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Peripheral Blood and Ovarian Levels of Sex Steroids in the Cyclic Hamster

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ABSTRACT

Changes in progesterone (P), testosterone (T), estrone (E_1) and estradiol-17β (E_2) in peripheral blood, ovary, corpora lutea (CL) and in the nonluteal portion of the ovary (NLO) were studied in the cyclic hamster by RIA. In addition, androstenedione (A) was measured, but only in serum. Serum P levels were the same on Days 1 (day of ovulation) and 2 at 0900 h (5 ng/ml), but declined markedly on Day 3 and on the morning of Day 4 (proestrus). After the LH surge on the afternoon of Day 4, serum and ovarian levels of P increased abruptly until 1900 h to 11–18 ng/ml and declined slowly thereafter. Peak T levels in both serum (209 pg/ml) and the ovary occurred between 1400–1500 h of Day 4, paralleling the rising P levels. However, T levels declined rapidly after 1600 h to undetectable levels by 1900 h, but there was a second peak of T by 2300 h in serum which was matched by the ovary.

Serum levels of E_1 and E_2 began to rise on Day 2, reaching peak values by Day 4 between 1200 and 1400 h (41 pg/ml and 152 pg/ml, respectively). Peak ovarian concentrations of E_1 and E_2 were reached on Day 4 at 0900 h. These high levels were maintained up to 1600 h of Day 4 and declined rapidly thereafter. The serum levels of E_1 were about one-third as high as E_2 and were not as drastically affected on the evening of Day 4.

Serum androstenedione ranged from 1–2 ng/ml, but changes throughout the cycle were not as striking as for the other steroids. However, increased levels of A were associated with decreased levels of estrogens and vice-versa. Therefore, the circulating levels of A probably reflect altered secretion rates of other steroids.

Administration of 5 μg LH on the evening of Day 4 (when all steroid levels are declining) caused significant increases in P and T but not E_2. Hence, the refractory period of estrogen synthesis on the evening of Day 4 cannot be attributed to insufficient levels of LH during the preovulatory period.

INTRODUCTION

There are a few isolated reports on circulating levels of progesterone (Leavitt and Blaha, 1970; Lukaszewska and Greenwald, 1970; Shaikh and Saksena, 1972; Blaha and Leavitt, 1974; Ridley and Greenwald, 1975) and estrogen (Shaikh, 1972; Baranczuk and Greenwald, 1973) in the cyclic hamster. However, blood levels of androstenedione (A) and testosterone (T) have not been reported. Similarly, the concentrations of progesterone (P), testosterone, estrone (E_1) and estradiol (E_2) in the ovary *in toto* and in luteal, as opposed to nonluteal compartments has not been documented.

The present paper was therefore designed to provide a comprehensive account of changes in sex steroids throughout the 4 day estrous cycle of the hamster by measuring all of the aforementioned steroids in serum and ovary.

MATERIALS AND METHODS

Adult female golden hamsters *(Mesocricetus auratus)* weighing 80–120 g and maintained on a 14 h light:10 h dark schedule (lights on: 0500 h–1900 h, CST) were used after 3 or 4 consecutive 4 day cycles with 6–8 animals in each group. Day 1 is defined as the day of ovulation. Hamsters were killed by decapitation on Days 1–4 at 0900 h and on Day 4 animals were also used at 1200, 1400, 1500 (proestrus), 1600, 1700, 1800, 1900 and 2300 h (estrus). The trunk blood was allowed to clot at 4°C for 24 h and serum was then collected and kept frozen until assayed. One

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ovary was weighed to the nearest 0.01 mg and stored at −20°C in vials containing 0.4 cc 95% alcohol. From the other ovary, corpora lutea (CL) and the nonluteal portion of the ovary (NLO) were separated, weighed and likewise stored in alcohol. However, due to the profound luteal regression on Day 4, it was not possible to dissect out CL at different times of Day 4 and therefore the entire ovary was used in animals killed from 1200 h and on.

Steroid Assays

The radioimmunoassays (RIAS) used for determination of steroids in sera and tissues were similar to procedures described previously for progesterone by Thorneycroft and Stone (1972), using specific antisera for progesterone (Surve et al., 1976), testosterone (Pang and Johnson, 1974), androstenedione (antiserum obtained from Dr. John Resko, Regional Primate Research Center, Beaverton, Oregon), estrone (Wright et al., 1973) and estradiol-17β (Exley et al., 1971). The testosterone antiserum cross reacted 58% with 5α-dihydrotestosterone and 2% or less with androstenedione. In the cyclic hamster, the preovulatory surge of androgen represents testosterone exclusively and no detectable amounts of DHT are present (Connor and Greenwald, unpublished). Hormone levels determined with this antiserum are therefore referred to as testosterone. The antiserum for androstenedione cross reacted 70% with 5α-androstane-3,17-dione and about 4% with dehydroepiandrosterone (DHEA). Hormone levels measured by this antiserum are collectively referred to as androstenedione. The antiserum for estrone cross reacts only 1% with estradiol.

For each steroid assay, quantities of sera, homogenized tissues and standards were diluted to 1.0 cc with distilled water and extracted with 2.0 cc anhydrous diethyl ether. For RIA of progesterone (P), 20 μl of sera, 1−5 μl of homogenized tissue and 5 μl (5.0−400 pg) standard P in methanol were assayed. For RIA of androstenedione (A), 20 μl of serum and 5 μl (4.0−1000 pg) standard A were used. For RIAs of testosterone (T), estrone (E_1) and estradiol (E_2), 300 μl of serum, 50 μl of homogenized tissues and 5 μl (2.0−300 pg) standard T, E_1 or E_2 in methanol were assayed. After extraction with ether, each tube was dried using a vacuum evaporator. The dry residue was suspended in 0.2 cc phosphate buffered gelatin (PBSG, 0.1 M phosphate buffer, pH 7.0), stirred on a vortex and incubated in a 45°C water bath for 20 min. To each assay tube, 0.1 cc antibody (dilution: 1/3000 for T; 1/50,000 for P and E_2; 1/3500 for A; and 1/25,000 for E_1) and 0.1 cc radiolabeled steroid in 0.1 M PBSG were added. The assay tubes were stirred on a vortex and incubated for 24 h at 4°C. Free and bound steroids were separated at 4°C by adding 0.8 cc PBSG containing 0.625% charcoal Norit A and 0.0625% dextran. The assay tubes were vortexed and incubated for 20 min. The tubes were then centrifuged at 1500 × g for 20 min. The supernatants were decanted into counting vials and 1.0 cc dioxane and 9.0 cc toluene counting solution (15 g omnifluor/gallon toluene) were then added. Each vial was counted for 10 min in a Packard Tri-Carb liquid scintillation counter, Model 3003.

The percentage recovery of internal standard from serum as well as tissues for all the steroids ranged from 80−90%. The lower limit of sensitivity for different steroid assays was as follows: P, 5.0 pg; A, 4.0 pg; T, E_1 and E_2, 2.0 pg. Serum from ovariectomized (6 weeks) and hypophysectomized (1 week before the autopsy) hamsters contained approximately 0.6 ng P/ml; 0.5 ng A/ml; 30 pg T/ml; 9.0 pg E_1/ml and 12 pg E_2/ml, respectively. The hormone levels for tissues are expressed in pg or ng/mg wet weight of tissue. More details of the assay procedures are given in a recent paper (Terranova and Greenwald, 1978).

Data were analyzed using Duncan's Multiple Range Test (Steel and Torrie, 1960) and where appropriate, Student's t test was employed. Differences were adjudged significant if $P<0.05$.

RESULTS

Changes in Progesterone (P) During the Cycle

On Days 1 and 2 of the cycle, serum P was relatively constant but the levels decreased significantly on the mornings of Days 3 and 4, with the nadir reached by 0900 h of Day 4. During proestrus (afternoon of Day 4), the first significant rise in serum P occurred at 1500 h and the levels continued to rise until 1800 h followed by a slow decline with time (Table 1, Fig. 1).

The ovarian concentration of P paralleled the serum levels. There was a significant rise in ovarian P at 1400 and 1500 h but, thereafter, the concentration was relatively stable until 1900 h when a significant decrease was observed (Table 2, Fig. 2).

Luteal concentration of P increased significantly on Day 2, followed by a precipitous decrease on Days 3 and 4. The highest value of P in the nonluteal ovary was on Day 1 with a sharp drop by the next day of the cycle. Thereafter, P concentration dropped progressively in the nonluteal ovary with the lowest values reached by the morning of Day 4 (Table 3).

Changes in Estradiol (E_2) During the Cycle

On Day 1 of the cycle, serum levels of E_2 were barely above the sensitivity of the assay (12 pg/ml). The levels doubled by Day 2 and subsequently tripled by Day 3. The highest serum E_2 values were found on the afternoon of Day 4, but showed no appreciable increase at 1500 h (when serum P abruptly increased). A striking progressive decline in serum E_2 began at 1700 h of Day 4, culminating at 2300 h in values approximating the values at Day 1 0900 h (Table 1, Fig. 1).

The ovarian concentration of E_2 reached its

TABLE 1. Steroid hormone levels in the peripheral blood serum of cyclic hamsters.

Day and hour of the cycle	Progesterone	Andro-stenedione	Testosterone	Estradiol	Estrone
	(ng/ml)		(pg/ml)		
Day 1 0900 h	5.1 ± 0.6 (8)	1.2 ± 0.1 (8)	73.2 ± 2.3 (7)	17.6 ± 1.2 (7)	10.8 ± 0.7 (7)
Day 2 0900 h	5.2 ± 0.3 (8)	1.4 ± 0.1 (8)	73.6 ± 8.4 (8)	31.9 ± 6.3 (8)	15.1 ± 0.8 (8)
Day 3 0900 h	1.5 ± 0.2 (8)[a]	1.3 ± 0.1 (8)	91.0 ± 8.0 (8)	89.3 ± 9.4 (9)[b]	31.1 ± 1.7 (8)[b]
Day 4 0900 h	0.6 ± 0.2 (8)[b]	1.4 ± 0.1 (8)	80.3 ± 6.2 (8)	93.3 ± 10.3 (9)	30.3 ± 3.1 (8)
Day 4 1200 h	1.4 ± 0.9 (4)	1.0 ± 0.1 (8)[b]	64.3 ± 3.5 (8)	152.0 ± 11.9 (6)[b]	29.1 ± 1.7 (8)
Day 4 1400 h	3.5 ± 1.4 (8)	1.2 ± 0.1 (8)	209.3 ± 18.4 (8)[b]	121.4 ± 14.9 (8)	40.9 ± 1.7 (8)[b]
Day 4 1500 h	11.0 ± 1.0 (9)[b]	1.5 ± 0.1 (8)[a]	156.4 ± 12.3 (8)[b]	158.3 ± 27.2 (7)	37.9 ± 1.7 (8)
Day 4 1600 h	14.0 ± 1.2 (8)	1.3 ± 0.1 (8)	141.3 ± 22.8 (7)	101.7 ± 13.7 (8)[b]	28.6 ± 1.3 (8)[b]
Day 4 1700 h	17.0 ± 1.5 (8)	1.7 ± 0.1 (8)[a]	77.6 ± 13.0 (8)[b]	53.2 ± 8.1 (6)[bd]	23.9 ± 1.5 (8)
Day 4 1800 h	18.2 ± 1.2 (8)	1.9 ± 0.2 (8)	70.2 ± 5.9 (8)	24.2 ± 3.1 (7)[c]	25.7 ± 3.7 (8)
Day 4 1900 h	16.5 ± 1.0 (8)	1.4 ± 0.1 (8)[b]	4.3 ± 0.0 (1)[e]	27.7 ± 3.1 (8)	21.5 ± 0.8 (8)
Day 4 2300 h	12.2 ± 0.8 (8)[b]	1.2 ± 0.1 (8)	62.4 ± 9.9 (8)[b]	19.7 ± 2.5 (8)	7.8 ± 1.6 (8)[a]

P values were calculated using Duncan's Multiple Range Test.

[a] $P<0.05$.

[b] $P<0.01$.

[c] 1 animal had undetectable levels of the hormone.

[d] 2 animals had undetectable levels of the hormone.

[e] 6 animals had undetectable levels of the hormone.

Figures in parentheses indicate number of animals.

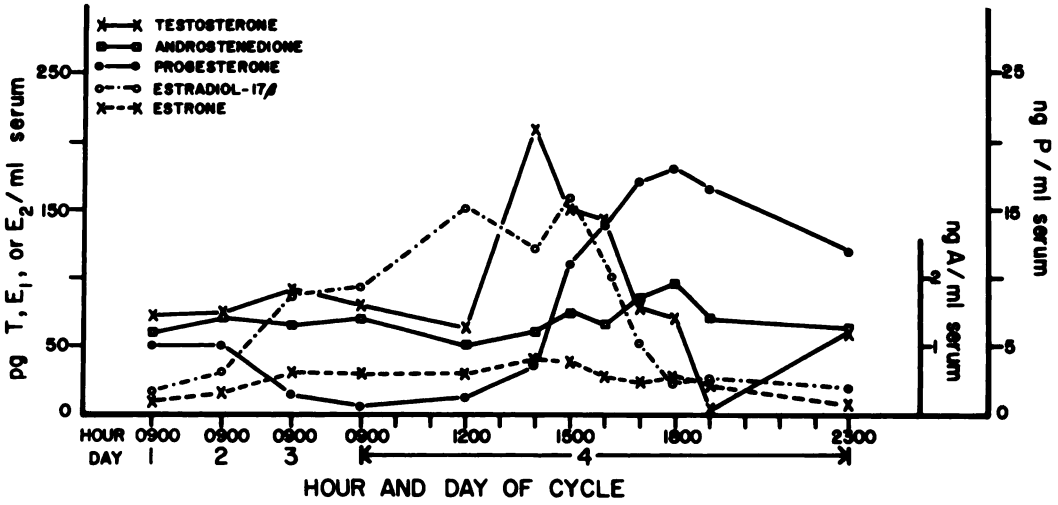

FIG. 1. Serum levels of steroid hormones in the cyclic hamster.

peak at 0900 h of Day 3, at which time the values remained at high levels until 1500 h of Day 4. The fall in ovarian concentration of E_2 was evident at 1600 h of Day 4; 1 h before serum levels of E_2 began to drop (Table 1, Fig. 1).

Luteal E_2 rose from the lowest values at Day 1 to a peak on Day 3 of the cycle. This was followed the next morning by a 50% reduction in luteal E_2 (Table 3). The concentration of E_2 in the nonluteal ovary shows a different pattern with increasingly high levels until Day 3. Comparison of the E_2 concentration in the

TABLE 2. Ovarian steroid hormone levels in the cyclic hamster.

Day and hour of the cycle	Progesterone	Testosterone	Estradiol	Estrone
	(ng/mg)	(pg/mg)		
Day 1 0900 h	8.8 ± 0.4 (7)	14.5 ± 1.5 (8)	3.6 ± 1.2 (7)	2.3 ± 0.3 (7)
Day 2 0900 h	8.2 ± 0.4 (8)	24.6 ± 2.6 (8)	67.0 ± 10.2 (8)	19.2 ± 3.9 (8)
Day 3 0900 h	4.3 ± 0.5 (8)	68.7 ± 12.1 (8)[b]	261.3 ± 68.8 (8)[b]	92.9 ± 15.7 (8)[b]
Day 4 0900 h	1.0 ± 0.1 (8)	58.0 ± 11.8 (8)	270.2 ± 48.5 (8)	141.9 ± 11.6 (8)[b]
Day 4 1200 h	0.7 ± 0.1 (6)	58.6 ± 14.7 (6)	173.6 ± 36.5 (5)	81.0 ± 16.5 (8)[b]
Day 4 1400 h	7.4 ± 3.2 (8)[b]	171.1 ± 14.9 (8)[b]	212.4 ± 18.6 (8)	77.8 ± 10.5 (8)
Day 4 1500 h	24.6 ± 2.2 (9)[b]	205.9 ± 10.0 (8)	229.1 ± 33.0 (8)	59.8 ± 11.4 (8)
Day 4 1600 h	29.3 ± 1.9 (7)	82.0 ± 16.6 (8)[b]	47.1 ± 9.2 (6)[bc]	10.2 ± 1.9 (8)[b]
Day 4 1700 h	28.9 ± 0.9 (7)	34.4 ± 1.9 (8)[b]	17.2 ± 6.0 (4)[d]	8.1 ± 2.2 (8)
Day 4 1800 h	30.7 ± 2.4 (8)	25.1 ± 1.4 (8)	7.0 ± 5.6 (2)[e]	4.4 ± 0.7 (8)
Day 4 1900 h	17.5 ± 0.9 (8)[b]	8.5 ± 2.3 (8)	5.6 ± 2.0 (8)	2.2 ± 0.7 (8)
Day 4 2300 h	11.2 ± 1.3 (8)[b]	11.5 ± 1.5 (8)	1.7 ± 0.5 (5)	1.0 ± 0.1 (8)

P values were calculated using Duncan's Multiple Range Test.

[a] $P<0.05$.

[b] $P<0.01$.

[c] 2 animals had undetectable levels of the hormone.

[d] 4 animals had undetectable levels of the hormone.

[e] 6 animals had undetectable levels of the hormone.

Figures in parentheses indicate the number of animals.

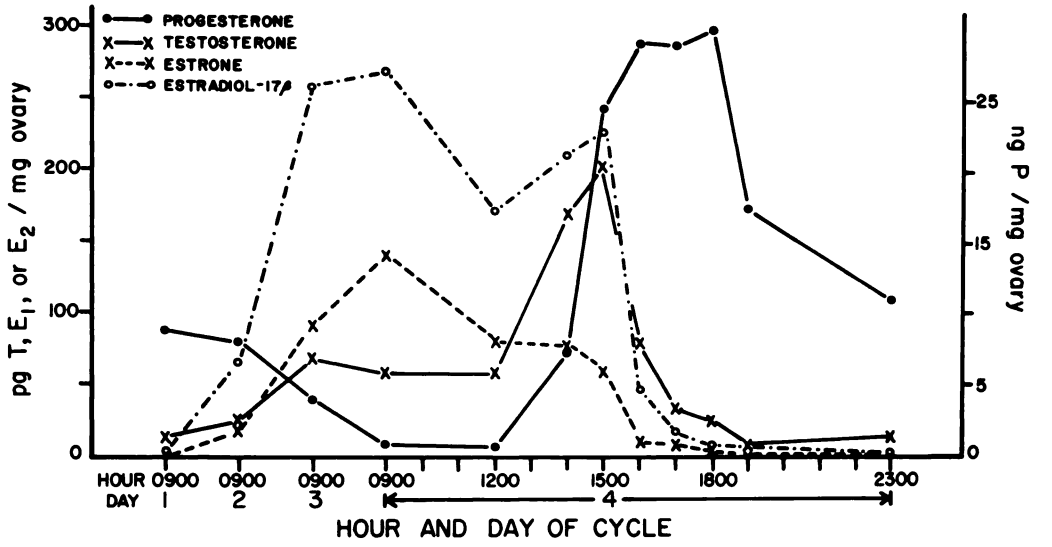

FIG. 2. Ovarian levels of steroid hormones in the cyclic hamster.

nonluteal ovary (Table 3) with the ovarian concentration (Table 2) indicates that almost all of the hormone produced by the ovary can be accounted for by the nonluteal compartment.

Changes in Estrone (E_1) During the Cycle

Changes in serum E_1 closely resembled the pattern of E_2, but E_1 levels were usually one-third to one-half the values for estradiol (Table 1, Fig. 1). The highest serum E_1 levels were reached on the afternoon of Day 4 at 1400 and 1500 h. This was followed by a significant decrease at 1600 h, but the decline in E_1 was not of the magnitude of the plummeting E_2 levels.

TABLE 3. Steroid hormones in corpora lutea and nonluteal ovary of the cyclic hamster.

Tissue	Day of the cycle (0900 h)	Progesterone	Testosterone	Estradiol
		(ng/mg)	(pg/mg)	
Corpora lutea	1	10.2 ± 0.9 (8)	21.7 ± 7.7 (5)	6.5 ± 1.9 (8)
	2	37.1 ± 10.4 (8)[b]	46.7 ± 9.9 (8)	31.8 ± 6.7 (8)[c]
	3	10.9 ± 1.7 (9)[b]	49.3 ± 9.5 (9)	75.0 ± 11.4 (6)[b]
	4	3.9 ± 0.1 (2)[b]	f	37.5 ± 11.9 (2)[a]
Nonluteal ovary	1	9.7 ± 0.6 (8)	5.8 ± 1.7 (7)	2.0 ± 0.8 (6)
	2	2.5 ± 0.3 (8)[d]	20.2 ± 2.1 (7)[d]	47.5 ± 4.8 (7)[d]
	3	1.5 ± 0.3 (8)[a]	47.1 ± 7.7 (8)[c]	246.4 ± 30.6 (7)[d]
	4	0.7 ± 0.1 (9)[ce]	37.4 ± 9.8 (8)[e]	205.6 ± 33.7 (9)[e]

P values were calculated using Student's *t* test, and with the preceding figures in the table.

[a] $P<0.05$.

[b] $P<0.01$ or 0.02.

[c] $P<0.005$.

[d] $P<0.001$.

[e] Entire ovary.

[f] Insufficient tissue was available to determine T.

The ovarian concentration of E_1 was similar to that of E_2 with progressive increases on Days 2–4 of the cycle (Table 2, Fig. 2). The peak in ovarian E_1 was at 0900 h of Day 4 and thereafter the levels fell abruptly in the face of maintenance of high serum levels of E_1 until 1500 h of Day 4.

Changes in Testosterone (T) During the Cycle

Serum T levels were stable at about 75 pg/ml from 0900 of Day 1 until Day 4 (Table 1, Fig. 1). An explosive increase in serum T occurred at 1400 h on Day 4. Subsequently, at 2 h intervals, the levels were approximately halved with the nadir reached at 1900 h. However, by 2300 h, serum T increased to levels comparable to the Day 1 0900 h values.

The ovarian maintenance of T closely reflected the serum pattern (Table 2, Fig. 2). The surge of serum T on the afternoon of Day 4 was paralleled by a 3–4 fold increase in ovarian T. An abrupt decline in ovarian T began at 1600 h with the lowest levels found at 1900 h and 2300 h.

The luteal concentration of T increased from Days 1–3, but the increase was not significant (Table 3). For the nonluteal ovary, there were significant increases in T on Days 2 and 3. Note from Table 3 that the nonluteal ovary contained 2–6 times as much E_2 as T.

Changes in Androstenedione (A) During the Cycle

Androstenedione was only measured in peripheral blood (Table 1, Fig. 1). Serum A ranged from 1–2 ng/ml and the levels were constant from 0900 h on Day 1–0900 h of Day 4. After a significant drop of serum A at 1200 h on Day 4, there was a significant increase at 1500 h. A further increase in serum A occurred at 1700–1800 h of Day 4, coinciding with the fall in both serum T and E_2.

Effects of Administration of LH at 1900 h Day 4 on Steroid Levels at 2300 h

After the LH surge, serum levels of LH revert to baseline values by 2200 h of Day 4 (Siegel et al., 1976). The final experiment was designed to determine whether exogenous LH could alter the normal pattern of steroidogenesis observed at 2300 h of Day 4. Hamsters were injected s.c. at 1900 h with either saline or 5 μg ovine LH (NIH, S-18).

The results in Table 4 indicate that adminis-

TABLE 4. Effect of 5 μg LH injected s.c. at 1900 h of Day 4 on steroid levels at 2300 h.

Treatment	Progesterone			Testosterone			Estradiol		
	Serum		Ovary	Serum[d]	Ovary		Serum		Ovary
	(ng/ml)		(ng/mg)	(pg/ml)	(pg/mg)		(pg/ml)		(pg/mg)
Saline controls[c]	12.2 ± 0.8	(8)	11.2 ± 1.3 (8)	...	11.5 ± 1.5	(8)	19.7 ± 2.5	(8)	1.7 ± 0.5 (8)
5 μg LH	17.9 ± 1.7[a]	(8)	17.7 ± 1.3[b] (8)	...	25.6 ± 2.0[c]	(8)	32.3 ± 6.6[ns]	(8)	3.1 ± 0.8[ns] (7)

P values were calculated using the Student's *t* test; comparing the saline treated control versus the LH treated.

[a] $P<0.01$.

[b] $P<0.005$.

[c] $P<0.001$.

[ns] Not significant.

[d] Insufficient serum was available for determination of testosterone.

[e] Values from Tables 1 and 2.

Figures in parentheses indicate the number of animals.

tration of LH significantly increased the levels of both P and T, but that the secretion of estradiol was not enhanced by the gonadotropin.

DISCUSSION

Figures 1 and 2 summarize the interrelationships of the steroids in blood and ovary which were measured in this study. In general, for any steroid, there is an excellent correlation between blood and ovarian levels. The most abundant steroids are progesterone and androstenedione which are present in ng quantities, as compared to the pg amounts of the other steroids.

Serum and ovarian levels of estradiol are also considerably higher than estrone and the former hormone consequently undergoes more dramatic changes during the course of the 4 day cycle. The levels of testosterone in serum match E_2 levels through the morning of Day 4, but ovarian levels of E_2 are considerably higher. Serum levels of both the C-18 and C-19 compounds are much higher in the hamster than in the rat (Dupon and Kim, 1973; Butcher et al., 1974; Smith et al., 1975). Conversely, serum progesterone is higher in the cyclic rat than in the hamster (Butcher et al., 1974; Smith et al., 1975).

During the first 3 days of the hamster cycle there is a clearcut inverse relationship between progesterone and estradiol, with the turning point occurring between Days 2 and 3. During the first 2 days of the cycle, progesterone is the dominant hormone with the CL as the principal source. This is demonstrated by the concentration of the hormone in the CL, especially on Day 2. However, the nonluteal ovarian concentration of P is also high throughout the cycle, which may be contributed by both the antral follicles and the interstitium, both of which are histochemically active for 3β-hydroxysteroid dehydrogenase (Saidapur and Greenwald, 1978). While progesterone serves in its own right as a hormone during the first 2 days of the cycle, its subsequent decline in both serum and ovary suggests its major role thereafter is as a prehormone for conversion to C-19 and C-18 steroids.

It is noteworthy that the CL, in addition to progesterone, contained appreciable amounts of estradiol and testosterone (Table 3). The latter steroids showed appreciable increases on Day 2 and, thereafter, paralleled the increases in the nonluteal ovary. Whether luteal E_2 and testosterone are synthesized *de novo* or represent diffusion from the nonluteal compartment is obviously not resolved by this study. However, the histochemical presence of 17β-hydroxysteroid dehydrogenase in hamster CL has been demonstrated (Saidapur and Greenwald, 1978).

The increase in E_2 on Day 2 and onwards correlates with the development of antral follicles and the appearance in them of 3β-HSD, 17β-HSDH, G-6-PDH and ICDH (Saidapur and Greenwald, 1978). The preantral follicles do not possess 17β-HSDH (Saidapur and Greenwald, 1978). Hence, the low serum levels of E_2 on Day 1 presumably do not represent a follicular contribution. *In vitro* incubation studies have indicated that antral follicles, in particular the granulosa cells, are the major sites of estrogen synthesis in the hamster (Makris and Ryan, 1975, 1977).

Serum levels of androstenedione fluctuate usually around a baseline value of 1 ng/ml. Increases and declines around this value usually are correlated inversely with changes in testosterone and estradiol. This suggests that androstenedione is serving mainly as an intermediary steroid and its concentration probably indicates whether steroidogenesis is proceeding beyond this point at one time or another. Note from Table 1 and Fig. 1 that the serum contains 10–12 times as much androstenedione as testosterone, suggesting that the latter steroid is rapidly being converted to estradiol.

The most dramatic changes in steroid levels, both in peripheral circulation and in the ovary occur on the afternoon of proestrus (Day 4). Previous studies have demarcated the onset of gonadotropin release on Day 4 beginning between 1200 h and 1400 h (Siegel et al., 1976). The preovulatory rise in progesterone at 1500 h is definitely LH-dependent (Norman and Greenwald, 1971) as is most likely the case for a number of the other steroid changes on Day 4.

Both testosterone and progesterone increased abruptly on Day 4 between 1200 h and 1400 h, whereas the already very high serum levels of estradiol were unaffected. The most dramatic changes observed began at 1400–1500 h of proestrus with an hour by hour decline in testosterone and estradiol in both blood and ovarian concentration. Estrone also fell, but not to the same extent as T and E_2. A similar fall in estradiol occurs after LH stimulation in the rat (Butcher et al., 1974; Katz and Armstrong, 1976; Hillensjo et al.,

1976) and sheep (Moor, 1974). It appears that LH is the key factor in arresting the synthesis of estrogen, but whether it is a direct action or mediated by progesterone or other steroids has not been established.

The present study shows that testosterone synthesis begins to return by 2300 h at a time when estrogen synthesis is still depressed. Administration of LH at 1900 h (Table 4) can stimulate significant increases in progesterone and testosterone, but not estradiol. Hence, the refractory period of estrogen synthesis cannot be attributed to insufficient levels of LH during the preovulatory period.

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