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Uterine Prostaglandin $F_{2\alpha}$ Content and 20α-Hydroxysteroid Dehydrogenase Activity of Individual Ovarian Compartments During Pseudopregnancy in the Rat

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ABSTRACT

To investigate relationships between prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and ovarian 20α-hydroxysteroid dehydrogenase (20α-OH SDH), uterine $PGF_{2\alpha}$ content and ovarian 20α-OH SDH activity were measured during pseudopregnancy in the rat. Quantitative cytochemical techniques were employed to assess 20α-OH SDH activity in individual compartments of the rat ovary.

The data indicate appreciable 20α-OH SDH activity in corpora of pseudopregnancy, in involuting corpora formed in estrous cycles prior to pseudopregnancy, and in the interstitial tissue of the ovary of the pseudopregnant rat. Although enzymatic activity remained unchanged in the latter two compartments between Days 6 and 15 of pseudopregnancy, a twofold elevation in corpora of pseudopregnancy 20α-OH SDH activity was evidenced between Days 11 and 15. The close temporal relationship between increased uterine $PGF_{2\alpha}$ content (beginning on Days 9–10, peak on Days 11–12) and increased 20α-OH SDH activity in corpora of pseudopregnancy provides further evidence implicating $PGF_{2\alpha}$ as the putative factor responsible for inducing luteal 20α-OH SDH activity during luteal regression in the rat.

INTRODUCTION

It is well documented that ovaries of several mammalian species elaborate two progestins, progesterone and 20α-hydroxypregn-4-ene-3-one (Simmer et al., 1963; Fajer and Barraclough, 1967). Since 20α-hydroxypregn-4-ene-3-one (20α-OH P) is invariably a less potent progestin than progesterone (Wilcox and Wiest, 1960; Wiest and Forbes, 1964), it has been considered a catabolite of progesterone (Talwalker et al., 1966; Wiest et al., 1968). The concept that catabolism of progesterone to 20α-OH P may constitute an important aspect of the luteolytic process (Wiest et al., 1968) was formulated to explain the observation that in rats and rabbits, decreased ovarian venous plasma progesterone levels during luteal regression are associated with a concurrent rise in 20α-OH P levels (Hilliard et al., 1969; Wiest et al., 1968). More recent studies have also shown that luteal regression in these species is associated with increased ovarian activity of 20α-hydroxysteroid dehydrogenase (Bast and Melampy, 1972; Strauss et al., 1972), the enzyme responsible for converting progesterone to 20α-OH P. At present, however, the primary stimuli or events responsible for activation of 20α-hydroxysteroid dehydrogenase (20α-OH SDH) and the subsequent catabolism of progesterone to 20α-OH P remain uncertain.

Although Wiest et al. (1968) consider LH and prolactin to be the primary regulators of ovarian 20α-OH SDH activity, there is evidence which suggests uterine prostaglandins are also important in this regard. For example, it has been shown that peripheral plasma as well as ovarian levels of 20α-OH P rise as progesterone levels fall in prostaglandin $F_{2\alpha}$-treated pseudopregnant rats (Pharriss and Wyngarden, 1969; Lau et al., 1979). Recent work has also shown that the onset of augmented 20α-OH SDH activity in pseudopregnant rats is delayed by hysterectomy (Bast and Melampy, 1972). Shaikh et al. (1977) report that the increase in peripheral plasma 20α-OH P/progesterone ratio at the time of parturition in the rat is preceded by increases in E and F prostaglandin levels in uterine venous plasma. This evidence suggests that prostaglandins that are elaborated by the

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uterus are responsible for the increased 20α-OH SDH activity seen during naturally occurring luteal regression.

In view of the above findings, it was reasoned that if uterine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the agent responsible for inducing ovarian 20α-OH SDH activity, a precise temporal relationship would be expected between uterine $PGF_{2\alpha}$ production and the onset of increased 20α-OH SDH activity. The present study was therefore conducted to investigate relationships between these two parameters in the pseudopregnant rat. Quantitative cytochemical techniques were employed to allow measurement and exact localization of 20α-OH SDH activity within the ovary.

MATERIALS AND METHODS

Mature Sprague-Dawley female rats weighing 140–160 g were maintained under controlled lighting conditions (12L:12D; lights on 0700 h) and given free access to food and water. Rats exhibiting three consecutive 4 or 5 day estrous cycles were caged with vasectomized males on the evening of a proestrous vaginal smear. The first day of a leukocyte-infiltrated smear was designated as Day 1 of pseudopregnancy. Vaginal cytology was examined daily to ensure that animals were pseudopregnant. Uteri and ovaries were excised under light ether anesthesia between 1000–1100 h on specified days of pseudopregnancy.

Uteri from 38 rats were assayed for $PGF_{2\alpha}$ content as follows. Uteri were excised and immediately placed in phosphate-buffered saline (pH 7.4) containing 10 μg/ml indomethacin to prevent prostaglandin synthesis during subsequent processing. Uterine slices weighing 0.1–0.2 g were homogenized in a mixture of 1.0 ml phosphate-buffered saline and 3.0 ml of an ethyl acetate:isopropanol:0.2N HCl (3:3:1) solution. $PGF_{2\alpha}$ content was determined by double-antibody radioimmunoassay as described by Levine and Van Vunakis (1970), Jaffe et al. (1971), Hickler (1968), and Caldwell et al. (1971). The procedure did not entail chromatographic separation as the only prostaglandin with significant cross reactivity to the $PGF_{2\alpha}$ antiserum is $PGF_{1\alpha}$. As the cross reactivity of $PGF_{1\alpha}$ with the antiserum was determined to be 28% (at 50% inhibition), it is presumed the assay provides a reliable estimate of alterations in $PGF_{2\alpha}$ levels.

Twenty rats were sacrificed for determination of ovarian 20α-OH SDH activity. Ovaries were rapidly trimmed of adherent fat and connective tissue and frozen in n-hexane chilled by a solid CO_2-ethanol slurry. After draining on lens paper, ovaries were placed in prechilled culture tubes and stored at −70°C prior to cryostat sectioning.

Fresh frozen sections used for enzyme analyses were cut at 10 μm in a Bright cryostat (Hacker Instruments, Inc.) with a cabinet temperature of −25°C. Alternate sections were stained with toluidine blue or hematoxylin and eosin for examination of ovarian morphology. The histochemical procedure for 20α-OH SDH analyses was a modification of that of Balogh (1964); briefly, it entailed the following steps: ovarian cryostat sections were incubated for 60 min at 37°C in a medium consisting of 0.5 mg/ml nitro-blue tetrazolium, 0.5 mg/ml 20α-hydroxypregn-4-ene-3-one (dissolved in dimethylformamide), and 2.0 mg/ml NADP in a solution of 22% polyvinyl alcohol in 0.05 M glycylglycine buffer (pH 8.0). Control sections were incubated either without substrate or without coenzyme as a check on nonspecific staining. Following incubation, sections were rinsed in distilled water for 30 min and mounted in an aqueous, nonreflective mounting medium (Zoller and Weisz, 1979). The amount of tetrazolium salt reduced was quantified using a Vickers M85 scanning microdensitometer at a wavelength setting of 580 nm. Absorbance readings were taken midway between the center and periphery of each corpus luteum using a field delimiting mask with an area of 81 μm^2, approximating the size of one cell. The same field delimiting mask was used to take interstitial tissue readings. The instrument was preadjusted to subtract automatically control or "blank" absorbancy values from readings obtained on sections incubated in the complete reaction medium. Ten readings were taken on luteal and interstitial tissue on each of three ovarian sections from each animal (with four animals per group). The mean of each set of readings was converted into absolute units of optical density (AU) by calibrating the instrument with neutral density filters. Three values per animal were then used for statistical analyses.

Enzyme data and logarithmically transformed radioimmunoassay data were first subjected to analysis of variance. When significant differences between group means were found, means were also compared using Duncan's new multiple range test (Duncan, 1957).

The histochemical reaction for 20α-OH SDH is based on the interconversion of progesterone and 20α-OH P. This enzyme, which has been purified and characterized (Wiest and Wilcox, 1961), is known to be NADP-dependent and favors the oxidation of 20α-OH P to progesterone under alkaline conditions. Histochemical and biochemical determinations of 20α-OH SDH activity are based on this reaction, namely the oxidation of 20α-OH P to progesterone in alkaline media. Histochemical assays differ from biochemical analyses, however, in the endpoint of enzymatic activity employed. Biochemical techniques utilize spectrophotometric measurement of NADP reduction, whereas the histochemical approach involves oxidation of NADPH by NADPH-diaphorase, which then transfers electrons to the ditetrazolium salt, forming an insoluble, colored diformazan.

Thus,a number of validation checks were required prior to undertaking the histochemical assay. First, we examined the distribution of NADPH-diaphorase in the ovary of the pseudopregnant rat, as the histochemical procedure is dependent on the diaphorase reaction. Appreciable diaphorase activity was evidenced in all corpora lutea and within the interstitial tissue, thus supporting the contention that lack of diaphorase activity probably does not constitute a rate limiting factor in the cytochemical assay of 20α-OH SDH. These observations agree with those of Pupkin et al. (1966) concerning the ovary of normal cycling rats.

Additional validation checks revealed that the rate of tetrazolium salt reduction is linear with respect to

incubation time (Fig. 1) and that the rate of tetrazolium salt reduction is also linear with respect to the volume of tissue present (Fig. 2). It was also established that 20α-OH P and NADP were provided in adequate concentrations and were not limiting factors in the reaction.

Our justification for using tetrazolium salt reduction as a useful and valid approach for quantification of 20α-OH SDH activity is based on the extensive studies notably by Altman (1972, 1976) and Butcher (1972), who have validated the utility of tetrazolium salt reduction for cytochemical assay of a number of dehydrogenases. Recently, these techniques have been utilized in the assay of one of the steroid dehydrogenases, namely 3β-hydroxysteroid dehydrogenase (Zoller and Weisz, 1979).

RESULTS

As illustrated in Fig. 3, uterine $PGF_{2\alpha}$ content was low (2.7 ± 0.8 and 6.7 ± 1.4 ng/gm) on Days 5–6 and 7–8 of pseudopregnancy, respectively. $PGF_{2\alpha}$ levels exhibited a significant rise (16.4 ± 4.4 ng/g, $P<0.05$) on Days 9–10 and a further elevation (23.4 ± 4.3 ng/g, $P<0.05$) on Days 11–12. Levels remained high on Days 13–14 (22.1 ± 4.0 ng/g), but then declined on Days 15–16 (4.7 ± 0.8 ng/g).

Measurable amounts of 20α-OH SDH activity were present in late regressing corpora lutea (formed in estrous cycles prior to pseudopregnancy), in interstitial tissue as well as in all corpora of pseudopregnancy (Table 1, Fig. 4).

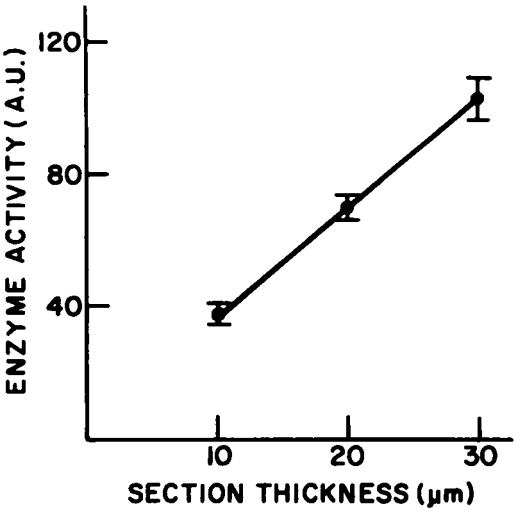

FIG. 2. Relationship between section thickness and 20α-OH SDH activity, determined from density readings of 20 areas of late regressing corpora, Day 6 of pseudopregnancy. Values were converted into absolute units of optical density (AU) and expressed as mean ± SD.

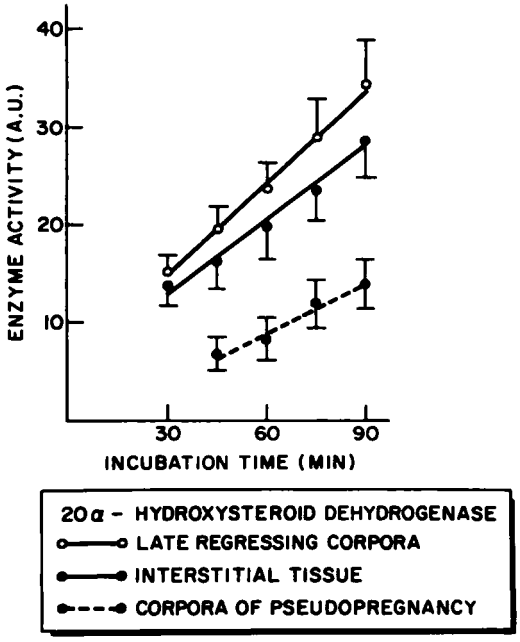

FIG. 1. Incubation-time profile of 20α-OH SDH activity of 10 μm sections of late regressing corpora, interstitial tissue, and corpora of pseudopregnancy, Day 6 of pseudopregnancy. Each point represents the average (± SD) enzymatic activity (formazan yield) expressed in absolute units of optical density (AU). At least 20 density measurements were made per unit area of the designated ovarian regions at each time interval.

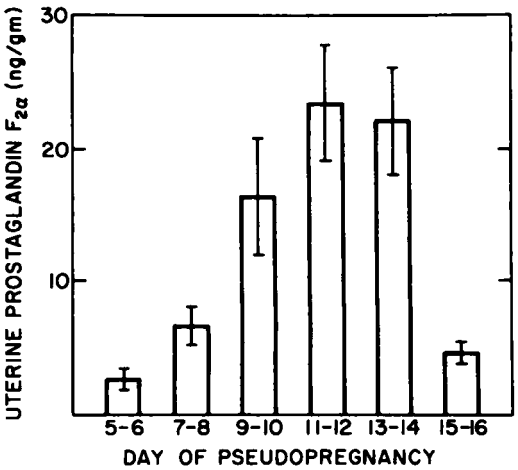

FIG. 3. Average (± SEM) uterine levels of prostaglandin $F_{2\alpha}$ (measured by radioimmunoassay of tissue homogenates) as a function of stage of pseudopregnancy. Each value is based on 6–7 determinations per time period.

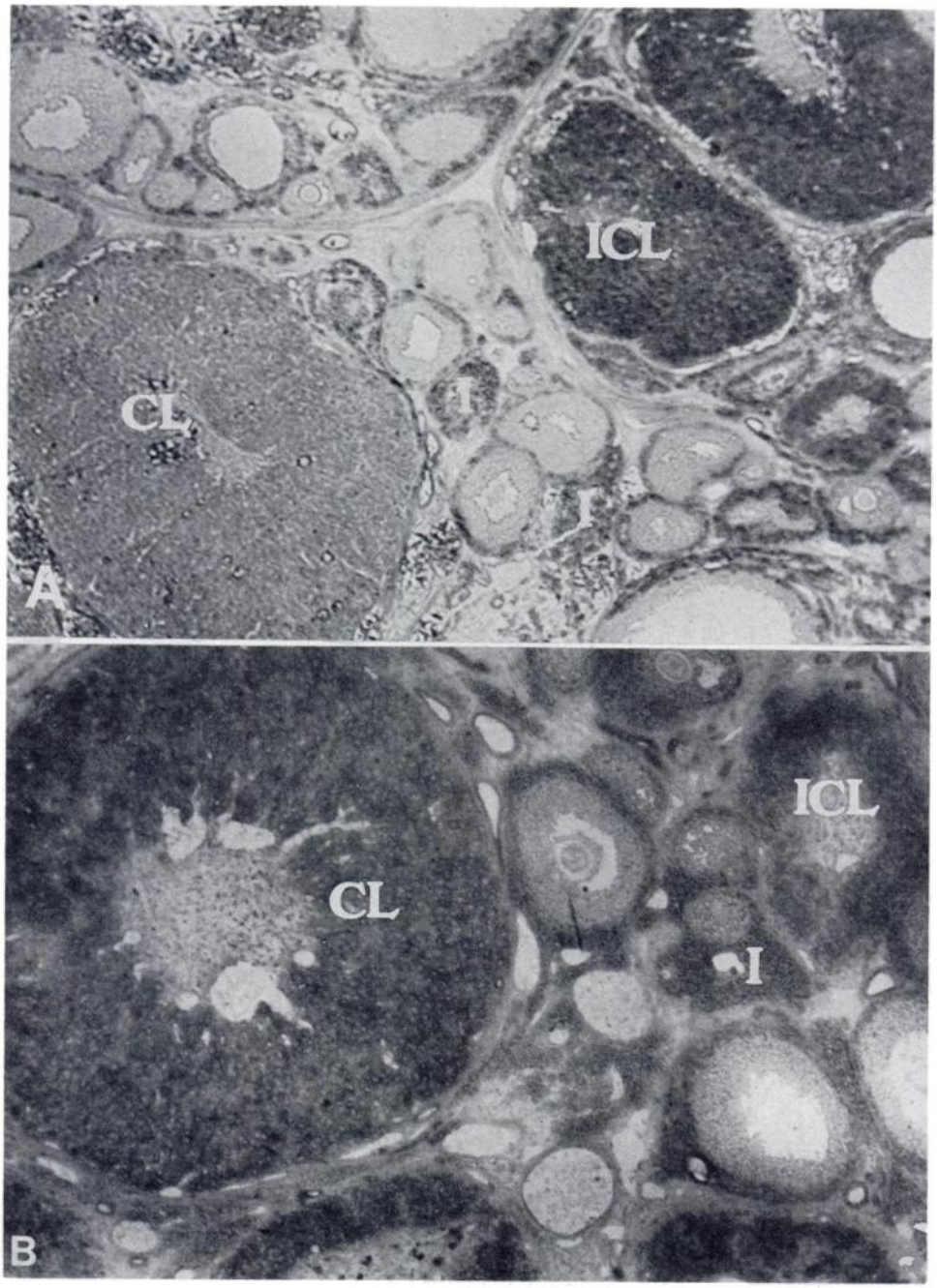

FIG. 4. A) Cryostat section showing 20α-OH SDH activity within rat ovary on Day 6 of pseudopregnancy. Moderate 20α-OH SDH activity is exhibited by corpora of pseudopregnancy (CL) Involuting corpora (ICL) show an intense reaction for the enzyme. Several pockets of interstitial cells (I) can also be seen in the photomicrograph. X125.

B) Portion of ovary excised on Day 15 of pseudopregnancy reacted for 20α-OH SDH. Corpora of pseudopregnancy (CL), now much smaller than corpora of pseudopregnancy, remain enzymatically active. Interstitial tissue (I) 20α-OH SDH activity is essentially constant from Days 6–15 of pseudopregnancy. X200.

TABLE 1. 20α-OH SDH Activity of individual compartments of the ovary of the pseudopregnant rat.†

	Day of pseudopregnancy				
	6	9	11	13	15
Corpora of pseudopregnancy*	19.2 ± 0.6[a]	21.0 ± 0.7[a]	21.7 ± 0.7[a]	28.7 ± 1.0[b]	40.2 ± 1.1[c]
Late regressing corpora	38.1 ± 2.0	39.2 ± 1.2	36.8 ± 1.2	35.8 ± 0.8	35.2 ± 1.9
Interstitial tissue	30.1 ± 0.9	30.0 ± 0.6	29.2 ± 0.8	31.4 ± 1.4	30.7 ± 0.9

†Absolute Units of optical density/81 μm^2 region ± SEM.

*Two means with different superscripts are significantly different at the 5% level. Duncan's new multiple range test.

On Days 6, 9, and 11, 20α-OH SDH activity was low and relatively constant [∿20 AU (absolute units)] in corpora of pseudopregnancy. On Day 13, enzyme activity in corpora of pseudopregnancy was significantly elevated (28.7 ± 1.0 AU, $P<0.05$). Activity continued to rise through Day 15 (40.2 ± 1.1 AU, $P<0.05$). Thus, a twofold elevation in corpora of pseudopregnancy 20α-OH SDH activity was evidenced between Days 11 and 15.

Although substantial 20α-OH SDH activity was present in late regressing corpora (∿37 AU) and in the interstitial tissue (∿30 AU), there was no alteration in enzymatic activity evidenced in these two compartments between Days 6 and 15 of pseudopregnancy (Table 1).

Based on the above data (and data from validation checks relating to the stoichiometry of the reaction), the coefficients of variation of enzymatic activity in absolute units of optical density were 0.13, 0.12 and 0.10 for corpora of pseudopregnancy, late regressing corpora, and interstitial tissue, respectively.

DISCUSSION

Quantitative cytochemical analysis of 20α-OH SDH activity within separate ovarian compartments of the pseudopregnant rat supports and extends previously reported biochemical studies of rat ovarian 20α-OH SDH levels. The observed twofold increase in corpora of pseudopregnancy 20α-OH SDH on Days 11–15 is in excellent agreement with the results of Bast and Melampy (1972), whose data indicate a two- to threefold increase in total ovarian 20α-OH SDH beginning ∿Day 11 of pseudopregnancy. Lacy et al. (1976) have shown that during luteal regression increased 20α-OH SDH activity occurs in the luteal and not in the nonluteal compartment of the pregnant rat ovary. An important aspect of the present study is the demonstration that this enhancement occurs only in corpora of pseudopregnancy, whereas activity remains unchanged in late regressing corpora and in the interstitial compartment.

Although others have considered LH and prolactin as the regulators of 20α-OH SDH activity, a body of evidence suggests uterine luteolytic activity may influence this enzyme. For example, $PGF_{2\alpha}$ administration results in an increase in serum 20α-OH P and a decline in serum progesterone in pseudopregnant rats (Lau et al., 1979). Furthermore, the increase in luteal 20α-OH SDH activity normally seen at the end of pregnancy in the rat is partially blocked by the administration of indomethacin (Strauss and Stambaugh, 1974). The close temporal relationship which was observed between increased uterine $PGF_{2\alpha}$ content (beginning on Days 9–10, peak on Days 11–12) and increased luteal 20α-OH SDH (Days 11–13) provides further evidence implicating $PGF_{2\alpha}$ as the putative factor responsible for inducing luteal 20α-OH SDH during luteolysis.

It is generally conceded that uterine $PGF_{2\alpha}$ has an important role in the initiation of corpus luteum regression in several mammalian species. If, in fact, $PGF_{2\alpha}$ is a primary mediator suppressing progesterone synthesis by the corpus luteum, one would anticipate that uterine $PGF_{2\alpha}$ production should be greater at the end of pseudopregnancy than in earlier stages. Increased uterine $PGF_{2\alpha}$ production prior to luteal regression has been shown in a number of species by analysis of uterine venous plasma and uterine homogenate $PGF_{2\alpha}$ concentration (Horton and Poyser, 1976). The onset of

elevated uterine $PGF_{2\alpha}$ content reported here concurs with the decrease in progesterone production seen on Days 10–12 in the pseudopregnant rat (Lau et al., 1979).

The only prior study found concerning estimation of uterine $PGF_{2\alpha}$ production in the pseudopregnant rat is that of Weems et al. (1975). The investigators reported greatly fluctuating uterine $PGF_{2\alpha}$ content, values being high on Days 9, 11, and 13 and low on Days 10 and 12. Also, $PGF_{2\alpha}$ levels were 20–40-fold higher than those reported here. Although these discrepancies remain unexplained, it may be noteworthy that Weems and associates failed to place the tissue in an inhibitor of prostaglandin synthesis after excision. One notable characteristic of prostaglandin synthesis is the rapid rate at which it proceeds. Kirton (1977) suggests that surgical trauma can induce prostaglandin synthesis and that abnormally high values can be obtained unless proper precautions (such as rapid freezing or placement in an inhibitor of prostaglandin synthesis) are taken.

For many years, the interstitial compartment of the ovary has been thought to possess endocrine capabilities. Ovarian interstitial cells of several species have ultrastructural and cytochemical characteristics of steroidogenic cells (Pupkin et al., 1966; Blaha and Leavitt, 1970; Carithers, 1976, Koering and Sholl, 1978; Taya et al., 1980); however, the exact nature of the secretory products of the interstitium remains uncertain. The interstitial tissue of the rabbit ovary contains 20α-OH SDH activity (Strauss et al., 1972) and is known to produce 20α-OH P (Solod et al., 1966; Keyes and Nalbandov, 1968). The data reported here indicated appreciable 20α-OH SDH activity in the interstitium of the ovary of the pseudopregnant rat and suggest that rat interstitial tissue is also a source of 20α-OH P.

Several earlier histochemical studies (Balogh, 1964; Pupkin et al., 1966) reported 20α-OH SDH activity only in involuting corpora of the cycling rat ovary; thus, it is generally held that 20α-OH SDH activity is not present in the interstitium of the rat ovary. However, Lamprecht et al. (1969) reported some evidence of histochemically demonstrable 20α-OH SDH activity in the interstitial tissue of the pseudopregnant rat. The observation of 20α-OH SDH activity localized in the interstitial tissue reported here is also in agreement with the biochemical demonstration of 20α-OH SDH activity in the nonluteal compartment of the ovary of the pregnant rat (Lacy et al., 1976).

The observation of elevated 20α-OH SDH activity in late regressing corpora lutea is in agreement with the histochemical studies of others (Balogh, 1964; Pupkin et al., 1966). The demonstration of 20α-OH SDH, 3β-hydroxysteroid dehydrogenase, and several carbohydrate-metabolizing enzymes in involuting corpora lutea of the rat ovary (Pupkin et al., 1966) has led to the proposal that these structures remain steroidogenically active for extended periods of time, their principal progestin secretory product being 20α-OH P (Wiest et al., 1968; Hilliard, 1973). Thus, it would seem that two compartments of the rat ovary, involuting corpora and interstitial tissue, contain the enzymatic machinery required for the production of 20α-OH P.

Although 20α-OH P has only slight progestational activity (Wiest and Forbes, 1964; Talwalker et al., 1966) and it has been hypothesized that production of this compound serves only to regulate the progestational potency of rat ovarian secretions (Wiest et al., 1968), there have been some suggestions of functional roles of 20α-OH P. It was originally believed that, in the rabbit, 20α-OH P acts to facilitate the release of LH following coitus (Hilliard et al., 1967). However, more recent studies have indicated ovarian hormones are not required for this release of LH (Goodman and Neill, 1976). The demonstration of two ovarian compartments apparently specialized for the production of 20α-OH P suggests the functional status of 20α-OH P merits further investigation.

One salient contribution of the current study is the demonstration of the utility of quantitative cytochemical techniques to assess steroidogenic enzyme activity within specific ovarian cellular compartments. One of the unique advantages of cytochemical methods is that they permit a more precise localization of enzymatic activity within the ovary than conventional biochemical techniques employing gross dissection and homogenization. Utilizing these techniques, we have demonstrated that there is a precise temporal correspondence between the onset of elevated uterine $PGF_{2\alpha}$ content (determined by radioimmunoassay of uterine homogenates) and increased corpora of pseudopregnancy 20α-OH SDH. Further, we have shown appreciable 20α-OH SDH activity in involuting corpora lutea and in the interstitial tissue of the pseudopregnant rat ovary, suggesting these two compartments serve to

produce a compound currently held to be an inactive metabolite of progesterone, 20α-OH P.

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