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Effects of Progesterone, Testosterone, and Cortisol on Hypothalamic Prolactin-Inhibiting Factor and Pituitary Prolactin Content*† (32707)

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Injections of estrogen, progesterone, testosterone, or cortisol increased pituitary prolactin content and initiated mammary secretion in rats (1). Progesterone (2) and cortisol (3) retarded mammary involution in postpartum rats after litter removal. Administration of androgen (4) or progesterone (5) also induced pseudopregnancy in rats. These observations indicate that these 3 steroids can induce pituitary prolactin release in rats.

Nicoll and Meites (6) reported that estradiol can directly stimulate pituitary prolactin release *in vitro,* whereas progesterone, testosterone, and cortisol had no effect on pituitary prolactin release *in vitro*. They suggested that progesterone, testosterone, and cortisol may stimulate pituitary prolactin secretion *in vivo* by acting on the hypothalamus or through other mechanisms.

The primary influence of the central nervous system (CNS) on prolactin secretion is inhibitory, and the presence of a prolactin-inhibiting factor (PIF) in acid extracts of rat hypothalami has been demonstrated (7). Several agents such as estrogen (8), reserpine (9) epinephrine (10), acetylcholine (10), and Envoid (11) were shown to reduce PIF content in the rat hypothalamus and presumably thereby to promote prolactin secretion. The present study was undertaken to determine the effects of progesterone, testosterone, and cortisol on hypothalamic content of PIF, on pituitary prolactin concentration and on the mammary glands of ovariectomized female rats.

Materials and Methods. Mature female Sprague-Dawley rats (Spartan Animal Farms, Inc., Haslett, Mich.) averaging about 170–190 gm were used for all steroid injections. They were housed in a temperature ($75 \pm 1°F$) and light (14 hours/day) controlled room. The diet consisted of Wayne Lab Blox pellets (Allied Mills, Inc., Chicago, Ill.). Pituitary donors for incubations were adult mature male rats of the same strain, weighing 220–250 gm each.

All rats were ovariectomized and injected subcutaneously once daily with a steroid 1–2 weeks later. Progesterone (P) was given in a daily dose of 10 mg in 0.2 ml of corn

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TABLE I. Pituitary (AP) Prolactin Concentration and Hypothalamic PIF Content of Control, Progesterone (P), Testosterone Propionate (TP), and Cortisol Acetate (CA) Treated Ovariectomized Rats (12/Group).

		AP assay			PIF assay		
Treatment	AP wt[a] (mg)	No. pigeons	Prolactin (IU/100 mg AP)[a]	Change (%)	No. pigeons	Prolactin released (IU/100 mg AP)[a]	Change (%)
Controls	10.8 ± 0.3	7	1.26 ± 0.11	51[b]	9	0.35 ± 0.09	68.5[b]
10 mg P	10.9 ± 0.3	7	1.91 ± 0.30		9	0.59 ± .10	
Controls	9.6 ± 0.2	6	1.85 ± 0.60	63[c]	6	0.38 ± 0.09	155[b]
2 mg TP	10.0 ± 0.4	6	3.00 ± 0.60		6	0.97 ± 0.33	
Controls	10.4 ± 0.4	5	0.97 ± 0.20	67[c]	7	0.28 ± 0.07	40[c]
2 mg CA	9.7 ± 0.5	5	1.62 ± 0.20		7	0.39 ± 0.08	

[a] Mean ± SE.
[b] $p<.05$.
[c] $p<.01$.

oil for 21 days, and testosterone propionate (TP) was injected in a dose of 2 mg daily in 0.2 ml of corn oil for 10 days. Control rats were injected with corn oil alone. Cortisol acetate (CA) was injected in a dose of 2 mg subcutaneously in 0.2 ml of saline daily for 10 days, and control rats were similarly injected with 0.2 ml of saline daily.

All rats were killed by guillotine on the day after the last injection, and the anterior pituitaries and hypothalami were removed. The posterior pituitary was discarded and the anterior pituitary was weighed, frozen, and stored at —20°C until assayed. The hypothalami including median eminences were collected in chilled 0.1 *N* HCl (10 hypothalami/2 ml) and frozen at —20°C until incubated a few days later. On the day of incubation the hypothalami were thawed and homogenized with a glass homogenizer and centrifuged at 12,000*g* for 40 min at 4°C. The supernatants were placed in protein free medium 199 (Difco Laboratory) and the pH was adjusted to 7.4 by adding a drop at a time of 1 *N* NaOH and testing with glass electrodes.

The PIF was assayed by the method of Kragt and Meites (12). Anterior pituitaries from adult male rats of the Sprague-Dawley strain were removed and each was hemisected. One half was placed in a control flask and the other half in an experimental flask. The equivalent of 3 anterior pituitaries (6 halves) was placed in each 25-ml Erlenmeyer flask containing 2 ml of medium 199. The neutralized hypothalamic extract (equivalent of 2 hypothalami per incubated pituitary) was added to the flask. Two flask pairs were used in each experiment. Incubations were carried out in a Dubnoff metabolic shaker (60 cycles/min) under constant gassing with 95% O_2 to 5% CO_2 at 37°±0.5°C for 4 hours. At the termination of incubation the pituitary halves were weighed and the medium was frozen at —20°C until assayed.

Prolactin was assayed in 5–8-week-old White-King squabs by the intradermal pigeon crop technique of Lyons (13) as modified by Reece and Turner (14). A direct comparison was made between the control and experimental samples by injecting the control sample over one side of crop sac and the experimental sample over the other side of the crop of the same bird. The responses in each bird were converted to IU from a standard dose-response curve established in the same breed of pigeons with NIH prolactin, as described previously (15). The results of assays of control and experimental preparations in each experiment were analyzed by the *t* test for paired observations. The right inquinal mammary gland was removed from each rat and prepared for gross and histological examination by standard methods (3).

Results (Table I). Prolactin concentration expressed in equivalents of IU/100 mg of wet

anterior pituitary gland showed that P, TP, and CA each increased prolactin concentration by 51, 63, and 67% respectively, over that of control ovariectomized rats. The mammary glands from control ovariectomized rats consisted of moderately developed ducts with few or no end buds. P increased duct and end bud development, whereas TP and CA each induced lobulo-alveolar growth and secretion.

Anterior pituitary halves incubated with hypothalamic extract from P, TP, and CA treated rats released an average of 68.5, 155.5, and 40.0% more prolactin, respectively, into the medium than corresponding pituitary halves incubated with HE from control ovariectomized rats. This indicates that the steroid treated rats had less PIF in their hypothalami. Neither P nor TP had any significant effect on body weight, whereas CA decreased body weight by approximately 64 gm. No significant change in pituitary weight was observed as a result of any hormone treatment.

Discussion. The present study shows that P, TP, and CA injections into ovariectomized rats significantly reduced hypothalamic PIF content, increased pituitary prolactin concentration, and promoted mammary development. The TP and CA also induced mammary secretion. The findings on pituitary prolactin concentration are in agreement with previous studies showing that the pituitaries of P, TP, and CA treated rats contain more prolactin than pituitaries of control rats (16–18). The results on hypothalamic PIF content at least partially explain how these steroids acted to increase pituitary prolactin concentration and to stimulate mammary growth. Since these steroids were previously found to have no direct effect on pituitary prolactin release *in vitro* (6), it can be concluded that they act through the hypothalamus *in vivo*. This does not exclude the possibility that P and testosterone may be converted to estrogens *in vivo* (19, 20) before acting on the hypothalamus.

In rats estrogen promotes prolactin release by decreasing hypothalamic PIF content (8) and by directly stimulating the pituitary (6). If P and testosterone are converted into estrogens *in vivo,* they could similarly promote prolactin secretion. Rothchild (2) proposed that a positive feedback occurs between P and prolactin secretion in the rat, and that P probably depresses CNS inhibition of pituitary prolactin secretion. The present results lend support to this idea, although the dose of P (10 mg daily) used was high. Since there is no conclusive evidence that cortisol can be converted into estrogens, it can be assumed that it acted as such on the hypothalamus.

Summary. The effects of P, TP, and CA on hypothalamic PIF content, pituitary prolactin concentration, and mammary development were studied in mature ovariectomized rats. Each of the 3 steroids significantly decreased hypothalamic PIF content, increased pituitary prolactin concentration and, induced mammary growth. The TP and CA also elicited mammary secretion. These observations indicate that the stimulatory effect of the 3 hormones on pituitary prolactin secretion is mediated through the hypothalamus.

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Inhibition of DNA Polymerase by β-D-Arabinosylcytosine and Reversal of Inhibition by Deoxycytidine-5′-triphosphate* (32708)

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The antitumor activity of β-D-arabinosylcytosine (ara-c)[2] has been well established (1). One site of action implicated has been inhibition of cytidine diphosphate reductase (2,3). Possible inhibitions by ara-c of the mammalian adenylate and guanylate reductases were also explored (4,5). The cytidine diphosphate and adenylate reductases were found to be inhibited by ara-c (3–5). The inhibitory effects on these 2 reductases, however, were minimal (3,4). In the case of the adenylate reductase, paradoxical drug effects were obtained with enzymes from the Ehrlich and Ca755 ascites tumors (4,5), i.e., inhibitions were reversed with increasing concentrations of drug. Since the ribonucleotide reductases have been shown to be regulatory enzymes for DNA synthesis (5,6,7), presumably, even minimal effects by ara-c on the reductases might eventually lead to profound inhibitory effects on DNA synthesis. Our previous study of the effects of ara-c on the incorporation of glycine-2-^{14}C, adenine-8-^{14}C, and orotic acid-6-^{14}C into the nucleic acids of the Ehrlich ascites tumor had implicated another site of inhibition, namely, the DNA polymerase of the Ehrlich tumor (3). In the study presented here, we have found that ara-c produced inhibitory effects on the utilization of thymidine-^{3}H for DNA synthesis by the Ehrlich ascites tumor. The DNA polymerase was found to be inhibited by ara-c, and this inhibition could be reversed by addition of deoxycytidine-5′-triphosphate to the medium.

Materials and Methods. Random bred female Swiss mice (Simonsen Laboratories, Gilroy, California) were each implanted with 7×10^5 Ehrlich ascites cells and used 5–6 days later for *in vivo* and *in vitro* metabolic studies.

Studies in vivo *and* in vitro. Mice were used for *in vivo* metabolic studies 6 days after implantation of tumor cells. Each mouse received either saline, ara-c, deoxycytidine, or ara-c plus deoxycytidine. One hour later each mouse received thymidine-^{3}H, 3 μmoles, 8.8 $\times$ 10^6 cpm/μmole (Schwarz BioResearch, Inc.) and metabolic utilization was allowed to occur for 1 hour. The fractionation and preparation of the Ehrlich ascites cell DNA has been described in detail elsewhere (3). For *in vitro* studies with Ehrlich ascites cell suspension, Swiss mice with 5-day implants were used as a source of cells. The incubation mixtures in 32 ml of Kreb's original Ringer phosphate medium, pH 7.4 (no $CaCl_2$), contained the following: glucose, 5.5×10^{-3} *M*; thymidine-^{3}H, 5×10^{-4} *M*, 8.8×10^6 cpm/μmole; and Ehrlich cells, 25 mg wet weight per ml of medium. Ara-c, 10^{-3} *M*, dissolved

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[2] The abbreviations used are: β-D-arabinosylcytosine, ara-c-DNA, deoxyribonucleic acid; dCTP, deoxycytidine-5′-triphosphate; TTP, thymidine-5′-triphosphate; TMP, thymidine-5′-monophosphate; ATP, adenosine-5′-triphosphate; dGTP, deoxyguanosine-5′-triphosphate; TCA, trichloroacetic acid; Tris buffer, tris(hydroxymethyl)aminomethane buffer.