THE PROSTAGLANDIN-FORMING CYCLOOXYGENASE OF OVINE UTERUS

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ABSTRACT

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Two experiments were conducted to characterize changes in the uterine prostaglandin-forming cyclooxygenase during the estrous cycle and to determine the uterine compartments where these changes occur. In experiment 1, cyclooxygenase activity of uterine microsomes from 26 ewes distributed over days 3, 8, 11, 13, 14 and 15 of an estrous cycle was higher on days 13 to 15 than earlier in the cycle ($P<.01$) with a maximum on day 14 ($P<.05$). Immunochemical equivalence points of the cyclooxygenase measured using cyclooxygenase antiserum did not vary during the estrous cycle indicating that increased activity on days 13 to 15 results from increased total cyclooxygenase protein. In experiment 2, cyclooxygenase activity of caruncular microsomes from 21 ewes distributed over days 3, 11 and 14 of an estrous cycle increased 3-fold on day 14 as compared to day 3 ($P<.01$). Immunohistofluorescence techniques failed to identify a specific caruncular cell type responsible for the increase in cyclooxygenase.

These results, which demonstrate a temporal and anatomical correlation between increases in uterine cyclooxygenase activity and reported changes in uterine secretion of $PGF_{2\alpha}$, indicate that luteolysis may be caused by an increase in the concentration of cyclooxygenase and, thus, increased conversion of arachidonate to PGH_2, the precursor of $PGF_{2\alpha}$.

DEDICATION

To My Parents

ACKNOWLEDGMENTS

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LIST OF ABBREVIATIONS

LH	luteinizing hormone
FSH	follicle stimulating hormone
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
CL	corpora lutea - corpus luteum
PGG_2	prostaglandin G_2
PGH_2	prostaglandin H_2
PGE_2	prostaglandin E_2
PGF	prostaglandins F
$^3H\text{-}PGF_{2\alpha}$	tritium-labeled prostaglandin $F_{2\alpha}$.
GPC	glycerophosphorylcholine
PGD_2	prostaglandin D_2
TxB_2	thromboxane B_2
PGI_2	prostacyclin
$6KetoPGF_{1\alpha}$	6-keto prostaglandin $F_{1\alpha}$
FITC	fluoroscein isothiocyanate
IgG	immunoglobulin G
Fab	antigenic determinant portion of IgG
RIA	radioimmunoassay

INTRODUCTION

Estrus marks the beginning of an estrous cycle and is followed by a quiescent period (diestrus) during which implantation of fertilized ova may occur. If pregnancy does not occur, sexual receptivity recurs. Ovine estrous cycles last 16 to 18 days and behavioral estrus, which usually lasts 24 hours, is termed day 0. Idealized hormone patterns for estradiol, progesterone and prostaglandin $F_{2\alpha}$ during an ovine estrous cycle are shown in Figure 1.

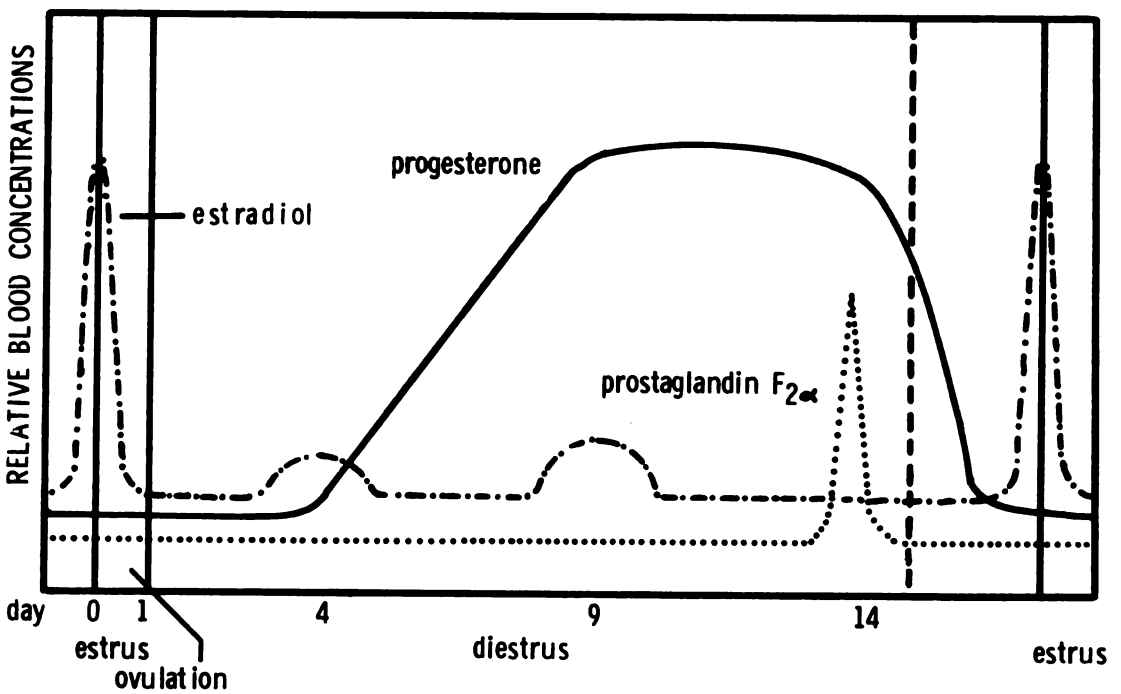

Figure 1. Idealized secretory patterns for estradiol, progesterone and $PGF_{2\alpha}$ during an estrous cycle of a ewe. The data represent mean daily levels.

A preovulatory surge of estradiol, secreted by follicles, initiates behavioral estrus. Two smaller peaks of estradiol also occur during diestrus as a result of follicular development occurring at those times. Following ovulation, follicles are transformed into corpora lutea (CL) which secrete progesterone rather than estradiol. Progesterone secretion increases from basal levels about day 4 and maximal secretion is reached about day 9 of an estrous cycle. During pregnancy corpora lutea play an important role by continuing to secrete progesterone. However, if fertilization does not occur, the CL degenerate (regress) and progesterone secretion declines (luteolysis), about day 14, leading to another estrus (1,2).

Luteal regression could occur automatically following a predetermined CL lifespan or following the depletion of a positive stimulus (luteotropin) of the CL. However, the available evidence favors the production of a negative stimulus (luteolysin) which causes luteal regression (3,4). Initial studies found the uterus to be a source of this luteolysin (5,6,7). Subsequently, exogenous prostaglandins were found to have a luteolytic effect and later studies reported endogenous $PGF_{2\alpha}$ in uterine tissues of ewes, guinea pigs, mares, gilts, rats and hamsters (7-15). Endogenous uterine concentration and secretion of $PGF_{2\alpha}$ increases from basal levels prior to the onset of luteolysis (8,16,17). Physiological studies have shown that exogenous progesterone and estradiol act in a sequential manner to increase uterine secretion of $PGF_{2\alpha}$ (18-20). Endogenous progesterone and estradiol may act in a similar manner, in ewes, since increased secretion of progesterone (days 4 to 12) precedes a small peak of estradiol around days 9 to 10 (19,21).

The biochemical mechanism by which progesterone and estradiol stimulate synthesis of $PGF_{2\alpha}$ is not known, but estradiol does increase cellular protein synthesis (22) and may act by increasing the concentration of prostaglandin biosynthetic enzymes (Fig. 2). The two major enzymes necessary for synthesis of $PGF_{2\alpha}$ are 1) a phospholipase, necessary for specific release of arachidonic acid from phospholipids (23,24) and 2) the prostaglandin-forming cyclooxygenase which forms prostaglandin endoperoxides (PGG_2, PGH_2) from free arachidonic acid (25,26). PGH_2 reductase may be responsible for the formation of $PGF_{2\alpha}$ from PGH_2. However, current evidence favors nonenzymatic formation of $PGF_{2\alpha}$ (132).

The studies reported here were designed to determine if a) fluctuations in uterine cyclooxygenase activity occurred during ovine estrous cycles and, if so, b) were these fluctuations due to the presence of a cyclooxygenase activator or to changes in the total amount of cyclooxygenase protein? Subsequent studies were designed a) to determine the uterine compartments responsible for the increase in cyclooxygenase as well as b) to determine the specific cell types responsible for the increase in cyclooxygenase.

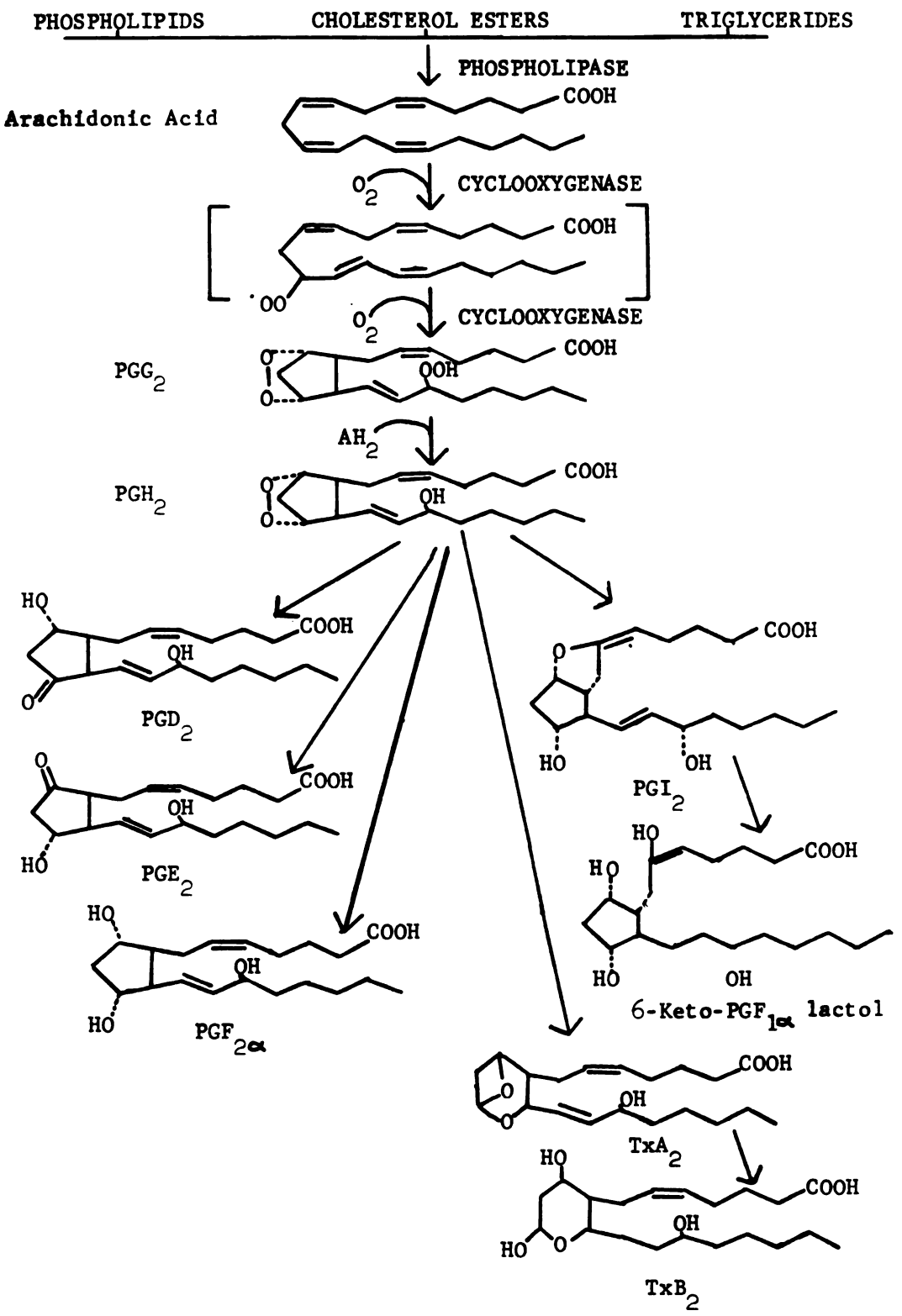

Figure 2. Biosynthetic pathways for prostaglandin formation.

REVIEW OF LITERATURE

Role of the Uterus in Control of Luteal Function

Loeb (27) demonstrated in 1923 that the uterus controlled the lifespan of corpora lutea in guinea pigs. In 1956, Wiltbank and Casida (28) found that complete hysterectomy prolonged the ovine or bovine estrous cycle. In later studies, unilateral hysterectomies were performed demonstrating local control of ovine corpora lutea by an adjacent uterine horn (29-31). Local control, defined as the effect of a uterine horn on the ipsilateral ovary, was also reported in pigs, cattle, guinea pigs, rats and hamsters (32-37). A systemic effect in which either uterine horn can affect corpora lutea on either ovary has been reported for rabbits (38) and horses (39) but will not be discussed in further detail.

Further evidence for a local action of the uterus on luteal function is derived from experiments with animals bearing autotransplanted organs. In ewes, one ovary was completely removed and the remaining ovary and/or uterus was transplanted to the neck and linked to a jugular-carotid pathway. The ovary and uterus must remain attached to each other for regular cyclic regression of corpora lutea to occur (40,41). When only the ovary was transplanted, corpora lutea were maintained for at least 22 days (the experiment was terminated on day 22) as opposed to normal regression around days 14 to 15 (42).

Identification of Uterine Luteolysins

Ligation of the uterine vein (43,44) resulted in prolonged luteal lifespan in ewes. Lyophilized ovine uterine venous plasma collected on day 14 postestrus and infused into the ovarian artery of ewes on day 8 postestrus shortened estrous cycles of those ewes by approximately six days (5). In contrast, jugular venous plasma collected on day 14 or uterine venous plasma collected on day 8 were not similarly active on day 8 postestrous ewes. Ovine uterine extracts or endometrial transplants, collected on days 14 to 15, shortened the length of pseudopregnancy in hamsters. Uterine extracts or transplants from ewes on days 3 to 9 postestrus did not affect pseudopregnancy in hamsters (6). McCracken et al. (7) used ewes bearing autotransplanted ovaries attached to the carotid artery to facilitate direct transfer of blood between experimental animals. Infusion of day 15 uterine venous blood from nonpregnant donor ewes to autotransplanted ovaries containing corpora lutea in recipient ewes resulted in a rapid decline in secretion of progesterone indicative of luteolysis. Donor blood from days 2, 6, 10 or 13 was not luteolytic. These observations provide evidence that the luteolytic factor is carried by the blood and that secretion is increased near the end of a normal estrous cycle.

Babcock (45) first suggested that prostaglandins might be the blood-borne luteolytic factor and Phariss and Wyngarden (46) proposed that $PGF_{2\alpha}$ was the specific prostaglandin responsible for luteolysis. Exogenous $PGF_{2\alpha}$ was luteolytic in guinea pigs (10,47), ewes (48,49) and pseudopregnant rats (46). Prostaglandin $F_{2\alpha}$ (1 mg/kg daily) was luteolytic when injected subcutaneously in rats (46). $PGF_{2\alpha}$ (0.25 mg

daily for three days) was luteolytic when injected into guinea pigs (10,47). Although approximately ten-fold less potent than $PGF_{2\alpha}$ (10,47) prostaglandin E_2 (PGE_2) was also found to be luteolytic.

Thorburn and Nicol (49) found 10 μg $PGF_{2\alpha}$/hr infused into the ovarian artery for three hours to be the minimal effective luteolytic dose in cyclic ewes. Similarly, 2 μg $PGF_{2\alpha}$/hr for 10-18 hours was luteolytic in ewes with autotransplanted ovaries (48). When infusions were made into the uterine vein, 40 μg $PGF_{2\alpha}$/hr for six hours was luteolytic in intact ewes (49). A similar effect was reported for the autotransplanted ovary following infusion of 20 μg $PGF_{2\alpha}$/hr for nine hours (50). These doses, and even 200 μg $PGF_{2\alpha}$/hr for three hours, had no luteolytic effect when injected into the jugular vein of cyclic ewes (50). These latter results can be attributed to inactivation of prostaglandins by the lung (51) and explain the importance of local utero-ovarian relationships in the control of the CL in most species.

If $PGF_{2\alpha}$ is an important endogenous luteolysin, inhibition of its synthesis should prolong the lifespan of corpora lutea. Guinea pigs injected subcutaneously with indomethacin (20 mg twice daily) (an inhibitor of PG synthesis) from day 7 until estrus occurred had estrous cycles lengthened by three days (52). Paraffin implants, containing indomethacin, and placed into a uterine horn, lengthened estrous cycles of guinea pigs by at least 30 days as compared with control implants (52,53). Indomethacin also blocked estradiol induced luteolysis in ewes (54).

Guinea pigs immunized against $PGF_{2\alpha}$ exhibited prolonged luteal function (55,56). Estrous cycles gradually lengthened and the longer cycles occurred in animals with higher antibody titers against $PGF_{2\alpha}$. Hildebrandt-Stark et al. (57) also reported prolonged luteal function as well as inhibition of ovulation in all guinea pigs immunized against $PGF_{2\alpha}$. Estradiol rose from 40 pg/ml on day 30 to 136 pg/ml by day 100 postestrus while progesterone declined to basal levels of 0.6 ng/ml. Active immunization of ewes against $PGF_{2\alpha}$ resulted in persistent corpora lutea and elevated progesterone (>2 ng/ml) within one month of the first inoculation (58). These experiments provide evidence that decreasing the synthesis and/or circulating concentrations of free $PGF_{2\alpha}$ results in prolonged lifespans of corpora lutea and that $PGF_{2\alpha}$ is important for luteolysis to occur.

Biosynthesis of $PGF_{2\alpha}$

For $PGF_{2\alpha}$ to be a physiological luteolysin it should be found in uterine tissues of normal animals near the end of the estrous cycle. In guinea pigs, the concentration of $PGF_{2\alpha}$ in uterine venous plasma increased on day 14, coinciding with a decline in jugular concentrations of progesterone (9). Similar results were seen using sows (59). In ovine uterine venous plasma, the concentration of $PGF_{2\alpha}$ rose from less than 2 ng/ml on day 3 to about 25 ng/ml on days 14 and 15, with the increase lasting about 24 hours (9,16,17,60). Wilson et al. (61) observed the concentration of $PGF_{2\alpha}$ in ovine endometrium to be greater on day 14 than on days 3, 8 or 11 of the estrous cycle. Prostaglandins F, as measured by radioimmunoassay (RIA), increased in bovine endometrial tissue on day 15 as compared to days 1-14 of the estrous cycle (62).

Local Transport of the Uterine Luteolysin to the Ovary

Transport of the luteolytic factor could occur via the oviduct, the lymphatics or the vasculature. Of these three systems, the lymphatics have been disregarded but not ruled out (63). Ligation or transection of oviducts had no effect on guinea pig (35) or rat (64) corpora lutea. Complete removal of the oviduct in ewes also did not prolong the estrous cycle (65). These results indicate that the luteolytic factor does not pass through the oviduct. On the other hand, selective ligation of the uterine and ovarian arteries and utero-ovarian vein did result in persistent corpora lutea. Kiracofe et al. (43) observed luteal maintenance after ligation of the uterine artery and uterine vein, but not after ligation of the uterine artery alone. Ligation of the uterine vein proximal to its junction with the ovarian artery caused luteal maintenance in four out of ten animals (44). These latter results indicate a positive role for the vasculature in luteolysis transport.

Prostaglandin Transport by a Local Venoarterial Pathway

Mapletoft et al. (66,67) surgically anastomosed or connected the utero-ovarian vein and the ovarian arteries in ewes. The effect of unilateral hysterectomy could be reversed by joining the uterine vein from an intact horn to the ovarian artery of an isolated ovary containing a corpus luteum. However, such anastomoses do not normally occur in intact animals. By tracing vascular pathways in the ovine and bovine uterus, Ginther et al. (33,68) demonstrated that the ovarian artery is convoluted and adherent to the surface of the utero-ovarian vein in the ewe (see Fig. 3). Careful surgical separation of these

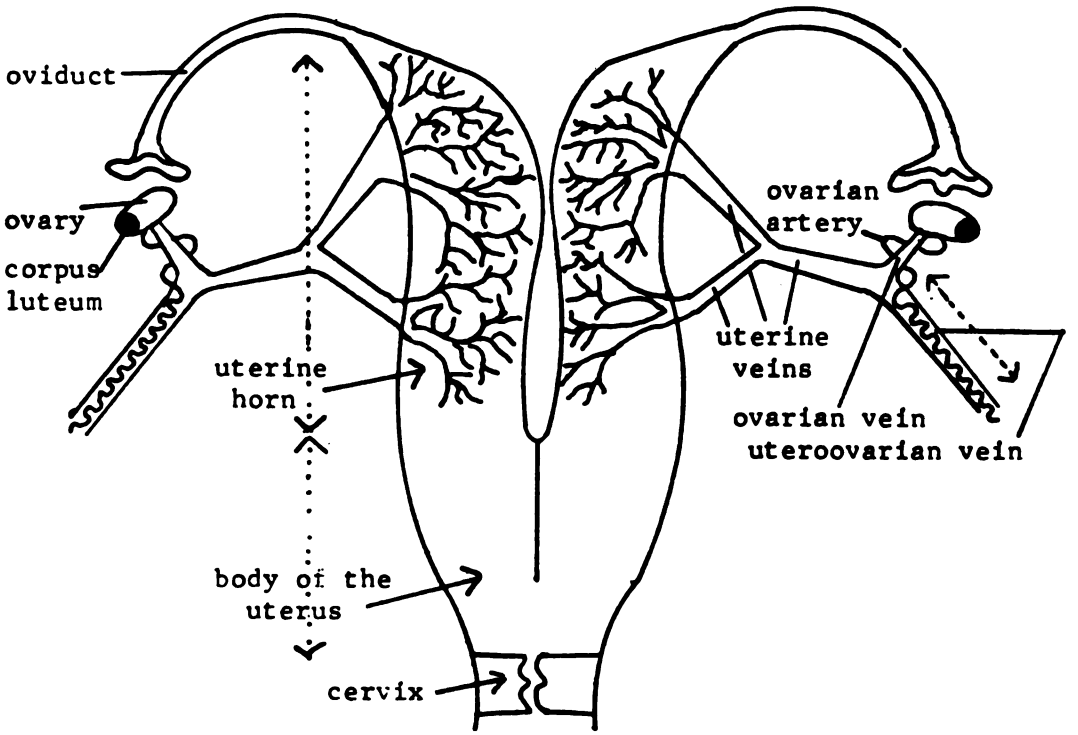

Figure 3. Representation of the ovarian and uterine vascular relationship in the ewe. The ovarian and uterine veins join to form the utero-ovarian vein. The ovarian artery is coiled and lays on the utero-ovarian vein.

two vessels without interfering with blood flow (confirmed by subsequent checks for patency) prevents corpus luteum regression, suggesting that prostaglandins diffuse out of the uterine vein into the ovarian artery (50). Evidence supporting such a countercurrent mechanism of transport was obtained in studies in which ^{3}H-$PGF_{2\alpha}$ was infused into the uterine vein and concentrations were measured in the ovarian and iliac arteries. McCracken et al. (7,69) reported as much as a 30-fold difference in the concentration of $PGF_{2\alpha}$ in the ovarian artery as compared to the peripheral control. Coudert et al. (70,71) were unable to confirm this finding. Land et al. (72) then demonstrated that the

concentration of $^{3}H\text{-}PGF_{2\alpha}$ in the ovarian artery increased in proportion to the log of the concentration of $^{3}H\text{-}PGF_{2\alpha}$ infused into the uterine vein. Thus, the available evidence appears to favor countercurrent transport of $PGF_{2\alpha}$ in cycling ewes.

Hormonal Control of the Secretion of $PGF_{2\alpha}$

Progesterone. Exogenous progesterone administered to ovariectomized ewes has yielded contradictory results. Progesterone (10 mg daily for four days) injected into ewes immediately following ovariectomy on day 4 of the estrous cycle reduced the concentration of $PGF_{2\alpha}$ in the endometrium (61). In apparent contrast, Ford et al. (18) found progesterone (3 mg twice daily for 5 days) to have no effect on uterine venous concentrations of $PGF_{2\alpha}$ in chronically ovariectomized ewes. Louis et al. (54) then observed PGF concentrations to increase in endometrium and uterine venous plasma following progesterone treatment (10 mg daily for 9 days) in ovariectomized ewes. The disagreement possibly reflects differences in dosage of progesterone and length of ovariectomy of the experimental animals.

In other species, no changes were observed in $PGF_{2\alpha}$ or PGE_2 in 7-day ovariectomized rats after treatment with progesterone (2 mg, days 8 and 9, and 50 mg, day 10) (73). Progesterone treatment for six days (1 mg daily) in mice ovariectomized for 14 days caused a 4-fold increase in uterine $PGF_{2\alpha}$ (74).

In intact ewes, progesterone decreased the length of cycles when administered early in the estrous cycle (75). The concentration of $PGF_{2\alpha}$ in ovine endometrium also increased on days 5 and 9 following progesterone treatment (40 mg daily) on days 0 and 1 of the estrous cycle (61). Recently, progesterone (40 mg) administered on days 0

and 1 of the estrous cycle has been shown to increase ovine uterine secretion of $PGF_{2\alpha}$ by day 8. These earlier peaks of $PGF_{2\alpha}$ apparently stimulated earlier luteal regression by approximately six days (76). In contrast, Ford et al. (18) reported no change in uterine venous concentrations of $PGF_{2\alpha}$ after progesterone treatment (10 mg daily) on days 1 to 5 of the estrous cycle. It does appear from the above experiments that progesterone may increase endometrial content but not immediate secretion of $PGF_{2\alpha}$.

Estradiol. Contradiction exists as to the effect of estradiol on uterine $PGF_{2\alpha}$ synthesis in ovariectomized ewes. Intramuscular injection of estradiol (12.5 ug twice daily for 5 days) increased uterine venous concentrations of $PGF_{2\alpha}$ in ovariectomized ewes (18). However, no such effect was seen following a nine-day estradiol treatment (15 ug twice daily) (54). McCracken et al. (3) demonstrated a 20-fold increase in uterine venous levels of PGF after a physiological dose of estradiol in ewes with autotransplanted ovaries. One nanogram per minute estradiol for two hours injected into the uterine artery increased PGF secretion from 5 to 100 ug/hr. Secretion of $PGF_{2\alpha}$ was also enhanced by treatment of anestrous ewes with estradiol (77).

Estradiol was luteolytic in intact ewes when administered on days 9 to 11 of an estrous cycle (78-81). Injection of estradiol (500 ug, days 9 and 10) increased ovine endometrial and utero-ovarian venous concentrations of $PGF_{2\alpha}$ (18). However, the same dose of estradiol on days 4 and 5 had no effect on concentrations of $PGF_{2\alpha}$. Blatchley et al. (8) demonstrated increased uterine venous concentrations of $PGF_{2\alpha}$ in guinea pigs treated with estradiol (10 ug, days 4-6). Treatment with estradiol on days 3 and 4 increased uterine

concentrations of PGF in cyclic hamsters (15). From the above evidence, estradiol appears to play an important role in increasing the concentration and secretion of uterine $PGF_{2\alpha}$ when administered during the later part of the estrous cycle.

Dosage of estrogen, method of administration, treatment schedule and stage of the estrous cycle are important factors in the luteolytic effect of estradiol. Greater than 250 ug estradiol must be given by an intramuscular injection on days 9 and 10 for a decrease in progesterone concentration to be seen in ewes (82). On the other hand, as little as 100 ug estradiol infused into the uterine artery over a 24-hour interval (but not a 12-hour interval) was luteolytic (82). Further studies on the luteolytic effect of estradiol have established that the uterus must be present at least 24 hours following the administration of estradiol (83). If hysterectomy is performed within 24 hours following estradiol administration, luteolysis does not occur.

If estradiol increases synthesis of $PGF_{2\alpha}$ decreasing free circulating estradiol should decrease synthesis of $PGF_{2\alpha}$ and lead to prolonged CL lifespans. Fairclough *et al.* (84) reported that treatment of ewes with estradiol antiserum did not prevent normal luteolysis. However, their treatment did prevent behavioral estrus and ovulation. Caldwell *et al.* (85) reported undetectable levels of PGF in ovariectomized ewes immunized against estradiol following treatment of these animals with progesterone and estradiol. X-irradiation of the ovary destroys primary and secondary follicles, resulting in a decreased synthesis of estradiol and prevented subsequent luteal regression (86).

Administration of Actinomycin D (50 ug) on day 11 of cyclic ewes maintained corpora lutea in a unilateral manner even with simultaneous administration of estradiol (87). Estradiol has long been known to stimulate cellular protein synthesis, and its luteolytic effect may be exerted through an increase in uterine prostaglandin synthetic enzymes.

Estradiol and Progesterone. Warren et al. (88) provided evidence that progesterone priming (pretreatment) is necessary for estradiol-induced luteolysis in intact ewes. Administration of estradiol on days 1 to 10 had no effect, while treatment with progesterone for four days in either the presence or absence of exogenous estradiol was necessary for a subsequent estradiol stimulus to cause luteolysis. Barcikowski et al. (19) reported progesterone priming to be important in increasing $PGF_{2\alpha}$ synthesis following exogenous or endogenous estradiol. Increased PGF concentrations in ovine uterine venous plasma were demonstrated following progesterone priming (10 mg, days 1-5) and estradiol treatment (0.5 mg, days 4 and 5) (18).

Both estradiol and progesterone were necessary for the in vivo secretion of $PGF_{2\alpha}$ in the guinea pig uterus (20). Progesterone priming followed by estradiol was also necessary for increased PGE and PGF synthesis in the cyclic rat (73).

In ovariectomized ewes, jugular venous concentrations of PGF increased if progesterone was given every other day for 11 days, followed by estradiol (50 ug) on day 13 (85). Uterine secretion of PGF also increased following progesterone (3 mg, daily for 5 days) and estradiol (125 ug on day 5) (18), as well as following progesterone (10 mg twice daily for 9 days) and estradiol (15 ug, twice daily, on days 1-9) (54).

As in ovariectomized ewes (18), subcutaneous injection of estradiol benzoate for three days in ovariectomized guinea pigs was as effective in increasing uterine concentrations of $PGF_{2\alpha}$ as progesterone priming for ten days followed by estradiol (89). In ovariectomized rats, estradiol alone was reported to predominantly control prostaglandin production, but progesterone exerted a small effect (73). A progesterone-estradiol regimen was more effective than estradiol-progesterone in increasing $PGF_{2\alpha}$ concentrations in uterine venous plasma of ovariectomized mice (74). Treatment of anestrous (noncycling) ewes with a regimen of progesterone and estradiol increased the caruncular concentration of PGF greater than 15-fold while whole uterine concentrations increased only 3-fold (90).

In summary, estradiol or progesterone administered alone may be luteolytic due to interaction of the exogenous hormone with endogenous hormone. Ovariectomy may further complicate exogenous hormone interactions, explaining the contradictory results achieved using this technique. It appears that progesterone priming followed by estradiol is the optimal method for exogenous stimulation of uterine $PGF_{2\alpha}$ synthesis. Endogenous hormone patterns support the theory that a similar effect is seen in vivo (19,21).

Oxytocin. Large doses of oxytocin (400-10,000 mU) injected intravenously increased peripheral venous levels of PGF in estradiol-primed anestrous ewes (77). An 11-fold increase in uterine secretion of $PGF_{2\alpha}$ followed administration of oxytocin to uterine explants (91). The greatest increase in $PGF_{2\alpha}$ occurred in tissues from ewes on day 15 of the cycle and was much greater in the endometrium than the

myometrium. In cyclic heifers, plasma concentrations of PGF increased following three days' administration of oxytocin and the greatest increase occurred on day 3 of the estrous cycle (92). Isolated strips of rabbit endometrium, pretreated with estradiol and progesterone for ten days, secreted $PGF_{2\alpha}$ in response to oxytocin stimulation (93). This stimulatory effect of oxytocin is not well understood and may be due to a true metabolic action or to a stimulation of uterine contractions which in turn cause PGF synthesis (93). Oxytocin is secreted early in an estrous cycle and affects ovum and sperm transport (1). The stimulation of PGF concentration by oxytocin is thus probably related to this transport rather than luteolysis.

Biochemical Regulation of $PGF_{2\alpha}$ Synthesis

While it is unclear how the uterine synthesis of prostaglandins is regulated, there are three possible steps at which the biosynthesis of $PGF_{2\alpha}$ could be controlled (Figure 2). The following literature review covers the regulation of prostaglandin synthesis which occurs during 1) phospholipase catalyzed release of arachidonate, 2) cyclooxygenase catalyzed formation of PGH_2 and 3) reduction of PGH_2 to prostaglandins, prostacyclin and thromboxanes.

Selective Release of Esterified Arachidonic Acid. Human platelets contain two different phospholipases (23,94). Phospholipase A_2 selectively hydrolyzed 1-acyl,2-arachidonyl glycerophosphoryl choline (GPC) in response to thrombin with a corresponding increase in free arachidonate. While ^3H-arachidonate was incorporated into all phospholipid regions, only ^3H-arachidonate in the diacyl GPC region decreased in response to thrombin. However, the specificity of the

lipase could be due to substrate compartmentation, since membrane asymmetry is well documented (95) or to substrate specificity similar to that for acyl CoA:monoacyltransferases (96). Blackwell et al. (97) have shown substantial arachidonate release from phospholipid pools of rabbit platelets after thrombin stimulation. Hong and Levine (98,99) reported arachidonate release in fibroblasts by bradykinin and thrombin as opposed to a serum or mechanical stimulus which released all lipids. These authors did not provide evidence for selective release of arachidonate, however.

Specific release of arachidonate as compared to linoleate or oleate by bradykinin was observed in isolated rabbit heart (24). Ischemia, a non-specific stimulus, caused release of all three acids. However, only arachidonate was efficiently incorporated into the diacyl GPC and release in response to bradykinin could be due to a diacyl GPC specific phsopholipase or to compartmentation of a lipase with the lacyl-2-arachidonyl GPC as well as an arachidonate specific phospholipase A_2.

Lipids, other than phospholipids, cannot be ruled out as a source of esterified arachidonate. The prostaglandin precursor may be derived from a cholesterol ester in the adrenal cortex (100), or from triglycerides in renal medullary interstitial cells which are enriched in arachidonic acid (101,102). However, Zusman and Kaiser (103,104) have shown mepacrine, an inhibitor of phospholipases, to reduce prostaglandin synthesis in renal interstitial cells by approximately 50%, suggesting that in these cells phospholipids are an important precursor for prostaglandin biosynthesis.

Phospholipase activity in platelets (105) and rat uterus (106,107) may be influenced by intracellular cyclic AMP or cyclic GMP levels, suggesting regulation of prostaglandin synthesis by phosphorylation-dephosphorylation of the phospholipase. Roberts et al. (91) suggested that oxytocin activation of ovine uterine prostaglandin synthesis occurs through a uterine lipase. However, these authors observed a considerable lag period occurring in the activation process while in previous instances described above, bradykinin or thrombin activation of the phospholipase occurred within minutes of the stimulus (23,24,94,98,99, 103,104).

Conversion of Arachidonic Acid to Prostaglandin Endoperoxides. Conversion of arachidonic acid to PGG_2 and PGH_2 is the most clearly defined step in prostaglandin synthesis. Samuelsson and Hamberg (108-110) found endoperoxide formation is initiated by abstraction of L-hydrogen at carbon number 13 and insertion of oxygen at carbon number 11. A series of intramolecular rearrangements results in addition of a second oxygen molecule at carbon number 15 and an endoperoxide formed between carbon numbers 9 and 11 (Fig. 2) (111-113). Samuelsson (114) proved that formation of this endoperoxide involves only a single oxygen molecule. A single enzyme, the prostaglandin forming cyclo-oxygenase, catalyzes the addition of both oxygen molecules to arachidonate and has been isolated by Hemler et al. (25) and Myamoto et al. (26). Heme is required by the cyclooxygenase (25) and an associated peroxidase activity for conversion of PGG_2 to PGH_2 was described (26). The purified enzyme is thought to have an $\alpha_2\beta_2$ structure and was found to be subject to time dependent self-destruction, activation by phenol

compounds, deactivation by glutathione peroxidase and inhibition by nonsteroidal antiinflammatory drugs (flurbiprofen, flufenamate) (115) similar to that observed in impure enzyme preparations (116,117). Smith and Wilkin (118) have shown the ovine uterine cyclooxygenase to be antigenically similar to the ovine vesicular gland enzyme. The ovine uterine enzyme also undergoes self-catalyzed destruction and inhibition by nonsteroidal and antiinflammatory drugs.

The cyclooxygenase, as stated, is activated by heme and phenol and deactivated by glutathione peroxidase and self-destruction (115-117,119). In addition, Chandrabose et al. (120) have indicated regulation may occur through an increase in total cyclooxygenase protein. These investigators have observed that dexamethasone treatment of cultured mouse 3T3 cells inhibits phospholipase activity while increasing the conversion of arachidonate to prostaglandins (therefore acting on the cyclooxygenase). This observation may be important to uterine studies where steroid treatment (progesterone and estradiol) also increases the concentration and secretion of $PGF_{2\alpha}$ (18-20).

Conversion of PGH_2 to Thromboxanes, Prostacyclins and Prostaglandins. Thromboxane derivatives were first identified by Hamberg and Samuelsson as products of arachidonate metabolism in human platelets following short-time incubations (30 sec) (121). Significant synthesis of thromboxane B_2 (TxB_2) was subsequently reported in lung, spleen and brain tissues (122-124). The more active precursor of TxB_2 was identified as TxA_2 and found to be identical to the previously reported rabbit aortic contracting substance (RCS) (121,125). A major effect of TxA_2 is to induce platelet aggregation at the site of a thrombosis (125).

More recently, Pace Asciak (126) and Moncada et al. (127,128) identified prostacyclin (PGI_2) as a major product in rat fundus and arterial microsomes, and PGI_2 has since been identified as a product in a number of tissues (129). While PGI_2 may be produced primarily by elements of the vasculature to prevent platelet aggregations in these tissues, Grenier and Smith (130) have shown that the renal collecting tubule cell synthesized PGI_2 and that production of PGI_2 represents a greater proportion of total products at low substrate concentrations. The product mix of other cells is thought to be regulated by cofactors of degradative enzymes (131-134). Myamoto et al. (26,134) reported the isolation of a glutathione activated PGE_2 isomerase and Hamberg and Samuelsson (112) reported an increase in PGE_2 synthesis by isolated platelets upon addition of glutathione. Sun et al. (135) reported a PGD_2 isomerase found in rat brain and polymorphonuclear leukocytes. Reducing agents are also known which favor PGF formation in bovine seminal vesicle microsomes, but these agents act nonenzymatically (132).

Before the identification of prostacyclins and thromboxanes, the uterus was thought to produce only PGE_2 and $PGF_{2\alpha}$. Since some of this early work involved identification by thin layer chromatography (TLC), it must be viewed skeptically, since the chromatographic mobilities of $6KetoPGF_{1\alpha}$ (the breakdown product of PGI_2) and TxB_2 are similar to those of PGE_2, PGD_2 and $PGF_{2\alpha}$ in most solvent systems. Recent studies have shown PGI_2 to be a major uterine product. PGE_2 was the major product of rat decidual microsomes followed by PGD_2, $PGF_{2\alpha}$ and $6KetoPGF_{1\alpha}$, the last of which cochromatographed with PGE_2. Rat myometrial microsomes were only 25% as active but produced only

6KetoPGF$_{1\alpha}$ instead of PGE$_2$ (136). Williams et al. (137,138), using bioassays, observed the rat myometrial PGI$_2$ activity to increase 18-fold by day 22 (the day of parturition), while decidual PGI$_2$ increased only 5-fold. 6KetoPGF$_{1\alpha}$ was then reported to be a major product of pseudopregnant rat, guinea pig and sheep uteri when measured in extracts of uterine homogenates (139,140). As yet, no total product characterization by homogeneous uterine cell cultures has been reported, and the product mix or factors governing prostaglandin formation in the PGF$_{2\alpha}$ producing cell are not known.

MATERIALS AND METHODS

Materials

Arachidonic acid was obtained from NuChek Preps, Inc., Elysian, MN. Sodium diethyldithiocarbamate, Tween 20 bovine hemoglobin and sodium pentobarbital were products of Sigma Chemical Co., St. Louis, MO. Flufenamic acid was purchased from Aldrich Chemical Co., Milwaukee, WI. Flurbiprofen (dl-2-(-2-fluoro-4-biphenylyl)propionic acid) was kindly supplied by Dr. Udo F. Axen of the Upjohn Co., Kalamazoo, MI. Protein A-Sepharose CL-4B was purchased from Pharmacia Fine Chemicals, and papain (35.7 units/mg) was purchased from Worthington Biochemical Corporation. Fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG, goat anti-rabbit whole serum and goat anti-rabbit IgG were obtained from Miles Laboratories, Inc. CM-Cellex was from Bio-Rad Laboratories. All other chemicals were reagent grade purchased from common commercial sources.

Methods

Preparation of Detergent-Solubilized Microsomes from Whole Uteri. Multiparous ewes were checked for estrus twice daily with the aid of vasectomized rams. After at least one cycle (16 to 18 days), ewes were assigned to be hysterectomized on days 3, 8, 11, 13, 14 or 15 (estrus = day 0). Hysterectomies were performed aseptically using Nembutal anesthesia. Uteri were cooled to 4° and trimmed free of mesometrium, oviduct and cervix. Uterine horns were separated, weighed and then minced into 3 volumes of 0.1 M tris-chloride, pH 8.0, containing 50

mM diethyldithiocarbamate and 0.1 mM flufenamic acid. Tissues were homogenized with a Tekmar rotary shear for 60 sec and then with two passes of a teflon-glass type homogenizer. The homogenat was centrifuged at 10,000 x g for 5 min and the resultant supernatant decanted and centrifuged at 100,000 x g for 75 min. The microsomal pellet was resuspended by homogenization in 1 ml of 0.1 M tris-chloride, pH 8.0, containing 10 mM diethyldithiocarbamate and 1% Tween 20. After 30 min at 4^{o} the sample was centrifuged at 200,000 x g for 30 min, and the resulting supernatant containing the solubilized enzyme was assayed for cyclooxygenase activity as described below.

Preparation of Detergent-Solubilized Uterine Microsomes from Caruncles, Noncaruncular Endometrium and Myometrium. Ewes were assigned to be hysterectomized on days 3, 11 or 14 postestrus. After anesthetizing the ewe, the uterus was immediately removed, cooled to 4^{o}, and trimmed free of mesometrium, oviduct and cervix. To randomize the dissection procedure, the uterine horns were cut into eight large segments of approximately equal size: a) four of the segments were dissected into three major fractions (caruncle, noncaruncular endometrium and myometrium); b) two of the uterine segments were dissected but the fractions not separated; and c) the remaining two uterine segments were left intact as whole uteri. Each of the five samples was minced in 5 volumes of 0.1 M tris-chloride, pH 8.0 containing 50

mM diethyldithiocarbamate and 0.1 mM flufenamic acid. The tissue was homogenized with two 15-20 second bursts of a Brinkman Polytron PT20ST homogenizer. The first 15 seconds of homogenization were at medium speed, the second 15 seconds at high speed. Solubilized microsomes were then prepared from each of the homogenates as described above and assayed for cyclooxygenase activity as described below.

Determination of Cyclooxygenase Activity. Cyclooxygenase assays were performed at 37° using a YSI Oxygen Monitor equipped with a voltage offset control essentially as described by Smith and Lands (116). Aliquots of solubilized uterine microsomes were added to oxygen electrode chambers containing 300 nmoles of arachidonic acid, 2 umoles of phenol, 2 nmoles of bovine hemoglobin and 15 umoles of EDTA in a final volume of 3 ml of 0.1 M tris-chloride, pH 8.0. Negative controls were performed by measuring the rates of oxygen uptake in the presence of two specific cyclooxygenase inhibitors, Flurbiprofen (141) and flufenamic acid (142) (10^{-4} M). Control rates were always less than 10% of experimental values. Further confirmation that oxygen uptake was a direct measure of cyclooxygenase activity came from observation of the self-catalyzed destruction kinetic phenomenon chracteristic of the enzyme (117). A unit of cyclooxygenase activity is defined as that amount of enzyme that catalyzes the uptake of one nmole of oxygen per min at 37°. Between five and ten separate rate measurements were made for each microsomal sample over a five-fold range of enzyme concentrations. These values included into means were within 10% of the mean.

Preparation of Antisera and Fab Fragments. Rabbit anti-cyclooxygenase serum monospecific for the enzyme from sheep vesicular gland and rabbit pre-immune serum were prepared essentially as described previously (118,143). Rabbits were immunized using a homogeneous sheep vesicular gland cyclooxygenase preparation (25). Rabbit IgG was isolated from both immune and pre-immune sera using column chromatography on Protein-A-Sepharose (144,145). Serum (10 ml) was applied to a column (1.1 x 4 cm) equilibrated at 4° with 0.1 M sodium phosphate, pH 7.0. The column was washed with 10 to 20 volumes of equilibration buffer. The IgG fraction (35-70 mg) was eluted with 1 M acetic acid and the eluant neutralized with concentrated NH_4OH. Isolated IgG was dialyzed overnight at 4° against 100 volumes of 0.1 M sodium phosphate, pH 7.0, and then stirred for 4 hr at 24° under a N_2 atmosphere in 0.1 M sodium phosphate, pH 7.0, containing 0.002 M EDTA, 0.01 M cysteine and papain (0.5 mg/100 mg IgG). The final IgG concentration was 5 mg/ml. Papain-digested IgG (Fab plus Fc fragments) was dialyzed 3 times for 12 hr each at 4° against 500 volumes of 0.02 M sodium acetate, pH 6.0. Fab fragments were isolated by column chromatography on CM-cellex (146). Immunochemical purity of the IgG and Fab fractions was assessed by Ouchterlony double diffusion analyses against goat anti-rabbit IgG and goat anti-rabbit whole serum. Single precipitin lines were observed for purified IgG and Fab preparations upon reaction with both anti-rabbit sera. The expected reaction of partial identity between IgG and Fab was also noted.

Immunochemical Equivalence Points. Aliquots (5-400 µl) of solubilized microsomes prepared from whole uteri (Experiment 1) and containing known levels of cyclooxygenase activity were added to a series of test tubes, each containing 25 µl of anti-cyclooxygenase serum (118). A parallel series of control incubations was performed in which 25 µl of rabbit preimmune serum was substituted for the anti-cyclooxygenase serum. All solutions were made to a final volume of 0.5 ml with 0.1 M tris-chloride, pH 8.0, containing 10 mM diethyldithiocarbamate and 1% Tween 20 and incubated for 24 hr at 4°. The resulting immunoprecipitates were sedimented by centrifugation at 2,000 x g for 30 min at 4° and the supernatants assayed for unprecipitated cyclooxygenase activity as described above. Values for supernatant cyclooxygenase activity measured in the samples containing anti-cyclooxygenase serum were corrected for nonspecific losses of enzyme activity caused by protein denaturation. These corrections were made by dividing the measured values by the fraction of activity recovered in each of the samples containing preimmune serum. Corrected values for supernatant activity were plotted versus added cyclooxygenase. Linear regression analyses were used to determine the x-intercept which represents the immunochemical equivalence point. This is the point at which a constant, maximum amount of cyclooxygenase protein is precipitated by 25 µl of anti-cyclooxygenase serum. The amount of cyclooxygenase activity precipitated at this point depends on the catalytic activity of the protein and varies depending on activation or inhibition of the enzyme.

To determine immunochemical equivalence points for the endometrial cyclooxygenase (Experiment 2), aliquots of solubilized microsomes derived from combining the caruncular and intercaruncular endometrium were used to provide enough activity to generate an immunochemical equivalence point as described above. In addition, 12.5 µl of anti-cyclooxygenase serum was used rather than 25 µl as used previously.

Immunohistochemical Procedures. Segments of uterine horns approximately 1 cm in length were cut approximately 5 cm from the tubouterine junction. Uterine and ovarian sections were embedded in 5% gum tragacanth and frozen on cork cylinders in hexane cooled to -60° in a dry ice-acetone bath. Cross sections (10 u) were cut on an Ames Lab Tek microtome at -20°, mounted on coverslips and dried at room temperature for at least 60 min prior to staining. Solutions of Fab fragments (0.3 mg/ml; equivalent to a 1:15 dilution of whole serum) were layered onto the dried sections and incubated for 30 min at room temperature. The coverslips were then rinsed twice for 5 min periods with phosphate-buffered saline. FITC-labeled goat anti-rabbit IgG (1:10 dilution in phosphate-buffered saline) was then layered over the sections for 30 min, the sections washed with phosphate-buffered saline as above, and mounted on microscope slides in glycerol. Fluorescence photomicroscopy was performed with a Leitz Orthoplan microscope equipped with an Orthomat camera using Kodak Ektachrome (ASA 160) or TriX-Pan (ASA 400) film.

Fluorescence quantitation was performed with a Leitz Orthoplan microscope equipped with a Keithley digital voltmeter. Relative fluorescence intensity was linear over a dilution range of 1:10 to

1:500 for solutions of FITC-labeled goat anti-rabbit IgG in phosphate-buffered saline. A 1:50 dilution of the FITC-labeled goat anti-rabbit IgG was used to standardize the voltmeter arbitrarily to 200 mV and was checked frequently to eliminate instrument variation. Fifteen to twenty fluorescence pulse readings were taken on small areas of each uterine cell type in cryostat sections stained with Fab fragments and FITC-labeled goat anti-rabbit IgG as described above. Mean fluorescence intensity and standard deviation were calculated for each uterine cell type on days 3, 11 and 14 of the ovine estrous cycle.

Statistical Analyses. Activity of the uterine cyclooxygenase among days of an estrous cycle from both experiments 1 and 2 were examined independently by analysis of variance. Differences among days of an estrous cycle were identified by single degree of freedom comparisons.

RESULTS

Cyclooxygenase Activity in Whole Uterus During the Ovine Estrous Cycle. Experiment 1 was conducted to determine if the specific activity of uterine cyclooxygenase fluctuates during the ovine estrous cycle. Because the cyclooxygenase is a membrane bound protein (147), a microsomal fraction was isolated. Treatment of the microsomal pellet with 1% Tween 20 solubilized greater than 95% of the cyclooxygenase. Removal of excess membrane using ultracentrifugation and assaying only a microsomal supernatant increased reproducibility of the enzyme assays. In each set of assays, cyclooxygenase activity measured over a five-fold range of protein concentration was linear with respect to added protein. Activities of the prostaglandin-forming cyclooxygenase in solubilized uterine microsomes from ewes on days 3, 8, 11, 13, 14 or 15 of the estrous cycle are shown in Table 1. Greater activities are found on days 13, 14 and 15 than on days 3, 8 and 11 ($P<.01$); cyclooxygenase activity was at a maximum on day 14 ($P<.05$) and was three-fold greater than found on day 3.

Plasma concentrations of progesterone increased from 0.72 ± 0.28 ng/ml on day 3 to 4.25 ± 2.14 ng/ml for the four ewes sacrificed on day 11. The five ewes sacrificed on day 14 had progesterone concentrations of 6.08 ± 3.48 ng/ml. Thus, no significant decrease in progesterone secretion had occurred prior to the increase in uterine cyclooxygenase activity.

Table 1. Uterine cyclooxygenase activities and immunochemical equivalence points at different stages of the ovine estrous cycle[a]

Days Postestrus	Cyclooxygenase Activity (units/g wet weight)	Immunochemical Equivalence Point (units precipitated/ 25 ul antiserum)
3	11.7 + 3.3 (5)[b]	13.9 ± 3.6 (4)[e]
8	14.9 ± 6.3 (4)[b]	10.4 ± 0.5 (2)[e]
11	19.1 ± 6.1 (4)[b]	14.1 ± 2.5 (4)[e]
13	23.4 ± 7.8 (4)[c]	12.3 ± 1.7 (3)[e]
14	33.5 ± 4.4 (5)[c,d]	11.2 ± 1.7 (3)[e]
15	27.4 ± 8.9 (4)[c]	14.1 ± 2.1 (4)[e]

[a]Values represent mean ± standard error for the number of animals in parentheses.

[b,c,d,e]Values within a column with different superscripts are significantly different.

Immunochemical equivalence points were determined on solubilized uterine cyclooxygenases prepared from sheep on different days after estrus. These analyses were performed to determine whether the increased enzyme activity observed on days 13 to 15 resulted from an increase in total cyclooxygenase protein or an activation of preexisting enzyme (Table 1). The techniques involved in determining immunochemical equivalence points are similar to those used in radioimmunoassays (RIAs) in that: (a) the amount of antiserum is held constant; (b) known but varied amounts of catalytic activity are added as with standard curves for hormones and (c) it is assumed that the anti-cyclooxygenase serum binds (and precipitates) cyclooxygenase protein

regardless of whether the enzyme is active, partially active or inactive in a manner analogous to the nondiscriminatory interaction of non-labeled and radiolabeled hormones with antiserum. This latter assumption is supported by the previous demonstration that native, heat-denatured and aspirin-inactivated cyclooxygenases are equally reactive with anti-cyclooxygenase serum (118). As with RIAs which employ charcoal as an adsorbent and in which supernatant radioactivity is measured, the activity of the cyclooxygenase is determined in the supernatant. The immunochemical equivalence point is that point at which a constant, maximal amount of cyclooxygenase protein is precipitated by a fixed quantity (25 µl) of anti-cyclooxygenase serum. The number of units of enzyme activity precipitated at the equivalence point is a measure of the number of catalytic units associated with a fixed amount of enzyme protein (i.e., a specific activity). For example, if on day 14 of the estrous cycle, preformed cyclooxygenase molecules had been activated three-fold by allosteric or covalent modification of the enzyme, the number of cyclooxygenase units precipitated at the equivalence point would have been increased from 13.9 (day 3, Table 1) to approximately 42 (although the quantity of cyclooxygenase protein precipitated would be the same). Since the same number of cyclooxygenase units were precipitated at the equivalence points for the enzymes on both day 3 and day 14 (and on other days of the cycle) (Table 1), metabolic activation of the cyclooxygenase must not have occurred. Instead, the three-fold increase in cyclooxygenase activity observed on day 14 must have resulted from a three-fold increase in the total number of cyclooxygenase molecules.

Anatomical Distribution of Changes in Cyclooxygenase Activity During the Estrous Cycle. Ovine uteri are easily dissected into three anatomical compartments: caruncles, intercaruncular endometrium, and myometrium. Solubilized microsomes were prepared from these three fractions and two positive controls: a) portions of whole uterus and b) portions of whole uterus which were dissected but then homogenized together. A three-fold increase ($P<.01$) in activity was noted for the caruncular cyclooxygenase on day 14 as compared to day 3 (Table 2).

Considerable cyclooxygenase activity was also present in the endometrial and myometrial microsomes, but no significant changes occurred among days 3, 11 or 14 of the estrous cycle. As shown in Table 2, a significant increase in activity did occur in whole uteri or uteri dissected but homogenized together ($P<.05$) among days 3, 8 and 11, but this 1.8-fold increase was less than that previously observed (Table 1). This difference is probably attributable to the use of more efficient homogenization techniques in the second experiment (a polytron homogenizer versus a combination of a rotary shearer and teflon-glass homogenizer). Uterine smooth muscle is a very tough fibrous tissue and the polytron homogenizer may have increased the percentage and efficiency of homogenization. Since the myometrium constitutes the greatest portion of the uterus by weight (50 to 60%) and because the cyclooxygenase activity of the myometrial fraction is the highest of all uterine subregions, the net effect would be to increase the overall cyclooxygenase activity in whole uterine preparations. This effect would be most noticeable on day 3 when caruncular activity is low; consequently, the differences in activities

Table 2. Cyclooxygenase activities in different anatomical subregions of the uterus on different days of the ovine estrous cycle[a]

Days Post-estrus	Number of Ewes	Cyclooxygenase Activity (units/g wet weight)					
		Caruncles	Noncaruncular Endometrium	Myometrium	Intact Horn	Dissected and Reconstituted Horn	Calculated[b]
3	7	9.5 ± 4.2[c]	18.2 ± 9.8[e]	27.5 ± 9.7[e]	19.2 ± 6.9[c,f]	20.0 ± 6.1[c,f]	21.8 ± 7.4[c,f]
11	6	16.6 ± 5.9[c]	18.2 ± 10.4[e]	27.1 ± 10.2[e]	23.6 ± 10.1[c,f]	23.9 ± 8.2[c,f]	22.8 ± 9.1[c,f]
14	8	25.7 ± 9.3[d]	21.7 ± 12.5[e]	33.5 ± 10.6[e]	34.8 ± 13.0[d,f]	36.5 ± 13.2[d,f]	31.6 ± 11.7[c,f]

[a]Values represent mean ± standard error.

[b]Values for the whole uterus calculated from the specific cyclooxygenase activities of the three uterine subregions and their respective wet weight contributions to the intact uterus (i.e., caruncles, 15-20%, noncaruncular endometrium, 20-30%, and myometrium, 50-60%).

[c,d,e]Values within 1 column with different superscripts are significantly different ($P<.05$).

[f]Values within a row with different superscripts are significantly different ($P<.05$).

between days 3 and 14 would be diminished. No significant differences in cyclooxygenase activities in a) whole uterus, b) whole dissected and reconstituted uterus and c) a "calculated" value for the whole uterus occurred among the three days studied (Table 2). Thus, the dissection procedure had no effect on the uterine cyclooxygenase activities.

Measurements of immunochemical equivalence points were made on solubilized cyclooxygenase from a combination of endometrial and caruncular microsomes of two ewes on day 3 and three ewes on day 14 of the estrous cycle and the values were the same (7.5 $\pm$ 0.7 vs 8.1 $\pm$ 3.0 units precipitated/125 ul antiserum). These data agree with those in Table 1, indicating that the three-fold increase in cyclooxygenase is due to an increase in the concentration of the cyclooxygenase protein and not to activation of the cyclooxygenase.

Immunohistochemical Localization of the Uterine Cyclooxygenase

An immunohistofluorescence procedure was developed in the kidney by Smith and Wilkin (118) which identifies specific cell types containing the prostaglandin-forming cyclooxygenase. A modification of this procedure was applied to ovine uterine and ovarian cross sections and almost all identifiable cell types stained positively for the cyclooxygenase on all days of the estrous cycle.

Fluorescence photomicrographs (Figure 4) show staining of uterine caruncles in adjacent uterine cross sections which are treated with Fab fragments prepared from anticyclooxygenase (Figure 4A) and preimmune (Figure 4B) sera, then FITC-labeled goat anti-rabbit IgG. Luminal epithelial cells, caruncular fibroblasts and capillary endothelial cells show increased fluorescence in the experimental

Figure 4. Fluorescence photomicrographs of a caruncle in a cross section of ovine uterus treated with (A) anti-cyclooxygenase Fab fragments or (B) preimmune Fab fragments, then fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG. Luminal epithelium (L), vascular endothelium (V) and caruncular fibroblasts (C). Dark splotches running from top to bottom in the left center of the micrograph are pigments. Magnification, 125X.

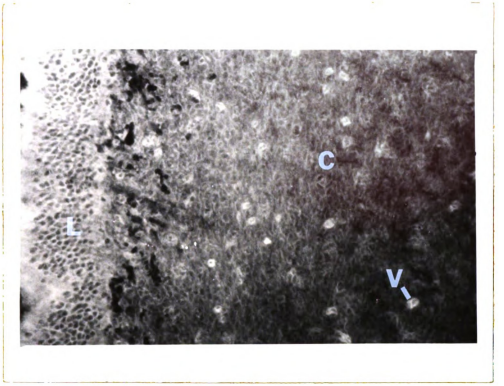

Figure 4A

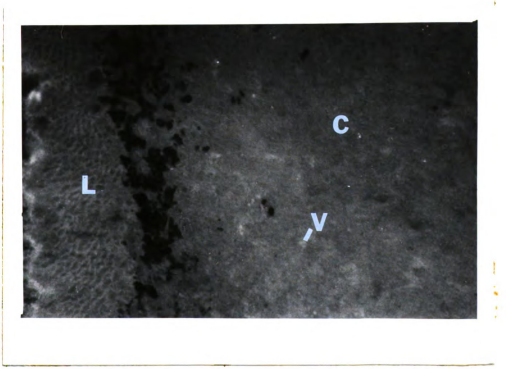

Figure 4B

(anti-cyclooxygenase) as compared to the control (preimmune) sections, indicating the presence of cyclooxygenase antigenicity. No cyclooxygenase positive staining is seen when using a preparation of anti-cyclooxygenase Fab fragments which are preincubated for 30 min at 4^{o} with sheep vesicular gland acetone powder (50 mg acetone powder per ml of Fab solution). This technique selectively absorbs cyclooxygenase antibody molecules and is a negative control for the cyclooxygenase staining.

Intercaruncular endometrium contains glandular epithelial cells, stromal fibroblasts and elements of the vasculature. Glandular epithelium, and arterial endothelial cells exhibit intense fluorescence when stained using immune (Figure 5A) as compared to preimmune (Figure 5B) Fab fragments. Uterine myometrium contains both longitudinal and circular smooth muscle fibers arranged in concentric rings and separated by elements of the vasculature (large arteries and veins). Circular smooth muscle cells stain positively for the cyclooxygenase as do the arterial endothelial cells, as seen by the increases in fluorescence in sections treated with immune (Figure 6A) as compared with preimmune (Figure 6B) Fab fragments. Longitudinal smooth muscle cells also stain positively for the cyclooxygenase as evidenced by an increase in cytoplasmic fluorescence intensity using Fab fragments from anti-cyclooxygenase (Figure 7A) serum compared with preimmune (Figure 7B) Fab fragments. In addition, discrete rings of positive cyclooxygenase staining surround the nuclei of these smooth muscle cells (Figure 7A). The presence of the perinuclear antigenicity is consistent with the possibility that cyclooxygenase may be associated with the nuclear membrane in certain cells (120,148).

Figure 5. Fluorescence photomicrographs of noncaruncular endometrium in a cross section of ovine uterus treated with (A) anti-cyclooxygenase Fab fragments or (B) preimmune Fab fragments, then FITC-labeled goat anti-rabbit IgG. Arterial endothelium (AE), arteriolar endothelium (aE), glandular epithelium (G), and endometrial stroma (E). Magnification, 312.5X.

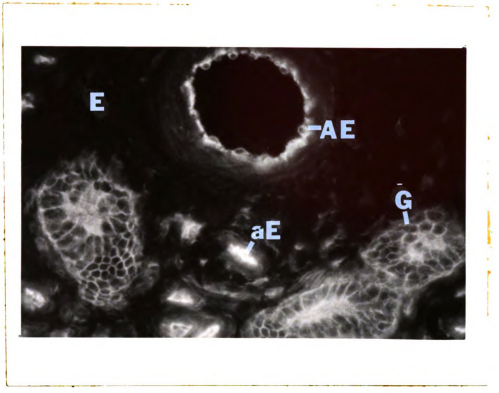

Figure 5A

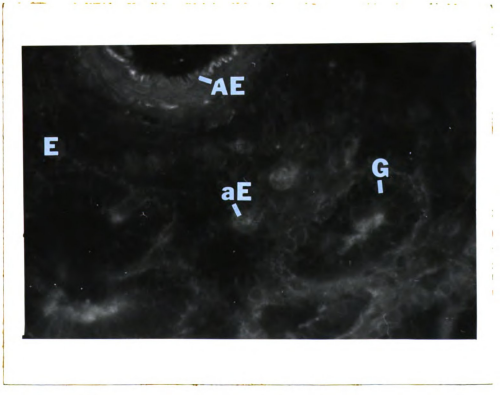

Figure 5B

Figure 6. Fluorescence photomicrographs of circular smooth muscle in a cross section of ovine uterus treated with (A) anti-cyclooxygenase Fab fragments or (B) preimmune Fab fragments, then FITC-labeled goat anti-rabbit IgG. Circular muscle fibers (CM) and arterial endothelium (AE). Magnification, 312.5X.

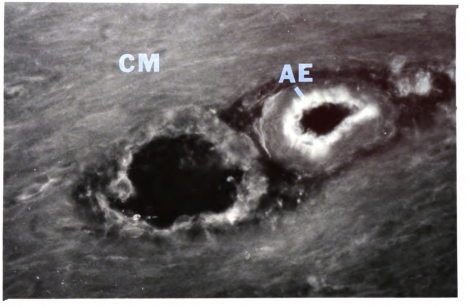

Figure 6A

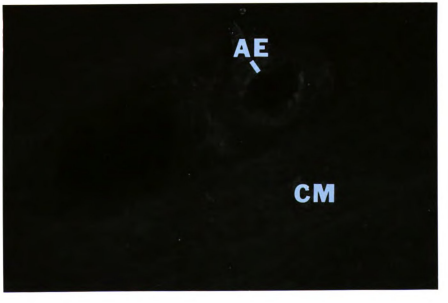

Figure 6B

Figure 7. Fluorescence photomicrographs of longitudinal smooth muscle in a cross section of ovine uterus treated with (A) anti-cyclooxygenase Fab fragments or (B) preimmune Fab fragments, then FITC-labeled goat anti-rabbit IgG. Bright circles are perinuclear staining in smooth muscle cells (LM). Mangification, 312.5X.

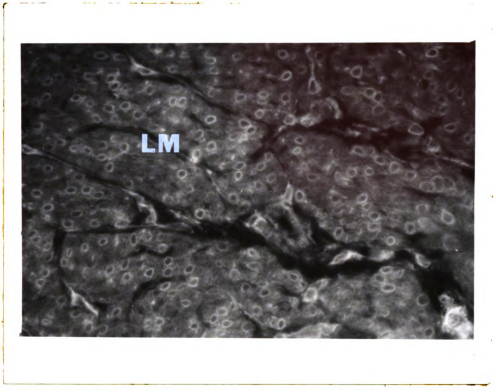

Figure 7A

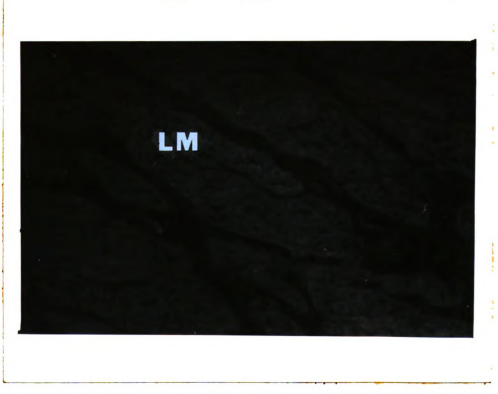

Figure 7B

In ovarian sections, small and large follicles, luteal cells, vasculature and ovarian stroma could be identified. Positive fluorescence staining for cyclooxygenase antigenicity is illustrated in small follicles and ovarian stroma in ovarian sections treated with immune (Figure 8A) but not preimmune (Figure 8B) Fab fragments. Positive fluorescence staining for the cyclooxygenase is also apparent in cells of a large follicle and arterial endothelium in similarly treated ovarian sections (Figure 9A,B). Cryostat sections of corpora lutea also stain positively for the cyclooxygenase as seen by the intense fluorescence when using immune (Figure 10A) as compared with preimmune (Figure 10B) Fab fragments. The presence of cyclooxygenase antigenicity in follicles and corpora lutea is consistent with the production of prostaglandin F from arachidonic acid measured in these tissues (149,150).

Immunohistochemical Quantitation of the Uterine Cyclooxygenase

Immunofluorescence positive staining in almost all uterine cell types is consistent with finding cyclooxygenase activity in all uterine compartments (Table 2). Each Fab fragment of rabbit anti-cyclooxygenase IgG can bind two cyclooxygenase molecules (2). Assuming that the binding of FITC-labeled goat anti-rabbit IgG to Fab fragments is proportional to the number of Fab fragments, the increase in cyclooxygenase protein observed on day 14 (Tables 1 and 2) should be measurable as an increase in cyclooxygenase fluorescence. Relative fluorescence intensities for the cyclooxygenase of six uterine cell types in tissue sections from ten animals distributed over days 3, 11 and 14 of the estrous cycle are shown in Table 3. Individual

Figure 8. Fluorescence photomicrographs of ovarian stroma in a cross section of ovine ovary treated with (A) anti-cyclooxygenase Fab fragments, or (B) preimmune Fab fragments, then FITC-labeled goat anti-rabbit IgG. Small or primary follicles (f), ovarian stroma (O). Magnification, 312.5X.

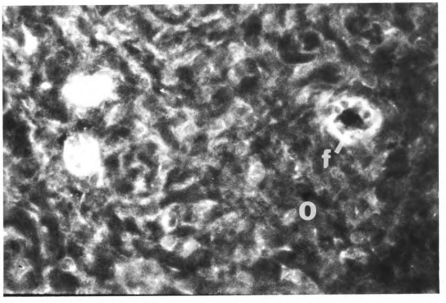

Figure 8A

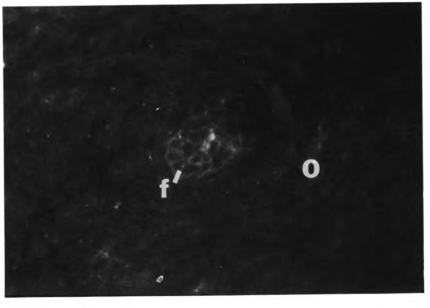

Figure 8B

Figure 9. Fluorescence photomicrographs of ovarian stroma in a cross section of ovine ovary treated with (A) anti-cyclooxygenase Fab fragments or (B) preimmune Fab fragments, then FITC-labeled goat anti-rabbit IgG. Large or tertiary follicles (F) (distorted during cryostat sectioning), arterial endothelial cells (AE). Magnification, 125X.

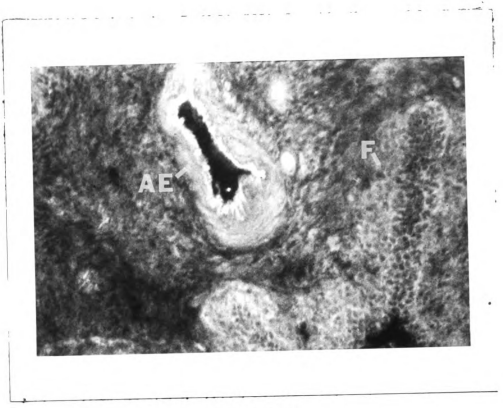

Figure 9A

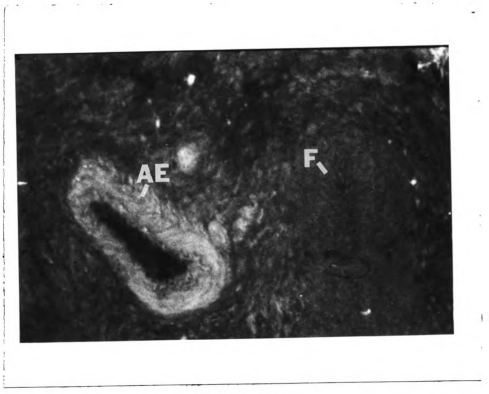

Figure 9B

Figure 10. Fluorescence photomicrographs of cross sections of ovine corpus luteum treated with (A) anti-cyclooxygenase Fab fragments or (B) preimmune Fab fragments, then FITC-labeled goat anti-rabbit IgG. Both photographs are of luteal cells only. Magnification, 125X.

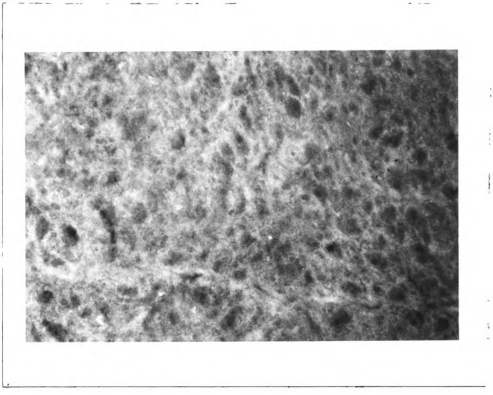

Figure 10A

Figure 10B

fluorescence measurements were made on small areas of the uterine cell types as illustrated in Figure 11. Fluorescence intensities are relative to a 1:50 dilution of the FITC-labeled goat anti-rabbit IgG, which was arbitrarily set to read 200 mV. Data in Table 3

Table 3. Relative cyclooxygenase immunofluorescence of selected ovine uterine cell types[a]

Days Postestrus			
Number of Ewes			
Relative Immunofluorescence			
lumen	47.3 ± 13.3	56.9 ± 9.8	48.5 ± 12.4
caruncle	32.4 ± 10.4	37.1 ± 4.0	37.3 ± 9.3
gland	94.1 ± 10.3	63.8 ± 11.8	61.3 ± 16.2
stroma	50.8 ± 8.3	65.5 ± 8.9	69.4 ± 17.0
longitudinal muscle	53.2 ± 20.1	60.0 ± 15.7	53.5 ± 17.2
circular muscle	23.2 ± 5.7	26.8 ± 6.1	32.0 ± 12.1

[a]Values represent mean ± standard error. Individual values used to calculate the mean were derived by subtracting background fluorescence using preimmune anti-cyclooxygenase serum from fluorescence using immune anti-cyclooxygenase serum as described in immunohistochemical procedures.

indicate measurable differences in the cyclooxygenase fluorescence between various cell types (i.e., glandular epithelium versus longitudinal smooth muscle) on each day of the estrous cycle. However, these data do not confirm the changes in cyclooxygenase activity presented in Tables 1 and 2. In fact, the only change in fluorescence is observed in the glandular epithelium, where a decrease in

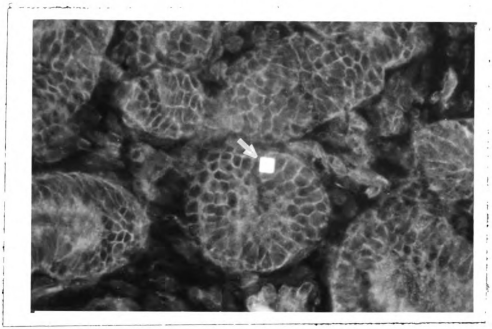

Figure 11. Illustration of the diaphragm size (arrow) used for measurements of relative fluorescence intensity in ovine uterine cross sections treated with Fab fragments of anti-cyclooxygenase serum and FITC-labeled goat anti-rabbit IgG. Magnification of tissue section, 312.5X.

relative cyclooxygenase immunofluorescence occurs on day 14 as compared with day 3. Since background or non-specific staining might be masking changes in the immunofluorescence antigenicity of the cyclooxygenase, relative fluorescence was measured using much greater dilutions of the Fab fragments and also the FITC-labeled goat anti-rabbit IgG. This procedure merely resulted in decreased fluorescence in all cell types until positive immunofluorescence was totally eliminated at high dilutions (i.e., 1:200) of the IgG. Triton X-100 or chloroform:methanol treatment of tissue sections should expose any cyclooxygenase binding sites "hidden" by membrane compartmentation. However, these treatments resulted in neither an increase in total cyclooxygenase fluorescence nor fluorescence evidence for cyclical changes in the cyclooxygenase concentration of any uterine cell types.

Attempts to prove that FITC-labeled goat anti-rabbit IgG binds to Fab fragments (bound to tissue cyclooxygenase) in a linear manner were unsuccessful. Mouse 3T3 cells have been reported to increase the cyclooxygenase protein concentration following dexamethasone treatment (120). However, we were unable to detect either an increase in prostaglandin synthesis measured using ^{3}H-arachidonate as a substrate nor an increase in cyclooxygenase antigenicity measured using immunofluorescence quantitation. Species differences exist in the cyclooxygenase antigenicity in renal medullary collecting tubule cells. However, differences in cyclooxygenase fluorescence intensity in bovine, rabbit and guinea pig collecting tubule cells were found to result primarily from differences in binding affinities, since the Fab fragments prepared from rabbit anti-sheep cyclooxygenase do not bind equally well to cyclooxygenase in these species.

The lack of changes in cyclooxygenase positive fluorescence does not appear to be a technical problem. Dilutions of the FITC-labeled goat anti-rabbit IgG (Figure 12) exhibit linear fluorescence over a wide range of dilutions which also encompassed the range of measurements in Table 3. While the fluorescence half-life is relatively short (approximately 30 seconds) (Figure 13), measurements were made on widely spaced cells in freshly stained tissue sections to eliminate fluorescence decay as an experimental variable.

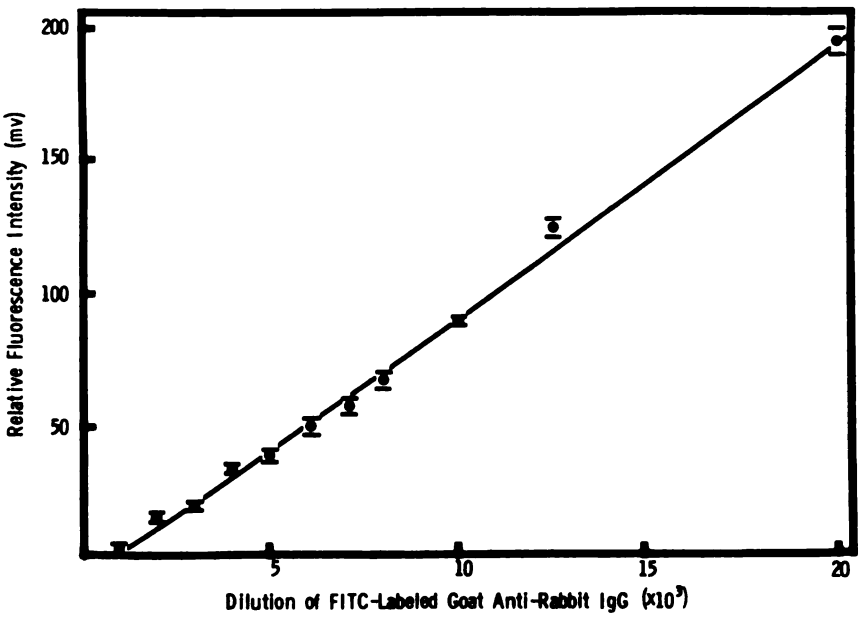

Figure 12. Relative fluorescence intensities of dilutions of FITC-labeled goat anti-rabbit IgG. A hemocytometer was used to maintain uniform solution volume. Each point represents mean ± standard error.

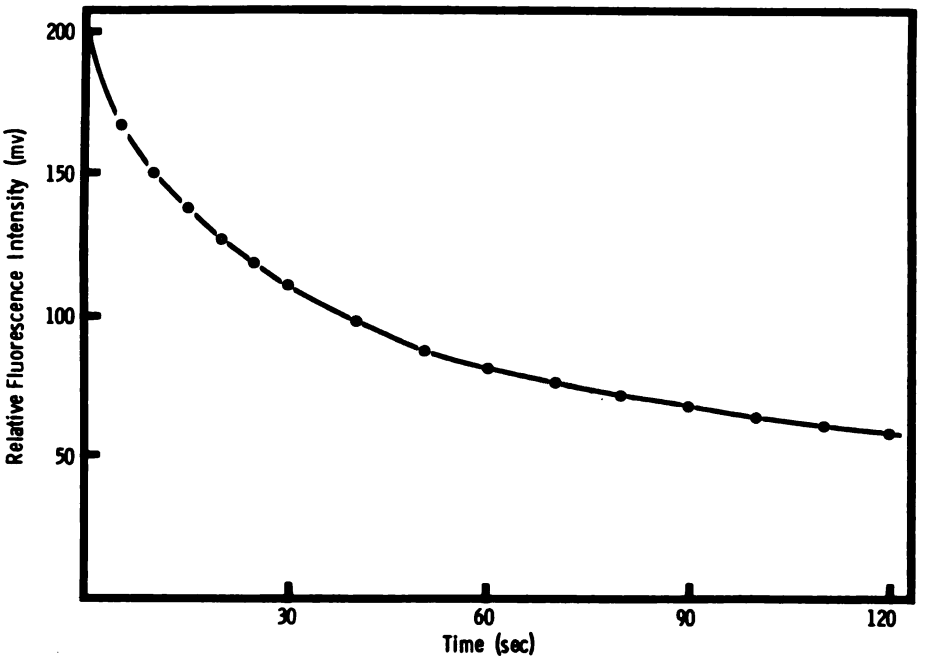

Figure 13. Decay of the relative fluorescence intensity of a 1:50 dilution of FITC-labeled goat anti-rabbit IgG during continuous excitation of the FITC-fluorophor by a Leitz fluorescence photomicroscope. Each point represents mean ± standard error.

DISCUSSION

Gas liquid chromatography-mass spectrometry and radioimmunoassay (RIA) have shown that concentrations of $PGF_{2\alpha}$ in utero-ovarian venous plasma increase from nondetectable levels on days 1 to 13 (less than 2-3 ng/ml) to approximately 22 ng/ml around days 14 to 15 in cycling ewes (9,16,17,60). This increase in the secretion of $PGF_{2\alpha}$ precedes or coincides with the onset of luteolysis as evidenced by decreased concentration of progesterone in jugular venous plasma (21). Results described here (Table 1) establish a positive correlation between increased activity of uterine prostaglandin-forming cyclooxygenase and these reported increases in $PGF_{2\alpha}$ secretion. It appears that increased cyclooxygenase activity is responsible, at least in part, for increased secretion of $PGF_{2\alpha}$.

The ovine endometrium is known to exert a luteolytic effect as demonstrated by the effect of endometrial extracts or implants from day 14 ewes on corpora lutea from cycling animals (6). Wilson et al. (61) observed the endometrial concentration of $PGF_{2\alpha}$ to be greater on day 14 than days 3-11 of the ovine estrous cycle. Recent studies have also identified uterine caruncles as a source of significant $PGF_{2\alpha}$ synthesis. Pexton et al. (60) reported stimulation of the concentration of $PGF_{2\alpha}$ by intrauterine devices to be greater in endometrium than caruncles. Robinson et al. (90) observed caruncles to contain the greatest concentration of $PGF_{2\alpha}$ in anestrous ewes treated with

progesterone and estradiol. Studies reported here (Table 2) indicate that only ovine uterine caruncles exhibit an increase in cyclooxygenase activity on day 14 as compared with day 3. Increased cyclooxygenase activity agrees well with the known onset of luteolysis and at least one of the cell types in the caruncle responsible for this increase should also be responsible for the luteolytic secretion of $PGF_{2\alpha}$.

One use for the immunofluorescence localization of the uterine cyclooxygenase (Figures 4-7) would be to provide a ready means of identifying a unique cell type responsible for synthesis of $PGF_{2\alpha}$ (luteolysin-forming cell). While ovarian cyclooxygenase antigenicity (Figures 8-10) can be correlated to PG synthesis in ovarian cell cultures (149,150), almost all uterine cell types contain prostaglandin-forming cyclooxygenase antigenicity (Figures 4-7). No visual differences were apparent in the positive cyclooxygenase immunofluorescence in any cell type among days 3, 8, 11, 13, 14 or 15 of the ovine estrous cycle. Immunochemical equivalence points (Table 1) indicated that an increase in cyclooxygenase protein occurred on days 13, 14 and 15 of the estrous cycle. Consequently, fluorescence intensity measurements on the uterine cyclooxygenase were performed to determine if cyclical changes in the cyclooxygenase fluorescence were occurring. Fluorescence quantitation (Table 3) did not reveal any significant changes in the cyclooxygenase specific fluorescence on any day of the estrous cycle studied.

Lack of changes in fluorescence measurements should not be the result of a technical problem since simple dilutions of FITC-labeled goat anti-rabbit IgG exhibit linear changes in fluorescence intensity (Figure 12). An assumption was made that binding was proportional to numbers of cyclooxygenase molecules. However, binding of FITC-labeled goat anti-rabbit IgG to Fab fragments and Fab fragments to cyclooxygenase is determined by a number of factors. One of the factors, such as steric hindrance, could prevent proportional binding, and thus increased fluorescence on day 14 of an estrous cycle. A positive control (a cell type with known changes in cyclooxygenase) could have been used to calibrate the fluorometer and correct for such unexpected factors. Since no such positive control was available, it is almost impossible to determine which parameters should be changes.

Because fluorescence techniques failed to identify a luteolysin-forming cell, we can only speculate as to the identity based on the cyclooxygenase activity studies. The dissected caruncular subregion contains luminal epithelium, fibroblasts and vasculature (endothelial capillaries) in addition to contamination (25% to 50% by weight) by endometrium (glandular epithelium, stroma and vasculature). The dissected endometrial subregion in turn is contaminated by circular smooth muscle fibers and the dissected myometrium is contaminated by small amounts of the endometrium. However, only in the caruncular subregion were changes in cyclooxygenase activity significant (Table 2). If the contaminating cells from the endometrium (i.e., glandular epithelium, stroma and arterial endothelial cells) (see also Figure 5) were responsible for the increase in activity, a significant change

in cyclooxygenase activity should also occur in the endometrial fraction where most of these cells are located. This was not observed (Table 2). Luminal epithelium can be discounted, as well, since this cell type lines both the endometrium and the caruncle. Endothelial capillaries are a source of cyclooxygenase, but these cells are known to synthesize PGI_2 rather than $PGF_{2\alpha}$. Thus, the only remaining cell type which should be the luteolysin forming cell is the caruncular fibroblast.

In cross sections of the dissected caruncular subregion, only about 50% of the tissue mass is estimated to be caruncular fibroblasts. Thus, for a three-fold increase in cyclooxygenase activity in whole caruncle to be noticeable, the cyclooxygenase activity in caruncular fibroblasts must increase six-fold by day 14 of the estrous cycle. This theory could account for greater secretion rate of $PGF_{2\alpha}$ by caruncular fibroblasts which would agree with reported changes in secretion of $PGF_{2\alpha}$ into utero-ovarian venous plasma (9,16,17,70).

The fact that immunochemical equivalence points of the uterine endometrial plus caruncular cyclooxygenase do not vary over the estrous cycle indicates that increased activity is due to increased concentration of cyclooxygenase protein and not increased catalytic efficiency of preexisting enzyme. There is precedence for such an effect, as seen by the reported increase in mouse 3T3 cyclooxygenase following dexamethasone treatment (120).

Estradiol may elevate the concentration of $PGF_{2\alpha}$ through an increase of uterine protein synthesis (107), especially since Actinomycin D, which blocks DNA transcription, can block the luteolytic effect of estradiol (87). In apparent contradiction, nuclear and cytoplasmic estradiol receptors in ovine endometrium decrease on days 6 and 10 as compared to days 0 and 3 of the estrous cycle (151). This would seem to indicate that in vivo sequential progesterone-estradiol stimuli are not responsible for elevating synthesis of $PGF_{2\alpha}$. However, high concentrations of estradiol are not necessary for stimulation of $PGF_{2\alpha}$ synthesis and, the receptor number is probably related to a requirement for increased binding following the preovulatory surge of estradiol.

Therefore, increased concentration of uterine cyclooxygenase could quite readily be a control point for uterine $PGF_{2\alpha}$ synthesis. This increased concentration of the cyclooxygenase (120) could merely be one of several factors responsible for increasing synthesis and secretion of $PGF_{2\alpha}$. However, the contribution of these other factors such as an increase in the arachidonate-specific phospholipase (23,24, 94,98) or an increased conversion of PGH_2 to $PGF_{2\alpha}$ (152) could not be determined with the present experimental design.

A model for $PGF_{2\alpha}$ synthesis by a luteolysin-forming cell is represented in Figure 14. Only the cyclooxygenase component of the biosynthetic pathway is represented as changing during the estrous cycle, because this is the evidence presently available.

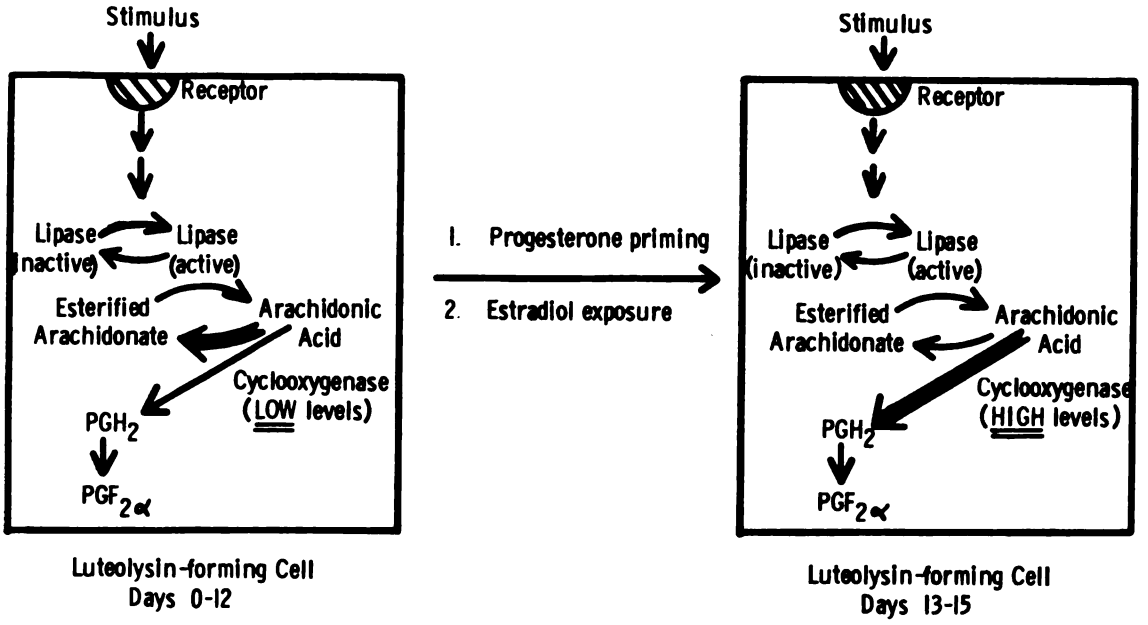

Figure 14. Model for regulation of concentrations of uterine cyclooxygenase and synthesis of luteolysin, $PGF_{2\alpha}$, in ewes.

The cyclooxygenase is proposed to compete with acyltransferases for available arachidonate. Early in the cycle most of the arachidonate is reesterified. Following sequential exposure of the uterus to progesterone-estradiol stimuli, the cyclooxygenase concentrations increase and convert more arachidonate to PGH_2, which is then reduced to $PGF_{2\alpha}$. However, this model is at best incomplete because some current evidence favors regulation of prostaglandin synthesis by phospholipase (23,24,94,97,98). In these systems, cyclooxygenase concentration is not a rate limiting step in the conversion of arachidonate to endoperoxides and the amount of prostaglandin synthesis is determined by activation of the phospholipase to cause release of more substrate. As more evidence is obtained on the

uterine regulation of prostaglandin synthesis (presumably following isolation of a $PGF_{2\alpha}$-forming cell type), it will eventually be determined if a hormonal stimulus occurs late in the cycle which increases arachidonate release to act in conjunction with the increase in cyclooxygenase to increase the synthesis of $PGF_{2\alpha}$.

REFERENCES

REFERENCES

1. Hafez, E.S.E. (1974) Reproduction in Farm Animals. Lea and Febiger, Philadelphia.

2. White, A., Handler, P., and Smith, E.L. (1973) Principles of Biochemistry, 5th ed., p. 1065-1069 and 1129-1130. McGraw-Hill, New York.

3. McCracken, J.A., Barcikowski, B., Carlson, J.C., Green, K., and Samuelsson, B. (1973) The physiological role of $PGF_{2\alpha}$ in corpus luteum regression. Adv. Biosciences 9:599-624.

4. Flint, A.P.F., and Hillier, K. (1975) Prostaglandins and reproductive processes in female sheep and goat, in Prostaglandins and Reproduction, ed. by S.M.M. Karim, p. 241-270. University Park Press, Baltimore.

5. Caldwell, B.V., and Moor, R.M. (1971) Further studies on the role of the uterus in the regulation of corpus luteum function in sheep. J. Reprod. Fert. 26:133-135.

6. Caldwell, B.V., Moore, R.M., and Lawson, R.A.S. (1968) Effect of endometrial grafts and extracts on the length of pseudopregnancy in the ovariectomized hamster. J. Reprod. Fert. 17:567-569.

7. McCracken, J.A., Carlson, J.C., Glew, M.E., Goding, J.R., Baird, D.T., Green, K., and Samuelsson, B. (1972) Prostaglandin $F_{2\alpha}$ identified as a luteolytic hormone in sheep. Nature New Biol. 238:129-134.

8. Blatchley, F.R., Donovan, B.T., Horton, E.W., and Poyser, N.L. (1972) The release of prostaglandins and progesterone into the utero-ovarian venous blood of guinea pigs during the oestrous cycle and following oestrogen treatment. J. Physiol. (Lond.) 228:69-88.

9. Goding, J.R. (1974) The demonstration that prostaglandin $F_{2\alpha}$ is the uterine luteolysin in the ewe. J. Reprod. Fert. 38:261-271.

10. Blatchley, F.R., and Donovan, B.T. (1972) The effect of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 upon luteal function and ovulation in the guinea pig. J. Endocrinol. 64:11P-12P.

11. Douglas, R.H., and Ginther, O.J. (1972) Effect of prostaglandin $F_{2\alpha}$ on length of diestrous in mares. Prostaglandins 2:265-268.

12. Hallford, D.M., Wetteman, R.P., Turman, E.J., and Omtvedt, I.T. (1975) Luteal Function in gilts after prostaglandin $F_{2\alpha}$. J. Anim. Sci. 41:1706-1710.

13. Guthrie, H.D., and Polge, C. (1976) Luteal Function and oestrus in gilts treated with a synthetic analog of prostaglandin $F_{2\alpha}$ (ICI 79,939) at various times during the oestrous cycle. J. Reprod. Fert. 48:423-426.

14. Saksena, S.K., and Harper, M.J.K. (1972) Level of F prostaglandin (PGF) in peripheral plasma and uterine tissue of cyclic rats. Prostaglandins 2:511-517.

15. Saksena, S.K., and Harper, M.J.K. (1972) Levels of F-prostaglandin (PGF) in uterine tissue during the oestrous cycle of hamster: Effects of estradiol and progesterone. Prostaglandins 2:405-411.

16. Bland, K.P., Horton, E.W., and Poyser, N.L. (1971) Levels of prostaglandin $F_{2\alpha}$ in the uterine venous blood of sheep during the estrous cycle. Life Sci. 10, Part 1:509-517.

17. Thorburn, G.D., Cox, R.I., Currie,W.B., Restall, B.J., and Schnieder, W. (1973) PGF and progesterone concentrations in the uteroovarian venous plasma of the ewe during the estrous cycle and early pregnancy. J. Reprod. Fert. Suppl. 18:151-158.

18. Ford, S.P., Weems, C.W., Pitts, R.E., Pexton, J.E., Butcher, R.L., and Inskeep, E.K. (1975) Effects of estradiol-17β and progesterone on prostaglandins F in sheep uteri and uterine venous plasma. J. Anim. Sci. 41:1407-1413.

19. Barcikowski, B., Carlson, J.C., Wilson, L., and McCracken, J.A. (1974) The effect of endogenous and exogenous oestradiol-17β on the release of prostaglandin $F_{2\alpha}$ from the ovine uterus. Endocrinology 95:1340-1349.

20. Nayler, B., and Poyser, N.L. (1975) Effect of oestrogen and progesterone on the *in vitro* production of $PGF_{2\alpha}$ by the guinea pig uterus. Br. J. Pharmacol. 55:229-232.

21. Baird, D.T., Land, R.B., Scaramuzzi, R.J., and Wheeler, A.G. (1976) Endocrine changes associated with luteal regression in the ewe: The secretion of ovarian oestradiol, progesterone and androstenedione and uterine prostaglandin $F_{2\alpha}$ throughout the oestrous cycle. J. Endocrinol. 69:275-286.

22. Gorski, J., Toft, D., Shyamala, G., Smith, D. and Notides, A. (1968) Hormone receptors: Studies on the interaction of estrogen with the uterus. Rec. Prog. Horm. Res. 24:45-72.

23. Bills, T.K., Smith, J.B., and Silver, M.J. (1977) Selective release of arachidonic acid from the phospholipids of human platelets in response to thrombin. J. Clin. Invest. 60:1-6.

24. Hsueh, W., Isakson, P.C., and Needleman, P. (1977) Hormone selective lipase activation in the isolated rabbit heart. Prostaglandins 13:1073-1091.

25. Hemler, M., Lands, W.E.M., and Smith, W.L. (1976) Purification of the cyclooxygenase that forms prostaglandins: Demonstration of two forms of iron in the holoenzyme. J. Biol. Chem. 251:5575-5579.

26. Myamoto, T., Ogino, N., Yamamoto, S., and Hayaishi, O. (1976) Purification of prostaglandin endoperoxide synthetase from bovine seminal vesicle microsomes. J. Biol. Chem. 251:2629-2636.

27. Loeb, L. (1923) The effect of extirpation of the uterus on the life and function of the corpus luteum in the guinea pig. Proc. Soc. Exp. Biol. Med. 20:441-444.

28. Wiltbank, J.N., and Casida, L.E. (1956) Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134-140.

29. Inskeep, E.K., and Butcher, R.L. (1966) Local component of utero-ovarian relationships in the ewe. J. Anim. Sci. 25:1164-1168.

30. Moor, R.M., and Rowson, L.E.A. (1966) Local uterine mechanisms affecting luteal function in the sheep. J. Reprod. Fert. 11:307-310.

31. Caldwell, B.V., Rowson, L.E.A., Moor, R.M., and Hay, H.F. (1969) The utero-ovarian relationship and its possible role in fertility. J. Reprod. Fert. Suppl. 8:59-76.

32. du Mesnil du Buisson, F. (1961) Regression unilaterale des corps jaunes apres hysterectomi partielle chez la truie. Ann. Biol. Anim. Biochem. Biophys. 1:105.

33. Ginther, O.J., and Del Campo, C.H. (1974) Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: Cattle. Amer. J. Vet. Res. 35:193-203.

34. Fischer, T.V. (1965) Local uterine inhibition of the corpus luteum in the guinea pig. Anat. Rec. 350:151.

35. Bland, K.P., and Donovan, B.T. (1969) Observations on the time of action and the pathway of the uterine luteolytic effect in the guinea pig. J. Endocrinol. 43:259-264.

36. Melampy, R.M., Anderson, L.L., and Kragt, C.L. (1964) Uterus and lifespan of rat corpora lutea. Endrocrinology 74:501-504.

37. Duby, R.T., McDaniel, J.W., Spilman, C.H., and Black, D.L. (1969) Utero-ovarian relationships in the golden hamster. I. Ovarian periodicity following hysterectomy. Acta Endocrinol. 60:595-602.

38. Keyes, P.L., and Bullock, D.W. (1974) Effects of prostaglandin $F_{2\alpha}$ on ectopic and ovarian corpora lutea in the rabbit. Biol. Reprod. 10:519-525.

39. Squires, E.L., Garcia, M.C., and Ginther, O.J. (1974) Effects of pregnancy and hysterectomy on the ovaries of pony mares. J. Anim. Sci. 38:823-830.

40. Harrison, F.A., Heap, R.B., and Linzell, J.L. (1968) Ovarian function in the sheep after autotransplantation of the ovary and uterus to the neck. J. Endocrinol. 40:xiii.

41. McCracken, J.A., and Baird, D.T. (1969) The study of ovarian function by means of transplantation of the ovary in the ewe. in The Gonads, ed. by K. McKerns, p. 175-209. Appleton-Century-Crofts, New York.

42. Goding, J.R., Harrison, F.A., Heap, R.B., and Linzell, J.L. (1967) Ovarian activity in the ewe after autotransplantation of the ovary or uterus to the neck. J. Physiol. (Lond.) 191:129-130P.

43. Kiracofe, G.H., Menzies, C.S., Gier, H.T., and Spies, H.G. (1966) Effect of uterine extracts and uterine or ovarian blood vessel ligations on ovarian function of ewes. J. Anim. Sci. 25:1159-1163.

44. Baird, D.T., and Land, R.B. (1973) Division of the uterine vein and the function of the adjacent ovary in the ewe. J. Reprod. Fert. 33:393-397.

45. Babcock, J.C. (1966) In Hansel, W. (ed.), J. Reprod. Fert. Suppl. 1:47.

46. Phariss, B.B., and Wyngarden, L.J. (1969) The effect of prostaglandin $F_{2\alpha}$ on the progesterone content of ovaries from pseudopregnant rats. Proc. Soc. Exp. Biol. Med. 130:92-94.

47. Blatchley, F.R., and Donovan, B.T. (1969) Luteolytic effect of prostaglandin in the guinea pig. Nature 221:1065-1066.

48. McCracken, J.A., Glew, M.E., and Scaramuzzi, R.J. (1970) Corpus luteum regression induced by prostaglandin $F_{2\alpha}$. J. Clin. Endocr. Metab. 30:544.

49. Thorburn, G.D., and Nicol, D.H. (1971) Regression of the ovine corpus luteum after infusion of prostaglandin $F_{2\alpha}$ into the ovarian artery and uterine vein. J. Endocrinol. 51:785-786.

50. Barrett, S., Blockley, M.A. de B., Brown, J.M., Cumming, I.A., Goding, J.R., Mole, B.J., and Obst, J.M. (1971) Initiation of the oestrous cycle in the ewe by infusions of $PGF_{2\alpha}$ to the autotransplanted ovary. J. Reprod. Fert. 24:136-137.

51. Piper, P.J., Vane, J.R., and Wyllie, J.H. (1970) Inactivation of prostaglandins by the lungs. Nature 225:600-604.

52. Horton, E.W., and Poyser, N.L. (1973) Elongation of oestrous cycle in the guinea pig following subcutaneous or intrauterine administration of indomethacin. Br. J. Pharmacol. 49:98-105.

53. Marley, P.B. (1973) Indomethacin lengthens the oestrous cycle of the guinea pig when given orally with oestrogen or when implanted in the uterine lumen. Prostaglandins 4:251-261.

54. Lewis, P.E., and Warren, J.E., Jr. (1977) The effect of indomethacin on estrogen induced luteolysis in the ewe. Prostaglandins 13:957-963.

55. Horton, E.W., and Poyser, N.L. (1974) Elongation of estrous cycle in the guinea pig following active immunization against prostaglandin $F_{2\alpha}$. Prostaglandins 5:349-354.

56. Poyser, N.L., and Horton, E.W. (1975) Plasma progesterone levels in guinea pigs actively immunized against $PGF_{2\alpha}$, hysterectomized or treated with intrauterine indomethacin. J. Endocrinol. 67:81-88.

57. Hildebrandt-Stark, H.E., Marcus, G.J., Yoshinaga, K., Behrman, H.R., and Greep, R.O. (1975) Influence of PGF autoantibodies on the estrous cycle of the guinea pig. Prostaglandins 9:231-242.

58. Scaramuzzi, R.J., and Baird, D.T. (1976) Estrous cycle of the ewe after active immunization against $PGF_{2\alpha}$. J. Reprod. Fert. 46:39-47.

59. Gleeson, A.R., Thorburn, G.D., and Cox, R.I. (1974) Prostaglandin F concentrations in the uteroovarian venous plasma of the sow during the late luteal phase of the estrous cycle. Prostaglandins 5:521-529.

60. Pexton, J.E., Ford, S.P., Wilson, L.,Jr., Butcher, R.L., and Inskeep, E.K. (1975) Prostaglandins F in uterine tissue and venous plasma of ewes with intrauterine devices. J. Anim. Sci. 41:144-153.

61. Wilson, L., Cenedella, R.J., Butcher, R.L., and Inskeep, E.K. (1972) Levels of prostaglandins in the uterine endometrium during the ovine estrous cycle. J. Anim. Sci. 34:93-99.

62. Shemesh, M., and Hansel, W. (1975) Levels of prostaglandin F (PGF) in bovine endometrium, uterine venous, ovarian arterial and jugular plasma during the estrous cycle. Proc. Soc. Exp. Biol. Med. 148:123-126.

63. Morris, B., and McIntosh, G.H. (1971) In Perfusion Techniques, ed. by E. Diczfalusy, p. 148-168. Karolinska Institutet for Reproductive Endocrinology, Stockholm.

64. Butcher, R.L., Barley, D.A., and Inskeep, E.K. (1969) Local relationship between the ovary and uterus of rats and guinea pigs. Endocrinology 84:476-487.

65. Stormshack, R., Lehman, R.D., and Hawk, H.W. (1967) Effect of intrauterine plastic spirals and HCG on the corpus luteum of the ewe. J. Reprod. Fert. 14:373-378.

66. Mapletoft, R.J., and Ginther, O.J. (1975) Adequacy of main uterine vein and the ovarian artery in the local venoarterial pathway for uterine induced luteolysis in ewes. Amer. J. Vet. Res. 36:957-963.

67. Mapletoft, R.J., del Campo, C.H., and Ginther, O.J. (1975) Unilateral luteotropic effect of uterine venous effluent of a gravid uterine horn in sheep. Proc. Soc. Exp. Biol. Med. 150:129-133.

68. Ginther, O.J., del Campo, C.H., and Rawlings, C.A. (1973) Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: A local venoarterial pathway between uterus and ovaries in sheep. Amer. J. Vet. Res. 34:723-728.

69. McCracken, J.A., Baird, D.T., and Goding, J.R. (1971) Factors affecting the secretion of steroids from the autotransplanted ovary in the sheep. Rec. Prog. Hormone Res. 27:537-582.

70. Coudert, S.P., Phillips, G.D., Faiman, C., Chernecki, W., and Palmer, M. (1974) A study of the utero-ovarian circulation in sheep with reference to local transfer between venous and arterial blood. J. Reprod. Fert. 36:319-331.

71. Coudert, S.P., Phillips, G.D., Faiman, C., Chernecki, W., and Palmer, M. (1974) Infusion of tritiated prostaglandin $F_{2\alpha}$ into the anterior uterine vein of the ewe. Absence of local venous arterial transfer. J. Reprod. Fert. 36:333-343.

72. Land, R.B., Baird, D.T., and Scaramuzzi, R.J. (1976) Dynamic studies of $PGF_{2\alpha}$ in utero-ovarian curculation of the sheep. J. Reprod. Fert. 47:209-214.

73. Castracane, V.D., and Jordan, V.C. (1975) Effect of estrogen and progesterone on uterine prostaglandin biosynthesis in the ovariectomized rat. Biol. Reprod. 13:587-596.

74. Saksena, S.K., and Lau, I.F. (1973) Effect of exogenous estradiol and progesterone on the uterine tissue levels of PGF ($PGF_{2\alpha}$) in ovariectomized mice. Prostaglandins 3:317-322.

75. Ginther, O.J. (1968) Influence of exogenous progesterone and the uterus on ovarian activity in sheep. Endocrinology 83:613-615.

76. O'Hobre, J.S., Lewis, G.S., and Inskeep, E.K. (1978) The effect of treatment with progesterone at estrus on the time of uterine release of $PGF_{2\alpha}$ in ewes. Proc. 70th Ann. Mtg. Am. Soc. Anim. Sci., Abst. 415, p. 381.

77. Sharma, S.C., and Fitzpatrick, R.J. (1974) Effect of estradiol 17β and oxytocin treatment on $PGF_{2\alpha}$ release in the anestrous ewe. Prostaglandins 6:97-105.

78. Stormshak, F., Kelley, H.E., and Hawk, H.W. (1969) Supression of the ovine luteal function by 17β-estradiol. J. Anim. Sci. 29:476-478.

79. Ginther, O.J. (1970) Length of estrous cycle in sheep treated with estradiol. Amer. J. Vet. Res. 31:973-975.

80. Hawk, H.W., and Bolt, D.J. (1970) Luteolytic effect of estradiol-17β when administered after midcycle in the ewe. Biol. Reprod. 2:275-278.

81. Kittock, R.J., and Britt, J.H. (1977) Corpus luteum function in ewes given estradiol during the estrous cycle or early pregnancy. J. Anim. Sci. 45:336-341.

82. Bolt, D.J. (1978) Influence of dose and duration of estrogen treatment on CL regression in ewes. Theriogenology 9:333-341.

83. Chakraboty, P.K., and Stormshak, F. (1976) Role of the uterus in estradiol-17β induced luteal regression in ewe. J. Reprod. Fert. 48:431-432.

84. Fairclough, R.J., Smith, J.F., and Peterson, A.J. (1976) Passive immunization against estradiol-17β and its effect on luteolysis, estrus and ovulation in the ewe. J. Reprod. Fert. 48:169-171.

85. Caldwell, B.V., Tillson, S.A., Brock, W.A., and Speroff, L. (1972) Effect of exogenous progesterone and estradiol 17β on prostaglandin F (PGF) levels in ovariectomized ewes. Prostaglandins 1:217-228.

86. Hixon, J.E., Gegenbach, D.R., and Hansel, W. (1975) Failure of prostaglandin $F_{2\alpha}$ to cause luteal regression in ewes after destruction of ovarian follicles by x-irradiation. Biol. Reprod. 13:126-135

87. French, L.R., and Casida. L.E. (1972) Effect of Actinomycin on corpus luteum regression in ewes. J. Anim. Sci. 37:1218-1221.

88. Warren, J.E., Jr., Hawk, H.W., and Bolt, D.J. (1973) Evidence for progestational priming of estradiol-17β induced luteal regression in the ewe. Biol. Reprod. 8:435-440.

89. Blatchley, F.R., and Poyser, N.L. (1974) The effect of estrogen and progesterone on the release of prostaglandins from the uterus of the ovariectomized guinea pig. J. Reprod. Fert. 40:205-209.

90. Robinson, J.S., Challis, J.R.G., Furr, B.J.A., Louis, T.M.m and Thorburn, G.D. (1976) Is the sheep corpus luteum subject to tonic inhibition during the luteal phase of the estrous cycle? Europ. J. Obstet. Gynec. Reprod. Biol. 4:191-199.

91. Roberts, J.S., McCracken, J.A., Gavagen, J.E., and Soloff, M.S. (1976) Oxytocin stimulated release of $PGF_{2\alpha}$ from ovine endometrium in vitro: Correlation with the estrous cycle and oxytocin receptor binding. Endocrinology 99:1107-1114.

92. Newcomb, R., Booth, W.D., and Rowson, E.A. (1977) Effect of oxytocin treatment on levels of prostaglandin F (PGF) in blood of heifers. J. Reprod. Fert. 49:17-24.

93. Small, M.G.P., Gavagan, J.E., and Roberts, J.S. (1978) Oxytocin stimulated production of $PGF_{2\alpha}$ by isolated endometrium of rabbit: Modulation by ovarian steroids. Prostaglandins 15:103-112.

94. Bills, T.K., Smith, J.B., and Silver, M.J. (1976) Metabolism of ^{14}C-arachidonic acid by human platelets. Biochem. Biophys. Acta 424:303-314.

95. Yeagle, P.L., Hutton, W.C., Martin, R.B., Sears, B., and Huang, C.-H. (1976) Transmembrane asymmetry of vesicle lipids. J. Biol. Chem. 251:2110-2112.

96. Hill, E.E., and Lands, W.E.M. (1970) Phospholipid metabolism, in Lipid Metabolism, ed. by S.K. Wakil, p. 185-277. Academic Press, New York.

97. Blackwell, G.J., Duncombe, W.G., Flower, R.J., Parsons, M.F., and Vane, J.R. (1977) The distribution and metabolism of arachidonic acid in rabbit platelets during aggregation and its modification by drugs. Br. J. Pharmacol. 59:353-366.

98. Hong, S.-C.L., and Levine, L. (1976) Stimulation of prostaglandin synthesis by bradykinin and thrombin and their mechanism of action on MC5-5 fibroblasts. J. Biol. Chem. 251:5814-5816.

99. Hong, S.-C.L., and Levine, L. (1976) Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. Proc. Nat. Acad. Sci. (USA) 73:1730-1734.

100. Chanderbahn, R., Hodges, V.A., Treadwell, C.R., and Vahouny, G.V. (1977) ACTH-induced synthesis of prostaglandin E_2 in adrenal cortical cells. Fed. Proc. 36:6714 (#2123).

101. Nissen, H.M., and Bojesen, I. (1969) On lipid droplets in renal interstitial cells. IV. Isolation and identification. Z. Zellforsch 97:274.

102. Muirhead. E.E.. Germain, G.S., Leach, B.E., Pitcock, J.A., Stephenson, P., Brooks, B., Brosius, W.L.,Jr., Daniels, E.G., and Hinman, J.W. (1973) Synthesis of prostaglandins by renomedullary interstitial cells (RIC) growth in tissue culture. Adv. Biosciences 9:341-347.

103. Zusman, R.M., and Kaiser, H.R. (1977) PGE_2 biosynthesis by rabbit renal medullary interstitial cells in tissue culture. J. Biol. Chem. 252:2069-2071.

104. Zusman, R.M., and Kaiser, H.R. (1977) Prostaglandin biosynthesis by rabbit renomedullary interstitial cells in tissue culture. J. Clin. Invest. 60:215-223.

105. Minkes, M., Stanford, N., Chi, M.M.-Y., Roth, G.J., Raz, A., Needleman, P., and Majerus, P.W. (1977) cAMP inhibits the availability of arachidonate to prostaglandin synthetase in human platelet suspensions. J. Clin. Inves. 59:449-454.

106. Kuehl, F.A., Jr., Cirillo, V.J., Zanetti, M.E., Beveridge, G.C., and Ham, E.A. (1976) Effect of estrogen upon cyclic nucleotide and prostaglandin levels in rat uterus. Adv. Prostaglandin and Thromboxane Res. 1:313-343.

107. Ham, E.A., Cirillo, V.J., Zanetti, M.E., and Kuehl, F.A., Jr. (1975) Estrogen directed synthesis of specific prostaglandins in the uterus. Proc. Nat. Acad. Sci. 72:1420-1424.

108. Hamberg, M., and Samuelsson, B. (1967) On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxidase. J. Biol. Chem. 242:5329-5335.

109. Hamberg, M., and Samuelsson, B. (1967) On the mechanism of the biosynthesis of prostaglandins E_1 and F_{1a}. J. Biol. Chem. 242:5336-5343.

110. Hamberg, M., and Samuelsson, B. (1967) Oxygenation of unsaturated fatty acids by vesicular gland of sheep. J. Biol. Chem. 242:5344-5354.

111. Nugteren, D.H., and Hazelhof, E. (1973) Isolation and properties of intermediates in prostaglandin biosynthesis. Biochem. Biophys. Acta 323:448-461.

112. Hamberg, M., and Samuelsson, B. (1973) Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. Proc. Nat. Acad. Sci. 70:899-903.

113. Hamberg, M., Svensson, J., Wakabayashi, T., and Samuelsson, B. (1974) Isolation and structure of two prostaglandin endoperoxidases that cause platelet aggregation. Proc. Nat. Acad. Sci. 71:345-349.

114. Samuelsson, B. (1965) On the incorporation of O_2 in the conversion of 8, 11, 14 eicosatrienoic acid to PGE_1. J. Am. Chem. Soc. 87:3011-3013.

115. Lands, W.E.M., Hemler, M., and Crawford, J. (1977) Characteristics of cyclooxygenase mechanisms. Prostaglandins 14:181-182.

116. Smith, W.L., and Lands, W.E.M. (1971) Stimulation and blockade of prostaglandin biosynthesis. J. Biol. Chem. 246:6700-6704.

117. Smith, W.L., and Lands, W.E.M. (1972) Oxygenation of polyunsaturated fatty acids during prostaglandin biosynthesis by sheep vesicular gland. Biochemistry 11:3276-3285.

118. Smith, W.L., and Wilkin, G. (1977) Immunochemistry of prostaglandin endoperoxide forming cyclooxygenase: Detection in rat, rabbit and guinea pit kidneys by immunofluorescence. Prostaglandins 13:873-892.

119. Cook, H.W., and Lands, W.E.M. (1976) Mechanism for suppression of cellular biosynthesis of prostaglandins. Nature 260:630-632.

120. Chandrabose, K.A., Lapetina, E.G., Schmitges, C.J., Siegel, M.I., and Cuatrecacas, P. (1978) Action of cortico-steroids in regulation of prostaglandin biosynthesis in cultured fibroblasts. Proc. Nat. Acad. Sci. 75:214-217.

121. Hamberg, M., Svensson, J., and Samuelsson, B. (1975) Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Nat. Acad. Sci. 72:2994-2998.

122. Hamberg, M. (1976) On the formation of thromboxane B_2 and 12 L hydroxy 5,8,10,14 eicosatetraenoic acid (12ho20:4) in tissues from guinea pig. Biochem. Biophys. Acta 431:651-654.

123. Hamberg, M., and Samuelsson, B. (1974) Prostaglandin endoperoxides. VII. Novel transformations in guinea pig lung. Biochem. Biophys. Res. Commun. 61:942-949.

124. Wolfe, L.S., Rostwaroski, K., and Marion, J. (1976) Endogenous formation of the prostaglandin endoperoxide metabolite, thromboxane B_2 by brain tissue. Biochem. Biophys. Res. Commun. 70:907-913.

125. Samuelsson, B. (1976) Metabolic transformation of arachidonic acid. Prostaglandins 11:431-432.

126. Pace-Asciak, C.R. (1976) A new metabolite of arachidonic acid. Formation of 6KetoPGF$_{1\alpha}$ by rat stomach. Experientia 32:291-292.

127. Moncada, S., Gryglewski, R., Banting, S., and Vane, J.R. (1976) A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (PGX) which prevents platelet aggregation. Prostaglandins 12:715-737.

128. Moncada, S., Gryglewski, R., Banting, S., and Vane, J.R. (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263:663-665.

129. Pace-Asciak, C.R. (1977) Oxidative biotransformations of arachidonic acid. Prostaglandins 13:811-817.

130. Grenier, F.C., and Smith, W.L. (1978) Formation of 6-Keto-PGF_{1a} by collecting tubule cells isolated from rabbit renal papillae. Prostaglandins 16:759-772.

131. Lee, R.E., and Lands, W.E.M. (1972) Cofactors in the biosynthesis of prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$. Biochem. Biophys. Acta 260:203-211.

132. Chan, J.A., Nagasawa, M., Takeguchi, C., and Sih, C.J. (1975) On agents favoring PGF formation during biosynthesis. Biochemistry 14:2987-2991.

133. Yoshimoto, A., Ito, H., and Tomito, K. (1970) Cofactor requirements of the enzyme synthesizing prostaglandin in bovine seminal vessels. J. Biochem. 68:487-499.

134. Myamoto, T., Yamamoto, S., and Hayaishi, O. (1974) Prostaglandin synthetase system - resolution into oxygenase and isomerase components. Proc. Nat. Acad. Sci. 71:3645-3648.

135. Sun, F.F., Chapman, J.P., and McGuire, J.C. (1977) Metabolism of prostaglandin endoperoxide in animal tissues. Prostaglandins 14:1055-1074.

136. Downing, I., and Williams, K.I. (1977) Differential prostaglandin production by microsomal fractions of rat pregnant uterus. Proc. B.P.S., July 13-15, 1977 p. 158P.

137. Williams, K.I., Sneddon, J.M., and Harney, P.J. (1974) Prostaglandin production by pregnant rat uterus in vitro and its relevance to parturition. Pol. J. Pharmacol. Pharm. 26:207-215.

138. Williams, K.I., Dembinska-Kiec, A., Zmuda, A., and Gryglewski, R.J. (1978) Prostacyclin formation by myometrial and decidual fractions of the pregnant rat uterus. Prostaglandins 15:343-350.

139. Fenwick, L., Jones, R.L., Naylor, B., Poyser, N.L., and Wilson, N.H. (1977) Production of prostaglandins by the pseudopregnant rat uterus in vitro and the effect of tamoxifen with the identification of 6-keto-prostaglandin $F_{1\alpha}$ as a major product. Br. J. Pharmacol. 59:191-199.

140. Jones, R.L., Poyser, N.L., and Wilson, N.H. (1977) Production of 6-oxo-prostaglandin $F_{1\alpha}$ by rat, guinea pig and sheep uteri, in vitro. Proc. B.P.S., January 5-7, 1977, p. 436P-437P.

141. Cook, C., and Collins, A.J. (1975) Prostaglandin synthetase activity from human rheumatoid synovial tissue and its inhibition by non-steroidal anti-inflammatory drugs. Prostaglandins 9:857-866.

142. Egan, R.W., Humes, J.L., and Kuehl, F.A., Jr. (1978) Differential effects of prostaglandin synthetase stimulators on inhibition of cyclooxygenase. Biochemistry 17:2230-2234.

143. Smith, W.L., and Bell, T.G. (1978) Immunohistochemical localization of the prostaglandin-forming cyclooxygenase in renal cortex. Am. J. Physiol. 235:F451-F456.

144. Kessler, S.W. (1975) Rapid isolation of antigens from cells with a staphylococcal protein A-antibody absorgant: Parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. 115:1617-1624.

145. Kessler, S.W. (1976) Cell membrane antigen isolation with the staphylococcal protein A-antibody absorbant. J. Immunol. 117:1482-1490.

146. Campbell, D.H., ed. (1979) Methods in Immunology: A Laboratory Text for Instruction and Research, 2nd ed. W.A. Benjamin, New York, p. 224-233.

147. Anggard, L., Bohman, S.O., Griffin, J.E., III, Larrson, C., and Maunsbach, A.B. (1972) Subcellular localization of the prostaglandin system in rabbit renal papilla. Acta Physiol. Scand. 84:231-246.

148. Tai, H.-H. (1976) Mechanism of prostaglandin biosynthesis in rabbit kidney medulla. Biochem. J. 160:577-583.

149. Shemesh, M., and Hansel, W. (1975) Stimulation of prostaglandin synthesis in bovine ovarian tissues by arachidonic acid and luteinizing hormone, Biol. Reprod. 13:448-452.

150. Erickson, G.F., Challis, J.R.G., and Ryan, K.J. (1977) Production of prostaglandin F by rabbit granulosa cells and thecal tissue. J. Reprod. Fert. 49:133-134.

151. Koligian, K.B., and Stormshak, F. (1977) Nuclear and cytoplasmic estrogen receptors in ovine endometrium during the estrous cycle. Endocrinology 101:524-533.

152. Wlodawer, P., Kindahl, H., and Hamberg, M. (1976) Biosynthesis of prostaglandin $F_{2\alpha}$ from arachidonic acid and prostaglandin endoperoxides in the uterus. Biochem. Biophys. Acta 431:603-614.