Estrogen-Stimulated Progesterone Synthesis by Rabbit Corpora Lutea *in Vitro*[1] (35616)

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There is increasing evidence from *in vivo* studies that estrogen exerts a direct luteotropic stimulus on progesterone biosynthesis in rabbit corpora lutea. Robson (1) reported that daily injections of estrogen maintained the normal morphological appearance of corpora in hypophysectomized rabbits. Similarly, Hammond and Robson (2) found that estrogen implanted adjacent to corpora lutea prolonged their life span, indicating a direct effect on the structural integrity of luteal cells. Recently, the tropic effect of estrogen was well demonstrated in hypophysectomized rabbits (3), in rabbits with X-irradiated ovaries (4), and in rabbits treated with antigonadotropic sera (5). The follicles, responding to LH stimulation, appear to be the major source of estrogen during pregnancy and pseudopregnancy and must be present for continued luteal function (6). In the absence of follicles, exogenous estrogen was shown to stimulate corpus luteum activity, whereas LH injections failed to do so (4). *In vitro* experiments have been employed with limited success. Armstrong (7) reported that, in a preliminary study, estradiol failed to stimulate progesterone synthesis by rabbit luteal tissue. Addition of LH to slices of isolated rabbit corpora lutea has been shown to increase progesterone production only slightly (8, 9). In spite of the failure to demonstrate an *in vitro* effect of estradiol, current evidence suggests that estrogen is the luteotropic hormone in the rabbit. The present *in vitro* study was undertaken to establish a direct effect of estrogen on progesterone biosynthesis in isolated rabbit corpora lutea.

Materials and Methods. Twenty-six pregnant Dutch Belted rabbits were utilized in two experiments consisting of two *in vitro* incubations per experiment. On day 11 of pregnancy (day 0 = day of mating) six to seven animals were sacrificed for each incubation by cervical dislocation, and the ovaries were removed. One-half of these animals received single intravenous injections of aminoglutethimide phosphate (Elipten), an inhibitor of cholesterol hydroxylation, 1 hr prior to sacrifice at a dose level of 50 mg/kg body weight. After removal the ovaries were placed in iced 0.9% saline and immediately removed to the laboratory where the corpora were dissected from the interstitial tissue, sliced, washed in cold saline, and randomly distributed to incubation vials. Numbers of corpora lutea ranged from 6–12 per rabbit. Luteal tissue samples were weighed on a Roller-Smith balance and weights per incubation vial were adjusted to within a 10-mg range. Approximately 55 mg of tissue were added to each vessel.

Incubations were carried out in a Dubnoff metabolic shaker for 2 hr at 37–38° in 2 ml of Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2. The buffer routinely contained 2 mg glucose per ml and 30 mmoles nicotinamide. In order to increase the solubility of estrogen in an aqueous incubation medium, estradiol 17-β (5 μg) was bound to serum proteins and added to the incubation vials utilizing a method similar to that of Newman and Zilversmit (10). Incorporation of the protein-bound steroid was accomplished by transferring ^{14}C-labeled and unlabeled estradiol 17-β, in known quantity

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TABLE I. Effect of Estradiol 17-β on Progestin Biosynthesis and Sterol Content of Rabbit Corpora Lutea *in Vitro*.[a]

	Incubated control		Estrogen	
	Incubation 1	Incubation 2	Incubation 1	Incubation 2
Number samples	5	6	5	6
Net progesterone synthesis (μg/g)	22.60 ± 3.24	37.50 ± 1.94	34.00 ± 2.90[b]	43.20 ± 2.21[b]
Net 20α-ol synthesis (μg/g)	1.22 ± 0.30	3.38 ± 0.41	2.38 ± 0.67[c]	4.32 ± 0.24[c]
Free sterol content (mg/g)		3.30 ± 0.33		3.10 ± 0.22[d]
Sterol ester content (mg/g)		7.00 ± 0.52		6.40 ± 0.65[d]

Analysis of variance of progesterone and 20α-ol synthesis

Source	*df*	Adjusted *MS* (progesterone)	Adjusted *MS* (20α-ol)
Incubation	1	864.00[e]	25.22[e]
Treatment	1	433.44[e]	6.07[f]
Interaction	1	50.52	.01
Error (from original AOV)	22	42.35	.95

[a] Each value is the mean for the incubation ± the standard error of the mean.
[b] Significantly different from incubated controls ($p < .01$).
[c] Significantly different from incubated controls ($p < .05$).
[d] Nonsignificant.
[e] $p = .010$.
[f] $p = .050$.

or activity to small pieces of filter paper. The paper was then incubated for 3 hr in serum obtained from a hypophysectomized–ovariectomized doe. After the incubation period a .1-ml aliquot of the serum containing the labeled estradiol was counted to determine the amount of estrogen transferred from the paper and presumably bound in the protein fraction. Ninety-five to one hundred percent of the labeled estradiol was absorbed by the serum. In this manner the amount of unlabeled estrogen in the serum could be closely approximated and added to the incubation vials in predetermined amounts. Usually .04–.06 ml of serum or serum–estradiol was added to the buffer medium. After the incubation period the samples were frozen on Dry Ice and stored in a freezer at −20° for subsequent analysis.

Methods used for extraction and purification of progesterone and 20α-hydroxypregn-4-en-3-one (20 α-ol) were carried out, with few modifications, as described by Armstrong *et al.* (11). Progesterone and 20 α-ol quantification was by gas–liquid chromatography, as previously reported (12). Sterols were measured spectrophotometrically using the Liebermann–Burchard reaction.

By definition, "net progesterone synthesis" is the difference between the number of micrograms of progesterone per gram tissue before and after incubation. Free and esterified sterol includes those unesterified and esterified Libermann–Burchard reactive sterols with the same *RF* value as cholesterol and cholesterol ester.

Aliquots of the steroids were assayed for radioactivity in a liquid-scintillation spectrometer, and corrections were made for procedural losses. An analysis of variance using unweighted means was utilized to test significance among the various treatment groups (13).

Results and Discussion. In the present

TABLE II. Progestin and Sterol Content of Rabbit Luteal Tissue after *in Vivo* Administration of Aminoglutethimide.[a]

	Control	Aminoglutethimide
Progesterone content (μg/g)	18.40 ± 1.45 (3)[b]	3.75 ± 0.78 (2)
20α-ol content (μg/g)	3.07 ± 0.67 (3)	1.05 ± 0.07 (2)
Free sterol content (mg/g)	3.50 ± 0.10 (2)	4.25 ± 0.14 (2)
Sterol ester content (mg/g)	6.40 ± 0.20 (2)	12.10 ± 0.21 (2)

[a] Each value represents the content in luteal tissue prior to *in vitro* incubation.
[b] Numbers in parentheses indicate number of observations.

study the increased synthesis of progesterone and 20α-ol demonstrates the direct luteotropic effect of estradiol 17-β under *in vitro* conditions. There were no significant changes in levels of cholesterol and cholesterol esters (Table I).

The net increase in progesterone of 11 and 6 μg/g of tissue for incubations 1 and 2, respectively, could be considered a small increase when contrasted with the response to LH of bovine (14), ovine (15), or rat (11) luteal tissues *in vitro*. However, the finding that sterol stores failed to reflect a conversion to progesterone after stimulation with estradiol might account for this minimal response in the rabbit. Cholesterol is a known precursor for progesterone biosynthesis (9, 16). It is also conceivable that the maximum steroidogenic response of rabbit luteal tissue to *in vitro* stimulation was attained. The significant increase in 20α-ol probably results from the increased synthesis of progesterone with its concurrent conversion to the hydroxylated steroid.

Surprisingly, the magnitude of the net increase in estradiol-stimulated progesterone production was similar to that reported for LH stimulation (8, 9). If estrogen is the "luteotropic" hormone of the rabbit as concluded from *in vivo* studies, one might expect a much greater response to estrogen stimulation than to LH. This paradox remains to be resolved.

Aminoglutethimide administered 1 hr prior to sacrifice resulted in a decrease in progestin content and an accumulation of sterols in luteal tissue *in vivo* (Table II). Comparable effects on steroid synthesis and sterol levels have been observed by Cohen (17) in the adrenal gland and by Wilks *et al.* (9) in ovarian tissue. It was anticipated that this enlargement of the steroidogenic cholesterol pool would effect a greater synthesis of progesterone with estradiol stimulation. However, amino glutethimide *in vivo* completely inhibited any *in vitro* steroidogenic action of gesterone (Table III). Although net progesterone synthesis was not different from untreated rabbits, LH stimulated *in vitro* progesterone biosynthesis following *in vivo* administration of aminoglutethimide (9). This would suggest that the biochemical effect of estrogen on steroidogenesis in rabbit corpora lutea differs from LH under *in vitro* conditions. As might be expected, with no change in progesterone levels, 20α-ol and sterol content remained unchanged by the estradiol treatment.

This report of a direct effect of estrogen on progesterone biosynthesis by rabbit corpora lutea supports the conclusions made on the basis of *in vivo* studies. The limited response of separated rabbit luteal tissue to *in vitro* procedures which have been utilized successfully for other species is difficult to understand. Whether this indicates an inadequate incubation system for rabbit corpora lutea or a unique steroidogenic control mechanism in the species is not known. Experiments are currently underway to define more precisely the luteotropic role of estrogen for steroid biosynthesis in the rabbit.

Summary. Rabbit corpora lutea were incubated with estradiol 17-β to verify a direct

TABLE III. Effect of *in Vivo* Aminoglutethimide Pretreatment on *in Vitro* Estradiol 17-β-Stimulated Progestin Biosynthesis and Sterol Content of Rabbit Corpora Lutea.[a]

	Aminoglutethimide		Aminoglutethimide + estrogen	
	Incubation 3	Incubation 4	Incubation 3	Incubation 4
Number samples	7	5	7	5
Net progesterone synthesis (μg/g)	34.90 ± 2.12	8.20 ± 0.50	34.10 ± 2.20[b]	7.20 ± 1.02[b]
Net 20α-ol synthesis (μg/g)	3.66 ± 0.35	0.14 ± 0.04	4.18 ± 0.20[b]	0.14 ± 0.03[b]
Free sterol content (mg/g)	5.90 ± 0.28	—	5.60 ± 0.36[b]	—
Sterol ester content (mg/g)	13.90 ± 0.63	—	13.80 ± 0.96[b]	—

[a] Each value is the mean for the incubation ± the standard error of the mean.
[b] Not significantly different from incubated controls.

stimulatory role of estrogen on progesterone biosynthesis. A significant increase in net progesterone and 20α-ol synthesis was obtained with no concurrent alteration in free and esterified cholesterol content of luteal tissue. Pretreatment with aminoglutethimide *in vivo* resulted in accumulation of cholesterol in luteal tissue, but estrogen failed to stimulate progesterone synthesis above control levels.

These results demonstrate a direct effect of estradiol on rabbit corpora lutea *in vitro*, but suggest the possibility of a unique steriodogenic mechanism for the species.

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