BIOLOGY OF REPRODUCTION 36, 729–739 (1987)

Evidence for the Preferential Utilization of Esterified Cholesterol in Progesterone Production by Rabbit Corpora Lutea[1]

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ABSTRACT

Our previous studies show that lipoproteins stimulate progesterone secretion by rabbit luteal cells in vitro and that estradiol modifies this effect. This study examines the relationship between estradiol and serum lipoproteins for progesterone production by rabbit corpora lutea in vivo. Using morphometric analysis, we determined that estrogen treatment of hysterectomized pseudopregnant (E-hyst) rabbits increased luteal lipid volume by mid-pseudopregnancy without altering serum progesterone levels. Treatment of E-hyst rabbits with 4-amino-3,4,pyrazola pyrimidine (APP) during early to mid-pseudopregnancy reduced serum cholesterol levels without decreasing serum progesterone concentrations. However, 3-hydroxy-3 methyl glutaryl-CoA reductase activity was increased. Thus, in the presence of exogenous estrogen, serum cholesterol is esterified and stored rather than converted directly into progesterone. APP-treatment of E-hyst rabbits during late-pseudopregnancy, when estrogen receptor levels are low, increased serum progesterone levels and reduced intracellular lipid content. Thus, stored lipid is the primary source of cholesterol for progesterone synthesis. In addition, estrogen, via estrogen receptor, is important in maintaining steady progesterone output despite fluctuations in serum lipoprotein levels. A working model for cholesterol utilization by rabbit luteal cells is presented, which suggests that stored cholesterol esters, derived from both endogenous and exogenous sources, is the key source or cholesterol for progesterone production. Furthermore, we propose that estradiol regulates the uptake and storage of cholesterol and its rate of metabolism into progesterone.

INTRODUCTION

The pioneering work of Brown and Goldstein (reviewed in Brown et al., 1979) identified serum lipoproteins as a major source of cholesterol for steroidogenesis. Their work describes the presence of specific cell-surface receptors for lipoproteins that mediate serum lipoprotein uptake and internalization. Once internalized, lipoprotein-derived cholesterol serves as 1) a major precursor for steroid synthesis; 2) a negative feedback regulator of both 3-hydroxy-3-methyl glutaryl (HMG)-CoA reductase, the rate-limiting enzyme of de novo cholesterol synthesis and lipoprotein-receptor synthesis; and 3) a positive activator of acyl CoA: cholesterol acyltransferase, which serves to esterify and store cholesterol within cells. Over the past seven years, multiple investigators, studying a variety of species, have extended the studies of Brown and Goldstein to demonstrate the importance of serum lipoproteins for steroid production by ovarian cells. These studies demonstrate that low-density lipoprotein (LDL) is the primary source of cholesterol in the human (Carr et al., 1981), cow (Savion et al., 1982), and hamster (Chang and Limanek, 1980), whereas the rat uses primarily high-density lipoprotein (HDL) (Christie et al., 1979; Azhar and Menon, 1981; Bruot et al., 1982). Specific receptors exist on the surface membranes of ovarian cells; these vary in number in corpora lutea throughout the menstrual cycle (Ohashi et al., 1982) and appear to be regulated not only by the intracellular concentrations of free cholesterol (Brown et al., 1979), but also by luteotropic hormones (Hwang and Menon, 1983; Gibori et al., 1984).

Accepted September 22, 1986.
Received June 26, 1986.
[1]Supported by HD-14871, HD-00447, DCB-415927 and University of Illinois CRB #973. Presented in part at the 5th Ovarian Workshop, Laramie, Wyoming, July 21-23, 1984.
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The one apparent exception to this generalization has been the rabbit corpus luteum. The controversy pertaining to the importance of serum lipoproteins for steroidogenesis by the rabbit corpus luteum originated with the study of Kovanen et al. (1978), who found that the rabbit corpus luteum contained high levels of HMG-CoA reductase compared to steroidogenic tissues from other species (i.e. 10- to 30-fold higher than rat ovarian tissue, Schuler et al., 1979; 1981). This suggests that de novo synthesis of cholesterol is the primary source of cholesterol for steroidogenesis in rabbit luteal tissue. However, both LDL and HDL can increase progesterone secretion by dissociated rabbit luteal cells in vitro (McLean and Miller, 1986), and (^{3}H) cholesterol from serum can be converted into steroid hormones by the rabbit ovary (Solod et al., 1966). In addition, estradiol, the luteotrophic hormone in the rabbit (Bill and Keyes, 1983), can suppress the acute stimulatory effect of LDL on progesterone secretion by dissociated rabbit luteal cells in vitro (McLean and Miller, 1986). Thus, despite high levels of HMG-CoA reductase in rabbit luteal tissue, both in vivo and in vitro data in the literature suggest that rabbit luteal cells can utilize serum-derived cholesterol for progesterone production.

The purpose of this study was to understand the relative importance of serum lipoproteins for progesterone production by the rabbit corpus luteum in vivo and to understand how estradiol might influence the uptake and utilization of lipoproteins by this tissue. The primary animal model used in these experiments was the estradiol-treated hysterectomized rabbit. In these animals, serum estradiol levels are elevated 2- to 3-fold, and progesterone secretion is maintained for at least 33 days. In contrast, corpora lutea from estradiol-treated intact rabbits have essentially regressed by Days 18–20 (Miller and Keyes, 1976). The mechanism through which estradiol sustains progesterone secretion in hysterectomized rabbits is not directly related to the level of estradiol receptors, since cytosol and nuclear estrogen receptor levels were low and not different by Day 20 in both control and estradiol-treated hysterectomized rabbits, despite elevated serum progesterone concentrations in estradiol-treated rabbits (Miller and Toft, 1983). In this study, we wished to determine if sustained estradiol treatment maintained serum progesterone in the hysterectomized rabbit by increasing the sensitivity to or the utilization of lipoproteins by the corpus luteum, since both LDL and HDL can stimulate progesterone secretion by dissociated rabbit luteal cells in perifusion culture (McLean and Miller, 1986), and the luteotrophic role of estradiol in the rat is associated with elevations in HDL receptors (Gibori et al., 1984). To this end, we compared the morphology of corpora lutea from control and estradiol-treated hysterectomized rabbits and studied the effect of 4-aminopyrazolo (3,4-d)-pyrimide (APP), which is known to lower lipoprotein synthesis by the liver (Morin et al., 1979), on the levels of serum progesterone in pseudopregnant intact and hysterectomized rabbits treated with estradiol.

MATERIALS AND METHODS

Animals

Mature female New Zealand White rabbits (3.5 kg) were obtained from Langshaw farms (Augusta, MI) and maintained in individual cages under controlled conditions with free access to lab chow and water. Pseudopregnancy was initiated in rabbits by an i.v. injection of 50 IU human chorionic gonadotropin (hCG) (Day 0). In one group of rabbits (estradiol-treated hysterectomized rabbits treated with APP on Days 10–15, see below), the hCG injection was preceded, by 4 days, with an i.m. injection of 40 IU pregnant mare's serum gonadotropin (PMSG). On Day 1, rabbits were anesthetized i.m. with 2.0 ml each xylazine (Rompun, 20 mg/ml) and ketamine (100 mg/ml), and hysterectomized through a mid-ventral incision; a 1-cm capsule filled with estradiol (Holt et al., 1975) was implanted at the base of the neck. Pseudopregnant non-hysterectomized rabbits were laparotomized on Day 1 to confirm ovulation. APP (25 mg/ml saline, pH 2.5) or saline was administered daily (10 mg/kg, i.p.) on Days 4–7 in pseudopregnant rabbits, and in estradiol-treated hysterectomized rabbits on Days 10–15 or Days 20–23. Blood samples were drawn from the marginal ear vein for the determination of serum progesterone and cholesterol. On Day 24, corpora lutea were dissected from the ovary, weighed, minced, and fixed for electron microscopic examination (see below). Other rabbits were hysterectomized on Day 1 and treated with empty implants filled with 1/2 cm or 1 cm crystalline estradiol. On Day 10, blood samples were drawn for progesterone, estradiol, and cholesterol measurement and the ovaries were fixed in situ for electron microscopic analysis (see below).

Electron Microscopy

Hysterectomized rabbits treated with 1/2- or 1-cm estradiol implants were anesthetized on Day 10 with xylazine and ketamine and 200 ml 3% glutaraldehye-3% paraformaldehyde fixative (in Hanks' Balanced Salts solution containing 50 mM HEPES and 2.2 mM collidine, pH 7.3) was injected into the dorsal aorta proximal to the ovarian arteries. Both ovarian veins were cut to allow fixative to perfuse the ovaries in situ. After perfusion, the ovaries were removed from the animal, and, while immersed in fixative, the corpora lutea were dissected from the ovary, sliced into 1-mm pieces, and left in fixative for an additional 2 h. Corpora lutea dissected from APP-treated animals on Day 24 of pseudopregnancy were similarly sliced into 1-mm pieces and left in fixative for 2 h. After fixation, luteal pieces were washed 3 times with 1% sodium cacodylate and postfixed in 2% osmium tetroxide and 3% potassium ferrocyanide for 2 h. Tissue was then washed three times distilled water, dehydrated through a graded ethanol series, infiltrated with araldite, and polymerized at 60°C for 72 h. Silver-gold thin sections were contrasted with aqueous uranyl acetate (Watson, 1958) and lead citrate (Sato, 1967) and examined at 3000X in an electron microscope.

Morphometric Analysis

Morphometric analysis was performed on micrographs by following the techniques of Weibel et al. (1966). Three or four micrographs, taken near the corners of each grid, were prepared from a grid containing entire luteal cells that had been sectioned through the nucleus. Micrographs from at least four animals were examined for each treatment. The point-counting method was used to determine, the percentage volume occupied by cell constituents. Identification of cellular components was according to the convention of Blanchette (1966).

Progesterone Radioimmunoassay

The concentration of progesterone in serum and tissue was determined by radioimmunoassay according to the protocol of Day and Birnbaumer (1980), using antisera (GDN #337, kindly provided by Dr. Gordon Niswender, Colorado State University) characterized by Gibori et al. (1977). Tritiated progesterone was added to each tube to correct for procedural losses that averaged approximately 30%. Results were calculated by using the logit-log weighted linear regression program of Rodbard (1980). The inter- and intraassay coefficients of variation are 5.9% (n=22) and 7.4%, 6.5% and 3.6% at 20%, 45% and 60% binding, respectively (n=10).

Estradiol Radioimmunoassay

Serum estradiol concentrations were measured by radioimmunoassay using a rabbit antiserum (GDN #930) raised against 17β-estradiol-11-hemisuccinate conjugated to bovine serum albumin (BSA, kindly provided by the Reproductive Endocrinology Program at the University of Michigan). The specificity of the antisera has been described by England et al. (1974). Each sample (1–3 ml) was extracted in 15 ml benzene and chromatographed on Sephadex LH-20 using a 9:1 benzene: methanol solvent system (Carr et al., 1971) prior to assay. Assay sensitivity was 0.2–0.3 pg and the midpoint of the standard curve was 12–15 pg. Each sample was corrected for procedural losses (recoveries averaged 87.6%) and results have been expressed as pg/ml serum.

3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase (HMG CoA Reductase) Assay

The activity of HMG-CoA reductase in rabbit corpora lutea was measured by the method of Kovanen et al. (1978) with slight modification. Rabbit ovaries were removed after cervical dislocation, the corpora lutea dissected on ice, and homogenized in buffer containing either 50 mM NaF or 50 mM NaCl (pH 7.0). The activity of HMG-CoA reductase observed in tissue prepared in the presence of NaCl was assumed to represent the total amount of enzyme present, whereas that observed in tissue prepared in the presence of NaF was assumed to represent the amount of enzyme in its active (dephosphorylated) form at the time the tissue was removed from the animal (Brown et al., 1979). The homogenate was centrifuged at 20,000 × g × 20 min and again at 100,000 × g × 60 min to isolate luteal microsomes. The microsomes were resuspended and HMG-CoA reductase activity was estimated in aliquots containing 15 and 30 μg of microsomal protein. The mevalonate formed was extracted into ether using sodium sulfate and separated on silica gel G thin-layer plates using acetone: benzene (1:1, v:v). The mevalonate spots were identified by exposing them to iodine vapor, scraped into scintillation fluid and counted.

The [^{14}C] radioactivity incorporated into [^{14}C]-mevalonate was calculated by using [^{3}H] mevalonate to correct for procedural loss (recovery averaged 45–50%). Enzyme activity was expressed as nmoles of mevalonate formed per min per mg protein (Bradford, 1976).

Other Materials

17β-[2,4,6,7-^{3}H] Estradiol, [1,2,6,7-^{3}H] progesterone, 3-hydroxy-3-methyl [3-^{14}C] glutaryl CoA (47.2 mCi/mmol) and [5-^{3}H] mevalonic acid triethyl ammonium (10.3 Ci/mmol) were purchased from New England Nuclear (Boston, MA); 4-amino pyrazolo (3,4-d)-pyrimidine (APP) was from Aldridge Chemical Co., Milwaukee, WI; glutaraldehyde, paraformaldehyde and araldite were from Fullum, Inc. (Schenectady, NY); toluene and scintillation cocktail 3a70b were from Research Products International (Mt. Prospect, IL); Scinti Prep I was from Fisher (Pittsburg, PA); and Silica Gel G plates were from Analtech (Newark, DE). Serum cholesterol was measured with a kit from Sigma Chemical Co. (St. Louis, MO.) by the cholesterol oxidase method. 3-Hydroxy-3 methyl glutaryl CoA, nicotinamide adenine dinucleotide phosphate (NADPH), glucose-6-phosphate dehydrogenase, glucose-6-phosphate, and all other biochemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Rompun was purchased from Bayvet, Shawnee, KA, and ketamine was from Parke-Davis, Morris Plaines, NJ. PMSG was from Orgenon (West Orange, NJ).

Statistical Analysis

Data are expressed as the mean ± SEM. Data were compared by Students *t*-test or analysis of variance, and when significant, differences between means of treatment groups were compared using Student-Newman Keuls multiple comparison test (Zar, 1974). A *p* value of less than 0.05 was considered significant.

RESULTS

Estradiol Treatment and Lipid Droplet Volume

Electron micrographs of luteal cells from control and estradiol-treated hysterectomized rabbits (1-cm estradiol implants) are shown in Figures 1A and B, respectively. The increase in the number of lipid droplets seen with estradiol treatment is apparent in these micrographs. When quantified by morphometric analysis, the cytoplasmic volume occupied by lipid droplets was increased significantly ($p<0.05$) by estradiol treatment (Fig. 2). Lipid droplet volume was increased to a similar extent with low (10.5 pg/ml) or high (17.8 pg/ml) levels of serum estradiol (Table 1). Despite increases in both luteal lipid droplet volumes and serum estradiol concentrations, serum progesterone concentrations did not differ in these animals (Table 1).

The Effect of APP Treatment During Early or Mid-pseudopregnancy

When APP was administered to rabbits between Days 4 and 7 (Fig. 3), or Days 10 and 15 (Fig. 4), serum cholesterol levels fell to a stable nadir by the second day of treatment. Despite significant reductions in cholesterol levels, serum progesterone concentrations were not altered by APP treatment during either early or mid-pseudopregnancy (Figs. 3 and 4).

To determine if HMG-Co A reductase activities in corpora lutea were regulated by exogenous cholesterol, estrogen-treated hysterectomized rabbits were treated with APP between Days 9 and 11, and luteal tissue was removed on Day 12 for the measurement of HMG-CoA reductase activity. As shown in Figure 5, APP treatment was associated with a prompt reduction in serum cholesterol. Despite this reduction in serum cholesterol, neither serum progesterone nor total (NaCl) HMG-CoA reductase activity was affected; however, the active form of HMG-Co A reductase (microsomes prepared in the presence of NaF) was elevated significantly ($p<0.05$) by APP treatment.

The Effect of APP Treatment during Late Pseudopregnancy

When APP was administered to estradiol-treated hysterectomized rabbits on Days 20–23 of pseudo-

TABLE 1. Serum steroids levels in estradiol-treated hysterectomized rabbits on Day 12 of pseudopregnancy.

Group	Progesterone	Estradiol
	(ng/ml)	(pg/ml)
Controls	20.4 ± 3.9*	4.0 ± 1.3
Low estradiol	24.0 ± 4.9	10.5 ± 1.2
High estradiol	20.8 ± 2.0	17.8 ± 1.3

*Mean ± SEM, n=7.

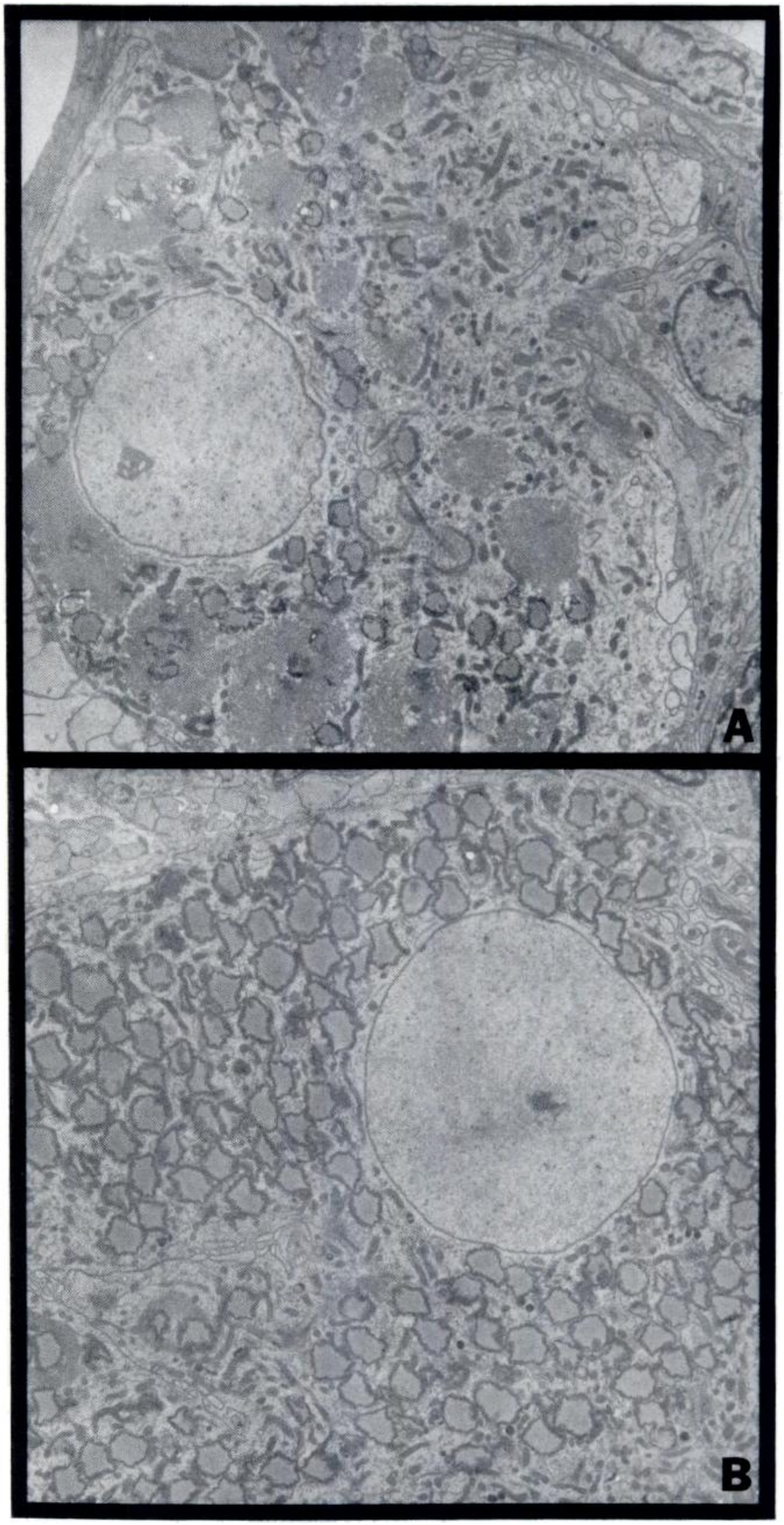

FIG. 1. Electron micrographs of luteal cells from control (*A*) and estradiol-treated (*B*) hysterectomized rabbits on Day 10 of pseudopregnancy. Micrographs illustrate an increase in the number and size of cytoplasmic lipid droplets with estrogen treatment. Initial magnification × 3000.

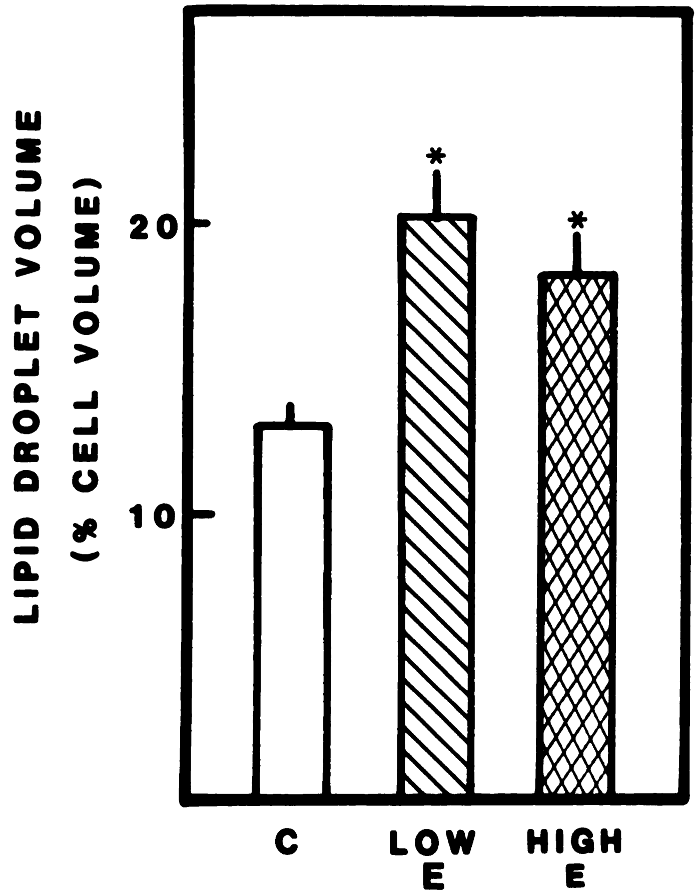

FIG. 2. Morphometric analysis of lipid droplet volume in luteal cells from control (C) and estrogen- (E) treated hysterectomized rabbits. Serum estradiol and progesterone concentrations in these animals are found in *Table 1.* Results are expressed as a percentage of cytoplasmic volume represented by the lipid droplet volume from 28 determinations (4 micrographs each from 7 animals per group; mean ± SEM). *Asterisks* represent a significant increase in lipid droplet volume compared to control animals.

pregnancy (Fig. 6), serum cholesterol levels fell after one day of treatment. Surprisingly, however, serum progesterone concentrations were elevated by Day 21 and remained elevated through the end of the experiment. Thus, serum progesterone concentrations in these animals were inversely related to the levels of serum cholesterol. The lipid droplet volume in luteal cells from these APP-treated animals was significantly reduced on Day 24 compared to the volume of lipid in saline-treated controls (Fig. 7).

DISCUSSION

The results of these and previous experiments from this laboratory suggest that serum lipoproteins can be used by the rabbit corpus luteum for progesterone production. However, we suggest that serum-derived cholesterol is not converted directly into progesterone, but is primarily esterified and stored in the form of lipid droplets within the cell prior to its metabolism to progesterone. In addition, our data suggest that the uptake and utilization of serum lipoproteins is regulated by estradiol, the principal luteotrophic hormone in the rabbit (Bill and Keyes, 1983). In this report, we show that estrogen treatment in vivo causes an accumulation of intracellular lipid droplets in corpora lutea without altering the concentrations of serum progesterone (Figs. 1 and 2, Table 1). The source of this excess lipid would appear to be from exogenous cholesterol since estradiol treatment did not increase luteal HMG-CoA reductase activity (McLean and Miller, unpublished results). Further support for this hypothesis is derived from our studies with APP in which the reduction in serum cholesterol in response to APP treatment during early to mid-pseudo-

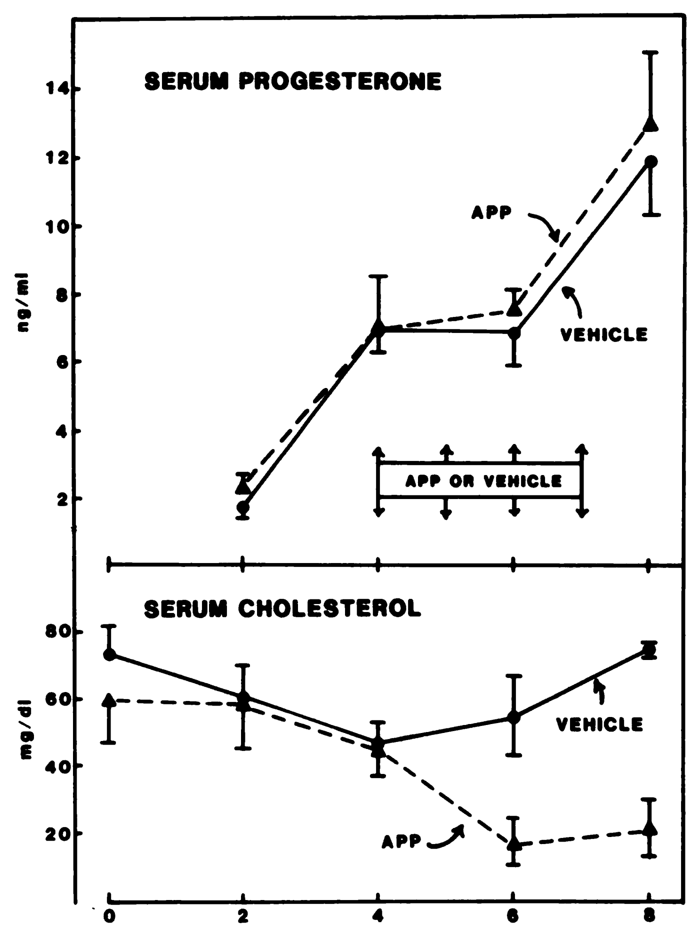

FIG. 3. The effect of APP treatment during early pseudopregnancy on the concentrations of *serum progesterone* and *cholesterol. APP* or *vehicle* was administered, i.p., between Days 4 and 7 to intact pseudopregnant rabbits. Points represent the mean ± SEM (n=4–5).

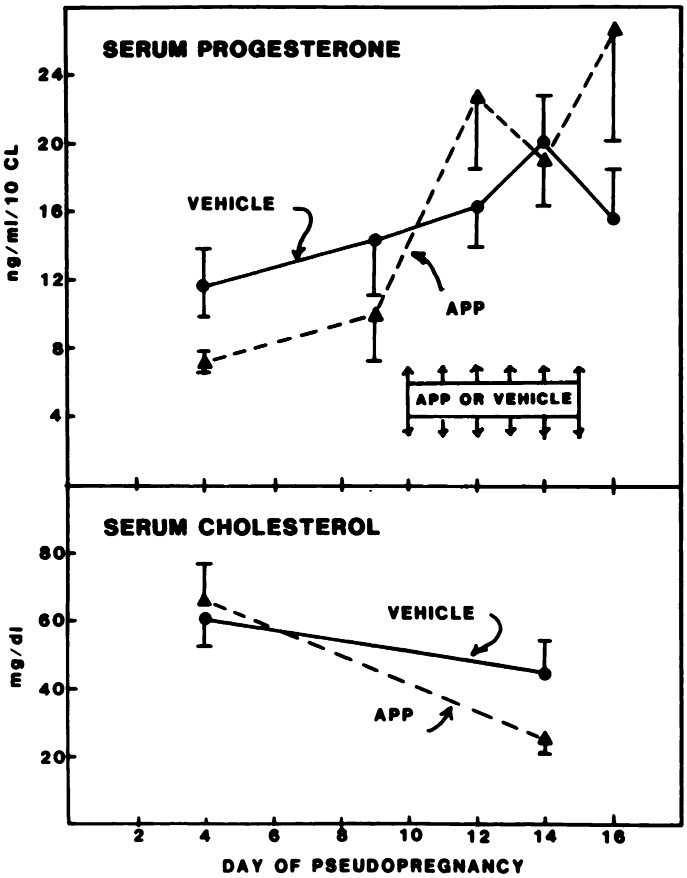

FIG. 4. The effect of APP treatment during mid-pseudopregnancy on the concentrations of *serum progesterone* and *cholesterol*. *APP* or *vehicle* was administered, i.p., between Days 10 and 15 to pseudopregnant rabbits that had been hysterectomized on Day 1 of pseudopregnant rabbits that had been hysterectomized on Day 1 of pseudopregnancy and treated with a 1-cm estradiol implant. Points represent the mean ± SEM (n=5 or 6).

pregnancy was accompanied by a reciprocal elevation in HMG-CoA reductase activity (Figs. 3 and 4), as has been seen in other tissues (Brown et al., 1979). Thus, it is clear that serum lipoproteins can be taken up by rabbit luteal cells in vivo and can alter the dynamics of cellular cholesterol metabolism. The accumulation of intracellular lipid in response to estradiol treatment suggests that estradiol may influence cholesterol uptake and storage, possibly through regulating the number of lipoprotein receptors.

When rabbits were treated with APP during early to mid-pseudopregnancy, serum progesterone concentrations were unaffected despite reductions in the level of serum cholesterol (Fig. 3 and 4). We propose that the maintenance of serum progesterone in these animals resulted from the increase in HMG-CoA reductase activity that followed APP treatment (Fig. 5). This suggests that rabbit luteal cells in vivo preferentially utilize intracellular rather than exogenous sources of cholesterol for progesterone synthesis, at least through mid-pseudopregnancy. This is in contrast to dissociated rabbit luteal cells in vitro, which are able to use lipoproteins directly for progesterone production (McLean and Miller, 1986). However, when rabbits were treated with APP during late pseudopregnancy (Days 20–23), the reduction in serum cholesterol was associated with an increase in serum progesterone (Fig. 6) and a decrease in luteal lipid droplet volume (Fig. 7).

These results also support the importance of serum cholesterol as a regulator of intracellular cholesterol dynamics and suggest that lipid droplet cholesterol was utilized for progesterone production in these animals. An important difference in these three groups of rabbits is that estradiol-receptor levels have fallen in the estrogen-treated hysterectomized rabbits by Day 20 of pseudopregnancy, despite sustained progesterone production (Miller and Toft, 1983). This suggests that estradiol, acting via the luteal estrogen receptor, is an important factor in maintaining a steady output of progesterone despite fluctuations in the concentrations of serum lipoprotein. A similar conclusion can be drawn from in vitro data in which it was found that estradiol not only sustained progesterone output by dissociated luteal cells but also attenuated the acute stimulatory effect of lipoproteins on progesterone secretion (McLean and Miller, 1985; 1986).

We have developed a working model to explain the mechanism through which estrogen might direct the utilization of cholesterol for progesterone production by the corpus luteum (Fig. 8). Two major hypothesis form the basis of this model. The first suggests that estradiol regulates the rate of progesterone production by controlling the rate of breakdown of esterified cholesterol. The second suggests that both endogenous and exogenous cholesterol sources contribute to progesterone production primarily by maintaining the pool of esterified cholesterol. Thus, this model proposes that there are two functional pools of free cholesterol that can be used for progesterone production. Pool # 1 is derived from the breakdown of cholesterol esters and provides the primary source of cholesterol for steroidogenesis. The second pool is derived from newly synthesized cholesterol and lipoprotein-derived cholesterol and is the primary source of free cholesterol available for esterification

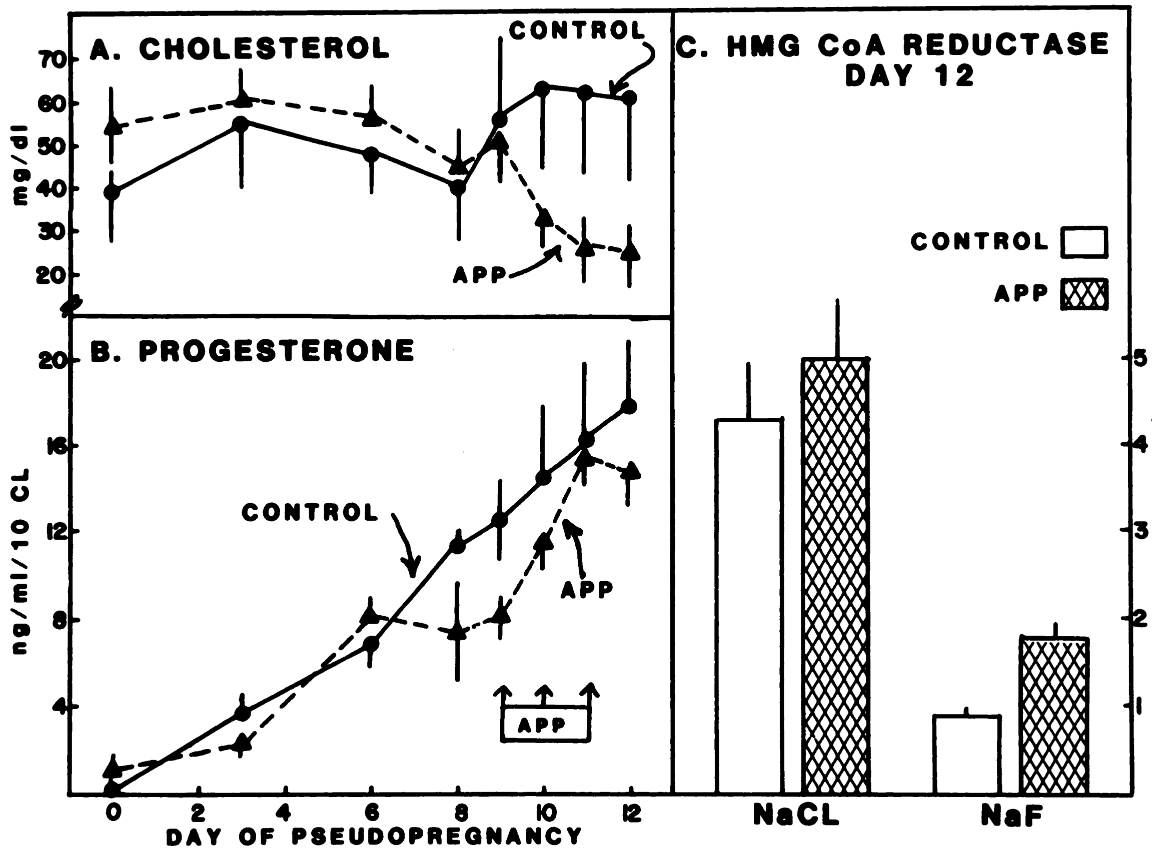

FIG. 5. The effect of APP on *HMG-CoA reductase activity* and the concentrations of serum *progesterone* and *cholesterol* in estrogen-treated pseudopregnant rabbits. *APP* or vehicle (*control*) was administered on Days 9–11, and corpora lutea were removed on Day 12 for the measurement of HMG-CoA reductase activity. APP treatment reduced serum cholesterol but did not alter serum progesterone levels. The active form of HMG-CoA reductase (NaF) was significantly elevated ($p<0.05$) with APP treatment. No change was observed in total (NaCl) HMG-CoA reductase activity. n=4 (controls) and 8 (APP).

and feedback regulation of HMG-CoA reductase and lipoprotein receptors. The concept, that a significant portion of free cholesterol might be shuttled into lipid droplet formation rather than directly into progesterone production, gains support from recent experiments of Veldhuis et al. (1985), who demonstrated that inhibition of Acyl CoA: cholesterol acyltransferase (ACAT) increased progesterone secretion by granulosa cells cultured in serum-free media. Thus, even when exogenous cholesterol is not available and progesterone secretion is low, ACAT activity can be an important regulator of progesterone output. In the rabbit corpus luteum, where serum lipoproteins apparently do not acutely influence the rate of progesterone production (at least during early and mid-pseudopregnancy), the regulation of ACAT activity, possibly by estradiol, might therefore provide a key step in controlling progesterone secretion. This is consistent with our observation that estradiol treatment of rabbits results in an increase in the number and size of luteal lipid droplets despite comparable serum progesterone concentrations.

This model suggests that estradiol controls not only the formation but also the breakdown of lipid droplets, by regulating either the activities of cholesterol esterase, the cholesterol side chain cleavage enzyme, or the level of cholesterol transport protein. As free cholesterol enters Pool #1, it is rapidly converted to progesterone. This is consistent with the observations of Holt et al. (1983) and Fuller et al. (1978). Fuller et al. (1978) found that estradiol stimulation of progesterone synthesis in vitro was not impaired by AY-9944, a potent inhibitor of cholesterol synthesis. This suggested that at least acute estradiol stimulation of progesterone synthesis is not dependent upon de novo cholesterol synthesis from acetate. In addition, Holt et al. (1983) found that when estradiol implants were removed from pseudopregnant rabbits to initiate luteal regression and replaced to stimulate progesterone production, the

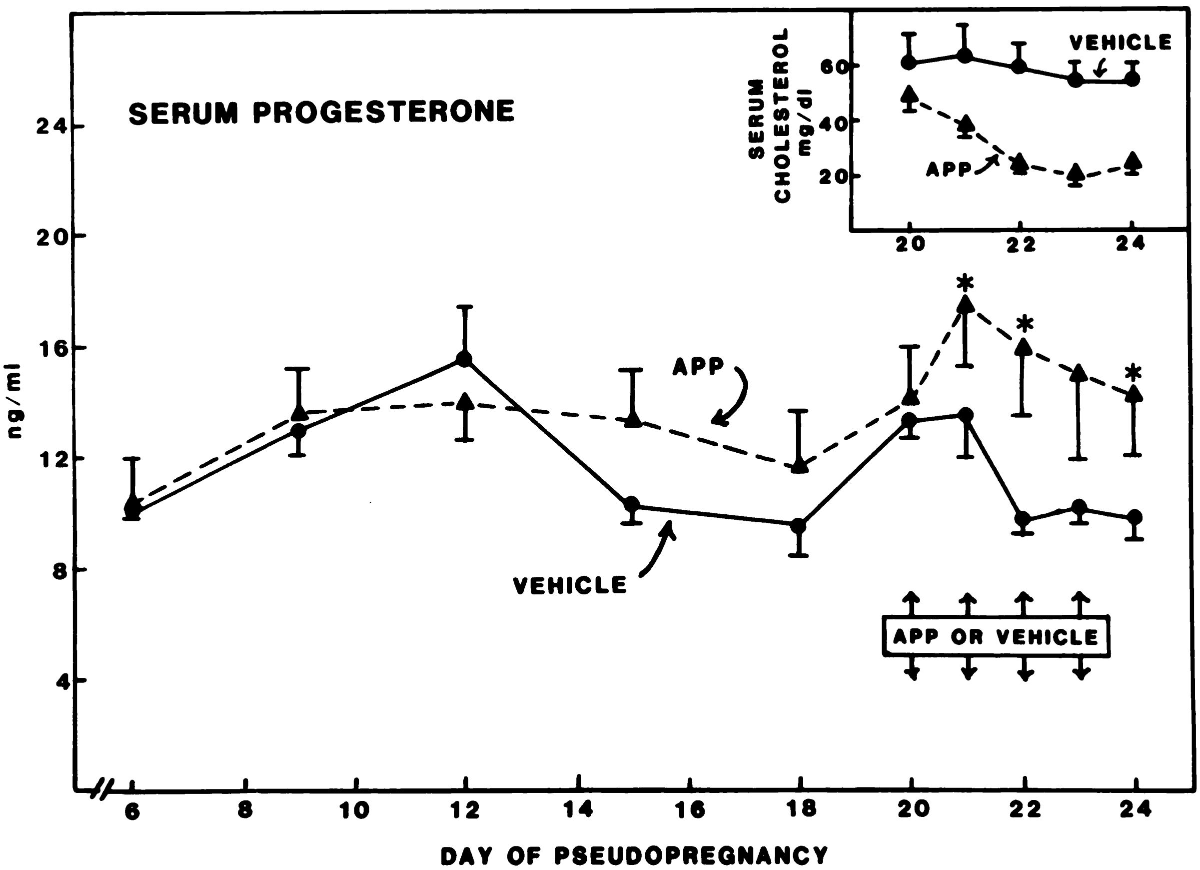

FIG. 6. The effect of APP treatment during late pseudopregnancy on the concentrations of serum progesterone and cholesterol. *APP* or *vehicle* was administered, i.p., between Days 20 and 23 to pseudopregnant rabbits that had been hysterectomized on Day 1 of pseudopregnancy and treated with a 1-cm estradiol implant. Points represent the mean ± SEM, n=6. *Asterisks* represent significant (p<0.05) elevations in serum progesterone in APP-treated rabbits compared to vehicle-treated rabbits on those same days of pseudopregnancy.

cholesterol and cholesterol ester content of rabbit corpora lutea was inversely related to serum progesterone concentrations. This suggests that the luteotropic action of estradiol is related to the metabolism of stored cholesterol. The ability of luteinizing hormone to stimulate progesterone production in vitro (McLean et al., 1987), even though it is not luteotropic in the rabbit in vivo, might also be related to its ability to cause the breakdown of lipid droplets, possibly through activation of cholesterol esterase, as has been found in rat luteal tissue (Behrman and Armstrong, 1969). In addition, estradiol may increase the number of lipoprotein receptors, as is known to occur in rat luteal cells (Gibori et al., 1984), and thus increase the uptake of cholesterol into the second pool.

The second pool of cholesterol is thought to be most important for progesterone production when cells are maintained in vitro with no added estradiol or when estrogen receptors are low (after Day 20 in estrogen-treated hysterectomized rabbits). In these situations, the model predicts that the esterification of cholesterol would be reduced. This would result in increased free cholesterol concentrations (2nd pool) which, in turn, might decrease HMG-CoA reductase activity through negative feedback (Brown et al., 1979). The concept of a second pool of cholesterol could account for the results of the APP experiments (see above). Thus, according to this model, when serum lipoprotein levels were reduced by APP treatment on Days 20–23 (i.e., when estradiol receptors are low), free cholesterol concentrations were also reduced. This, in turn, resulted in release from inhibition of HMG-CoA reductase and increased progesterone production. Since both luteal lipid droplet volume (Fig. 7) and the wet weight of corpora lutea from APP-treated rabbits were also reduced compared to controls (data not shown), and luteal

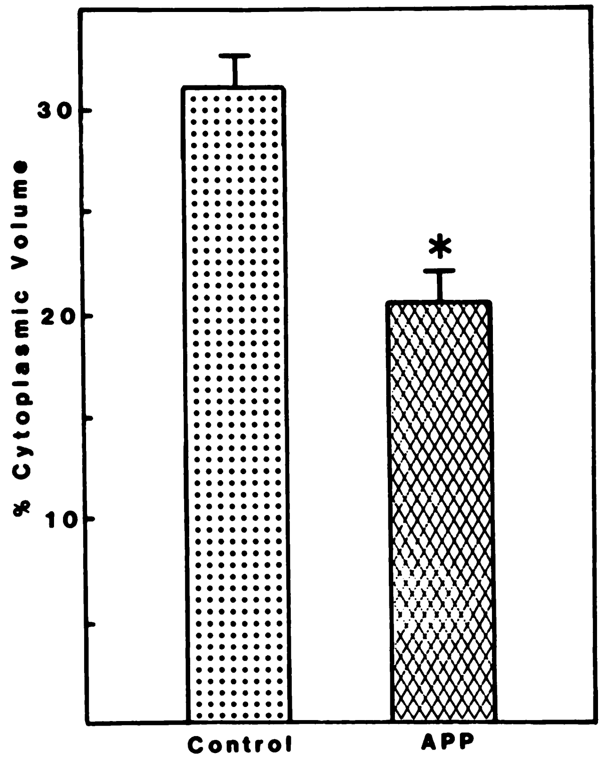

FIG. 7. Morphometric analysis of lipid droplet volume in luteal cells on Day 24 of pseudopregnancy from estrogen-treated hysterectomized rabbits treated with or without APP between Days 20 and 23. Serum progesterone and cholesterol levels in these animals are shown in *Figure 6*. Results are expressed as the percentage of cytoplasmic volume represented by the lipid droplet volume from 22 (*control*) or 17 (*APP*) determinations (3–4 micrographs each from 5–6 animals per group; mean ± SEM). *Asterisk* represents a significant (p<0.05) decrease in lipid droplet volume in the APP-treated animals compared to control animals.

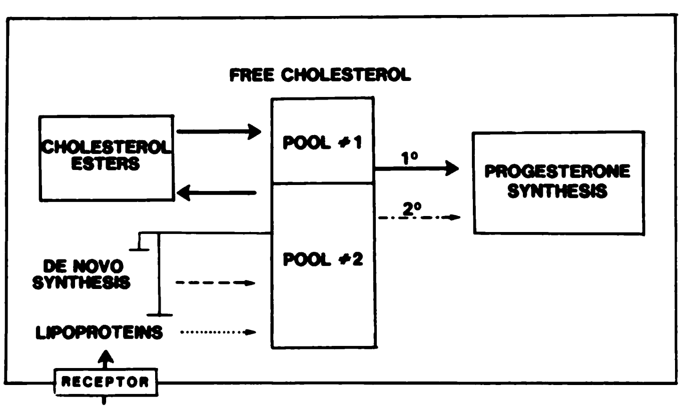

FIG. 8. A working model for the utilization of lipoprotein-derived and de novo-synthesized cholesterol by rabbit luteal cells in the production of progesterone. *Heavy lines* represent the primary pathway of cholesterol uptake, storage, and metabolism, which is proposed to be regulated by estradiol as mediated through the estradiol receptor. *Interrupted* and *thin lines* represent a secondary pathway of cholesterol utilization that occurs when estradiol or estradiol-receptor levels are low. See *text* for discussion.

weight has been related to the content of lipid droplets, it is possible that esterified cholesterol was also used as a precursor for progesterone synthesis. This suggests that cholesterol esterase might also exhibit at least basal activity when estradiol levels are low. Thus, the results of this study indicate that lipoproteins play an important though indirect role as a cholesterol source for luteal progesterone production in the rabbit. As the principal luteotropin in this species, estradiol regulates the uptake and storage of this cholesterol source primarily into lipid droplets. This provides a stable pool to insure a steady rate of progesterone output independent of fluctuation in the levels of lipoprotein.

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