was the most effective for causing mammary tumor regression in rats with DMBA-induced mammary cancers, despite the greatly elevated serum-prolactin values produced by this dose of estrogen. Thus the inhibition of mammary tumor



TEXT-FIGURE 1.—Effects of daily injections of EB, 20 μg, or of prolactin (1 mg of NIH ovine prolactin, 28 IU/mg), or of both, on growth of DMBA-induced mammary cancers in Sprague-Dawley female rats. *Note* that EB inhibited growth, whereas prolactin stimulated it. Administration of prolactin together with EB prevented EB from inhibiting tumor growth [Meites *et al.* (*16*)].

growth by this dose of estrogen cannot be attributed to a decrease in prolactin secretion, but rather to an interference with the peripheral action on the mammary tumor. How large doses of estrogen inhibit the action of prolactin on mammary tumor growth is not clear, but it may block binding of prolactin to receptor sites on, or in, the mammary tumor cells or otherwise prevent prolactin from acting on the mammary tumor cells.

Relation of Blood Prolactin Levels to Development and Growth of Mammary Tumors in Rats

In a recent study (Nagasawa and Meites, unpublished data), we measured serum prolactin in rats with established DMBA-induced mammary tumors for about 6 months. Despite the continuous growth in size and increase in number of mammary tumors in these rats, serum levels of prolactin remained relatively constant and within the normal range. This study appears to demonstrate that DMBA-induced mammary tumors can develop and grow in rats in the presence of *normal* serum prolactin levels. However, there is considerable evidence that a reduction in blood prolactin below normal levels results in decreased mammary tumor growth, whereas an elevation in blood prolactin values above normal levels results in increased mammary tumor growth. A few examples will be cited.

Spontaneous mammary tumors frequently appear in old female rats and may reach an incidence of 50% or greater in female Sprague-Dawley rats 2 years old or older. These tumors usually appear in a rat as a single benign adenoma or fibroadenoma and differ in their characteristics from the DMBA-induced multiple adenocarcinomas per rat. Bilateral lesions were placed in the median eminence of normal female Sprague-Dawley rats at 10 months of age and they were killed 25 weeks later (16.5 months of age). Placement of such lesions in the hypothalamus of rats has resulted in a rapid and prolonged rise in serum-prolactin values (*3*). Control rats were given sham lesions. Table 2 shows that 4 of 21 control rats developed 4 tumors, whereas 12 of 23 rats with lesions in the median eminence developed 20 tumors or an average of 1.6 tumors per rat. It can be seen that serum-prolactin concentration was more than 3 times as great in the rats with lesions than in the control rats. Thus, many more mammary tumors were produced in female rats before the age of optimal spontaneous mammary-tumor development by increasing serum levels of prolactin. Similar early induction of mammary tumors was achieved in Sprague-Dawley female rats by grafting multiple pituitaries underneath the kidney capsule (*2*).

In carcinogen-induced mammary adenocarcinomas in rats, the effects of an increase in prolactin secretion were somewhat different than for the spontaneous benign mammary tumors. If prolactin levels were increased before DMBA administration and continued before the appearance of mammary cancers, development of mammary cancers was inhibited. Thus an increase in prolactin by placement of lesions in the median eminence (*10*, *11*), grafting of extra pituitaries underneath the kidney capsule (*17*), administration of reserpine (*18*) or a norethynodrel-mestranol combination (*19*) all

TABLE 2.—Effects of median eminence lesions on development of normal and neoplastic mammary tissue and serum prolactin levels in female rats (from Welsch *et al.*, 1970)

Treatment*	Total number of rats	Final body weight (g)	Serum prolactin levels (mμg/ml)	Average mammary gland ratings	Number and percent of rats with tumors	Total number of tumors
Controls, sham lesion	21	360± 7†	50. 9± 9. 6†	3. 1±0. 1†	4(19%)†	4†
Median eminence lesions	23	451±19†	179. 8±23. 9†	4. 2±0. 2†	12(52%)†	20†

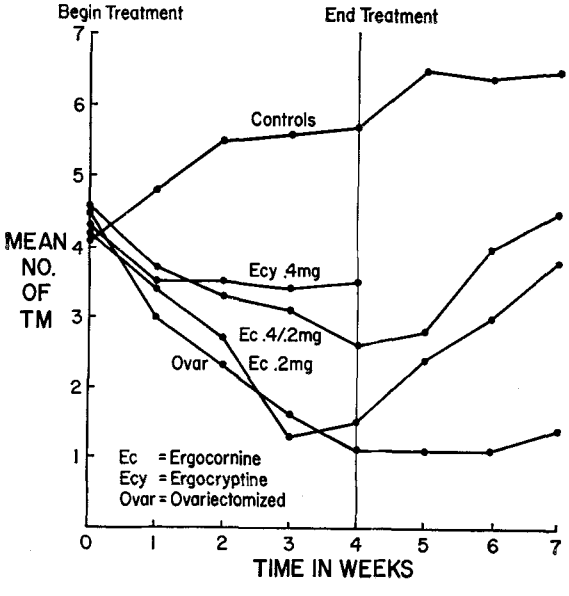
*All rats were killed 25 weeks after placement of median eminence or sham lesions. Final body weight, serum prolactin levels, and average mammary gland ratings are represented as the mean value ±SE.
†$P<0.001$.

resulted in a decreased incidence of mammary tumors when given before administration of DMBA in Sprague-Dawley rats. Apparently stimulation of mammary growth by prolactin protects the mammary epithelium from the action of the carcinogen. This also demonstrates that development of normal mammary tissue and mammary tumors do not necessarily proceed simultaneously, and that different mechanisms may operate to control each type of growth.

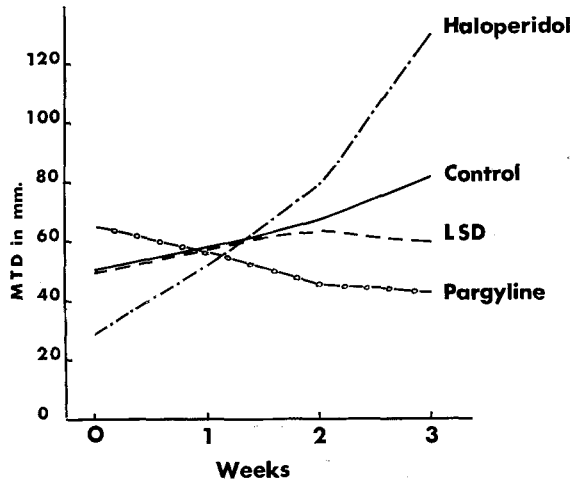
After mammary adenocarcinomas appeared as a result of carcinogen treatment, an increase in prolactin secretion resulted in enhanced growth whereas a decrease in prolactin secretion resulted in inhibition of mammary cancer growth. Thus placement of lesions in the median eminence (*10, 11*), grafting of multiple pituitaries underneath the kidney capsule (*17*), injections of reserpine (*18*) or a norethynodrel-mestranol combination (*19*), all increased growth of DMBA-induced mammary cancers. On the other hand, administration of drugs that reduce blood prolactin levels resulted in decreased growth of these mammary cancers. Several ergot drugs have been shown to be particularly effective in inhibiting growth of DMBA-induced mammary cancers. The effects of injecting ergocornine and ergocryptine for 4 weeks on rats with DMBA-induced mammary cancers (*20*) are shown in text-figure 2. Both drugs inhibited mammary cancer growth during the 4-week treatment period, and tumor growth promptly resumed after treatment was terminated. These 2 drugs also inhibited growth of spontaneously developed mammary tumors in old female Sprague-Dawley rats (*21*). Ergot drugs depress prolactin secretion by a direct inhibitory action on the mammary gland (*22, 23*) and by increasing hypothalamic prolactin-inhibiting factor (PIF) activity. Other drugs that inhibit prolactin secretion and depress mammary tumor growth include iproniazid, pargyline, and LSD, whereas haloperidol and reserpine stimulate prolactin secretion and mammary tumor growth (text-fig. 3).

CONTROL OF PROLACTIN SECRETION

In view of the importance of prolactin in mammary tumor development and growth in the rat, the major factors that control its secretion must be considered. The mammalian anterior pituitary



TEXT-FIGURE 2.—Inhibition of DMBA-induced mammary cancer growth by daily injections of ergocornine and ergocryptine. Doses are indicated for each drug. *Note* resumption of cancer growth after termination of ergocornine treatment (*20*).



TEXT-FIGURE 3.—Effects of daily injections of prolactin (1 mg NIH ovine prolactin, 28 IU/mg), iproniazid (2.5 mg/100 g body weight), or pargyline (2.5 mg/100 g body weight) on growth of DMBA-induced mammary cancers. MTD = mean tumor diameter (Quadri *et al.* unpublished data).

can synthesize and release sustained high amounts of prolactin after severance of hypothalamic connections, in contrast to the marked reduction in secretion of the other 5 anterior pituitary hormones. Under most conditions the hypothalamus inhibits secretion of prolactin through production of PIF (*24*). PIF release into the hypothalamo-pituitary portal vessels appears to be controlled mainly by the amounts of catecholamines (perhaps principally dopamine) released from nerve endings in the median eminence and perhaps elsewhere in the hypothalamus. A rise in catecholamine concentration and turnover in the hypothalamus resulted in increased release of PIF and in reduced pituitary release of prolactin, whereas a reduction in catecholamine activity resulted in decreased release of PIF and increased release of prolactin (*25*). Hypothalamic catecholamines stimulated release of luteinizing hormone releasing factor and follicle-stimulating hormone releasing factor, resulting in increased release of luteinizing hormone and follicle-stimulating hormone (*26*). Thus the effects of catecholamines on release of the 2 gonadotropins are opposite to those on prolactin.

Many drugs such as reserpine, chlorpromazine, haloperidol, alpha-methyl-para-tyrosine and others have been shown to decrease catecholamine and PIF activities in the hypothalamus and to evoke elevations in serum prolactin values; drugs such as l-DOPA, the precursor of dopamine, monoamine-oxidase inhibitors such as iproniazid or pargyline, increased hypothalamic catecholamines and PIF and reduced serum-prolactin levels (*26*). The suckling stimulus increased prolactin release by decreasing hypothalamic catecholamines and PIF (*24*), whereas high levels of blood prolactin (*24*) or injections of ergot drugs (*23*) increased hypothalamic catecholamines and PIF and reduced pituitary prolactin release. The ability of prolactin to inhibit its own secretion is of considerable interest, but there is as yet no conclusive evidence that this constitutes an important mechanism for control of prolactin secretion.

In addition to hypothalamic regulation, some hormones and drugs can act directly on the anterior pituitary to alter prolactin secretion. Estrogen acts directly on the pituitary to stimulate prolactin release, but in addition acts on the hypothalamus to decrease PIF activity (*24*). Estrogen therefore increases prolactin secretion by dual mechanisms. Ergot drugs directly inhibit pituitary release of prolactin (*22*) and also act on the hypothalamus to increase PIF activity, thus depressing prolactin release via the pituitary and hypothalamus. Thyroxine and triiodothyronine act directly on the pituitary to increase prolactin release (*24*) but apparently have no effect on hypothalamic PIF levels. The thyroid hormones therefore appear to act only on the pituitary to increase prolactin secretion. Pentobarbital inhibited prolactin release by a direct action on the pituitary and acted on the hypothalamus to decrease PIF activity (*27*). Apparently this accounts for the early increase and subsequent prolonged inhibition of prolactin release produced by pentobarbital (*28*).

It is apparent that direct manipulation of the brain (by hypothalamic lesions, electrical stimulation, implantation of hormones or drugs, etc.), or alteration of hypothalamic function by drugs or environmental stimuli, can evoke profound changes in prolactin secretion. Therefore the question may be asked whether some mammary tumors (and other endocrine-dependent tumors) may not have their origin in malfunction of the hypothalamus. This question cannot be adequately answered at present, but we have reported some evidence of fundamental changes in hypothalamic function of

old female rats that were associated with a rise in prolactin secretion and increased incidence of spontaneous mammary tumors (*29*). Prolactin secretion is mainly under direct control by the hypothalamus, and estrogen secretion is under the indirect control of the hypothalamus via a releasing factor or factors which regulate LH and FSH secretion. By the use of appropriate drugs and hormones, the release of prolactin (and of estrogen) can be altered to inhibit development and growth of mammary tumors in rats. Whether these observations are relevant to the problem of human breast cancer remains to be investigated.

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