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Synergism between Prolactin and Ovarian Hormones on DNA Synthesis in Rat Mammary Gland[1] (38770)

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The synergistic effects between prolactin and ovarian hormones in the development and growth of the mammary gland are well established (1–3). Estrogen has been shown to induce growth of the ductal epithelium, and progesterone to stimulate lobuloalveolar development (4). In the absence of the pituitary, however, the ovarian steroids have little or no effect (2, 3). Prolactin treatment alone increases in hypophysectomized–ovariectomized animals the total DNA content of mammary tissue (5), while *in vitro* experiments have yielded conflicting results (6–9). Prolactin and progesterone combined showed increased [^{3}H]thymidine incorporation in an *in vitro* system, while prolactin alone, or in combination with estrogen, did not have any effect (7).

The present experiment was designed to study the influence of prolactin in combination with estradiol or progesterone on [^{3}H]thymidine incorporation in the mammary gland of hypophysectomized–ovariectomized rats.

Materials and Methods. Virgin female Sprague–Dawley rats (Hormone Assay, Chicago) were hypophysectomized on the 56th day of life and ovariectomized on the 58th day. Two days after ovariectomy the animals were divided into six groups (Table I) and received the following daily subcutaneous injections for 5 days: Group I, control with vehicle, sesame oil only; Group II, estradiol-17β, 1.0 μg/100 g body wt.; Group III, progesterone, 3.0 mg/100 g body wt.; Group IV, prolactin, (ovine, NIH, 26.4 IU/mg), 1.0 mg/100 g body wt.; Group V, estradiol, 1.0 μg/100 g body wt., plus prolactin, 1.0 mg/100 g body wt.; Group VI, progesterone, 3.0 mg/100 g body wt. plus prolactin, 1.0 mg/100 g body wt. Steroids were dissolved in sesame oil, and prolactin in alkaline saline (pH 8.0). Estradiol or progesterone was injected at 9 AM, and prolactin injected in half-doses twice daily at 9 AM and 5 PM. The animals were maintained with constant access to food and water in a 12 hr/12 hr light–dark environment.

Twenty-four hours after the last steroid hormone treatment, a single dose of 100 μCi/100 g body wt. of [^{3}H]thymidine (New England Nuclear), sp act 40–60 mCi/mole, was administered ip. Four hours after the [^{3}H]thymidine injections, under ether anesthesia, the inguinal pair of mammary glands were excised and immersed in phosphate-buffered 2.5% glutaraldehyde (pH 7.4) for 18 hr. Completeness of hypophysectomy was assured in each case by examination of the sella turcica.

After fixation the tissue was processed through alcohol and xylene, and immersed in unpolymerized glycol methacrylate with four infiltration washes, 12 hr each. The glands were subsequently embedded in gelatin capsules with fresh glycol methacrylate, and polymerized at 40° for 48 hr.

Two-micron sections were cut on an LKB-Huxley ultramicrotome, using a glass knife. The sections were mounted on glass slides, dipped in photographic emulsion (Kodak NTB-3), dried overnight, and exposed in light-proof boxes over Drierite for 7 days. The slides were then developed, fixed, and stained with methylgreen pyronin. An average of 2000 epithelial cells per animal were counted in different sections at representative levels of the gland for the assessment of [^{3}H]thymidine incorporation which is expressed as the labeling index, that is, the

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TABLE I. Effect of Estradiol, Progesterone, and Prolactin on [^{3}H]Thymidine Incorporation in Ductal and Alveolar Epithelium of Hypophysectomized–Ovariectomized Rats.

Group	Treatment[a]	Ductal epithelium Mean ± SE[b, c]	Alveolar epithelium Mean ± SE[b, c]
I	Control (3)	0	0
II	Estradiol (3)	0	0
III	Progesterone (3)	0.1 ± 0.01	0.1 ± 0.02
IV	Prolactin (3)	3.5 ± 0.5	1.6 ± 0.1
V	Prolactin + estradiol (5)	9.1 ± 2.8	1.1 ± 0.3
VI	Prolactin + progesterone (3)	12.1 ± 1.0	10.3 ± 1.9

Numbers in parentheses indicate animals per group.

[a] Five-day treatments with estradiol-17β (1.0 μg/100 g body wt.), progesterone (3.0 mg/100 g body wt.), or prolactin (1.0 mg/100 g body wt.) per day.

[b] Labeled cells expressed as percentage of total population. About 2000 cells per animal were counted.

[c] Due to the small number of animals per group, statistical analysis was not performed.

percentage of labeled cells of the duct or alveoli. Background level was 1.5 silver grains/1000 square microns. Nuclei with three or more silver grains were counted as labeled.

Results. Results are summarized in Table I. Control animals and animals treated with estrogen alone did not incorporate measureable amounts of [^{3}H]thymidine. No difference was observed in the general glandular morphology between the control and estrogen-treated animals, both groups having atrophied, regressing, and sparse epithelium as compared to animals receiving combined hormone treatments (Figs. 1–2).

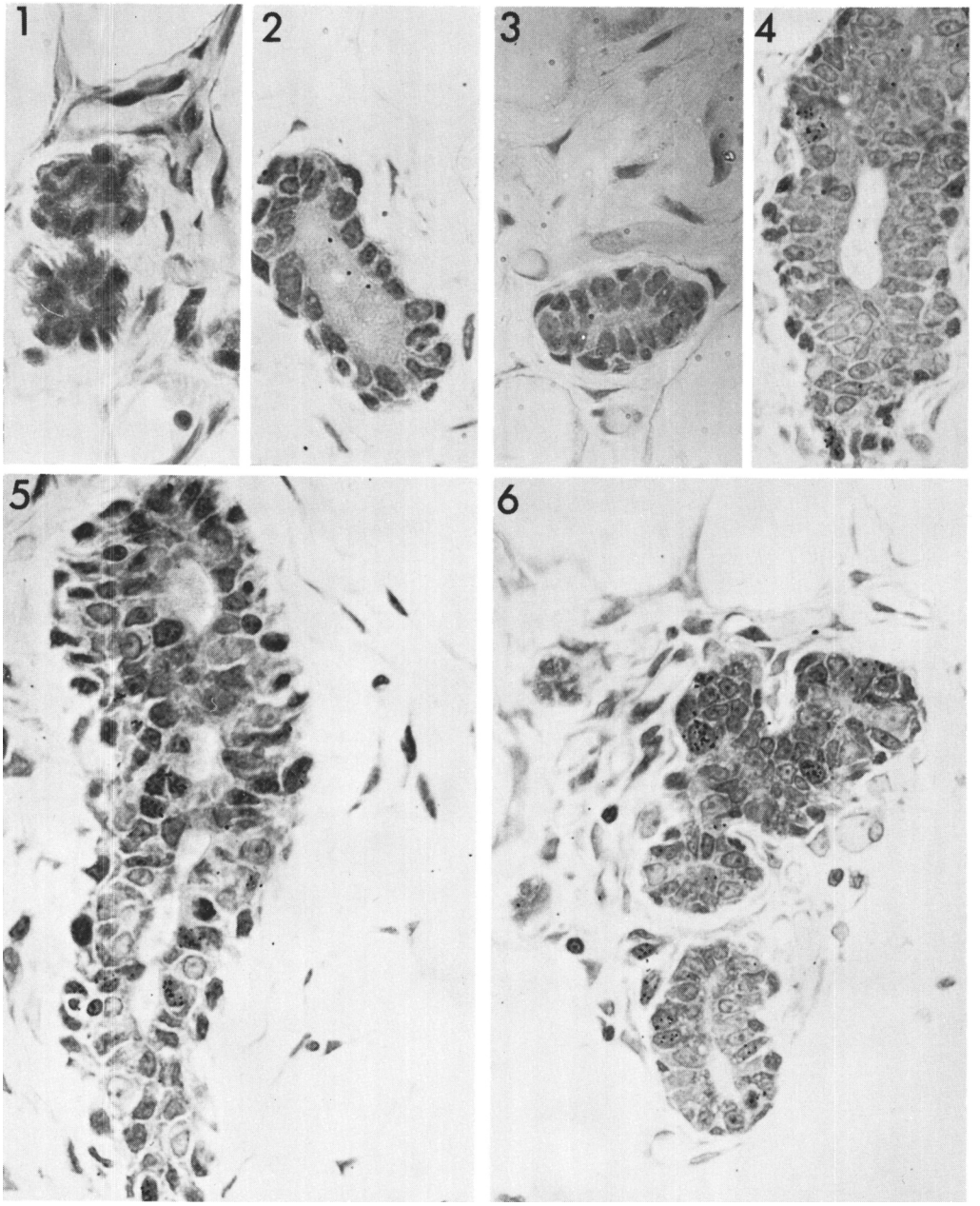
Animals which received progesterone alone showed a slight (0.1%) increase in labeling of both ductal and alveolar epithelium. The only difference in morphology was that individual cellular atrophy appeared not as severe (Fig. 3) as compared to controls and estrogen-treated animals.

The labeling index of animals which received prolactin increased by 3.5% in the ductal epithelium and by 1.6% in the alveolar epithelium. While prolactin maintained alveolar structure better than control animals, there was no remarkable proliferation of the glandular structure (Fig. 4). The combination of prolactin and estrogen, however, caused marked increases both in [^{3}H]thymidine incorporation and in the general morphology of the ductal epithelium, although there was variability of individual responsiveness. Estrogen and prolactin in combination stimulated a 9.1% increase in labeling index of the ductal epithelium, and while the ductal elements showed extensive proliferation, there was no effect on alveolar differentiation (Fig. 5).

The most pronounced effects on mammary structure was seen in the animals treated with prolactin and progesterone in combination. Animals receiving these hormones together had striking elevations of both the ductal (12.1%) and alveolar (10.3%) epithelium labeling indices. There was proliferation of both ducts and alveoli (Fig. 6).

Discussion. The results of the present study demonstrate that both estrogen and progesterone act synergistically with prolactin in stimulating DNA synthesis in rat mammary epithelium. The synergistic effects between prolactin and estrogen were exerted primarily on the ductal epithelium, whereas the effects of progesterone and prolactin were seen in both ductal and alveolar structures. Animals treated with prolactin alone showed a slight increase in [^{3}H]thymidine incorporation, more pronounced in the ducts.

This autoradiographic study further supports the earlier reports of the effects of these hormones on the mammary gland. The results demonstrate that prolactin potentiates the effects of estrogen and progesterone on mammary gland [^{3}H]thymidine incorporation. The synergism between prolactin and progesterone agrees with the results ob-



FIGS. 1–6. Autoradiograms of mammary glands of untreated and hormone-treated hypophysectomized rats, 4 hr after injection of [^{3}H]thymidine. The ductal epithelium in the control (Fig. 1) and in the estradiol (Fig. 2) and progesterone (Fig. 3) pretreated animals is hypoplastic and hypotrophic, with no or very little [^{3}H]thymidine uptake, while after pretreatment with prolactin alone (Fig. 4) some nuclear labeling is visible. Combined pretreatment with estradiol and prolactin causes hyperplasia and increases [^{3}H]thymidine incorporation, primarily in the ductal epithelium (Fig. 5), whereas pretreatment with progesterone and prolactin stimulates cellular proliferation and [^{3}H]thymidine uptake in both ductal and alveolar epithelium (Fig. 6). Exposure time 7 days. Two microns, ×480. Stained with methylgreen pyronin.

tained in *in vitro* studies (6, 7) which, however, did not show synergism of prolactin with estrogen. Since the animals in this experiment were not adrenalectomized, the possibility of involvement of traces of adrenal estrogen or progestin may not be excluded. It is not known whether the slight increase in labeling index obtained in animals treated with prolactin alone is due solely to prolactin, or to the synergistic effects of adrenal hormones with prolactin. The presence of residual adrenal steroids could also explain why synergism between prolactin and estrogen was observed in vivo but not in vitro.

Estrogen binding to mammary tissue is enhanced by prolactin *in vitro* (10), which could explain the mechanism by which prolactin enhances the effect of estrogen on DNA synthesis. Similar prolactin-induced increases in progesterone receptors, however, has not been reported so far. Our results suggest that the mechanism of prolactin potentiation is similar for both steroids, however, preferentially effecting different cell populations of the mammary gland, analogous to the uterus (11).

Summary. Autoradiography with [^{3}H] thymidine has been used to measure the rate of DNA synthesis in the mammary epithelium of hypophysectomized–ovariectomized rats under the influence of estradiol, progesterone, and prolactin. Controls and animals treated with estradiol did not increase [^{3}H]thymidine incorporation, while progesterone alone had a slight stimulatory effect. Prolactin alone stimulated some [^{3}H]thymidine uptake in ductal and alveolar epithelium, but when combined with either estradiol or progesterone synergistic effects were observed. Estradiol with prolactin stimulated incorporation primarily in the ductal epithelium, whereas progesterone with prolactin stimulated both ductal and alveolar epithelium.

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