Differential Effects of Estrogen and Prolactin on DNA Synthesis in Organ Cultures of DMBA-Induced Rat Mammary Carcinoma[1] (36201)

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Estrogens have long been known to enhance the development and growth of mammary carcinoma in a variety of experimental animals (1). These steroids have been implicated as one of a number of potentially oncogenic factors in human breast cancer (2). Hyperpituitarism has also been implicated in the development and growth of experimental mammary carcinoma (3). A variety of experiments have indicated that prolactin may be the principal pituitary hormone involved in this process (4–7).

Ovariectomy-adrenalectomy of mammary carcinoma-bearing rats causes prompt tumor regression, which can be reversed by concurrent administration of prolactin (7). Hypophysectomy of mammary carcinoma-bearing rats also evokes immediate tumor regression, but the administration of estrogen does not reverse this regression (8). Since estrogens are known to stimulate pituitary prolactin secretion (9), it has been suggested that these steroids are mammary oncogenic primarily as a result of their ability to influence prolactin secretion (10).

Organ culture techniques have been used to preclude involvement of the complex hormonal interrelationships existing *in vivo* and to evaluate and compare direct effects of hormones on tissue growth. Thus, the purpose of this study was to determine and compare the capacity of estrogen and prolactin to promote DNA synthesis in organ cultures of 7,12-dimethylbenzanthracene (DMBA)-induced rat mammary cancers. This experimental mammary tumor system closely resembles human breast cancer, as judged by significant morphological and physiological criteria (11, 12).

Materials and Methods. Mammary carcinoma were induced in female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, MI) by the administration of DMBA, as previously described (13). Tumors used for organ culture were in weeks 5 to 10 of development and were approximately 1.5 cm in diameter. Immediately following aseptic removal, the tumors were cut into explants (approx 1 mm^3) and placed on wire grids in small (10 × 30 mm) Falcon disposable petri dishes containing 2 ml of medium 199, Earle base (Difco Labs., Detroit, MI). Medium 199 was supplemented with penicillin G (50 IU/ml), insulin (5 μg/ml) (bovine pancreas, 22.5 IU/mg), and corticosterone (1 μg/ml). Insulin and corticosterone were added to the media to aid in the maintenance of explant viability (14, 15). Estrogen-17β and prolactin (NIH-S8-ovine, 28.0 IU/mg) were added to the media in amounts indicated in Figs. 1 and 2. Media was sterilized by passage through Millipore filters (0.45 μ).

The study was divided into 2 separate experiments: Expt. 1 (Fig. 1) consisted of 4 groups. Three hundred sixty explants were excised from 3 mammary carcinomas (120 explants/tumor) and equally divided among the 4 groups (90 explants/group). Expt. 2 (Fig. 2) consisted of 7 groups. Six hundred thirty explants were excised from 3 mammary carcinoma (210 explants/tumor) and equally divided among the 7 groups (90 explants/group). Each experiment was performed 3

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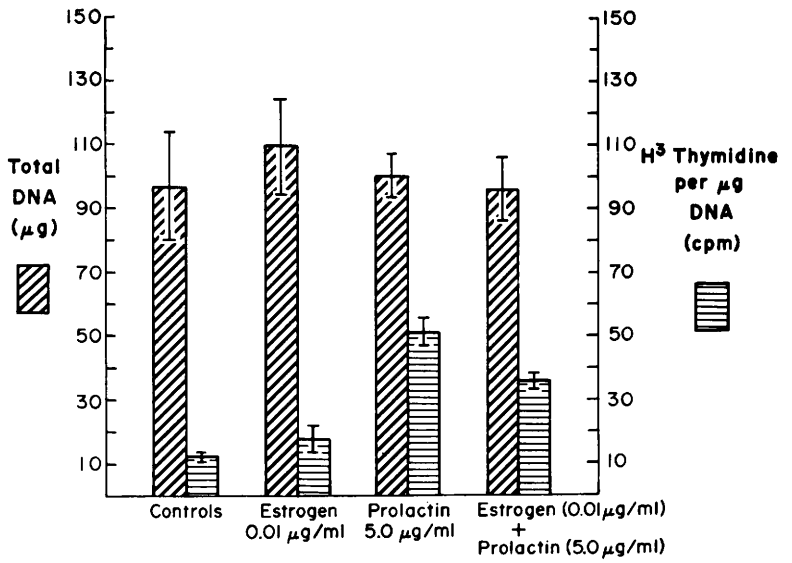

FIG. 1. Effect of estrogen and prolactin on DNA synthesis of organ cultures of DMBA-induced rat mammary carcinoma: ^{3}H-thymidine incorporation per microgram of DNA; significance levels are: $p < .01$, controls (C)/P, C/P + E, E/P, and E/E + P; $p < .05$, P/E + P; p = NS, C/E. No significant difference among groups in total DNA synthesis was observed.

times, the means shown in Figs. 1 and 2 were derived from the 3 separate cultures in each experiment.

Each culture assembly consisted of 3 small petri dishes containing 10 explants/dish. These were placed into large petri dishes (100 × 15 mm Falcon disposable) containing water-saturated filter paper. Covers were placed on the large petri dishes, but were not used on the smaller petri dishes containing the explants. The petri dishes were placed in a small gassing chamber housed in an incubator at a temperature of 37°. The chamber was continuously infused with gas (95% O_2:5% CO_2) during the incubation period.

Explants were cultured for 5 days. Media

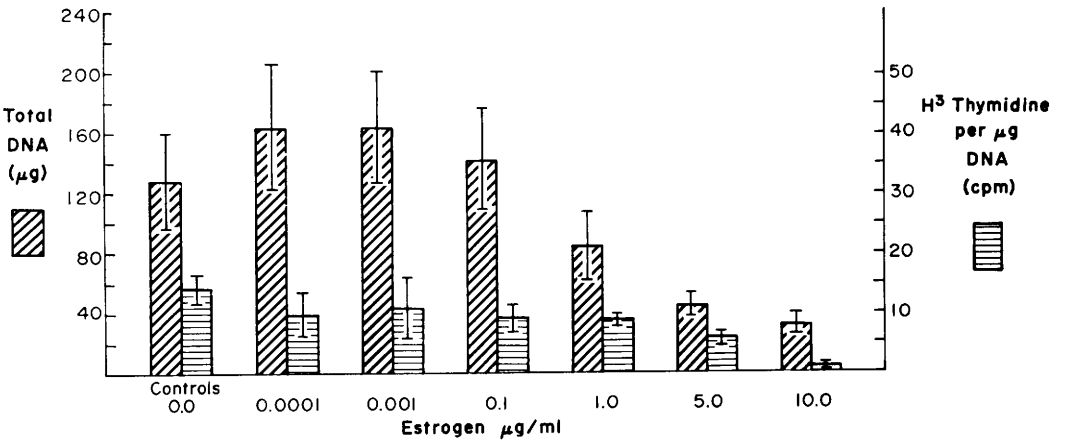

FIG. 2. Effect of varying levels of estrogen on DNA synthesis of organ cultures of DMBA-induced rat mammary carcinoma: ^{3}H-thymidine incorporation per microgram of DNA; and total DNA significance levels are: $p < .01$, controls/E(5.0 μg/ml) or E(10.0 μg/ml). No significant differences among other groups were observed.

was changed on days 2 and 4. On day 5, 4 hr prior to termination of the culture, ^{3}H-thymidine was added to the explants in a final concentration of 0.5 μCi/ml. Termination of the cultures involved quick removal of the explants from the media and storage in 0.15 *M* KCl at −15°. for DNA analysis (within 1 week). DNA synthesis was estimated by measuring ^{3}H-thymidine incorporation into DNA by a filter paper technique (16). DNA was extracted and quantitatively analyzed by a modification (16) of the colorimetric method of Burton (17). The data were analyzed statistically by two-way analysis of variance.

Results. When prolactin was added to synthetic media containing explants of mammary carcinoma, a highly significant ($p<.01$) increase in ^{3}H-thymidine incorporation into DNA was observed when compared to the controls (Fig. 1). Under identical experimental conditions, estrogen was without significant effect. The combination of estrogen and prolactin caused a highly significant ($p<.01$) increase in ^{3}H-thymidine incorporation relative to the controls, although this increase was significantly ($p<.05$) less than in those cultures containing prolactin but lacking estrogen. This inhibitory effect of estrogen on prolactin activity was consistently observed in each of the 3 cultures. None of the hormonal treatments significantly influenced total DNA.

The ineffectiveness of estrogen, at the 0.01 μg/ml level, in promoting DNA synthesis, provoked further inquiry to determine whether higher or lower dose levels of estrogen were equally ineffective (Fig. 2). At levels of 0.0001, 0.001, 0.1, or 1.0 μg/ml, estrogen was totally ineffective in influencing DNA synthesis. Significant ($p<.01$) *inhibition* of ^{3}H-thymidine incorporation into DNA and significantly ($p<.01$) reduced levels of total DNA were observed in cultures containing 5.0 or 10.0 μg/ml of estrogen.

Discussion. The results of this study demonstrate that: (i) prolactin actively stimulates DNA synthesis in organ cultures of DMBA-induced rat mammary carcinoma; (ii) estrogen, at physiological dose levels, is without effect and, at pharmacological dose levels, is inhibitory to DNA synthesis; and (iii) estrogen suppresses prolactin-induced DNA synthesis.

The observed stimulatory effect of prolactin on DNA synthesis provides *in vitro* evidence that prolactin is an important hormone in promoting growth of rat mammary carcinoma. Two recently reported *in vivo* studies (6, 7), in progress at the time of our study, provide further evidence for the singular importance of prolactin in murine mammary tumorigenesis. Administration of prolactin to adrenalectomized-ovariectomized rats bearing DMBA-induced mammary carcinoma (7) or to adrenalectomized-ovariectomized-hypophysectomized rats bearing mammary carcinoma (6), surgical procedures which normally induce prompt tumor regression (11), resulted in stimulation of tumor growth.

The total lack of a stimulatory effect of estrogen *in vitro* on DNA synthesis of mammary carcinoma lends credence to the hypothesis that estrogens are mammary oncogenic primarily as a result of their ability to influence prolactin secretion (10). The pituitary dependency of estrogen in promoting growth of mammary carcinoma is emphasized in the study of Sterental *et al.* (8), who demonstrated that the administration of moderate doses of estrogen to intact mammary carcinoma-bearing rats markedly stimulated tumor growth but was *without effect* when administered to hypophysectomized tumor-bearing rats. Although we have been unable to demonstrate an estrogen-induced stimulation of DNA synthesis *in vitro,* this hormone may be actively involved in enhancing other cellular processes (18).

Inhibition of DNA synthesis of cultured mammary carcinoma by pharmacological doses of estrogen and suppression of prolactin-induced stimulation of DNA synthesis by a lower dose level are 2 possible mechanisms by which the chemotherapeutic doses of the steroid may be oncolytic. Turkington and Hilf (19) were also able to demonstrate estrogen-induced inhibition of DNA synthesis in cultured C3H mouse mammary carcinoma, although the steroid was without effect on cultured rat R3230AC mammary carcinoma. Evidence of a direct estrogen interaction with cultured mammary carcinoma, resulting in significant modification of cellular proliferation, had not been previously provided. The

suppressive effect of estrogen on prolactin-induced DNA synthesis is an interesting observation. Although the antagonistic effects of excessive doses of estrogen on prolactin activity in normal mammary development, growth, and lactation *in vivo* are well established, the mechanism remains obscure (20). This antagonism may be direct, i.e., interference by the steroid of cellular replication, as indicated in this study, rather than via the pituitary as previously suggested (21).

Summary. Explants from mammary carcinoma of DMBA-treated Sprague-Dawley rats were cultured for 5 days in medium 199 containing insulin (5 μg/ml) and corticosterone (1 μg/ml). Estradiol-17β (0.0001–10.0 μg/ml) and/or ovine prolactin (5μg/ml) in various combinations were added to the media. Media were replaced at 2 and 4 days. ^{3}H-thymidine (0.5 μCi/ml) was added to each group of cultures 4 hr prior to termination and its rate of incorporation into DNA was used in the assessment of DNA synthesis. A highly significant increase in incorporation of ^{3}H-thymidine into DNA was obtained in the prolactin-treated cultures. Estrogen, at the lower levels (0.0001–1.0 μg/ml) or at the higher levels (5.0–10.0 μg/ml), was without significant effect or inhibitory, respectively, to DNA synthesis. These data demonstrate an important role for prolactin in stimulating DNA synthesis *in vitro,* in contrast to estrogen, which was consistently found to be without stimulatory effect.

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